## CONSERVATION OF

THE GENETIC RESOURCES OF FISH: PROBLEMS AND RECOMMENDATIONS

Report of the Expert Consultation on the Genetic Resources of Fish Rome, $9-13$ June 1980

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 Problems and RecommendationsReport of the Expert Consultation on the Genetic Resources of Fish Rome, 9-13 June 1980

## CORRIGENDUM

1. Page 32, 7.4, paragraph 2, second and fifth lines: Replace the words "aquatic parts" with "aquatic parks".
2. Page 39, Appendix 1, List of Participants: Delete Dr. Fred Allendorf (unable to attend) and insert:

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1196 Gland, Switzerland
3. Page 41, Appendix 2, paragraph III: Replace "Service:" with "Other services:".
4. Page 43, Appendix 3, paragraph 3, first line: Replace "UNEP and Unesco" with "UNEP, FAO, IUCN and Unesco".

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## Preparation of this Report

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#### Abstract

The report reviews arguments for the preservation of the genetic resources of fish and various strategies for accomplishing this in both marine and continental waters. It reviews the significance of genetic diversity, especially within populations and subpopulations, to the viability of aquatic ecosystems and to the fitness of fish populations, and discuss the various means by which genetic impoverishment comes about. The report emphasizes the importance of maintaining breeding populations of an effective size of at least 50 for short-term fitness and of at least 500 for long-term survival; and of avoiding "genetic bottlenecks" created by reduction of breeding populations to small size for one or more generations.

The report also considers available technologies for the monitoring, preservation and enhancement of genetic resources in closely managed fish stocks. It concludes by presenting recommendations addressed to five different groups: these are international organizations, governments, aquaculturists and fishery managers, conservationists and research scientists.


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## 1. INTRODUCTION

### 1.1 Historical Background of International Action

The need for conservation of fish genetic resources has been recognized by fishery scientists and aquaculturists for some time, especially in relation to overfishing of natural stock, the effects of largescale alterations to river systems, and domestication of species through aquaculture. The FAO World Symposium on Warm Water Pond Fish Culture (Rome, 1966) stressed the importance of genetic selection and hybridization in improving fish varieties for culture and noted problems of excessive inbreeding on carp farms. Need for an international system of designating strains and stocks was also recognized. In 1971 , FAO established an ad hoc Working Party on Genetic Resources of Fish, which reviewed progress in genetic selection for fish farming, identified priority areas for research and made several recommendations for the conservation of fish genetic resources. Included were suggestions that methods for doing so needed urgent study, that a catalogue of threatened genetic resources of potential use to aquaculture should be prepared, and that the collection of wild species of potential usefulness should be undertaken. These actions have not taken place, largely because there has been no consensus on the criteria that are needed to limit such work to manageable proportions. The FAO Technical Conference on Aquaculture, held in Kyoto, Japan, 26 May to 2 June 1976, reaffirmed the need to maintain the genetic diversity in artificially propagated stocks, noted that indiscriminate transfer of fish and shellfish have, in some cases, had adverse effects on indigenous stocks, and called for increased research on fish genetics as there was a substantial lack of information on this subject, making the formulation of selective breeding programmes difficult.

One strategy for conserving the diversity of natural populations of fish and other organisms is to set aside aquatic reserves. The International Union for Conservation of Nature and Natural Resources (IUCN) has been active in promoting marine parks, and with the cooperation of the Government of Japan, the World Wildlife Fund and UNEP, held an International Conference on Marine Parks and Reserves in Tokyo, 12 to 14 May 1975. This conference reviewed the conclusions and recommendations of earlier regional and world conferences concerned with the marine environment and proposed, inter alia, that IUCN and other concerned agencies "develop a coordination strategy to satisfy forthcoming requests for assistance /To developing countries in the establishment of marine parks/", and "set up teams to undertake appropriate surveys to enable systems of marine parks and reserves to be based on the best available data, to assist in the formulation of appropriate conservation policies relating to marine parks and reserves, and to identify those projects suitable for bilateral and/or other technical cooperation programmes".

The United Nations Conference on the Human Environment, Stockholm, 1972, produced recommendations emphasizing, inter alia, the need for conservation of all genetic resources. In its third session (1975) the Governing Council of the United Nations Environment Programme requested, among other things, the preparation of an overview on the problems of the conservation of genetic resources as part of an overall report on the conservation of genetic resources. The Food and Agriculture Organization, through a cooperative project with UNEP (FP/1108-75-01) prepared a paper which included a brief review of the problems and requirements for action on fish resources (UNEP/PROG./4). This overview has now been revised and produced by UNEP as UNEP Report No. 5 (1980), "An Overview of Genetic Resources". The report emphasized that the basic constraint is lack of knowledge and recommended, among other things, the need for a mechanism for monitoring changes in the genetic diversity of fish populations, for promotion of research directed at creation of knowledge on the genetics of fish which would assist in a more applicable definition of genetic impoverishment in fish species and for promotion of research on appropriate methodologies for conservation.

During discussions held in FAO, Rome, in June 1979, it was agreed that the variety of needs and problems associated with this aspect of genetic conservation could not be adequately reviewed and assessed by a single individual. It was proposed that a group of experts be convened to accomplish this review and propose a balanced and practical programme of action.

It was then agreed that FAO would organize an expert consultation, in cooperation with UNEP, and with the participation of Unesco and IUCN, to be held in Rome, 9-13 June 1980.

### 1.2 Objectives and Terms of Reference

The purpose of the consultation, as stated in a prospectus sent to prospective participants, was:
(1) to consider in depth the problems necessitating the conservation of fish genetic resources;
(2) to develop a scientific consensus on the needs and on a strategy to solve these problems;
(3) to propose an action plan and appropriate methodology to carry it out.

Subsequently, it became evident that the consultation needed to focus primarily on the scientific aspects of the problem, considering especially the nature of the threats to genetic diversity of fishes, both among and within species, methodologies for monitoring and assessing change in genetic diversity, and identifying feasible actions which might be taken to conserve diversity. The development of a comprehensive and coherent action plan for implementation by governments was seen as a subsequent step. Thus, in the course of the consultation, the experts were urged to address recommendations to several different kinds of people or institutions: scientists, conservationists, fishery managers and aquaculturists, governments and international organizations.

### 1.3 Nature Conservation Versus Genetic Resource Preservation (GRP)

The objective of nature conservation is to preserve for posterity as much as possible of the earth's biological and ecological diversity. The justifications of such an attitude span a very broad range of human thought and experience - from divinely inspired ethics to short-term economic and political gain. At the one extreme some people argue that morality dictates the right of all species to persist and evolve, and that man's power to alter and destroy the biosphere does not give him the ethical licence to do so. On the other hand, there are those who argue from the standpoint of human welfare - that the preservation of biological diversity is only common sense, and that the prudent strategy is to keep the options open by preserving genetic diversity in order to sustain production in agriculture, forestry, fisheries and other natural products and to minimize the threats of ecological disasters such as climatic alteration, erosion, siltation, desertification and pollution.

While there is no "best" approach or justification, there are appropriate ones. Genetic resource preservation is one such tactic. The approach taken by proponents of Genetic Resource Preservation is pragmatic. As a matter of human welfare, it is essential that production of natural resources increases. Fish are an important source of protein and other valuable organic products. Therefore, the protection and exploitation of options for the improvement of fisheries and aquaculture is a high social priority. The accomplishment of these goals depends to a large and increasing extent on technology and science and it is the role of genetics in this effort to which this report is directed. An interest in and knowledge of the role of genetics in the enhancement of fisheries production does not necessarily make one a conservationist. But such knowledge will make a more effective soldier in the battle to feed the rapidly growing human population.

### 1.4 An Important Distinction: Extinction Versus Genetic Impover ishment

It is convenient and heuristic to distinguish two processes by which genetic resources are lost; these are (1) the extinction of a species and (2) the reduction of genetic variation within a species. The former process, once it occurs, is qualitative, final and irreversible. The latter process is a matter of degree, and is to some extent reversible.

This report discusses aspects of both processes because both are clearly relevant to the maintenance of options in fisheries. Nevertheless, the relevance to exploitation of these two processes depends on the stage of development, its intensity and the degree of human control in the fish extraction enterprise. These problems will be dealt with throughout the report.

A realistic perspective on the process of extinction and its current status is essential for those in the resource fields. Of special import is the current change in rate of extinction. Barring periods of dramatic climatic swings such as the Pleistocene, both the origin and extinction of species have probably gone on at similar rates for hundreds of millions of years. Now, however, due to habitat destruction by man, extinction rates are probably three or more orders of magnitude higher than ever
before (Myers, 1979; IUCN-UNEP-WWF, 1980), while the rate at which new species of large organisms appear, especially in the tropics, is approaching zero (Soule, 1980). Hence for groups of organisms that occupy disappearing habitats, it is not likely that organic diversity will ever again reach its present levels.

For fishes the situation may not appear to be so severe, but one must specify the place and the group. Habitat destruction in the oceans is not yet appreciable. At least no significant or detectable increase in extinction rates has been observed in the oceans (although populations have been extinguished from overfishing and pollution). For other aquatic habitats, however, the situation is already deteriorating rapidly. The issue boils down to one of disparate time scales. The time scale for pollution, exploitation or development is measured in years, decades or centuries, whereas that for speciation is usually measured in much longer intervals.

For example it may only require ten years to dam a river, thus creating a habitat inimical to the existing riverine fauna dependent on periodic flooding or moving water. On the other hand, the lifetime of the resulting reservoir will be too short by thousands of years for the evolution of lacustrine forms of fish.

Similar arguments could be made for the effects of pollution and siltation on rivers, lakes, estuaries, lagoons and reef habitats. Obviously, it is naive to entertain the sanguine expectation that evolution of new species will compensate for the loss of biological variety during the next few decades.

### 1.5 The Case for Genetic Resource Preservation in Fish

The assumption that underlies the discussion in this section is that the manner in which a resource is exploited is as important in the long run as the degree to which it is exploited. More explicitly, the way in which fish populations are managed will largely determine the success of the enterprise. This comes as no surprise to fisheries biologists, but there is a dimension to management that has been relatively ignored - the genetic dimension. One of the objectives of this report is to underscore this dimension and by so doing, to anticipate and thereby prevent the very serious loss of options that has already occurred in agriculture (National Academy of Sciences /ŪS//, 1972; Heslop-Harrison, 1974), and to a lesser extent in animal husbandry (Frankel and Soule, 1981; $\overline{\mathrm{F} A O}, 1975$ ).

### 1.5.1 Nutritional arguments for Genetic Resource Preservation

Fish flesh is an important source of high grade animal protein. With a growing world population and rising expectations for living standards, the pressures on fish both as a source of food and for its byproducts can only increase in the future. At present fish and aquatic animals constitute 17 percent of the total animal protein in the human diet. This statistic conceals wide regional differences in fish consumption. For example, 32 countries obtain 34 percent or more of their animal protein from seafood. On the African continent, ten countries obtain over 40 percent of their protein from fish. In 21 countries on the same continent over half of the fish landed comes from inland lakes and rivers, and in 13 , the fish supply is entirely from inland sources.

The potential for further growth of natural marine and freshwater fisheries is limited. Already the total world harvest is $15-20$ million tons lower than it might have been had fishing been strictly controlled through scientific management, and 25 valuable fisheries have been seriously depleted by overfishing. Other factors which limit the contribution of marine fish to the diet include problems of distribution, storage and cultural inhibitions to its consumption.

Intensive fish farming can, in principle, overcome two of these limitations. Fish farming activities may often be sited near to the markets in which the product is sold. They also avoid the depletion of biological resources except where still dependent on the collection of seed from the wild. Fish farming not only allows the products to be delivered directly to their markets but it also represents both a renewable and inexhaustable resource. Aquaculture is still in a relatively early stage of development and the production of new strains and races of food fishes will require careful management of available genetic resources. The lesson learnt from extensive breeding in crop plants and in domestic animals is that a narrowing in the genetic base of the species is inevitable. In such processes, genetic determinants which are likely to be lost at an early stage include those controlling disease-resistance
and fitness in marginal environments. It is hence prudent for breeders to be especially mindful of the need to protect and preserve, at the earliest stage possible, broad genetic diversity within those species most likely to come under intensive breeding pressure.

### 1.5.2 Economic arguments for Genetic Resource Preservation

Fisheries not only provide a significant portion of the protein available for human consumption they are also an economically significant activity, providing jobs and investment opportunities, and, for many countries, a means of improving the balance of international trade.

Certain species of fish are, by virtue of special or unusual biological features, useful as experimental animals and as a source of biochemical and pharmacological agents. Often species and genetic traits to be preserved are not the same as those for food fishes. Since only a handful of the 25000 plus species of fish have been subject to scientific scrutiny the extinction of any species may represent a potential loss of an economic resource.

Substances isolated from fish and other aquatic animals are already widely used in medical research. For example, tetradotoxin (TTX), a toxin isolated from the puffer fish, Tetraodon immaculata, is used in neurophysiological research as a specific sodium channel blocker and is playing an important part in reaching an understanding of the basic ionic mechanisms of nervous transmission.

Another class of compound used as a research tool is luminescent ${ }_{2}$ proteins. For example, Aqyeorin from a jellyfish species has been widely used to monitor the $\mathrm{Ca}^{2+}$ concentration in cells. $\mathrm{Ca}^{2+}$ serves as an important signal in transmitter/hormone release, and in excitation contraction and excitation-secretion coupling. An understanding of such processes is important in the development of new drugs and in the treatment of disease. It is likely that fish toxins, hormones, glycoproteins and naturally occurring polypeptides constitute a large and relatively unexplored reservoir of compounds of equal pharmacological interest.

Other natural products, notably oils and waxes, have long established industrial uses, e.g., for vitamin enhancement of animal feedstuffs. New applications for these byproducts might include substitute natural bases for medications and cosmetics. In many countries fish meal is already widely used for feeding domestic animals and as a major source of fertilizer.

In cases where species are threatened with extinction, establishing the importance of natural products remains an issue of high priority. The economic and social benefits provided by such discoveries, however, go far beyond the immediate value of the fish as an economic resource. The history of natural resource utilization tells us that the ultimate value of any genetic resource may not be appreciated at the present time.

Nor is it sufficient to stave off extinction by preserving a small handful of individuals. It is not uncommon that the genetic determinants are not evenly distributed within the species - in quantitative or even qualitative terms. This presents a strong argument for preserving broad genetic diversity within a species, if its socio-economic potential is to be realized.

Another important area of economic activity relates to sport fishing. Increasingly, freshwater sport fisheries are 'scientifically' managed and restocked from hatchery populations. Freshwater fishing in countries such as Ireland and marine sport fisheries in various tropical countries are a major factor in the development of tourism. Thus sport fisheries can increase employment and support and stimulate a wide range of ancillary economic activity. The creation of classes of marine reserves where controlled fishing is permitted may provide a valuable way of safe-guarding the species diversity of coastal areas while providing a good economic return from anglers and day visitors.

In North America, Europe and South-East Asia, the ornamental aquarium trade is a major industry. For example, the wholesale turnover of tropical fish in Florida (U.S.A.) alone in 1974 was in excess of $\$ 30$ million (Courtenay et al., 1974). Many of the most popular species come from the Amazon basin and the Great Lakes of Africa. These fish of ten occur in small discrete sub-populations which live in highly specialized or localized habitats and are thus extremely vulnerable to extinction.

Properly guided and encouraged, the culture of such species by hobbyists could be one way of preserving this resource for future generations. However, owing to the danger of inbreeding, in situ conservation is much more likely to be successful. Salt water coral reef fishes provide similar potential for income as well as similar problems for the long-term protection of the resource.

Certain species of fish are now being seeded into lakes and rivers for use in the control of weeds and insect pests. This may have significance in the elimination of vector-borne disease. While this is socially and economically desirable attention should be paid to the effects on indigenous fish populations (see Section 5.3).

In summary, in order to keep our economic options open, it is desirable to implement aggressive programmes that operate on both levels of genetic resource preservation - programmes that (1) minimize the rate of species extinction by mitigating habitat disruption and that (2) minimize the erosion of genetic variability of species in no immediate danger of extinction, especially of those which already play major economic roles in human survival.

### 1.5.3 Ecological arguments for Genetic Resource Preservation

The principle ecological argument for the preservation of fish genetic resources is, in a sense, the logical converse of the preceding arguments. It is as follows: Stability of ecological systems and the maintenance of biological (taxonomic) diversity is a universally acknowledged value, although social and economic considerations may often be granted a higher priority. One important means of maintaining stability and diversity is the maintenance of fitness in species, particularly the dominant, high trophic level consumers. The depletion or extinction of such species constitutes the loss of genetic resources and is a threat to the integrity of ecosystems.

Habitat protection is the best way to insure the survival of ecologically important fish species. Habitat destruction is particularly disastrous when local endemic species are involved because the elimination of an entire species is an irreversible process (see Section 1.4). We expect this to be an increasing problem, particularly in tropical areas where endemics with narrow habitat requirements abound. While some decay in water quality and habitat diversity can be tolerated, there is probably a point at which even partial destruction of habitat integrity can reach genetic and ecological thresholds that result in the precipitation of extinctions. Some tropical ecologists believe that the extinctions of certain key species will bring about a rather sudden cascade of linked extinctions (Gilbert, 1980; Terborgh and Winter, 1980). In summary, a concern for survival of species, and the development of appropriate preservation programmes and guidelines, will indirectly benefit man by protecting entire ecosystems and all their attendant economic and ecological values (World Conservation Strategy, 1980).

## 2. LEVELS AND METHODS OF GENETIC RESOURCE PRESERVATION IN FISH

From the point of view of the preservation of the genetic resources of fish, four main levels of concern and strategy can be identified (Table 1): (1) oceanic systems, (2) continental waters (both fresh and marine), (3) aquaculture and (4) stock enhancement programmes. The last two levels of concern are directly controlled by man, whereas the first two can be regarded as uncontrolled systems, although human activities can have major impacts on them.

### 2.1 Human Impacts on Aquatic Habitats

There are a number of characteristics which clearly differentiate oceanic systems from continental waters as far as genetic resource preservation is concerned. Firstly, the much greater size of oceanic systems, in terms of both physical dimensions and biomasses involved, make them much more difficult to manage. It must be remarked, however, that a few of the larger river basins (e.g., in South America and Africa) and their exploited fish biomass approach oceanic dimensions.

The effects of meteorological and especially hydrological cycles are usually much more crucial in continental waters where they have a strong influence on fish populations - especially as far as reproductive migrations or strategies are concerned. The combination of these characteristics means

## Table 1.

Potential Genetic Problems
Sub-population extinction
Sub-population extinction
Species extinction
Genetic erosion
Restocking
Controlled breeding
Hybridization
Cryopreservation
Controlled breeding
Hybridization
Cryopreservation
หวołs paэnpoxiu! fo uo!
The scope of genetic resource preservation in fish

> Establish reserves
Scientific managem
> Tagging and monitoring
> Scientific management of reserves
Controlled breeding
Hybridization
Cryopreservation
Controlled breeding
Hybridization
Cryopreservation
Introgression with wild stock
Level
Oceanic fisheries
Continental habitats
Stock enhancement
that both natural and man-induced stresses have a relatively greater impact in continental systems. Included in this group are intertidal habitats, coral reef systems, brackish waters and fresh waters.

Biologically, these conditions give rise to two further distinctions which are relevant to genetic resource conservation: (a) continental areas have very much higher numbers of endemic and locally distributed species and sub-populations than oceanic systems, and (b) the lower impact of stresses on pelagic oceanic species, and their high fecundity, make their extinction unlikely. At low abundance levels their exploitation will be uneconomical, whilst in enclosed continental systems many species could, and indeed have, disappeared.

In oceanic systems, the main changes are produced by the direct but selective exploitation through fisheries. Such exploitation can have two different effects on a considered species or population. One is a direct effect on the population structure of the target species by selective removal of a particular sector. The other is an indirect effect on non-target species, where the fishery may affect the food chain by, for example, upsetting established interactive relationships.

In continental waters the same effects can occur but of ten to a much greater degree and whole populations can become extinct, solely through over-fishing. Here, however, other important stresses occur, notably pollution, disruption of life history cycles and disruption of ecosystem structure. Examples include the introduction of foreign predators, competitors and pathogens.

Among the different kinds of pollution, air-borne pollution leading to acid precipitation probably has had the most devastating (and sometimes selective) effect on fish populations particularly in southern Scandinavia and the north-eastern and north-central U.S.A. The direct pollution of waters through the discharge of various chemicals (some of them mutagenic) as wastes or the run-off of agricultural chemicals is also of major importance. Land-use may also have highly undesirable effects such as the production of very acid soils (and run-off) and the silting of streams.

Another serious human impact is the disruption of biological (in most cases reproductive) cycles in fish populations as a consequence of building dams or other obstructions on rivers where migratory species occur. Dams may not only prevent the migration to upstream spawning grounds, but they may also change rivers into semi-lacustrine habitats quite unsuitable for stream species. Ecological barriers are also common and may be caused by zones of pollution in the lower reaches of rivers preventing the migration of various species.

The introduction of exotic species may also be included as a factor of importance to endemic fish populations (see Section 5.3). They may lead to the introduction of disease, higher levels of predation, or so affect the ecosystem (e.g., by competition for food) as to cause the extinction of local species. It is very rare to find introductions which have filled a totally empty niche, especially in the tropics, so introductions are highly likely to result in changes in the endemic populations.

Compared to oceanic and continental systems, aquacultural, hatchery and stock enhancement programmes are subject to an even greater degree of human control by definition. Within such systems, direct environmental and genetic management can and should be imposed and should only be limited by economic criteria and technological sophistication.

### 2.2 Ways and Means of Genetic Resource Preservation

As shown in Table 1, a strategy for genetic resource preservation is largely dictated by the type of aquatic system. This section briefly outlines the approaches relevant to the various kinds of systems. Technical details are described in Section 5.

### 2.2.1 Oceanic systems

For any oceanic fish species, one of the first requirements for the formulation of a preservation policy must be a distribution map and the identification of any distinct sub-populations which there may be. Distribution data will probably be available from relevant fisheries, but the detection of subpopulations may have to be carried out by the use of one or more of the 'labelling' techniques available
(e.g., tagging, the identification of meristic characters, electrophoresis, etc.). The importance of detecting sub-populations is that, although the danger of the extinction of oceanic species through overfishing is probably very small (the assumption being that the fishery will disappear before the species), it is perfectly possible that a unique sub-population could disappear. (Incidentally, the practice of a rushed, published description of a local population as a new sub-species, merely for the purpose of forestalling development, is a legalistic device that will probably prove counter-productive, since it is likely to be abused.)

Significant changes within an exploited population can only be detected through a continuing programme of monitoring designed to study the overall population structure, its composition and its change. The decline in any part of the population can probably only be controlled by regulation of the fishery, either through reduction of exploitation in space or time, or by altering the nature of the gear used.

The effect of fishing on the genetic diversity of oceanic populations is discussed below (Section 3.5). If there is evidence of undesirable genetic changes in any species because of selection imposed by the fishery, it would be ideal to try to reverse the trend by appropriate management tools.

### 2.2.2 Continental waters

One of the enormous problems related to smaller enclosed systems, especially in the tropics, is the identification of the species involved. In parts of Africa, Asia, and especially in South America, large numbers of endemic and very locally distributed fish species occur and many are undescribed. Thus an essential pre-requisite to any broad programme of genetic resource preservation is a proper taxonomic study of the fish species occurring in each area and a full check-list of these species, indicating the status of each, and, if possible, its significance in ecological, economic, scientific and social terms. Such lists would provide the basis of any international list of endangered species to be included in the Red Data Book published by the IUCN.

Because of the high probability of the loss of genetic diversity in any species taken into culture (Section 3.4), the best method of maintaining this diversity is to conserve self-maintaining populations in natural habitats. The normal procedure here would be to establish nature reserves (lakes, river basins, estuaries, coastal lagoons, coral reefs, etc.) which already contain one or more populations of the fish species or communities concerned. These reserves could well be multi-purpose areas, conserving several other types of habitat and communities in addition to the fish resource, e.g., National Park of Sabana Grande, Venezuela.

In some situations an individual species or its habitat may be so threatened that the only option is to collect stock and transfer this to an aquaculture system, or preferably, an alternative suitable location to create a new population. Ideally this new location should be within a nature reserve or in an area not liable to man-made pressures. The refuge so created can later be used to re-stock the original habitat if conditions there improve. This strategy is being used at present in both Canada and Scotland (Maitland, 1979) to preserve local populations of whitefish (Coregonus). Aquaculture techniques may be used on a short-term basis to enhance local stocks which are subject to temporary recruitment problems.

By definition, it is essential that man-induced stresses be strictly regulated in any established nature reserve. This includes control not only of fishing effort and gear, water quality degradation, barriers to migration, etc., but also of introduction of exotic fish or any other species likely to have a harmful impact on the ecosystem (see Section 3.8). The wild trout and charr watch concept, as recently proposed by Regier and Powers (1979), may prove to be one useful way of developing international monitoring schemes for important species or groups of fish.

Once aquatic reserves are established, scientific management to prevent degradation and loss of diversity is absolutely essential. In terrestrial ecosystems, management of nature reserves is a new and often contentious discipline, but the high rate of extinctions for vertebrate species in terrestrial reserves requires the immediate attention of managers and consultants (Frankel and Soule, 1981). Fishery and aquatic reserve managers would be well advised to consider this problem at the outset.

## 3. GENERAL PRINCIPLES OF GENETIC RESOURCE PRESERVATION

### 3.1 The Importance of Genetic Variation

Genetic variation is the raw material in species populations which enables them to adapt to changes in their environment. New genetic variation arises in a population from either spontaneous mutation of a gene or by immigration from a population of genetically different individuals. Alternate forms of a particular gene (or locus) are called "alleles". The number and relative abundance of alleles in a population is a measure of genetic variation, sometimes termed "heterozygosity". Genetic variation is a measure of a population's ability to adapt to environmental change or stress, and thereby to survive.

Population geneticists have spent several decades establishing the importance of genetic variation in natural populations. It is known, for example, that the response to natural selection by experimental populations is accelerated by mutation-inducing radiation and/or the introduction of genes from different strains. In terms of the preservation of genetic resources, we can expect that benefits would be derived from maintaining the maximum level of genetic variation in a strain as well as by maintaining multiple strains that could serve as additional sources of genetic information by hybridization. It follows that the loss of genetic variation for whatever reason (e.g., prolonged selection, inbreeding, isolation) will result in a decrease of the potential adaptability of a population.

It appears that the benefits of multigene heterozygosity are universal in outbreeding organisms (see Soule, 1980, for a review). In several organisms, including some fish species, individuals possessing the most genetic variation have been shown to have better survival rates or higher relative growth rates. Relatively heterozygous individuals appear to be more resistant to environmental perturbations during development. Clearly, genetically variable populations have many advantageous characteristics that are absent from genetically impover ished ones.

Over the past several years a rather large body of evidence has accumulated on the biochemical differences between alleles of genes coding for metabolically important enzymes. These biochemical differences emphasise the relationship between genetic and functional diversity. The functional properties of different alleles often reflect a biochemical and genetic adaptation to life in a heterogeneous environment. The extent, however, to which the states of physiological and behavioural traits at the whole animal level can be correlated with genetic and biochemical data is still uncertain. Most workers in the field agree that a proportion of protein polymorphisms have no direct or measurable effect on viability or some other aspect of fitness. Nevertheless, there is significant evidence from studies of individual genes, organisms and populations to substantiate the importance of genetic variation to population adaptability.

### 3.2 Effects of Inbreeding

The selection of small numbers of parents (Section 4.3) can reduce genetic variability. Equally serious is the fact that brood stock may be continually selected from closely related, perhaps full sib individuals. This leads to generation after generation of inbreeding of closely related individuals which very often results in homozygosity for unfavourable genes. The overall result is inbreeding depression.

Inbreeding depression is the loss of fitness (e.g. vigour, viability, fecundity) in connection with the loss of genetic variation due to homozygosity. The evidence that inbreeding is harmful is copious and virtually universal (Allendorf and Utter, 1979; Kincaid, 1976a, b; Kirpichnikov, 1972, and Kosswig, 1973).

A general view of the effects of inbreeding and its relationship to the conservation of genetic resources can be found in Soule (1980) and is treated more extensively by Frankel and Soule (1981). Quoting from the former: "A survey of inbreeding experiments leads to the generalization that increasing the inbreeding coefficient by 10 percent induces a $5-10$ percent decline in a particular reproductive trait." Note that an F value (inbreeding coefficient; see Section 4.2) equal to 10 percent approximates the amount of inbreeding that would theoretically occur in a population of five adults
breeding at random for a single generation, or in a population of 25 adults breeding at random for five generations.

A 5-10 percent decline in fecundity might not appear to be very serious (especially when dealing with such fecund animals as fish) but if the effects of inbreeding depression on other traits (such as viability) are also considered this amount of inbreeding can lower reproductive potential as a whole by 25 percent (e.g., in fowl and swine). Gjederm (1974) showed that a 10 percent increase in the inbreeding coefficient in rainbow trout can result in a 10 percent decline in hatchability, and a 24 percent decline in viability of fingerlings. It should be noted that such independent effects are multiplicative in their impact on total, absolute survival and reproduction. Thus the unavoidable conclusion is that relatively small amounts of inbreeding can do tremendous damage to the reproductive potential and productivity of a fish stock.

Expected inbreeding depression is related to the current inbred state of a population. For example, a very small and isolated lake population of a certain fish species may be highly inbred because of its inherent demographic structure. The inbreeding of such a fish would not be expected to display an inbreeding depression as large as from a previously outcrossed group.

In some breeding systems (e.g., those using gynogenetic techniques, Section 6.2) inbreeding is the goal and its associated "depression" is an undesirable but expected result for which compensatory breeding programmes can be utilized. Using quantitative methods of Nace et al. (1970) and assuming an average recombination frequency of about 0.1, Nagy et al. (1979) estimate that one gynogenetic generation is equal to 10-12 generations of full-sib mating. In many other breeding and brood stock selection programmes, practical logistic and economic management considerations can and have resulted in the inadvertent selection of brood stock in a way which causes close inbreeding. Unfortunately, once it has occurred, inbreeding depression is not reversible except by hybridization.

### 3.3 Monitoring and Measuring Genetic Variation

Technologies exist for a direct assessment of the genetic properties of a population. In some species the genetic basis of variation in some visible characters (e.g., colour patterns) can be established by breeding experiments, and the characters, or phenotypes, can be used to directly assess gene frequencies in populations. Traits such as colour patterns are typically controlled by a small number of genes (one to three). As such, those genes may not be representative of either overall genetic variation or population structure, but probably reflect fine-scale ecological or social structuring such as family or age-class recognition. Although these characters can be conveniently assessed in a population, they will too of ten give misleading or unrepresentative information on genetic variation.

Electrophoresis of proteins has been widely applied for the direct study of genetic variation in fish populations. The importance of electrophoresis to the study of fish genetics resides in the ability to directly estimate genetic relationships from its results, and also because variation of electrophoretically detectable genes is of ten correlated with variation of other genes. To the extent that such a correlation is widespread among fish species, electrophoretic variation can be a general estimator of genetic variaton.

The "state of the art" is to use electrophoretic techniques to analyze genetic variation in natural populations because, among other things, electrophoretic variation is "taxonomically congruent" with morphological variation in interpreting phylogenetic and evolutionary relationships (Mickevich and Johnston, 1976). However the use of electrophoretic variation analysis requires some qualifications regarding aquaculture. At this time there is no direct evidence in the literature to indicate that either qualitative or quantitative allozymic variation is indicative of potential economic performance in such characters as food conversion rates, tolerance to temperature extremes, low dissolved oxygen tension, etc. Because of this, caution must be exercised in using the level of allozymic variation as the only criterion for choosing stocks for desirable physiological, nutritional, or other related production performances. There is some evidence of a correlation between electrophoretic variation, meristic variation, and developmental stability in nature (Soule, 1980). It is theoretically possible to develop the use of qualitative variation as predictors or indicators of quantitative performance in laboratory or hatchery populations but this involves complex breeding systems probably beyond the practical scope of most aquaculture breeders (Soller, et al., 1976).

There are situations in which it may be desirable to electrophoretically estimate genomic variability and then to use these data as a base-line for comparing the genetic effects of a particular pattern of stock breeding or exploitation. For example, when exploitation of a species can be anticipated, base-line information on the genetic variation of pre-exploited stocks would be desirable. This would allow some direct assessment of the genetic consequences of exploitation by continuous monitoring of exploited stocks, or populations.

When re-introduction of a locally extinct population is contemplated, earlier base-line information might allow a closer matching of the introduced fish to the original population. Proper genetic matching would increase the likelihood of successful re-introduction. When base-line data is not available (the usual case), direct genetic assessment of the potential parental stocks for re-introduction allows an intelligent choice of stocks for introduction. Other things being equal, populations of maximum electrophoretic variation should be selected for introduction because this probably increases the likelihood of evolutionary adaptation to a novel environment.

A similar genetic monitoring of cultured species would be desirable to assess genetic changes that result from a particular culture scheme. It may be important to maximize outbreeding in a stock, in which case electrophoretic variation would be an important tool for monitoring the breeding programme.

There are certain dangers associated with the absence of genetic monitoring. In the southwestern United States, a major breeding programme was undertaken some years ago in order to produce sterile males of the screw worm fly for introduction into the wild populations (Bush et al., 1976). During the breeding programme, a particular allele of the electrophoretically detectable gene dglycerophosphate dehydrogenase was accidently selected probably by natural selection in the culture population. This enzyme is important in flight metabolism. The properties of the selected allele militated against flight in the wild of the introduced, sterile males. Knowledge of the biochemical properties of the alleles and electrophoretic monitoring would have avoided this unfortunate situation.

We do not wish to imply or recommend that every exploited fish or every cultured stock be monitored in this way. Not only would this be expensive, but unless such studies are properly designed and controlled, the data are not likely to be very useful. We do, however, suggest that several carefully designed monitoring programmes be set up, and that these be coordinated to maximize their utility.

### 3.4 Population Genetic Structure in Relation to Exploitation and Extinction

Fishes probably surpass all vertebrate groups in their variety of social structures and kinds of life histories. It is not surprising therefore that some controversy has arisen regarding the significance of such variables as population structure, dispersal, and genetic drift, particularly with regard to genetic integrity of populations. At one extreme there are species like the American eel in which the adult population is spread out over thousands of kilometres, yet this species apparently verges on being a single random breeding (panmictic) population. At the other extreme there are hundreds of species which are territorial, which have demersal eggs with parental protection and which have very limited vagility. In species of this latter type with a localized and fragmented type of population structure, the neighbourhood size or the local population could be as low as 100 , and there may be very limited gene flow between the local populations. As a consequence of this diversity, it is hazardous to generalize about demographic, georgaphic and genetic structural characteristics of fish. Each species must be examined as a unique case, even recognizing the possibility for intra-specific variation in population structure.

A good knowledge of the population structure in the management of fisheries cannot be exaggerated, whether the purpose of the management is exploitation or preservation or both (as should often be the case). Only when stocks are properly defined can the fishery be managed optimally. For example, one might conceivably decide to artificially enhance a pink salmon fishery by introducing fry from a hatchery. But unless one knew that odd and even year pink salmon were genetically distinct populations, the resulting hybridization could cause significant genetic change and a decrease in fitness in both odd and even year stocks.

Even very closely related sympatric species can have very different population structures, and it is dangerous to generalize from one to the next (Allendorf and Utter, 1979). In the rainbow trout in the Pacific northwest, the genetic data differentiate the populations into eastern and western groups, the major division coinciding with the crest of the Cascade Mountains. Many early workers had concluded that the principal basis for genetic separation of rainbow trout populations was anadromy and time of return to fresh water. Allendorf and Utter, however, emphasize that the taxonomic units based on electrophoretic data correspond to geographic groupings rather than to the above behavioural characteristics. They emphasized the importance of glacial events in dividing these populations historically.

In coho salmon, however, glacial events are not considered to have played a major role in the present subdivision of the populations in the Pacific northwest. The discontinuous distribution of (certain) transferring alleles among coho salmon populations cannot be directly explained on the basis of glacial events.

In the chinook salmon the coastal populations appear to be genetically distinct from the inland populations in both Oregon and Washington for the fall-run salmon, but the line of geographical demarcation is different from that of rainbow trout. This information has significant management potential because it could allow the determination of the major areas of origin of ocean caught fish.

Hence, among three closely related species in the same region, there is absolutely no correspondence in the geographic distribution of racially distinct populations, i.e., the geographic barriers that separate sub-populations in one salmonid species are not relevant in another. Management generalizations based on the distribution of populations in one species could prove disastrous if applied to other species.

Population structure is thus a useful guide for a priori priority ranking with regard to genetic resource preservation, at least with regard to the extinction potential of local populations. Geographic range alone is quite useful. The majority of species which reproduce in estuaries, river systems in the temperate zones, and in coastal pelagic zones are relatively widely distributed and fairly numerous. Species residing in tropical floodplain rivers, extreme environments such as shallow desert lakes or salt lakes may be far less numerous and typically have rather limited geographic ranges.

Many such "local" species are relatively vulnerable to habitat disruption. Both from the standpoint of biological conservation and from that of genetic resource preservation. Species which are widely distributed and relatively numerous warrant less attention than do the species with very limited distribution and small population sizes. We do not mean to imply that genetic depletion is only a threat to local endemics.

The potential for genetic depletion can also be quite high for a single reproductive unit of a broadly distributed species. The obvious distinction between the two cases is that the extinction of the endemic cannot be reversed, whereas the recolonization of a reproductive habitat upon loss of the more broadly distributed species is possible. For example, the Japanese sardine Sardinops melanosticta following a massive population collapse and range contraction, has recolonized the Japan Sea from refugia on Japan's east coast. Another example is the successful, artificial reintroduction of Atlantic salmon in small coastal rivers in the northeastern U.S.A.

In the latter case, the genetic characteristics of the replacement population should be a relevant concern (Section 6.4). For example, failure to consider the particular ecological adaptations of replacement or enhancement of stock has caused severe management problems in bob-white quail (Clarke, 1954). In Scandinavia some introductions of Arctic charr and whitefish into lakes already populated by conspecifics has had deleterious consequences (Svardson, 1979).

It is a moot point whether a naturally or artificially recolonized stock becomes as well adapted as the original stock. The point is to maximize the chances of a successful restocking or recolonization by taking into account all of the relevant genetic and ecological variables.

## 4. CRITERIA FOR MINIMUM POPULATION SIZES

### 4.1. Criteria and Time Scale

The establishment of minimum viable population sizes is one of the principal goals of preservation genetics. In arriving at such minimum sizes it is necessary to consider all aspects of the biology of the species involved, not just the genetic. Other important criteria would include the demography and life history of the species, and certain ecological variables, for example, the probability and severity of catastrophe.

Very little in general can be said about the latter subject because the nature and consequences of catastrophe are highly dependent on the life history of the species and particularly on the kind of environment in which it lives. For instance, fishes living in shallow bodies of water in regions subject to extreme drought are likely to have a high probability of extinction. Also, they may go through severe bottlenecks with significant frequency (see Section 4.3). At the opposite extreme, deep sea species are unlikely to be exposed to physical events which bring about a collapse in population size.

Given sufficient demographic information, it is sometimes possible to produce estimates of minimum viable population sizes for a species. But even when the demographic information is at hand, the genetic approach is relevant and could be the dominating consideration, assuming that the minimum sizes based on a genetic criterion are smaller than those based on demographic or ecological criteria.

The "time scale of survival" is a useful device for structuring a discussion of preserving genetic variation. Somewhat arbitrarily, there are three problems or issues:

1. a short-term issue is immediate fitness - the maintenance of vigour and fecundity during an interim holding operation, usually in an artifical environment, such as when breeding domesticated or semi-domesticated fish stocks. (If, however, breeding is expected to continue for more than $\mathrm{N}_{\mathrm{e}}$ (see Section 4.2) generations, the programme, in effect, becomes a long-term operation.); ${ }^{\text {e }}$
2. the long-term issue is adaptation - the persistance of vigour and evolutionary adapatability of a population in the face of a changing environment;
3. the third issue is evolution in the broadest sense, i.e., speciation, or the creation of evolutionary novelty (Soule, 1980). For our purposes, the first and second issues are the most relevant and the third is the least relevant.

From the above discussions on heterozygosity and inbreeding in both natural and captive populations (see Section 3), we can conclude that excessive loss of genetic variability, particularly inbreeding, can and must be avoided. The issue, however, is a quantitative one and the above qualitative discussions are not very helpful in providing specific guidelines to minimum viable population size. We must examine this point in greater detail.

### 4.2 Survival of Captive Populations

Captive populations will tend to be small and potentially subject to the deleterious effects of inbreeding. By trial and error, animal breeders have discovered the magnitude of inbreeding that can be tolerated by domestic animals before the lines begin to decline in fitness. (In discussing inbreeding, it is convenient to use the inbreeding coefficient, F , which is a quantitative measure of the magnitude of inbreeding. In a population that is totally outbreeding, $F=0$. For a population that is totally inbreeding, $F=1.0$.)

A general rule is that the per generation rate of inbreeding should not be higher than one to three percent (Franklin, 1980; Soule, 1980). Higher rates of inbreeding fix deleterious recessive genes too rapidly for selection to eliminate them, and the vigour and fertility of the line decreases.

The lower inbreeding rate of one percent $(F=0.01)$ is preferred because:

1. poultry and mammal stocks have been partially purged of deleterious genes over the milennia which allows them to tolerate higher rates of inbreeding than wild outbreeding species;
2. animal breeders can safely ignore some inbreeding and random loss of genes. In constrast, conservationists wish to preserve the "wildtype".

How does this basic "one percent rule" translate into population size? The rate of loss per generation of heterozygosity due to inbreeding as measured by F is equal to $1 /\left(2 \mathrm{~N}_{e}\right)$, where $\mathrm{N}_{e}$ is the effective population size. ("Effective population size" is the size of an idealized population. The definition of $\mathrm{N}_{\mathrm{e}}$ is cumbersome, but the population must have an equal sex-ratio and individuals must mate at random. A number of additional "ideal" characteristics could be stated. For our purposes, it is important to note that in practice $\mathrm{N}_{\mathrm{e}}$ is nearly always smaller than the actual number of breeding individuals.) Thus, $\mathrm{N}_{\mathrm{e}}$ must equal at least 50 if the inbreeding rate is to be kept below the one percent level.

However, even when $F=0.01$, the loss of genetic variation is appreciable after a few generations, and a gradual attrition of genetic variation cannot be prevented. Eventually, the population will become virtually homozygous, the time depending on $\mathrm{N}_{\text {. }}$. This is why the one percent rule must be viewed as short-term criterion. A population held in check at $\mathrm{N}_{e}=50$, will lose about one-fourth of its genetic variation after 20 to 30 generations, and along with ${ }^{e} i t$, much of its capacity to adapt to changing conditions. Thus, if it is desired to maintain a particular stock for longer than this, it will be necessary to increase its $N_{e}$. A rough rule of thumb is that $G$ is approximately equal to $N_{e}$, $G$ being the number of generations the stock is likely to retain its fitness at a relatively high level.

The above information is necessary but not a sufficient basis for the conservationist or the fisheries' biologist to conserve short-term fitness, or to maintain short-term fitness in captive populations of fish. The reason is that the effective population size is not a simple phenomenon and is affected greatly by variation in sex ratio, population size through time, by a non-random distribution of progeny among families, and other aspects of the breeding system. To the extent that any of these effects occur, a larger absolute population size must be maintained to achieve a desired $\mathrm{N}_{\mathrm{e}}$. Section 4.3 summarizes these complicating factors.

### 4.3 Population Influences on Effective Population Size

When populations decline or "crash", the survivors constitute a genetic "bottleneck" in the history and evolution of the population. Any deviation in the genetic makeup of these survivors from the gene pool of the original population will be reflected in future generations. More particularly, if the progenitor's gene pool is less diverse than that which existed in the original population, future generations will have a corresponding deficit in genetic diversity.

If the minimum population size is very small, due either to normal fluctuations or to an environmental changes or catastrophes, it is tantamount to squeezing the genetic variability of the source population through a very narrow channel and eliminating a significant amount of this variability. Bottlenecks inevitably accompany the establishment of a captive stock for breeding purposes.

Prevention or further genetic erosion or a recovery to the original level of genetic variation depends greatly on how fast the population grows to a moderate size or several hundred or more. If a preserved population is subject to fluctuations in numbers (as it most probably will be), the influence of the minimum absolute size on effective population size is more relevant to preservation of genetic diversity than the average absolute size.

The loss of genetic variability concomittant with the bottleneck event has both qualitative and quantitative aspects. Qualitatively, specific alleles may be lost and if they are lost it is very unlikely that they will be replaced by mutation as long as the population remains small. Quantitatively, the variability for specific traits will be reduced and the mathematics in the loss of the variance of quantitative traits have been described by Falconer (1960) and others. The qualitative effect is usually
greater than the quantitative one; that is, the loss of alleles, especially low-frequency alleles, is much greater than is the loss of genetic variance per se. Incidentally, several workers have pointed out that the number of founders in a colony, so long as it is greater than about five individuals, is not nearly as important as the long-term maintenance size of the colony (Nei et al., 1975; Denniston, 1978). That is, a single bottleneck event followed by rapid growth to a large size, say $2 \mathrm{~N}_{\mathrm{e}}$ greater than 500 , does relatively little damage, compared, that is, to a chronically small $\mathrm{N}_{\mathrm{e}}$.

We know from experience with resistance to pesticides in anthropods that some alleles that occur at very low frequency in natural populations (and which are likely to be lost during a bottleneck) can be very important. Such alleles can mean the difference between survival and extinction. The same thing probably applies to resistance genes in general. Therefore, it would be expected that populations of fish passing through bottlenecks might not be noticeably affected until a disease epidemic swept through the population. Only then would the loss of these resistance genes be detectable.

Several other factors determine $\mathrm{N}_{\mathrm{e}}$. Among these are the sex ratio; $\mathrm{N}_{\mathrm{e}}$ is lowered by deviations from an equal sex ratio. Another of the characteristics of a genetically ideal population is that the number of progeny are randomly distributed among families. When this condition does not hold, for example when the reproductive output of a few families is especially great, $\mathrm{N}_{\mathrm{e}}$ will be lowered. It is incumbent on persons dealing with captive populations, for purposes of either preservation or culturing, to be aware of these effects and to maintain N at a level that will maintain the fitness of their stocks. In some cases, it will be desirable to consult with a population geneticist, especially if the breeder is in doubt about the estimation of $\mathrm{N}_{\mathrm{e}}$.

### 4.4 The Maintenance of Long-term Fitness

Long-term preservation requires rather large population sizes, large enough so that an equilibrium will be maintained between the loss of genetic variability due to drift and selection and its generation from mutation. When $2 \mathrm{~N}_{\mathrm{e}}$ is a large number, say greater than 500 or 1000 , the effect of drift will be negligible compared to that of weak selection. When 2 N is small, say less than 100 , the randomization of gene frequencies between generations will not only fix many loci, it will also counteract all but the strongest deterministic forces, particularly directional selection, thus, to a large degree precluding adaption by natural selection.

The consequences of small population sizes might be thought to be irrelevant when considering the genetics of fishes in an aquatic reserve, or similar ecosystem. However, there will always be some populations in a natural ecosystem, particularly large predators, which have quite small numbers (such as groupers on an atoll, pike or muskies in a lake). The loss of such keystone predators from a natural community can have serious effects on the diversity of prey species, as has been documented by many workers, particularly with marine invertebrate systems (Paine, 1966; Harper, 1969).

Franklin (1980) argues that a minimum effective size of 500 is needed to preserve useful genetic variation, because:

1. The relevant phenotypic traits in conservation are quantitative (polygenic). For such traits the average effect of a gene is small, and most of the genetic variation is additive.
2. Weak directional or stabilizing selection does not erode additive genetic variation at a significant rate;
3. The significant evolutionary forces, therefore, are mutation, and genetic drift. That is, if a population is below some threshold size, it loses variation by drift at a faster rate than it gains variation by mutation.

Franklin derives his number from the work of Lande (1976) on bristle number variation in Drosophila. The evidence is meagre but Franklin believes his number (500) is about the right order of magnitude. Simple theory also yields this number as mentioned above.

It is necessary to caution again that the employment of any number is subject to all the same qualifications given in the preceding section for short-term preservation, namely that any effective size translates into a much larger number of breeding adults, when dealing with real, not ideal, populations. In addition, this recommendation ignores genetic differences between species. Conservation questions of this kind must also be considered on a case by case basis.

## 5. THE COMPONENTS OF GENETIC IMPOVERISHMENT IN FISH POPULATIONS

Human activities can cause the erosion of genetic variation and the extinction of fish via several mechanisms. These include:
(1) Pollution and other environmental changes that stress a population and cause differential mortality, extinction, or both;
(2) Fishing pressure which can favour some genotypes over others;
(3) Artificial selection and domestication which can result in conscious or unconscious inbreeding and genetic impoverishment;
(4) The introduction of exotic species and diseases.

In this section the above factors are reviewed in the light of recent experience in fisheries and aquaculture.

### 5.1 Effects of Pollution and Other Stress Factors

Pollution of different kinds (e.g., eutrophication, toxins such as mercury, DDT, PCB) have had disastrous effects on many fish communities. In most western countries toxins in single fish species have made them unfit for human consumption. In some cases legislation has been successful in reversing this situation. For example, a large part of Lake Vånern, Sweden (the third largest lake in Europe) has been blacklisted, but the situation has improved by changing the water purification procedures of the pulp mills.

The paramount problem in Scandinavia and elsewhere has been airborne acid rains ( $\mathrm{SO}_{2}$ ) from industries using fossil fuels as a source of energy. For example, large parts of southern Norway and southwestern Sweden have become almost devoid of fish due to acid rains originating from Great Britain and the Federal Republic of Germany. Species of fish differ in their response to acidification. Roach (Leuciscus rutilus) and most salmonids are extremely sensitive to decreasing pH . The addition of lime has been tried as an "artificial breathing" agent ( 1 million Swedish Kroner a year in Sweden), but it is obvious that this problem cannot be solved without some kind of international negotiation. Several charr populations have recently become extinct due to their sensitivity. The primary effect of acidification is apparently on the early reproductive stages.

Pollution by nutrients (especially $\mathrm{PO}_{4}$ ) from human communities has been most disastrous to densely populated areas in temperate, sub-tropic and tropic areas. Despite this, in arctic or alpine areas with extremely oligotrophic environments, eutrophication by adding nutrients has been promoted as a method of increasing productivity. This is now being studied experimentally in Scandinavia as well as in Canada (Milbrink and Holmgren, 1981; Schindler and Fee, 1974).

In some cases, "nutritional pollution" has been successfully treated. In all these cases, the construction of purification plants has resulted in significant improvement in water quality. In Lake Washington (U.S.A.), the spawning run of Pacific salmon species is now restored and natural spawning is occurring. Likewise, there is now a natural recolonization of native species in the lower Thames in England. The construction of purification plants in all communities around Lake Malaren in Sweden has made it possible to reintroduce Baltic salmon and sea-run brown trout into a stream that discharges through Stockholm.

Hydroelectric power development has also affected the fish populations in impounded lakes and the river stretches between the power plants. An important observation in Scandinavian waters is that there are sub-populations of salmonids that are affected in different ways. For instance, brown trout is often represented by different populations in different sites within a stream, apparently with little interbreeding. During the construction of dams the populations spawning in the outlet area become extinct. Arctic charr and whitefish are often represented by two or more discrete populations which are affected in different ways. On the whole, pelagic populations are less affected than benthic ones.

There are only a few rivers remaining in Sweden which support natural spawning of salmon. In the Baltic the majority of salmon originate from Swedish hatcheries. The smolts released have been carefully selected, so as to represent the original native stocks. There is evidence for genetic change, however, mainly toward smaller size and earlier homing (earlier maturity), this is probably an effect of overfishing.

The landlocked salmon of Lake Vänern are represented by two sub-populations which do not seem to interbreed. These are: the "Klarälven" salmon, which spend three years in the River Klaralven and return for spawning after three to four years, and the "Gullspångsälven" salmon which spend two years in the River Gullspångsälven and return from the lake after four to five years. The growth rates of the two populations are strikingly different. Tagging experiments with reared smolt have shown that after 40 months in the lake the salmon from Gullspångsålven attain a weight of about 5 kg , whereas the salmon from Klaralven reach only 2.5 kg . They are also segregated spatially during their life in the lake, suggesting an inherent capacity for both growth and habitat selection.

Both populations are endangered (Figure 1), mainly by hydroelectric development. The fastgrowing population from the River Gullspångsälven is very close to extinction, but efforts are being made to save it, both by hatchery operations and introductions into other lakes.

### 5.2 Effects of Fishing

Overfishing for Baltic salmon has resulted in slower growth, smaller size at maturity and early homing. Very similar results were obtained by Gwahaba (1973) in the case of Tilapia nilotica in Lake George, Uganda (Figure 2). He claimed that the restricted size of nursery zones, fish killing storms and predation have been factors in the decreased propagation, but an increased commercial fishery appeared to be the main reason for the changes.

Turner (1976) provides another example of ecosystem change by intensive fishing on Lake Malawi cichlids. Whereas at the beginning the catch was dominated by large species, small species now predominate. This example dramatically shows the effects of "overfishing" on an entire fish community. It is predictable that in the case of continuous fishing with nets of unsuitable meshes, the larger species may be threatened with extinction, in part, because the more abundant smaller species can outcompete the small stages of the former large ones.

Similar observations were made comparing discrete populations of Arctic charr which are subjected to different exploitation rates. Figure 3 illustrates that the esteemed fast-growing population matures earlier than the slow-growing, unexploited one (Nilsson and Filipsson, 1971). In this case the difference is no doubt genetically determined (Nyman, 1972).

### 5.3 Effects of Exotic Fish Species

### 5.3.1 Active introduction of food fish

Since the early 1970's much information has accumulated on the serious consequences of introductions of exotic (foreign) fish on native ichthyofauna (e.g., Walford and Wicklund, 1973). For example, many formerly fishless, deglaciated lakes were stocked by the Lappish people and European settlers in Scandinavia and in North America by the Indians and European settlers. As hatchery technology has improved, the practice has spread.


Figure 1. Catches of land-locked salmon in Lake Vänern, Sweden, 1881-1977. From Nilsson, 1979.


Figure 2. The percentage of females of Tilapia nilotica with mature ovaries at different lengths. The maturity curve for the 1971-72 survey ( O ) is compared with the one drawn earlier by Fry and Kimsey (1960), (©).



Figure 3. Differences in age and length of two population of Arctic charr at maturity in Lake Ovre Bjorkvattnet, Sweden. The ordinary charr are faster growing and heavily exploited. From Nilsson and Filipsson, 1971.

The rate of such introductions has greatly increased in recent years. According to Everett (1973) rainbow trout (Salmo gairdneri) was introduced to the Lake Titicaca Basin in Peru and Bolivia in 1942. In the 1950's the following trout species were also introduced: S. trutta, Salvelinus fontinalis, S . namaycush and the atherinid Basilichthys bonariensis (Villwock, 1972). By 1972 two of the most valuable (largest) species of the endemic genus Orestias in Lake Titicaca became practically extinct by predation or food competition from the trout species. Sporozoon parasites which were introduced passively together with the exotic species account for the dramatic population declines in the majority of endemic species of Orestias (Frey, 1975).

Another well known example is that of Lake Lanao on the island of Mindanao in the Philippines. According to Villaluz (1966: cited by Frey, 1969) personnel associated with the State University of Marawi City on Mindanao introduced Clarias batrachus (Siluridae), Ophiocephalus striatus (Ophiocephalidae) and Tilapia mossambica (Cichlidae). Even more serious was the accidental introduction of Glossogobius giurus (Gobiidae) at the same time. According to Frey at least some benthic and pelagic species of the endemic genus Barbodes (Puntius) have become rare as evidenced by their virtual disappearance from the local fish markets.

Both these species groups, the endemic Orestias in Lake Titicaca, as well as the Barbodes in Lake Lanao, were the principal economic and nutrition resources of the local residents. These cannot be replaced by the introduced species (Villwock, 1972) because the introduced species are not readily accepted by the local population. The effects on the genetic resources of the endemic species of Orestias or Barbodes are irreversible. Some species are evidently extinct and even those which may have survived have probably been diminished to one or a few small, disjunct populations.

### 5.3.2 Introductions of sport fishing and ornamental fish

Game fishermen and aquarists have been responsible for numerous cases of introductions which have led to detrimental changes in the native fauna. Studies in Scotland and Scandinavia have shown that native species may become extinct by ecological competition with exotic ones. For example, Svardson (1979) described the effect of introductions of species of whitefish (Coregonus) and consequent extinction of the Salvelinus alpinus complex in Scandinavia. As a result of competition between the two forms shown in Figure 4 the size at maturity, age, weight and gill-net catch of charr decreased continuously resulting in its eventual extinction around 1965 (Nilsson, 1967).

Hybridization between native and related, introduced species (Svărdson 1979; Moyle, 1976) also has serious consequences on genetic resources. In the second half of the 19 th century different trout species were introduced into California, confusing the already complex situation in the native trout fauna (Hoopaugh, 1974: cited by Moyle, 1976). Similar events have been reported by Moyle (1976) for species of chub (Gila) and sticklebacks (Gasterosteus). The most frequent result is swamping of the genome of the native species. The large number of hybridization events reported from Florida were caused by aquarists or the aquarium fish industry (Courtenay, et al., 1974). Twenty exotic species and five hybrid populations have established themselves as breeding populations.

### 5.3.3 Introductions for weed and insect control

Moyle (1974) reported on fish introductions in California for weed and insect control. In addition to several killifish and two species of Tilapia (T. mossambica, T. zilli), the mississippi silverside (Menidia audous, Atherinidae) was introduced illegally in 1967. Since then it has become the most abundant and widespread species. The Tilapia as well as Menidia have affected the relative abundance of the different fish and the total number of zooplankton organisms to which native fish were highly adapted, although it is difficult to predict the ultimate consequences for the native species.

Both food competition and predation on the larvae by exotics of native species have often led to extinction. Within less than a decade, mosquito fishes (Gambusia, Mollienisia) introduced into southern European countries and North Africa were responsible for the decline of the native Aphanius sp. population (Cyprinodontidae) (Villwock, 1977 and unpublished data). Similarly, Cyprinodon in the Colorado River system is endangered by direct competition of introduced live-bearing forms.


Figure 4. Decrease in the gillnet catch of charr (Salvelinus alpinus) as the catch of the introduced whitefish (Coregonus sp.) increased, Lake Västansjö, North Sweden. From Svärdson, 1976.

### 5.3.4 Accidental introductions

Accidental introductions have occurred in several ways. Among these are uncleaned gillnets ("egg pollution") and the escape of bait (Johannes and Larkin, 1961). Another danger is the escape of stocked and exotic species from aquaculture ponds, for instance by flooding during rainy seasons. Introductions have also occurred via canals or tunnels used for shipping and power plants. The most famous case is that of the landlocked sea lamprey (Petromyzon marinus) which entered the Upper Great Lakes through the Welland Canal. Similar movements have occurred into the Mediterranean Sea since the opening of the Suez Canal.

### 5.3.5 The biological consequences of introductions

Many introductions have been economically or aesthetically successful. For example, most species of North American salmon, as well as the black bass, have been introduced into European fresh waters, and several Oncorhynchus species have been introduced into the Northern Russian marine waters and to the Baltic. Kokanee (Oncorhynchus nerka) has been introduced into Scandinavian freshwaters. Although most of these introductions have failed, black bass in Germany and rainbow trout in Great Britain have been successfully established. Brown trout and carp from Europe have been introduced into North America, New Zealand and most alpine areas where Europeans have settled. The introductions of lake trout (Salvelinus namaycush), which began in Fennoscandia in the 1960's, seem to have been successful in some Swedish and Finnish waters.

Little is known about the impact of these introductions on the genomes of the species, but where intense culturing and domestication is practised, for instance in rainbow trout, genetic change is inevitable. The same should be true for brown trout in Europe and elsewhere.

A very intense study on the effects of transplanting Coregonus and Salvelinus species has been carried out in Sweden and Italy (Svärdson, 1979; Nilsson, 1978; Berg and Grimaldi, 1966; Nyman, 1972). Briefly it has been shown that introductions of "exotics" have four possible results:
(1) extinction of "ecological homologues". The most drastic example has been the extinction of Arctic charr (Salvelinus alpinus complex) by introductions of certain whitefish stocks;
(2) hybridization with concomitantly profound effects of the genetics of the original fish population;
(3) failure of the introduction, in part, because of competition from established resident species;
(4) coexistance, which means that the introduced species has found a "vacant niche" in the community, with an interactive niche segregation as abvious result.

Figure 5 illustrates a common result of the introduction of species in northern Scandinavia and their impact on the zooplankton community. Apparently the grazing of zooplankton plays a great part, the most efficient zooplankton feeders becoming dominant (Nilsson and Pejler, 1973; Svärdson, 1976).

Introductions of exotic prey species is now a common practice, especially in North America and Scandinavia. In Canada and Sweden, for instance, introductions of glacial relicts (especially the crustacean Mysis relicta) have been practised for several decades (Fürst et al., 1978). Such introductions have on the whole seemed successful. In many cases, however, a strong impact on the native zooplankton community has been observed.

### 5.4 Effects of Artificial Selection

Artificial selection is usually associated with desirable genetic changes. However, in many cases the outcomes of artificial selection may be neither desirable nor predicted. This is especially true when brood stock selection and management lead to loss of genetic variation. By their very nature, specific examples of genetic impoverishment due to such practices are not generally available. Nevertheless,



Heterocope saliens Daphnia longispina Polyartemia


Heterocope saliens Daphnia galeata


Heterocope saliens Daphnia longispina


Heterocope appendiculata Daphnia cristata

Figure 5. Model of the "dimensions" of the niches of brown trout (Salmo trutta), Arctic charr (Salvelinus alpinus) and whitefish (Coregonus sp.) in allopatry and sympatry, and the dominant species of zooplankton. (After Nilsson and Pejler, 1973)
there is sufficient background knowledge to warrant caution. For example, genetically based performance under one set of conditions (i.e., hatchery) may not be correlated with performance under a different set of conditions (stream, lake, natural area). If the goal is to release stock in a different environment from that where they are bred, then the brood stock selection practices must be designed to avoid unconscious selection and inbreeding.

In this regard, the accessibility of brood stock to human managers does not ensure its genetic superiority. For example, it has been shown that large carp that are chosen from a population of identically aged individuals do not necessarily represent superior genotypes (Wohlfarth and Moav, 1969). Such individuals may represent an exposure to a favourable set of environmental conditions which magnify a small, initial, non-genetic, size differential into a large one. The use of a brood stock composed of such individuals would lead to little or no genetic progress, and could, if the number of selected individuals is small, lead to inbreeding and genetic drift (Section 3). Moreover, large individuals could represent behaviourally aggressive phenotypes under genetic control. The choice of such individuals could lead to an undesirable general level of agressiveness in the population.

In some cases brood stock selected from culture units containing mixed year classes (as is probable in undrained tropical ponds) probably represent a mixture of both superior and inferior genotypes. Consequently little genetic progress, and even erosion, can be expected by the haphazard selection of brood stock. For example, in the case where only gravid females from undrained, net harvested ponds are used for brood stock, they may represent both large young animals (superior genotype) and large older ones (inferior genotype). In addition, it may be that genetically inferior (i.e., slow growing but "net avoiding" males) are reproducing more of ten with these females.

It is in part for reasons such as these that Tilapia pond culture has failed in some tropical areas. In harvesting Tilapia ponds it is not uncommon to select the large fish and to return the small ones. This procedure favours the reproduction of small and slow-growing fish, and it has been shown experimentally that such selection can produce a genetic shift in the population toward genetically smaller (and less desirable) fish. The problem in Tilapia is compounded by mouth breeding and defence of young until they reach a size safe from cannibalism. The result is overpopulated ponds with stunted individuals. Cannibalism can be genetically and economically advantageous in some aquaculture systems.

### 5.5 Domestication

Domestication is defined as a genetic selection process mediated by geographic and reproductive isolation, inbreeding, and small population size to produce profound and desirable evolutionary changes in a genetic stock. Domestication presumably involves at least the ability to live under artificial conditions through most parts of the life cycle. It further would usually be expected to include genetic adaptation such as to crowding, handling, and artificial diets. Genetic improvement, in the sense of enhanced growth, modification of body form and some loss of fear-flight behaviour are also usually assumed. The anthropological aspects of this are well documented (Zeuner, 1963), but not much scientific scrutiny has been directed at the domestication process itself because of the limited opportunity to study, document, and control the phenotypic and genotypic changes that occur in the domestication of livestock, poultry, and companion animals. The opposite is true in aquaculture.

There are probably a number of reasons why man has not domesticated as many fishes as he has mammals, birds and plants. Because of the difficulties of transporting live fish from place to place over land, domestication could only have proceeded in sedentary cultures. There is indeed evidence of fish culture in Sumeria contemporaneous with very early stages of agricultural development, pre-dating the reported beginnings of aquaculture in China in 600 B.C.

It is also likely that attempts to apply the criteria for selection learned in animal and plant breeding to fish or to cold-blooded vertebrates generally were counter-productive. Whatever the reason, and with the notable exception of the goldfish, ornamental carp, and perhaps the common carp, few fish could be considered domesticated even though some strains of trout, for example, are clearly much more adapted to hatchery conditions than their wild counterparts (Moyle, 1969; Hines, 1976). Other species like the Chinese carps, Indian carps, Tilapia sp. and American catfishes are becoming domesticated.

As pointed out in Section 5.4 culture conditions themselves, along with the conscious selection imposed by man, will foster domestication. By and large the outcomes of this process are not only desired but predictable. Workers might be cautioned, however, that, history rarely reports failures in such attempts. In this regard researchers, culturists and breeders should develop the perspective that these efforts represent, in many cases, the initial steps of incipient domestication of a new animal group. Their scientific scrutiny should be designed to monitor and document this process.

It is recognized that in view of the inevitable operation of natural selection in culture environments (Section 5.4) that propagation without domestication is, perhaps, impossible. However, in some circumstances breeding without genetic change is the goal, as in the case of stock enhancement programmes of natural fisheries, in which it is desirable to preserve the inherent, undomesticated genotypes. Thus care must be taken to avoid the development of traits that have historically charracterized domestication (e.g., loss of awareness of predators, agressivity, irritability). Such losses may prove useful in brood stock management because these traits result in increased accessibility and tractability to human management. However, development of such traits will be counterproductive to the goals of stock enhancement of natural fisheries.

In some cases of stock enhancement, behavioural genetic change under domestication is useful and desirable. For example, impounding of areas in coastal reclamation projects or inland reservoirs can create vast areas of semi-natural aquatic habitat useful for aquaculture. In such situations, the loss of migratory and other inappropriate social behaviours may be desirable.

It might be expected that the scientific knowledge now available will result in relatively rapid domestication of aquatic species as suggested by Moav et al. (1978). Techniques such as gynogenesis are likely to facilitate the domestication process dramatically. The normally high fecundity of fish species has been cited as contributing to rapid domestication. Whether or not this happens, it is surely essential, as was pointed out in the recommendations of the 1976 FAO Technical Conference on Aquaculture held in Kyoto, that fish breeders provide very careful documentation of the breeding history of strains undergoing domestication. This documentation, as now recognized by plant and mammal breeders, will be of great importance when it later becomes desirable to regain genes, for example, for disease resistance, which have been lost in the domestication process. It has also been proposed (Malecha et al., 1980) that there is an opportunity in the domestication of aquatic species to study the early stages of the process of domestication itself, in a way no longer possible with existing livestock.

## 6. TECHNIQUES FOR THE PRESERVATION AND ENHANCEMENT OF GENETIC RESOURCES IN CLOSELY MANAGED FISH POPULATIONS

The methods for preserving genetic resources in fish are outlined in Table 1, Section 2. In this section those methods that are appropriate for aquaculture and stock enhancement are discussed in more detail.

### 6.1 Artificial Selection of Broodstock

Artificial selection of broodstock is broadly defined as the conscious selection of an individual or genetic group for the purpose of providing progeny for culture or stock enhancement. This selection can be based upon the performance of an individual (individual selection), of its progeny (progeny selection), of its ancestors (pedigree selection), of its contemporary relatives or on the potential of a useful combination with a parent of another genotype (hybridization selection). In all cases selection is practised in order to improve the performance of the progeny group above that of a progeny group from unselected parents.

There are many selection methods available by both the professional and non-professional aquabreeder (Moav, 1979). The proper application of the methods in a breeding programme can lead to useful and desired economic, aesthetic and ecological outcomes while still maintaining the genetic variability in the population. However, improper application of artificial selection or its application without sufficient background knowledge can lead to a deterioration of the genetic base.

Useful outcomes, both experimental (Cherfas, 1969) and economic (Kirpichnikov, 1973; Moav and Wohlfarth, 1973) have been documented for artificial selection in aquaculture. In these particular cases, undesirable outcomes have either been economically inconsequential or predicted (as in the case of experimental systems). In many cases, however, there have been undesirable results. This happens when simple brood stock selection and management per se leads to artificial selection.

The use of controlled mass rearing hatchery techniques dramatically increases the survival of larvae, post larvae and fry above that realized in nature. In theory, higher survival rates should increase the genetic variability available for natural and artificial selection, because they can "open up" the genotype of a genetic group in so far as new genetic combinations are allowed to survive. In the long term, however, culture conditions impose their own "natural" selection on the genotype (Doyle and Hunte, 1980; McCauley, 1978). This may or may not lead to a desirable outcome and must be monitored carefully.

Propagation of fish stocks in hatcheries for the establishment of a selected strain takes anywhere from ten to 50 generations. Fish breeders, therefore, might be tempted to maintain a minimum number of individuals. It is thus essential that they carefully weigh the costs of decreasing fitness and genetic variability against the economic advantages of maintaining such small numbers of individuals. As discussed in Section 4, fitness is expected to decrease so long as the effective population size of a brood stock is less than 50, and genetic variation in quantitative traits is expected to leak away unless the size is on the order of 500 . If breeders choose to ignore these guidelines, they should do so in the knowledge that the genetic health to utility of their lines could be endangered.

### 6.2 Artificial Methods of Reproduction

Methods of artificial propagation, including the use of hormones, in vitro fertilization and development, are in wide use in aquaculture. For some species artificial propagation is necessary for life cycle control. In others, such propagation techniques are more efficient than natural breeding methods. The ways in which artificial propagation can affect the maintenance and study of genetic variation include:

1. Increase of genetic variability and genetic understanding. Numerous broods from different breeding pairs and from single maternal and multiple paternal (and visa versa) crosses allow not only the avoidance of inbreeding and bottleneck effects but the integration of selection operations with proper experimental designs. As pointed out in the report of the FAO Ad hoc Working Party on Genetic Resources of Fish (FAO, 1972) this integration allows both an improvement of stocks and a contribution to the knowledge of the genetic control of the production characters.
2. Aids in the conservation of stocks, strains, species and other genetic groups. Artificial propagation and life cycle control allows the conservation aqua-breeder to maintain valuable or potentially valuable stocks for current and future use.
3. Allows the development and use of specialized breeding methods. Gynogenetic procedures have been developed with the use of artificial propagation techniques. These procedures are used to create and maintain highly inbred lines which can be used to create and preserve useful homozygous genotypes. By maintaining numerous such homozygous lines in a population, overall genetic variability can be preserved despite the fact that each line represents severely reduced variability. Crosses of the lines can create heterozygous variability which may be economically useful because of hybrid vigour and/or because of the favourable non-heterotic combination of genotypes. Heterosis of $30-40$ percent above that of the homozygous, gynogenetic lines can be expected in crosses of these lines.
4. Allows the efficient maintenance of effective population size. As discussed in Section 4 maintaining a minimum effective population size is essential for the conservation of genetic variability. Under natural conditions effective population size (Section 3.4) can vary widely. Through the use of artificial propagation methods man can control the effective population size most simply through the control of the numbers of breeding males and females.
5. Aids in interspecific hybridization. Normally, behavioural and anatomical differences preclude interspecific or intrageneric crosses between species which otherwise are reproductively compatible at least for the creation of $\mathrm{F}_{1}$ progeny. The use of artificial propagation techniques, especially those involving in vitro manipulation of gametes and in vitro culture of developing embryos in species which have maternal brooding, would greatly aid in developing interspecific hybrids.

### 6.3 Hybridization and Heterosis

Hybridization (or, cross breeding) can be divided into two categories: (1) intraspecific: crosses between strains, stocks, land races, geographic populations within a species; (2) interspecific: crosses between species. Hybridization is practised to achieve either of two favourable outcomes. These are: (1) heterosis or hybrid vigour, which is defined in a broad sense as an increased performance or value (to the aquabreeder) of progeny above the average of the parental performances or value, and (2) nonheterotic effects which is the performance of the progeny as the result of simple combination of parental genotypes.

Specific hybridization is useful in producing a wide variety of new genetic combinations. Improvements in production above that attained for "land race" strains have been realized in extensive and intensive fish farming (Bakos, 1979; Wohlfarth and Moav, 1972; Yant, 1976). The expected heterotic effect can be increased using highly inbred parental lines (see Section 6.2). The desired outcome is the homozygosity of useful genes in a highly standardized and uniform product.

Interspecific hybridization is used not only to search for heterotic effects but also to search for favourable combinations of genotypes that control traits and performances that do not vary within a species to a large degree. For example, new kinds of social and feeding behaviour, better adaptation to environmental extremes in natural and controlled systems, and better adaptation to new husbandry systems can be realized by interspecific crosses. In addition, interspecific hybridization can be used to create desirable sterile or monosex progeny groups which also display favourable production performance. For example, a sterile triploid hybrid between the big head and grass carp has been developed (Marian and Krasznai, 1978). This sterile hybrid can be introduced to ecosystems for weed control with no danger of overpopulation or crossing with wild populations. The creation of monosex broods of Tilapia sp. brought about by interspecific hybridization is being intensely researched (Pruginin et al., 1975). As yet no one combination has consistently resulted in a monosex brood. However, crosses leading to highly skewed sex ratios are possible and are being utilized.

Interspecific hybridization is not rare in nature (Hickling, 1968; Hubbs, 1955; Schwartz, 1972; Slastenenko, 1957). However, to date most human attempts at interspecific hybridization in fishes have been directed at exploited Salmonid (Chevassus, 1979), Cyprinid (Bakos et al., 1978), and Centrarchid (Childers, 1971) fishes. However, in all fishes, unexploited species represent a vast repertoire of behavioural, physiological, nutritional and other adaptations which may prove useful in making larger impacts in genetic improvement than would be obtained through the use of intraspecific genetic manipulation. The development and use of artificial propagation and in vitro techniques will greatly aid in this endeavour.

### 6.4 Enhancement of stocks

There are numerous examples of activities based on the concept of augmentation of natural population production by introduction of hatchery raised, "cultured" juvenile fishes into natural systems. Varying levels of success have been achieved. In some cases, for example, the early attempts to augment cod (Gadus morrhua) production in the North Atlantic Ocean have not been obviously positive. In closed systems such as lakes, inland seas, and reservoirs, however, there have been obvious improvements in the productivity of various species.

It is apparent that natural fish stocks undergo genetic alteration through addition and deletion of genetic material. Although quite a lot of attention has been given to effects of competition between "wild" and hatchery raised fish, for example in salmonids, very little has been given to changes which may result to the genomes of the wild stock by mixture with artificially raised stocks. In some cases parental material is taken directly from the population into which the hatchery fish are subsequently
released. In this case, the principle hazard would be the possible loss of genetic diversity through longterm inbreeding of the parental stock. Where there is relatively low rate of natural reproduction or where very few spawners are used to provide most of the recruitment, random loss of genetic variation (genetic drift) would increase. When, as is often the case, one hatchery is used to provide seed for stocking many lakes, the effects are much more likely to be important. Flick and Webster (1976) have reported higher survival of both wild and hybrid domestic strains of brook trout after release in small natural lakes compared to pure hatchery strains. In salmonids and carps, quite a number of distinct hatchery strains have been developed.

Genetic differences have been demonstrated between fish populations in different streams of the same river basin, between nearby lakes, and between sub-populations within the same body of water (Section 3.4). There is, however, little direct evidence concerning the degree to which such variation among stocks affects "fitness" in the different habitats. Recent biochemical and physiological studies have shown, however, the existence of correlations between gene frequencies and environmental variables, such as temperature (Place and Powers, 1979; Powers, et al., 1979, Koehn, 1969; Merritt, 1972). Thus, local ecotypic adaptations of fish populations are to be expected and the genetic and ecological consequences of intraspecific transfers may be undesirable.

### 6.5 Cryopreservation of Genetic Resources

In order to maintain high levels of genetic variability in cultured fish, it would be necessary to maintain large numbers (i.e., hundreds) of breeders. This is expensive and of ten not feasible. The introduction of the cryopreservation of gamets which can be used when necessary, thereby reducing the genetic erosion that invariably results from inbreeding, allows one to freeze the sperm of numerous male donors from all possible strains.

Essentially the method involves stripping sperm, from males, diluting the sperm with appropriate extenders and life protectors, then freezing in liquid nitrogen or on dry ice followed by liquid nitrogen. The exact methodology is somewhat different for each species (e.g., Horton and Ott, 1976; Rosenthal et al., 1978). Once the method has been refined for a given species, sperm from thousands of donor males can be kept for years thereby preserving the genetic resources for (1) cultured fish stocks, (2) unique natural populations, and/or (3) species that are threatened. At present methods for freezing fish eggs or embryos are not technically feasible but research in that area is being actively pursued (e.g., Whittingham and Rosenthal, 1978).

## 7. RECOMMENDATIONS

Considering the broad terms of reference given to the Consultation, and the diversity of recommendations which arose from the discussion, the participants agreed to group their recommendations according to the differing audiences to which each was primarily addressed. The groups agreed upon are as follows:
(1) International Organizations
(2) Governments
(3) Aquaculturists and Managers of Fisheries
(4) Conservationists
(5) Research Scientists

Much of the immediate impetus for holding the Consultation came from the continuing work of the United Nations Environment Programme (UNEP) to develop a global strategy for the conservation of genetic resources. For this reason the Consultation gave particular attention to activities that are needed to develop and implement a global strategy of conservation of fish genetic resources. Many of the following recommendations, in all categories, were formulated to contribute to the development of a global strategy, as proposed by UNEP, for long-term conservation. The first set of recommendations, addressed to the international organizations, is particularly aimed at developing institutional support for programmes of genetic resources conservation. In this instance, additional details are provided in the appendixes. Similarly those for scientists are primarily concerned with providing support for such strategies.

The group also considered more immediate actions for effectively managing the present use of aquatic resources. Recommendations concerning such actions are mostly grouped under those directed toward governments and to individual fishery managers and aquaculturists.

### 7.1 Recommendations Primarily for International Organizations

1. FAO, through cooperative programmes with other international agencies, should promote "grass roots" awareness of genetic preservation concepts in the fishery/aquaculture communities at regional and national levels. These objectives could be met on the short term by development and distribution of training manuals and information documents which need to be followed up immediately by workshops.
2. Biological criteria for the design and management of aquatic reserves need to be defined from genetic, ecological and demographic principles. A consultation of experts, with varied applied, experimental and theoretical backgrounds representing the critical areas of expertise, should be convened to define biological criteria for the design and management of aquatic reserves for preserving natural genetic stocks of fish and other aquatic organisms. The consultation should include genetic, ecological and demographic principles in their considerations.
3. It is recommended that international centres be established, preferably on a regional basis, where corps of experienced researchers would (1) assemble basic biological and genetic data on exploited and potentially exploitable fish stocks, and (2) disseminate such information through catalogues, newsletters, etc., in order to provide more direct mechanisms at the international level for the resolution of biologically important problems and conflicts, particularly regarding the exploitation of fish species. Special attention would be given to sub-species components of resources shared by two or more nations, and to species which may be considered for introduction into different drainage basins or across national boundaries (see Appendix 2).
4. Unesco and perhaps other international organizations such as FAO and IUCN, should consider establishment of a programme of education and training, at both national and regional levels, on genetic resource conservation/preservation in fish and other aquatic organisms. As a basis for its educational work, the programme should assemble baseline information (a) on the diversity and vulnerability of aquatic genetic resources, (b) on procedures for identifying vulnerable species and population, and (c) on appropriate methods assuring that information regarding vulnerability and direct threats comes to the attention of agencies competent to act (see Appendix 3).
5. Acid rain is at present a particularly serious threat to genetic resources of aquatic organisms and a problem requiring international action. FAO and other private and public international organizations should encourage governments to negotiate appropriate protocols for the control of the amount of sulphur that is discharged into the atmosphere.
6. The many international organizations for the regulation of fish stock exploitation (e.g., EIFAC, ICES, Great Lakes Fishery Commission, etc.) are encouraged in their efforts to prevent the extinction and genetic deterioration of valuable stocks.

### 7.2 Recommendations to Governments

1. Introductions of new species into aquatic systems has often had serious consequences on existing resources. Governments which do not now have mechanisms to ensure that an objective analysis of risks precedes the introduction of an aquatic organism into national waters should take immediate steps to establish such mechanisms. Genetic, behavioural and ecological data, as well as potential for introduction of disease, should be included in the risk analysis. In this connexion, governments should be aware that the probability of escape of cultivated aquatic species (even those kept only for research purposes) is so high that intent to confine imported aquatic animals does not obviate the need for such risk assessment.
2. Governments should consider urgently the establishment of fresh-water and marine reserves following principles which have been established for land reserves (see 7.1, Recommendation 2 and 7.4, Recommendation 2).
3. Governments should insist that the potential impacts of planned hydroelectric and irrigation, and other development projects upon fisheries and fish genetic resources be evaluated at the earliest stages of consideration of such projects to ensure that there is opportunity to examine appropriate alternatives.
4. Environmental alteration (by pollution, siltation and erosion, etc.) is generally a more important threat to the preservation of fish genetic resources than their direct exploitation. Governments wanting to protect these resources should make every effort to ensure that environmental damage to natural waters is minimized.

### 7.3 Recommendations to Aquaculturists and Fishery Managers

1. Fish breeders should be concerned for the continuing fitness (viability, vigour, fecundity) of their stocks, and should maintain the effective population size, $\mathrm{N}_{\mathrm{e}}$, of the stocks at 50 or more for short-term breeding and culture programmes, and much more (ca. 500) for the protection of genetic variability within lines. Inbreeding techniques should be used only for specific genetic goals and only when strict genetic control is possible and can be used in conjunction with other selection programmes.
2. Research aquabreeders and culturists should collect founder stocks from as wide a distribution as possible within the species range in their efforts at domestication. This is to ensure that domestication, at least in its initial stages, be based on the broadest genetic base as possible. These stocks should also be subjected to a wide variety of genetic analyses to determine that the founder group represents, more or less, the same species and that the individuals are chromosomally (genetically) compatible.
3. Research on artificial propagation techniques, including those for in vitro fertilization and development, should be given high priority in order to aid programmes of genetic preservation during the initial stages of domestication. The use of specialized breeding techniques, such as gynogenesis, are being successfully applied in domesticating some species of fish and should be further developed as a part of efforts to bring new species or genetic groups under domestication.
4. The efforts of aquabreeders, culturists and researchers should be directed not only at preserving and maintaining the present domesticated stocks of strains in culture units but also at preserving and maintaining the wild relatives of these genetic groups as valuable reservoirs of genetic variation. Further, it is important to document the process of domestication including the source and history of wild stocks.
5. Research aquabreeders, culturists and fishery managers should be cognizant of the limitations of current methods for estimating genetic variation especially insofar as these techniques may be used for determining a priori which species, geographic population or groups are to be selected for domestication or for restocking natural waters.
6. Hatcheries carrying out introduction or restocking programmes in natural areas should be cognizant of the fact that there may be genetic changes of natural ecosystems which result from using inbred populations. Such stocks are suitable only for "put and take" fisheries, and then only if they are not likely to breed with native stocks. They should also recognize the possible genetic and ecological consequences on natural fish stocks and fisheries of the use of genetically biased brood stocks collected from one area to generate seed for restocking another area.
7. Aquabreeders, research aquaculturists and managers of natural fisheries should direct some of their efforts toward developing methodologies, proce dures and genetic breeding systems for the generation of suitable genetic groups for stocking deteriorated natural fisheries (especially inland ones). This effort should also involve methods to assess the productivity and survival of properties of the released group.
8. Morphological, meristic and electrophoretic techniques should be used where appropriate to survey commercially exploited fish species to determine specific indicators of populations within each species. When populations can be identified by unique phenotypes or gene frequencies, they should be monitored to prevent overfishing of specific groups thereby reducing the probability of eliminating the genetic resources of unique populations.
9. Previous groups have recommended that there be established an international system for designating strains and stocks of fishes used in aquaculture and hatcheries. Whereas some may feel it is still impractical to do this, we believe that breeders should begin this process for certain fish, for example, common carp, rainbow trout, Tilapia nilotica, and that the discussions could begin at a future international fish breeders or aquabreeders meeting. The FAO Plant Genetic Resources office could provide background based on their broad experience in the preservation of geneticaly and geographically defined natural races.

### 7.4 Recommendations Primarily for the Conservation Community

1. There should be increased effort directed at identifying populations of exploited or endangered species, at solving the major taxonomic problems existing in some parts of the world (especially South America and Africa), and at understanding the role of fish species in the ecosystems in these areas.
2. A series of international meetings should be planned over the next few years in order to define where and how marine and aquatic parts (Biosphere Reserves) should be established. Each meeting or working group should bring together regional and scientific expertise on a specific habitat. These should include, for example, mangrove, coral reef, brackish water, large tropical river and large tropical lake habitats. To facilitate the development of marine and aquatic parts, a working group should be established to discuss and determine sizes and geographic form of natural aquatic genetic reserves taking into account ecological, demographical and genetic criteria (see also Section 7.1, Recommendation 2 and Section 7.2, Recommendation 2).
3. Assistance should be given to fish hobbyists and aquarium managers in maintaining stocks of rare or endangered species, as is already being done in zoos of birds and mammals, giving full attention to the need to maintain adequately large populations of each species.
4. Consideration should be given to developing national or international monitoring schemes for fish, such as the wild trout and char watch at present being organized.
5. There is a general need for identifying important geographic areas, species and their distributions in order to provide background for making decisions about where, what and when to begin any preservation programmes for potentially threatened fishes. Conservationists should cooperate with such agencies as the IUCN in cataloguing threatened fish genetic resources.

### 7.5 Recommendations Primarily for the Scientific Community

1. Physiological "races" have been recognized for many years, yet we do not know whether physiological differences among fish populations are representative of genetic differences. Physiological variation is influenced by environmental variables making the genetic bases of these variables difficult to elucidate. Research on this problem is urgently needed.
2. Monitoring the genetic variability of both natural and cultured fish populations would be advantageous for a number of reasons. However, it is not known if the different methods of ascertaining genetic variability are representative of genomic variance per se. Therefore, research in this area is also highly recommended. Correlations between electrophoretic, meristic and morphological variation should be examined in numerous fish species to determine which methods provide unbiased estimates of genetic variation within species. These studies should be supported, when possible, with artificially induced changes in genetic variation via inbreeding studies.
3. Genetic (electrophoretic) monitoring of several fish species and populations during the entire course of their exploitation is deemed desirable for several reasons: e.g. (1) the possibility exists that similar patterns of change will be detected in different exploited populations; (2) biochemical correlates of changing age or size class structure might be found and used as indicators of overexploitation.
4. Recent advances in genetic engineering techniques suggest that transfers of genetic information between unrelated fish species will be possible within the foreseeable future. Research in this area is to be encouraged because such developments will broaden the genetic resource base for fisheries.
5. Research should be accelerated on the cryopreservation of fish sperm, ova and embryos.
6. Extensive ecological and systematic (taxonomic) surveys are required in tropical regions where large fractions of ecosystems are poorly understood and a large fraction of species are undescribed.
7. Research is needed on the controllability of sterile hybrids in relation to their impact on the ecosystem. It is particularly important to assess the possible genetic "leakiness" of "sterile" hybrid stocks and their possible impact on wild populations.

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## APPENDIX 1

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## APPENDIX 2

Proposal for Regional Centres of Aquatic Genetic Resources Information

Aquatic ecosystems and resources are of critical important to the human population. To better utilize these resources there must be more emphasis on obtaining basic information, providing intelligent advice, and promoting cooperative efforts to preserve the available genetic resources for posterity. At the international level there must be direct mechanisms for the resolution of biologically important problems and conflicts, particularly regarding the exploitation of fish species. At present there is little opportunity for such clearly needed research as, for example, identification of sub-species components of resources shared between two or more nations. We propose the development of regional research centres where a corps of experienced researchers could act in both applied and tutorial roles. The fellowships, secondments and training opportunities could provide adequate manpower to operate a research facility on a seasonal basis. The proposed programme must include a centralized and/or regional centre for:
I. Archiving species, sub-species, genetic and simple meristic data; general life history, habitat and physiological data.
II. Information dissemination service:

1. Newsletters
2. Data catalogues
3. Documenation
4. List of experts to respond to enquiries about genetical, biological and ecological implications of genetic preservation and options.
III. Service: Catalogue of species for introduction; their sources; ecological limitations; and as transport mechanism which "records" introductions as well as facilitating later evaluation of the general impacts of the introductions.

APPENDIX 3<br>Proposal for a Programme of Education on Genetic Conservation for Aquatic Resources

An international programme is needed for education and to provide baseline information from which to evaluate the diversity and vulnerability of genetic resources in fish. Before we can begin such evaluations or activities to preserve endangered genetic resources certain questions must be answered. These questions vary with species, geography and environmental regime. A few regions and habitats are well studied particularly in the temperate zones. For example, in the California current there has been a long history of systematic evalutions of species abundance at all ages, and in many cases from egg to adult. Such background information provides a basis for identifying small populations with limited distributions which might be vulnerable to events leading to genetic depletion. Several small endemics are already under protection in this system (e.g., Garibaldi), primarily through legislation and development of preserves (e.g., La Jolla marine preserve).

In order to preserve the genetic resources of vulnerable fish populations, programmes for identifying potentially threatened or vulnerable species need to be implemented; monitoring programmes need to be devised and begun; archival methods and information retrieval and dissemination systems need to be provided. For maximum impact these need to be institutionalized at the international level.

The role of the United Nations agencies such as UNEP and Unesco seems appropriate for such a programme. The basis of the programme would be the development of training courses on the national and regional levels. The first level would, by design, involve employees of the fishery institutions at the lower technical levels so that they might remain in the operational or active sectors of their institutions for sufficient time to promote continuity of any monitoring and identification programmes.

The training course should include introductory level exposure to general ecological and genetic principles. This could take the form of a series of lectures and field exercises which would be the basis of the identification of vulnerable resources and subsequent monitoring schemes. The field exercises would not only be designed to teach collecting and monitoring techniques, but would also be a source of baseline information.

A second level of training workshops could be held at the regional level. Here it would be appropriate to use natural biogeographic features such as drainage systems to define the regions, and/or shoreline or substrate characteristics for the marine system (e.g., mangrove or coral reef systems). The objectives at these workshops would be to familiarize regional experts in the techniques of identification and a classification of fish and in the estimation of genetic and ecological vulnerability in fish, in order to begin the evaluationof the specific regional resources which look to be most vulnerable. The technical level of the trainees need not be particularly high, but familiarity with basic mathematics, biology and chemistry, would be required.

The advantages of a two-level training scheme would include: (1) the accumulation of basic information about the fish resources of the regions; (2) direct contact with national institutions for involvement in the programme objectives and means; (3) communication networks could be created at the level of most relevance (the field staff or operational level); (4) the programme can be promoted and operationally implemented at the outset, rather than this being separated in time.

The impact could be direct on both national and international programming and funding levels. At the national level there should be increased emphasis on all aspects of fisheries research, for example, in the general areas of: taxonomy; toxicology; genetics; ecology; physiology; behavioural biology; biochemistry; developmental or early life history studies. The above are suitable programmes to be initiated by UNEP and other UN bodies and would provide opportunities for young scientists and students from Third World developing countries to train in the national and regional workshops, and in appropriate cases, perhaps at the Ph.D. and graduate level in the more industrialized countries.

Certainly there are medium-term objectives involved where skills and knowledge may best be transferred directly. This could best be resolved through development of international research educational exchange programmes.



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