ACTION BEFORE EXTINCTION

An International Conference on Conservation of Fish Genetic Diversity

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ACTION BEFORE EXTINCTION

An International Conference on Conservation of Fish Genetic Diversity

Edited by:

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An Overview of *Action Before Extinction*

Brian Harvey [1]

Setting the Stage

The idea for an international workshop on fish genetic conservation suggested itself in 1994 when I was reviewing global salmon gene banking for the Canadian Department of Fisheries and Oceans. Preparing the report (Harvey 1994) involved a lot of correspondence with contacts I already had, like the Norwegian Directorate for Nature Management, Washington State University, the National Institute of Aquaculture in Mie, Japan, and others. But it also involved meeting new people and learning about new programs, like the gene banks in Finland, Iceland, and Sweden, and the huge investment in genetic conservation that had gone on in the former Soviet Union until about 1992. Everyone I talked to or corresponded with agreed that we should all meet.

But these were only the salmon people. I had begun my research career in the early 1980s on tropical species, and by 1994 World Fisheries Trust had become involved in several fish genetic conservation projects in developing countries where we were dealing with the technical and policy ends of gene banking a variety of migratory fishes in several South American countries. Everyone we worked with had the same problems and concerns as the salmon people farther North: selection of stocks, national and local policies (or, more commonly, the lack of them), technology, access and ownership, standards, data management, when to begin a gene banking program, even the public and professional perception of gene banking.

Clearly there was some global "common ground" here. In a very general way, Article 9 of the Convention on Biological Diversity had already acknowl-

"By 1994 there had already been several international meetings on fish genetic conservation, including FAO's Expert Consultations in Rome in 1980 and 1992, and an ICLARM-FAO workshop in Rome in 1995"

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"By 1997, even the protection of habitat was no guarantee of fisheries recoveries"

edged an important role for *ex situ* conservation of genetic resources, even if it was plant genetic resources that were probably uppermost in the minds of those drafting it. By 1994 there had already been several international meetings on fish genetic conservation, including FAO's Expert Consultations in Rome in 1980 (FAO 1981) and 1992 (FAO 1992), and an ICLARM-FAO workshop in Rome in 1995 (Pullin and Casal 1996). The Rome meetings laid the conceptual foundation for global fish genetic conservation, and it was significant that the last two included representatives from the plant genetic resource community in recognition of the vast policy experience they could bring to bear on the problem. But there had to date been little opportunity for the people and institutions who were actually doing fish gene banking around the world to get together and compare notes, and none at all for them to do so in the company of policy makers and funders. This was the need that *Action Before Extinction* was intended to fill.

"By 1997 we certainly knew that aquatic biodiversity was eroding, but we were becoming acutely aware that there would be no simple explanations for why this was happening, and we were feeling less and less confident that we knew how to stop it."

Fisheries crises as a backdrop

By the time organization of *Action Before Extinction* began in earnest, several other things had become clear. First, the outlook for global fisheries was even grimmer than in 1992, and by 1997 freshwater biodiversity had become a much-debated topic, serving as the theme for the Third Meeting of the Subsidiary Body on Scientific, Technical and Technological Advice (SBSTTA). Just as clear was the realization that, in many countries, a drastic loss in aquatic genetic diversity by no means meant the automatic establishment of a gene banking program on anything like the scale in agriculture; despite the fact that delegates to SBSTTA in 1997 actually recommended establishment of fish gene banks, there was (and remains) debate about the proper contribution of *ex situ* genetic conservation, and in more than a few instances there was evidence of outright concern that *ex situ* conservation was an abdication of a higher responsibility, namely the protection of habitat. But then, by 1997, even the protection of habitat was no guarantee of fisheries recoveries: mounting evidence from around the world was beginning to force managers to accept that the effects of climate change on fish behaviour had now to be considered too. In Canada, huge declines in ocean survival of some salmonids were beginning to be considered as important as habitat loss and overfishing in causing the disappearance of stocks.

In short, by 1997 we certainly knew that aquatic biodiversity was eroding, but we were becoming acutely aware that there would be no simple explanations for why this was happening, and we were feeling less and less confident that we knew how to stop it.

Action Before Extinction was conceived in the untidy environment of global fisheries in the 90s, with the aim of putting practitioners and policy makers together for three days to review past achievements, discuss future imperatives, and perhaps even agree on some present actions. As organizers, World Fisheries Trust tried to keep the meeting from being all-policy, a role much better played three months later at the ICLARM-FAO Bellagio conference (Pullin et al. in preparation), or all-technique (that was taken care of by the special session on cryopreservation at the World Aquaculture Society Annual Meeting, held on the same dates as *Action Before Extinction*). In WFT's view, fish genetic conservation encompassed far more than *ex situ* preservation, was as important for conservation as for aquaculture, and certainly wasn't just a matter of collecting some samples of fish sperm and freezing them.

"In WFT's view, fish genetic conservation encompassed far more than ex situ preservation."

Participants and venue

The best way to keep the agenda balanced was of course to invite the right mix of people. We decided to try and cater to the following broad categories:

- *Established fish genetic conservation programs* (Ananiev, Chebanov, Harvey, Isaksson, Kopeika, Piironen, Ponniah, Thorgaard, Walso);
- *Genetic conservation programs in early or planning stages* (Diaz, Foresti, Godinho, Wada, Zaniboni);
- *Agencies and funders* (Bartley, Braga, Davy, Fischer, Fortier, Pullin, Raymond, Windsor).

Naturally, assignment to any one of these categories is restrictive and imprecise for several participants, and impossible for Chris Wood and Blair Holtby, whose paper on defining conservation units had generic, overall relevance. And of course the list was incomplete; although the scope of fish genetic conservation is still fairly limited, keeping the number of participants below 25 meant ignoring many other potentially excellent contributors. China, for example, is not represented, nor is Africa.

Holding the meeting in Vancouver meant gathering in a place where interest in salmon issues is intense and fisheries debate is a daily (often rancorous) media event. Canada, although respected internationally for the quality of its fisheries research, has seen its share of fisheries catastrophes in the past decade. The costly demise of the Northern cod still looks irrevocable, and, despite the closure of the commercial fishery for Atlantic salmon, escapements for many stocks continue to worsen—another example of a fishery that has not responded to a ban on fishing. To maximize the opportunity for exchange of ideas, we also invited interested local biologists, man-

"Canada, although respected internationally for the quality of its fisheries research, has seen its share of fisheries catastrophes in the past decade."

agers, First Nations and NGOs to attend; these people, who are listed in Appendix 1, did not present papers but took part in all discussions.

In February 1998, when the meeting was held, the continued decline in ocean survival of BC coho salmon was much in the news. A month after the workshop the Government of British Columbia announced a living gene bank for steelhead salmon and, as this chapter is written in May, fishing communities are in turmoil over an outright ban on coho harvest that will profoundly affect fishing for other salmonid species as well. *Action Before Extinction* could hardly fail to be relevant in this climate, a fact to which the wide variety of sponsors willing to underwrite the meeting also attests. To further underscore the local relevance of what was, after all, an international meeting, the keynote address that opened *Action Before Extinction* was delivered by Hon. John Fraser, who is not only Canada's Ambassador for the Environment but has also contributed enormously over the years, both officially and privately, to the understanding and resolution of the many problems that beset Canadian fisheries.

The participants in *Action Before Extinction* spoke in their personal capacities. Their views do not necessarily represent or constitute the official position of their organization. The papers presented in these Proceedings are excellent and discussion was lively. My overview introduces each paper and then summarizes the discussion that followed each presentation, as well as general discussion that occupied the final afternoon, according to a half dozen or so recurring themes.

"The costly demise of the Northern cod still looks irrevocable, and, despite the closure of the commercial fishery for Atlantic salmon, escapements for many stocks continue to worsen—another example of a fishery that has not responded to a ban on fishing."

Summary of Papers

The meeting was organized so that authors representing agencies and funders first presented an overview of fish genetic conservation; they were then followed by presentations that described genetic conservation programs of greater or lesser magnitude in a number of countries. A general discussion, based on themes that became evident during the presentations and the brief discussion that followed each, occupied most of the final day of the meeting. My overview follows roughly the same sequence.

Opening addresses

The meeting opened with addresses from **Hon John Fraser**, Canada's Ambassador for the Environment, and **Jamie Alley**, Director of Fisheries, B.C. Ministry of Fisheries. Mr. Fraser spoke of what he termed the "disconnect" between the facts that science can provide to society and the story that is often presented to the public, and he provided many examples from the areas of climate change and fisheries. He also provided a valuable synopsis of comments on the status of Pacific salmon stocks, particularly coho, provided by the federal Department of Fisheries and Oceans Science Branch.

Mr. Alley provided an overview of management of B.C. fisheries from the perspective of the Province of British Columbia and listed a number of new provincial initiatives for inventorying fishery resources and protecting habitat to promote *in situ* conservation of biodiversity.

Agencies and funders

Presentations by **Roger Pullin, Devin Bartley** and **Ruth Raymond** set the stage by highlighting the interdependencies in philosophy, methods, and in some cases policy, between the international plant, animal and fish genetic resource communities. The International Centre for Living Aquatic Resource Management (ICLARM—Pullin) and the International Plant Genetic Resources Institute (IPGRI—Raymond) are both members of the Consultative Group on International Agricultural Resources (CGIAR); as such, both work closely with the Food and Agriculture Organization of the United Nations (FAO—Bartley) to promote, regulate and develop policies for collection, management and sharing of the world's genetic resources. Any eventual global coherence to fish genetic conservation will depend on joint planning, coordination and sharing of expertise among these key agencies, and indeed *Action Before Extinction* carried on a tradition, begun with the FAO Expert Consultation on Fish Genetic Resources in Grottaferrata in 1992, of including all three institutions in international discussion of fish genetic conservation.

Roger Pullin and co-authors, in attempting to place aquatic genetic conservation in a global context, suggest a very broad definition of gene bank, namely "any collection of genetic material kept to ensure the future availability of that material for conservation, study or production purposes." Their paper compares gene banking for plants and aquatic organisms, in terms of what has been done and what can be done in the future. ICLARM's own involvement with gene banking aquatic organisms is described for the Genetic Improvement of Farmed Tilapia (GIFT) project as well as establishment of several marine protected areas. The authors argue that protected areas should be used as *in situ* gene banks or "gene parks", and that they should be set up sooner rather than later.

Devin Bartley describes how fish gene banks can be used to implement articles of the FAO Code of Conduct for Responsible Fisheries and the Convention on Biological Diversity. He cites the precautionary approach to development as important in deciding on acceptable levels of risks and "trigger points" for establishing gene banks for particular stocks. As first hand experience he describes the FAO gene banking project for ship sturgeon *Acipenser nudiventris* in the Caspian Sea, where habitat cleanup efforts are unlikely to be in time to prevent catastrophic loss of biodiversity.

Ruth Raymond argues that plant, animal and fish genetic resources have enough in common to warrant common legal and policy frameworks. Her paper is particularly important in providing the rationale for fish genetic conservation to "learn from" several decades of accumulated experience with plants—although there are clearly many historical and technical differences. Those interested in developing local and national policies on the utilization and exchange of fish genetic resources will find the paper's discussion of bilateral and multilateral arrangements very useful and will find lessons with obvious applicability to fish. Pros and cons of restrictive exchange arrangements, such as are tending to emerge from many countries as a result of the Convention on Biological Diversity, are discussed. Her paper should be essential reading for fisheries policy makers.

Brian Davy (International Development Research Centre, IDRC) presented a funder's view of aquatic biodiversity and described specific programs and projects that promote conservation of fish genetic diversity. Within IDRC's overall mission of "empowerment through knowledge", aquatic genetic conservation is contained in the Sustainable Use of Biodiversity program along with agricultural biodiversity, and the emphasis is on ensuring food security and equitable sharing of the benefits of genetic resources. IDRC's programs have a strong community flavour and in this area focus on the intersection between wild genetic diversity, capture fisheries and aquaculture. IDRC funds research, promotes development of policies for sharing biological diversity, and funds meetings like the present one (IDRC was the lead sponsor of *Action Before Extinction*).

The World Bank is a lending institute with a responsibility to help borrowing countries comply with provisions of the CBD, including those that relate to freshwater biodiversity. With a total water resource portfolio of US $33 billion over the last decade there are many opportunities for the Bank to invest in conservation of freshwater biodiversity, and projects that specifically target this area are showing up in increasing numbers in the Bank's portfolio. **Isabel Braga** outlines the kinds of impact on freshwater habitat that result from most water development projects and recommends that genetic diversity should not decline as a result. To achieve this, she argues that scientists be involved in project planning; that genetic diversity considerations be incorporated into project planning right from the start, not as after-the-fact mitigation; and she provides a "Recommended Approach" to the *in situ* conservation of freshwater biodiversity in development projects. She also gives some examples of past and present World Bank initiatives in conserving freshwater biodiversity, including direct investments in conservation, workshops, publications, inclusion of freshwater biodiversity in lending activities, and support for strategic global partnerships like the Global Water Partnership and the World Water Council.

The North Atlantic Salmon Conservation Organization (NASCO) is a treaty organization that contributes to conservation and management of Atlantic salmon stocks in Canada, Denmark, the European Union, Iceland, Norway, the Russian Federation and the United States. **Malcolm Windsor** and **P. Hutchinson** describe the importance of genetic diversity in survival of Atlantic salmon and the variety of threats—especially the threat of genetic contamination from farmed salmon—to which the species is presently subjected. They stress the precautionary or conservative approach, especially in the light of continued stock declines despite cessation of commercial fishing (this has been the experience with Canada's Atlantic salmon). Examples of this approach include establishment of gene banks such as the Norwegian Atlantic Salmon Gene Bank. NASCO's own guidelines for establishing and operating salmon gene banks are included in the paper—an example of multi-party standards that aid in coordinating activities of the various members of NASCO. These standards will be of interest to those interested in establishing a gene banking program.

An indigenous perspective on conserving genetic diversity of Pacific salmon is provided by **Fred Fortier,** representing the Shuswap Nation Fisheries Commission and the B.C. Aboriginal Fisheries Commission. Salmon stocks in Shuswap traditional territory have experienced severe declines in the past decade, with many coho stocks in particular being at critically low escapements. Fortier describes the various costs of these declines, from lost fishing opportunity to the financial cost of maintaining a gene bank of selected stocks. The Shuswap Nation, with technical assistance from World Fisheries Trust, began a gene banking program in 1993; cryopreserved sperm from six stocks is currently held in trust pending an action plan for its use in rebuilding stocks. Fortier's paper provides important insights into indigenous attitudes toward stewardship and exploitation of biodiversity, and proposes policies for the conservation and management of genetic diversity. (All fishing on Thompson and Skeena coho stocks, including those in Shuswap territory, has been declared closed by the Department of Fisheries and Oceans as this is written. The aims of the Shuswap program are similar to those of the Nez Perce Tribe in Idaho, USA, who are pursuing a long-term gene banking program for chinook and sockeye salmon in tributaries of the Columbia River—Eds.).

Finally, a change of scene from salmon was provided by **Carlos Fischer** of the Brazilian Ministry of Natural and Renewable Resources (IBAMA), who described the regulation of fishing in Brazil, the growing recognition of the importance of freshwater biological diversity, and the belief that maintenance of habitat is essential and that regulation of fishing alone cannot be counted on to halt declines in numbers of fish (see also **Godinho**, this volume, for a discussion of the ineffectiveness of fishing closures in Brazil). The scope of the problem is huge, not only in terms of species (Brazil has more than 3,000 freshwater fish species) but also from the standpoint of a regulatory structure in which, up till 1989, each State set its own standards for gear size, catch, reporting and enforcement; there were 28 such regulations for the Amazon basin alone. His paper describes IBAMA's post-1990 efforts to re-orient fisheries management according to hydrographic basin and to include input from all groups that use the resource. Sport fishing is a big industry, representing, for example, 75 per cent of the catch in the Pantanal region. There are also more than 2,500 unregulated pay-fishing ponds in Brazil, compounding the threat of introduction of exotic species that already exists from aquaculture. IBAMA is currently working with local governments, fishermen and industry to develop fisheries management strategies that recognize these threats.

Established fish genetic conservation programs

The "established" fish genetic conservation programs represented at *Action Before Extinction* do not comprise a complete global list, but they include most of the better-known ones (which is not to say that significant programs were not overlooked). Programs in Russia (Ananiev, Chebanov, Kopeika) and Norway (Walso) have been established for a decade, and the gene banks in Finland (Piironen) and Iceland (Isaksson) took the lead from Norway's. Gene banking in Canada (Harvey) and the USA (Thorgaard) is more recent. The National Bureau of Fish Genetic Resources in India is the oldest national program, having been in operation since 1983.

The Norwegian Gene Bank Program for Atlantic Salmon is described by **Øyvind Walsø.** It has operated since 1986 and was established to preserve and eventually re-establish the genetic diversity of Norwegian salmon stocks threatened by acid rain, parasite infestations, water development projects and escaped farmed salmon. Initially a frozen sperm bank only, the gene bank has expanded to include three "living gene banks" or broodstock stations. The stations facilitate conservation of genetic diversity by using frozen milt and by maintaining several year classes. Mating schemes in the living gene banks incorporate the use of samples of cryopreserved milt that now number over 6,000 individuals from 155 stocks. The cost for roughly a decade's operation of the program has been about CAD$14.6 million, a substantial figure that primarily represents expenditures on the living gene banks and was justified without hesitation by the author as a demonstration of the deep significance of salmon to Norwegians.

Jorma Piironen's paper on genetic conservation of endangered fish stocks in Finland is a lucid description of how another Nordic country has come to grips with the problem of conserving aquatic biological diversity at risk from habitat alteration, overfishing, unplanned stocking and disease. Only two of eighteen original Atlantic salmon populations remain in

Finland, and sea trout have declined similarly. His paper clearly lays out the roles the State has decided to play, as well as those it has not, and is explicit about the role of State-controlled fish culture in Finland as the means to preserve fish genetic diversity. In accord with the Finnish national plan of action to preserve biodiversity there are State-run living and cryopreserved gene banks for a variety of species including Atlantic salmon, char, sea trout, brown trout, whitefish and grayling, all with the aim of establishing breeding stocks in which the genetic makeup is as close as possible to that found in the remaining wild fish. Frozen milt is incorporated in breeding schemes as a means of further broadening the genetic base. As an example, the milt banking program and the associated living gene bank for Tana (Tenojoki) River salmon is described in some detail, including practical linkages with the Norwegian Atlantic Salmon Gene Bank.

Fish genetic conservation in another Nordic country, Iceland, is described by **Árni Ísaksson.** In general, and partly due to a ban on ocean harvest of salmon since 1932, Icelandic stocks are in good condition. Acid rain has not affected Icelandic rivers as it has Norway's, and the greatest concern is that straying of farmed stocks will be detrimental to wild ones which are the basis of a large sport fishing industry. To this end a small gene bank of cryopreserved salmon sperm was established by the Reykjavik Angling Club in 1989, but its activities have declined since sea cages were removed from the areas of greatest concern.

In India as in Norway and Finland there is a national plan specifically for preservation and sustainable use of fish genetic resources. The mandate and activities of the Indian National Bureau of Fish Genetic Resources are described in detail by **A.G. Ponniah,** who, like Pullin and Raymond, makes comparisons to plant genetic resource conservation. Pros and cons of fish gene banking (including living and cryopreserved banks) are discussed, along with the ways in which such banks can be used in a practical way to conserve and sustainably use Indian fish genetic resources. Species selected for gene banking are chosen on the basis of economic importance and endangered status, and cooperation between the private sector, local communities and the State is encouraged in NBFGR programs. The bureau also has a significant research function that includes genetic analysis of wild and cultured stocks so that decisions about what to conserve are made rationally. The NBFGR milt gene bank has operated since 1989 and uses low-cost field cryopreservation methods.

Genetic conservation of the rich and in many cases threatened fish fauna of the former Soviet Union is reviewed by **Eugeny Kopeika** (Ukraine), **Valentin Ananiev** and **Michail Chebanov** (Russia); the latter concentrates specifically on sturgeon status and recovery efforts. Papers by Ananiev and Kopeika concentrate on the use of cryopreservation methods, developed in

Russia and the Ukraine, to conserve genetic diversity from a wide variety of species; they argue that cryopreservation is a cheap and practical way of preserving genetic diversity while efforts to remedy the root causes of its loss continue. This strategic approach was embodied in the Low Temperature Gene Bank of Marketable, Rare and Endangered Species, begun in 1990 and comprising cryobanks in several locations that accumulated a considerable number of accessions until the collapse of the Soviet Union and subsequent funding crises made it extremely difficult to continue, and in some cases led to the loss of stored samples of genetic material.

Chebanov's paper describes genetic conservation of sturgeon in Russia, all species of which have now been placed in Appendix II of CITES. Existing methods of repopulation focus on captive breeding which, Chebanov argues, must be informed by a thorough understanding of local population structures. Effects of dam construction on sturgeon in the Sea of Azov are described, along with programs for enhancement that include living and cryopreserved gene banks. Together, the papers from the former Soviet Union paint a vivid picture of a complicated fish fauna under threat, significant investment in ex situ conservation theory and practice, and an acute need for funding to keep even some of these conservation programs alive.

Brian Harvey relates the experiences of World Fisheries Trust in *ex situ* banking of fish genetic resources in Canada and South America. Since 1993, World Fisheries Trust has used portable field equipment to cryopreserve sperm from wild fish. Programs in Canada include collaborative field work with various First Nations in British Columbia and a two-year pilot gene banking program for the federal Department of Fisheries and Oceans. In South America, WFT has worked on adaptation of field genetic conservation methods to migratory fishes in Colombia and Venezuela, and is presently managing a three year project to train Brazilian biologists from government, academia and the private sector in genetic conservation theory and practice. WFT is also active in policy development with various agencies in Canada.

Gary Thorgaard and co-authors provide a picture of *ex situ* fish genetic conservation in the United States against a complicated management backdrop in which several agencies may have conflicting responsibilities—not unlike the situation in some parts of Brazil. As examples they discuss gene banking programs for Columbia River salmon, Colorado River fishes and sturgeon. Their point—that complexity of government structure tends to make it unclear who is ultimately responsible for preventing extinction, and that divided responsibility leads to confusion and inaction—is one that surfaced frequently in other papers. They also bring up two other common themes of the conference: that gene banking is perceived to be costly, and that some biologists see gene banking as an abdication of a more basic responsibility to protect habitat.

Genetic conservation programs in early or planning stages

Since about 1990 the effects of pollution and habitat alteration (especially dams) on large migratory fish species in South America have stimulated more and more local interest in genetic conservation, especially as captive breeding for restocking forms such an important part of recovery efforts. Two countries with an exceptionally rich migratory fish fauna, Brazil and Colombia, are represented in *Action Before Extinction*, and their technical papers (policies in Brazil have already been covered, by Carlos Fischer), give readers unfamiliar with the subject a fascinating first look at a problem of enormous magnitude.

Papers by **Hugo Godinho** and **Evoy Zaniboni** describe the fish themselves, their habits, threats to which they are presently subject and measures that are being taken to conserve their genetic resources and habitat. These two papers cover two different geographic areas: Godinho's concentrates on the São Francisco basin, already much-developed for hydroelectric power generation, and Zaniboni's on the Uruguai, where major development is planned but not yet underway. Both authors talk about reproductive patterns and the effects of dams, and particularly about the practice of restocking reservoirs and the difficulty of ensuring that such programs have access to a wide range of genetic diversity at a time when it is becoming harder and harder even to locate broodstock. Gene banking of cryopreserved sperm from a variety of migratory species is one tool that is now being introduced in both these areas.

Silvio Toledo and **Fausto Foresti** present an overview of Brazilian research in conservation genetics at the State University of São Paulo and the University of São Paulo; their paper concentrates on studies aimed at understanding the genetic makeup of populations of migratory fish using cytogenetic, allozyme and DNA techniques. Results of their studies are provided to fishery managers and aquaculturists and help set a rational course for hatchery programs and restocking, much as the work described by Wood and Holtby (this volume) is used to help determine management strategies in Canada. Note especially the genetic analysis of pacu *Piaractus mesopotamicus* populations in the Pantanal wetlands to identify *in situ* gene banks for the species—this is the broader application of gene banking mentioned by Pullin and co-authors.

Colombia shares both migratory species and problems with Brazil; the Magdalena River, for example, has shown a 78 per cent decline in landings over the past two decades. **Jaime Diaz** provides an excellent overview not only of the Colombian resource and the threats it faces, but also of Colombian legislation and policy that affect conservation. The importance of the Andean Decision of 1996, "Common Regime of Access to Genetic Resources" is rightly highlighted as crucial in the design of genetic conservation programs. The paper is especially useful in that ongoing policy development in Colombia is also described; policy will affect the strategies that are developed for fish genetic conservation and the sustainable use of those resources. Actual on-the-ground efforts at *ex situ* conservation using captive broodstocks and cryopreservation are just beginning in Colombia.

In Japan, certainly one of the world's most-developed countries, habitat loss and a very well developed aquaculture industry have clearly contributed to the loss of fish genetic diversity. However, while there is considerable technical expertise, activity in fish genetic conservation has to date primarily been in order to ensure continued seed production for aquaculture. Recently, though, as **Katsuhiko Wada** describes, a number of research programs funded by the Environmental Agency of Japan have begun to look at the genetics of endangered wild populations of several species of fish. There are several collections of cryopreserved fish sperm at universities and private aquaculture companies in Japan, but no coordinated national program.

"For some, a fish gene bank is analagous to an agricultural seed bank; others are comfortable including broodstock collections as 'living' gene banks; some suggest the term is very broad and includes 'collections' of organisms within aquatic protected areas."

Defining conservation: how do we decide what to conserve?

Every paper presented at *Action Before Extinction* talks about threatened populations of fish and the pros and cons of a variety of methods for conserving their genetic resources. Several refer to *ad hoc* guidelines or criteria that provide the framework for making choices about which species or populations to select for conservation. But none offer a model or a line of reasoning that could be applied broadly, in many different situations, to ensure that there is a solid scientific basis for selecting one population over another. The paper by **Chris Wood** and **L. Blair Holtby** attempts this, taking Pacific salmon as a well researched example. Their thesis is that surveys of natural genetic variation can guide decisions about sampling design for two very different *ex situ* conservation objectives: archiving genetic diversity for the species as a whole, and preserving local adaptations for the restoration of wild populations. Strategies for archiving are discussed using genetic survey data for sockeye salmon.

Restoration of salmon runs means conserving local adaptations; Wood and Holtby argue that choosing the appropriate population unit to satisfy this objective means determining the spatial scale of local adaptations, and they propose terms for defining population (and conservation) units according to empirical estimates of gene flow based on actual genetic survey data. By way of a case study of this approach, they describe studies that have led to definition of population units of coho salmon in the Skeena River in northern British Columbia, where declines in numbers continue (all harvest on these and other B.C. coho stocks has been banned in 1998—Eds.), and relate their own conclusions to IUCN Red Book criteria. Although Wood and Holtby warn against the substitution of theoretical predictions for empirical evidence of local adaptation ("gumboot biology"), their paper has obvious and profound relevance for the rational development of any genetic conservation program.

Summary of Discussion, Common Themes and Recommendations

Individual presentations were followed by ten-to-fifteen minute discussions, and the final afternoon of the conference was devoted to discussion of common themes that had made themselves known as the presentations went along. What follows is a summary of discussion under these thematic headlines.

Definitions: What is genetic conservation? What is gene banking?

Action Before Extinction was not intended to be a gene banking meeting; whether it turned out to be depends on your definition of the term. For some, a fish gene bank is analagous to an agricultural seed bank; others are comfortable including broodstock collections as "living" gene banks; some suggest the term is very broad and includes "collections" of organisms within aquatic protected areas. The first two examples could be considered *ex situ* genetic conservation, and the latter *in situ*, but, as Pullin pointed out, the definition of a gene bank for fish may need to be different from that for plants. **The approach most participants seemed comfortable with was to think instead of "genetic conservation", not gene banking, and use the definitions of genetic resources, *ex situ* conservation, *in situ* conservation and protected area as provided in the Convention on Biological Diversity.** The term "gene bank" means different things to different people and for some has connotations of sterile archives of biodiversity; "genetic conservation" is a less loaded, and already well defined, term. Following the CBD definitions has the added benefit of reference to a widely ratified document where the supporting role of *ex situ* conservation is unequivocal, a role that was endorsed by all participants.

Assistance from the plant genetic resource community

Broad overall similarities between plant genetic resource conservation and fish genetic resource conservation came up repeatedly during the meeting, and all participants recognized the enormous experience pool on the plant side. However, as Raymond, Bartley and Pullin pointed out, the two fields are anything but identical, and fish genetic conservation programs in the years to come will need to be selective about where they can learn and borrow from the plant people. Standardized data management is one area where fish genetic conservationists can get some immediate help; tools used by participants ranged from custom accessions management software like SpermSaver to various spreadsheet adaptations. In terms of technologies for genetic conservation there appeared to be much less crossover.

Probably the most fruitful area for learning from plant genetic conservation is in policy development, where there have been decades of international discussion and negotiation over just the same issues of access, ownership, compensation and sharing that fish genetic conservation is only now beginning to confront. **Participants recommended that a Working Group representing the fish genetic resource community establish formal, functional relationships with the plant genetic resource community. This would likely most easily be accomplished by using IPGRI as an entry point.**

"Probably the most fruitful area for learning from plant genetic conservation is in policy development, where there have been decades of international discussion and negotiation over just the same issues of access, ownership, compensation and sharing that fish genetic conservation is only now beginning to confront."

Who is responsible for conserving fish genetic resources?

Gary Thorgaard put it succinctly: divided responsibility for fish genetic resources leads to confusion and inaction; often, the resources simply do not get conserved. Many views on responsibility were presented, from clear State responsibility (for example the Nordic countries) to the confused situations with multiple agencies and regulations in Canada, the US and Brazil. Other participants referred to the Tragedy of the Commons, where a common property is allowed to disappear for lack of leadership. **In practical terms, participants recognized that to wait for consensus on collection and management of fish genetic resources within and between nations will take too long; what is now an environmental issue will rapidly become a food security issue, and action must be taken immediately.** Some kinds of national policies can, in fact, even work against conserving and using genetic resources, as recent experience has shown in countries that have created policies that severely limit the exchange of genetic resources.

Is gene banking an admission of failure?

Many participants referred to the view that gene banking is an admission that management has failed to protect the resource. Along with this sentiment go perceptions of gene banking as mitigation, as a license to despoil the environment, and as diverting funds away from habitat preservation. This view seems most commonly voiced by fisheries managers; it is not usually held by scientists or the public. Several participants referred to cases where managers resisted gene banking for these reasons, and all felt a need to respond to the fear that gene banking is an alternative to good management rather than a part of it. **Participants agreed that, in accordance with Article 9 of the Convention on Biological Diversity, *ex situ* genetic conservation supports rather than replaces *in situ* conservation, and is an example of using the precautionary approach to conservation.** Ísaksson pointed out that, in Iceland, concerns about a variety of risks stimulated formation of an Atlantic salmon gene bank that clearly embodies the precautionary approach—in this case gene banking went ahead even though the risks were still unclear. In the end, the following statement was accepted: **Gene banking is an admission of uncertainty.**

"Divided responsibility for fish genetic resources leads to confusion and inaction; often, the resources simply do not get conserved"

Uses of ex situ collections of genetic resources; when to collect

Participants agreed that, however defined, gene banking is an important tool for archiving useful genetic adaptations for study and future use; for permitting the rebuilding of endangered populations using the widest possible genetic base; and for facilitating the development of breeding programs for enhancement and aquaculture, especially culture of native species. Only the first and last of these functions are analogous to plant genetic resource conservation and use; rebuilding populations is unique to fish. Also in contrast to plants, preservation of fish genetic diversity is particularly urgent in light of the very limited development of breeding programs at a time when genetic resources are threatened in many areas.

The two most commonly cited uses of genetic conservation—rebuilding threatened populations and improving breeding programs for aquaculture—tend to segregate into "conservation" and "commercial" applications, and there was discussion on whether such a segregation is a good thing. It was pointed out that, as in plants, wild genetic resources may have use down the road for aquaculture, although it's not likely that society will fund collections on that basis. As Piironen pointed out, genetic resources are controlled by the State in Finland no matter what their eventual use; Walso argued for a separation of commercial and conservation gene banks. Funding, though, may only be obtainable when both functions are integrated, the strategy being followed in India. Pullin argued that, in situations where fish genetic resources are vanishing at unprecedented rates, for example the Philippines, the international community has a responsibility to ensure their conservation; this responsibility is also contained in the Convention on Biological Diversity.

"Participants agreed that... gene banking is an important tool for archiving useful genetic adaptations for study and future use; for permitting the rebuilding of endangered populations ...and for facilitating the development of breeding programs"

Technology, cost, data management and standards

Participants treated the meeting as primarily a non-technical one, although many provide technical details of broodstock management and cryopreservation methods in their written papers. Standardization of cryopreservation methods is probably too much to hope for in the near term but there are many methods that work for a variety of species; previously untried species, for example the migratory fishes of South America, have proved amenable to experimentation. Little has been published on the results obtained with different collection and mating schemes used by living gene banks, and several participants noted the need for such information as national programs are planned. There was little in the way of technical information on *in situ* conservation in aquatic protected areas.

"In short, cryopreservation can be cheap or expensive depending on how it's done."

About the cost of fish genetic conservation there are obviously different experiences and perceptions. Living gene banks or captive broodstock collections are acknowledged to cost much more than cryopreserved gene banks, and expenditures for some countries (Norway, India, Philippines) were provided by participants. The cost of cryopreservation, on the other hand, depends on *where* it is done (centrally or in the field), *how* it is done (with simple field equipment or using a programmable controlled-rate cooler) and even *who* does it (local trainees or consultants). Collection of wild genetic material seems to demand a field technique, as it is clearly impossible to carry sophisticated equipment into the bush in any country, and the logistics even of transporting chilled sperm collected in the field to a central cryopreservation facility, as is reported by Thorgaard and co-authors, break down if the distance is too great. **In short, cryopreservation can be cheap or expensive depending on how it's done.**

Participants recognized the need to standardize the data on genetic material held in fish gene banks, especially when such resources begin to be shared between countries. As noted earlier, the plant genetic conservation community can provide models, for example the SINGER database developed at IPGRI; World Fisheries Trust's SpermSaver software was developed with input from IPGRI and uses standard gene banking terminology to catalogue accessions as they move in and out of a gene bank. Perhaps the first step to standardizing data reporting is the one recommended by Pullin, **namely the cataloguing of all known *ex situ* and *in situ* collections of fish genetic resources.** Such a project would make abundantly clear the variety of needs of different organizations and profitable ideas on standardizing data management are certain to suggest themselves.

"As more and more fish genetic resources are collected...there is a need to develop codes of conduct and practice for such collections."

As more and more fish genetic resources are collected and held in living and cryopreserved gene banks there is a need to **develop codes of conduct and practice for such collections.** Some models exist; there is a code of conduct for collection of plant germplasm as well as published gene bank standards, and NASCO has already developed international guidelines (code of practice) for collections of cryopreserved fish sperm. Participants agreed on the need for such codes; again, compiling a list of known gene banks may be the first practical step.

Threats to fish genetic diversity

The litany of threats to fish populations is extensive and well known; most of the papers in these Proceedings start out with some sort of recitation. Nevertheless there was some general discussion about the growing sense of urgency to conserve fish biodiversity, and in particular whether the effects of climate change on fish should be considered an exceptional stressor. In the end, participants agreed that the global complexity of the threats facing fish populations made it unwise to single out any single stressor in a meeting like this one. However, there remains a lively debate over the effect of climate change in specific cases; in British Columbia, for example, there is much scientific and media attention presently being paid to the controversial thesis that ocean warming is altering migration patterns of Pacific salmon and may be the explanation for record low marine survival.

"Currently the most comprehensive and long-running genetic conservation program is the Norwegian Atlantic Salmon Gene Bank."

Case studies of fish genetic conservation

For many participants, a few good case studies that consider pros, cons and outcomes would go a long way toward helping make decisions in their own countries about what kind of genetic conservation program to adopt. Currently the most comprehensive and long-running genetic conservation program is the Norwegian Atlantic Salmon Gene Bank. In operation for ten years, it comprises both living and cryopreserved banks and is beginning to compile a "track record" of conservation. There is also a history of banking Atlantic salmon in Finland and in other Nordic countries. Atlantic salmon provides the closest thing to a case study of genetic conservation, although many participants pointed out that as a luxury fish it is hardly representative of global fisheries, and several suggested common carp or Nile tilapia. Nevertheless, there is an informative history around Atlantic salmon throughout its range: protracted fishery declines, a variety of stressors, experience with the effects of limiting harvest, and complementary *in situ* and *ex situ* conservation.

"Managers need trigger points for genetic conservation and they need criteria for selection."

What to conserve and when to start

One of the thorniest problems facing managers designing a genetic conservation program is what to conserve, and when to start. True to the title of the meeting, participants urged action before it is too late (in line with the precautionary principle), but there is no getting around the fact that the tools for making the actual decisions about which species or populations to conserve, whether *in situ* or *ex situ*, need work. **Managers need trigger points for genetic conservation and they need criteria for selection.** Neither exists for fish, but there are potential models. IUCN criteria provide a starting point, and Allendorf *et al* (1997) have developed criteria for selecting salmon conservation units based on the IUCN model. Wood and Holtby, whose paper in this volume concerns using data on genetic variation to identify conservation units, suggested that the IUCN criteria were likely most applicable to fish when dealing with closed populations and could be used to trigger *ex situ* conservation.

"Where local and indigenous communities are involved in providing access to genetic resources, they will almost certainly wish to develop policies that protect their own rights and interests, which may be different from the State's."

The need for policy

With a few exceptions (Diaz, Fortier, Harvey), presentations did not include reference to any local or national policy that enables the preservation of fish genetic resources, although all participants were asked to include such references where possible. While such policies undoubtedly exist in a general sense, there still seem to be few examples of policy explicitly written for *ex situ* conservation—however, participants were able to share many examples where there was overlapping or conflicting management of fish populations. Such policies are, however, going to be needed, especially as various nations interpret the Convention on Biological Diversity in terms of exchange of genetic resources and benefit sharing. Where local and indigenous communities are involved in providing access to genetic resources, they will almost certainly wish to develop policies that protect their own rights and interests, which may be different from the State's.

In many countries the lack of policy may simply mean that genetic conservation does not occur. All participants agreed on the need for policy, and encouraged the efforts of the international workshop *Toward Policies for Conservation and Sustainable Use of Aquatic Genetic Resources* (Pullin et al. in prep.). They also agreed that to wait until policies were in place before acting to conserve genetic resources would in many cases be to wait too long. **This might, in fact, be fairly said to be the message finally emerging from the meeting—yes, there is a need for better criteria and for policy, but there are many cases where fish genetic diversity will vanish irrevocably unless action is taken now, and the longer we wait, the more expensive (financially and biologically) it will get.**

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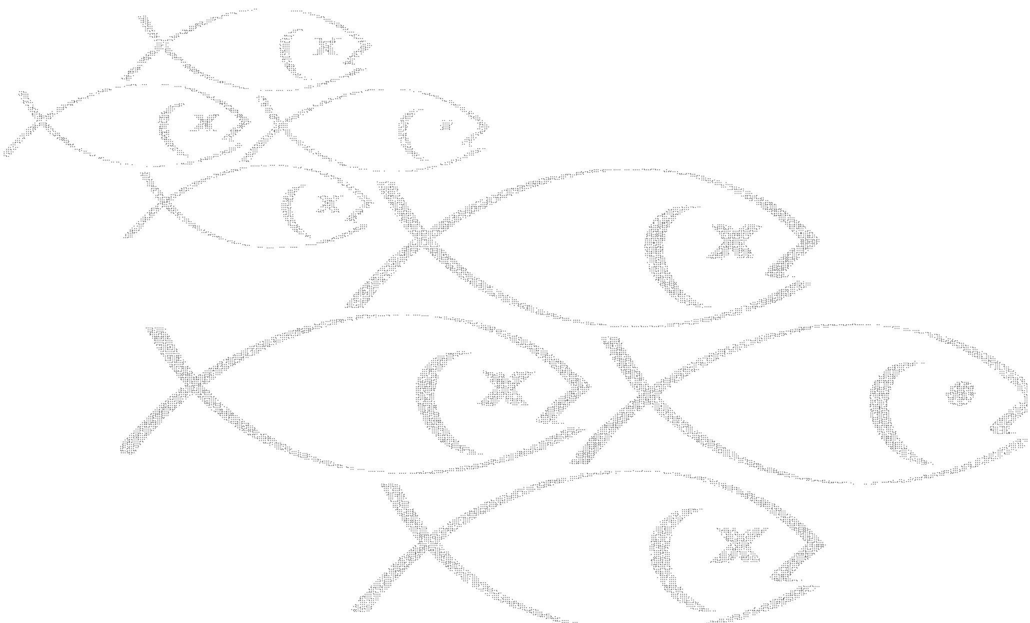

Keynote Address

Fisheries Fact and Fiction: Science and Spin Doctors

The Honourable John A. Fraser [1]

PHOTO CREDIT: MIKE HENDRY

Ladies and Gentlemen,

I want to make it very clear that in this august company, I approach this subject with as much humility as I can muster and certainly with some diffidence because you are all, to one degree or another, experts in your scientific backgrounds and disciplines. My discipline was law; but one thing good lawyers remember from what they were taught is that they have to deal in facts, not 'information'.

In my lifetime, there have been thousands of so-called 'information offices' open around the world; some in the West, some in the Soviet Bloc. Much of the information that emanates is propaganda, much of it is the consequence of spin doctors putting on the best appearance they can, especially when the information comes from governments. They couch things in language which is so antiseptic and so wrapped in what we used to call 'comfortable words' that one could almost think that things were as spin doctors

"But what we are dealing with here, as people trained in science, are facts. Facts are reality, not supposition, not rhetoric, not theory and above all not 'comfortable words'".

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wished us to think: that in every day and every way the world was getting better and better.

"While it would be folly not to take cognisance of what we have done well...it is surely also folly to pretend that things are different from what they are, to try to make them better than they are."

But what we are dealing with here, as people trained in science, are facts. Facts are reality, not supposition, not rhetoric, not theory and above all not 'comfortable words'. There is, however, both in terms of the science and in terms of the task of the politician to inform and lead, a terrible 'disconnect', not just here in my country but around the world. I am assigned each year to take a principal part in Canada's participation in the Commission for Sustainable Development (CSD). As you know, the Commission for Sustainable Development was established after the Rio Conference to monitor whether or not we were doing what we said we would do. One of the ways it was hoped that we could monitor this was that each country would on its own file a report each year, and that report would, for better or for worse, set out how each country was doing in its efforts to obtain sustainable development according to the commitments that we all made at Rio. Unhappily, too often those reports are put together by people whose instructions are to make it look as good as they can. While it would be folly not to take cognisance of what we have done well (because we had done some things very well over the years), it is surely also folly to pretend that things are different from what they are, to try to make them better than they are. Again, the 'disconnect' between the reality and what people are saying.

The Commission on Sustainable Development has been meeting each year, trying to bring together governments to deal with what we talked about at Rio and for decades before. But again, when we finished the five-year review at the United Nations, only months ago last spring, the result was not what we had hoped for and, even with all the things that we have accomplished, the considered opinion (and here the CSD and the UN were starting to move to reality) was that we have lost ground globally, not gained it.

"What I want to do is keep us reminded of the necessity to deal with facts, not rhetoric, not spin doctors' notions but what has actually happened."

What I want to do is keep us reminded of the necessity to deal with facts, not rhetoric, not spin doctors' notions but what has actually happened. When it comes to biodiversity I think what we are seeing is that we are going to have to pay far more attention than we have. The old word 'ecology' which, for my generation was still a bit of a mystery, really leads inevitably to biodiversity because what it teaches us is that if the life system of this planet begins to disintegrate the consequence is not just one species going extinct or one stock of fish failing to return to its native river in British Columbia; it means the complete unravelling of that miraculous web that ties it all together.

It is true, as some people like to point out, that there has always been change. But what we are facing today is a rapidity of change and a diminishing of biodiversity in an alarming, indeed an extraordinarily unsettling way. It is all very well to say that ten to twenty thousand years ago in the Ottawa valley, the capital city of Canada was under a kilometre of ice and, after all, the world survived and so did the human species. But what we are facing now is the prospect of rapid climate changes which, while admittedly partly due to natural phenomena, we can now measure the unprecedented increase of greenhouse gases in the atmosphere over the last 50, 100 and 150 years. This is an arithmetical fact that you cannot get around. CO_2 is doubling and will triple and will go beyond that if we do not do anything about it.

This man-made increase in greenhouse gas emission is, according to most of the best scientists in the world, affecting our climate. This has been denied by some journalists, some of them in Canada; it has been denied by some industrialists, some of them in Canada; it has been denied by a few scientists who put their own particular spin on the interpretation but back away from the fact that their views were considered by many more of their scientific colleagues and rejected.

What we had at Rio was a very late but significant recognition, at least by those who were there representing their governments, that we have a problem that is not a figment of somebody's imagination. It wasn't made up by Greenpeace to raise funds, it is not the consequence of some conspiracy of left wing subversives to destroy the market economy. And yet, for the last five years since Rio, we have heard a great deal of 'information' on climate change put out by people that support the status quo which has been an appalling abdication of any responsibility to reality. I have touched on climate change because part of what you will all be considering today, tomorrow and the next day you will not be able to speak about without consideration of climate change.

But climate change is not the only problem we have. When Europeans first came to this continent they saw what former US Secretary of the Interior Stuart Udall called, in his remarkable book *The Quiet Crisis*, the myth of superabundance, the myth that there was always something more to take in the next valley, in the next watershed, that there would always be more fields to plow. There was no consideration at all, at least in the early years, that there had to be some limits on exploitation and that we had to live in harmony with the great natural world we were exploiting.

We in North America have been extremely critical of ourselves, and for good reason, but some countries still insist on saying that they don't need to do anything because the developed counties started the industrial revolution, that we started the pollution and it is up to us to fix it. Again, that is spin doctor nonsense. I have listened to it, and so have others. For the most part it doesn't get challenged, but it needs to be because intellectually it is nonsense, and pragmatically it is folly. The fact of the matter is that if you just take greenhouse gases, the so-called developing world will produce, within fifteen to twenty years or maybe sooner, over half the greenhouse gas emissions on the globe.

"If the life system of this planet begins to disintegrate the consequence is not just one species going extinct or one stock of fish failing to return to its native river in British Columbia; it means the complete unravelling of that miraculous web that ties it all together"

"Some figures show that at least 70 per cent or more of the fisheries in the world are at their maximum exploitation or declining."

"The Ambassador's role is not to be a knight on a white charger hurling epithets and massive darts of negativism at everybody that is failing to do what some of us think perhaps they ought to be doing."

Here in British Columbia, we have taken a terrible public beating for the alleged misuse of our forests. Some of it has been legitimate criticism, but there are other places that ought also to be looked at. We are also very concerned about our fisheries. Why shouldn't we be? We have misunderstood and mismanaged the Atlantic cod to the point where, even as recently as two weeks ago, scientists seemed to be concluding that the cod are not recovering—and we may not even know why. In British Columbia we have lost an abundance that we once took for granted, especially with anadromous fish. So we have reasons to be concerned. But if one goes around the world, and considers the reports of the UN agencies, the fact of the matter is that it is happening everywhere. Some figures show that at least 70 per cent or more of the fisheries in the world are at their maximum exploitation or declining.

The position of Ambassador for the Environment of Canada was established after Rio to do four things:

1) To make sure that Canada keeps the commitments that we made at UNCED;
2) To make sure that everybody else keeps their commitments;
3) To identify the things that we did not achieve at Rio and the things that we discovered need attention since Rio, and
4) To work collaboratively and act in a liaison capacity with the provincial governments, the academic community, non-governmental organizations, the industrial and technological sector of our country, and the public generally.

The Ambassador's role is not to be a knight on a white charger hurling epithets and massive darts of negativism at everybody that is failing to do what some of us think perhaps they ought to be doing. It is, instead, to explain and promote Canadian policy within and outside the country.

For a moment I want to remind the Canadians here today, and perhaps inform others, about the Canadian position on biodiversity. You know that the Secretariat of the Biodiversity Convention is presently in Montreal. In 1992, Canada became the first industrialised nation to ratify the UN Convention on Biological Diversity. The theme for the fourth meeting of the Conference of the Parties to this Convention, which will take place in May in Bratislava, happens to be freshwater biodiversity and we will be well represented there. The theme for the Conference of the Parties which took place in November 1995 was "Marine and Coastal Biodiversity" and we were well represented there. Out of that came what has been called the Jakarta Mandate, supported by Canada, which led to the creation of an international roster of experts on marine and coastal biodiversity. That roster of experts met in March 1997 and the report of the first meeting of this group will also be tabled for discussion at the meeting in Bratislava. In that report were proposed elements for a three-year working-plan that will cover, among other things, the evaluation of the 'precautionary approach', innovative marine and coastal area management, marine and coastal living resources, marine protected areas, mariculture and alien species. The effectiveness of those recommendations and whatever actions might be agreed upon afterwards is going to depend on the determination of everybody there to deal with facts, not fancy.

And when you are going to deal in facts, you have to get down to the places where these things are happening. And that is sometimes embarrassing for countries. It has been embarrassing for Canada. But nonetheless, unless we are prepared, all of us, to put the facts on the table at these international fora, we are not going to be able to take the action that is necessary. This has nothing to do with one place seeming to be superior to another. It has everything to do with facing the facts. It has everything to do with, I suppose, the scientific approach. Here in British Columbia, we are shortly going to announce the establishment of the Pacific Salmon Fisheries Conservation Council. It is to be an independent council established by both the provincial and federal governments. It is not there to manage the fishery. It is not there to allocate fish between competing gear types. It is there to report to governments on the state of the stocks and their habitat. The thinking behind the Conservation Council is not to manage the fishery, but to have an independent body that reports, at least to the degree that it is possible to do so, what has actually happened up and down this coast and in our rivers.

"For years, here on this coast, we have listened to so much self-serving rhetoric from different groups that it has been extremely difficult for the public to know what is the truth. The 'disconnect' is really quite extraordinary."

Now it is not true to say that nobody in the Department of Fisheries and Oceans knew anything about this until the council was set up. That is not so. But it is correct to say that for years, here on this coast, we have listened to so much self-serving rhetoric from different groups that it has been extremely difficult for the public to know what is the truth. The 'disconnect' is really quite extraordinary. On the one hand, the media report almost daily on local and international fishing collapses, but a magazine targeting the sport fishing market in British Columbia is still capable of publishing an article inviting anglers to flock to our coasts for the fishing experience of their lives!

Here again is the disconnect, which has got to be considered extraordinarily alarming by any sane person, between what we are getting from the science side and what we are getting from the "every day and every way the world is getting better and better" side. This is a disconnect which confounds intelligence and flies in the face of any kind of legitimate intellectual inquiry. It certainly doesn't seem to be consistent with what science is telling us. I asked the Department of Fisheries and Oceans to give me some background for my address to you today. And I said this is a meeting made up not of people who are likely to be wandering around waving placards, and not people who are searching for a microphone to condemn the Prime Minister or the President of the United States for problems we've got with the Alaskans over the salmon fishery but for people who are primarily trained in science, trained to look at what the realities are. I want to read just a couple of pages of some material that was put together for me by one of our top fishery scientists, Dr. Dick Beamish, here in British Columbia. Dr. Beamish writes:

"Here again is the disconnect, which has got to be considered extraordinarily alarming by any sane person, between what we are getting from the science side and what we are getting from the 'every day and every way the world is getting better and better' side."

> "Although the word biodiversity is commonly used, it means different things to people depending on their background. Scientists want to relate biodiversity to ecosystems and managers want to know how to preserve and protect animals and plants of commercial interest. There is an urgent need to find scientifically acceptable definitions and measurements of biodiversity. But if past experience with similar issues is any lesson, definitive answers are not just over the horizon. It is clear, though, what concerns us. The general public are worried about the health of the ocean. They hear stories of overfishing; they see images of pollution and the destruction of marine life. For the average Canadian, biodiversity represents the need to preserve our marine habitats or ecosystems.
>
> The concern is real but the management methods are almost unknown. The truth is that no-one knows how to preserve and protect full ecosystems. The new Oceans Act (a Canadian piece of legislation) requires that we learn, but it must be made clear that ecosystems management is a 'learn as we go' process. That's the most important problem we have in retaining our biodiversity: we are not sure how to do it. Some strong leadership should quickly focus our attention and, like many issues, we will find that we have useful knowledge that was already available. However, a broader understanding of the factors that regulate the abundance of fish stocks will provide better management and will protect ecosystems.
>
> On the west coast of Canada the specific biodiversity issues are the declining abundance of wild coho and chinook salmon, the declining abundance of total salmon catch, the introduction of exotic species (e.g. Atlantic salmon), and the very poor understanding of ecosystems. The coho situation in British Columbia in 1998 is extremely serious. Coho abundance has been declining in the 1990s as a result of low survival in the ocean. Marine survivals have declined from 15 to 20 per cent in the 1970s to less than 2 per cent in 1997. The release of large numbers of hatchery coho has not stopped the decline in abundance. Equally alarming is the relatively high percentage of hatchery fish; estimates in 1997 indicated that hatchery fish could represent 65-75 per cent of the population.
>
> It is difficult to define what we mean by 'wild coho' and it is equally difficult to know how many truly 'wild coho' are left. The crisis in 1998

results from this uncertainty and the possibility of higher marine mortalities because of El Niño. A similar situation exists in Washington and Oregon. Unless the trend is reversed, wild coho stocks will become extinct. Reversing the trend will not simply result from eliminating fishing; we need to protect fresh water habitat and understand the impact that hatchery fish may have on wild stocks in fresh water, in the oceans, and in the fishery.

There is also concern about the declining abundance of salmon in the total catch—that is, salmon of all species. The recent total returns are well below the average catches for this century. Since 1920, the average catch of all species has been 68,000 metric tons for about 22,000,000 salmon. In 1995, 1996, and 1997, the total catch was 44,000 metric tons, and in terms of numbers, 14,000,000 (1995), 9,500,000 (1996), and 12,500,000 (1997). It is expected that the catches in 1998 could be even lower, possibly the lowest ever. The low abundance could result in extinctions, if overfishing occurs at the same time as an extreme environmental change.

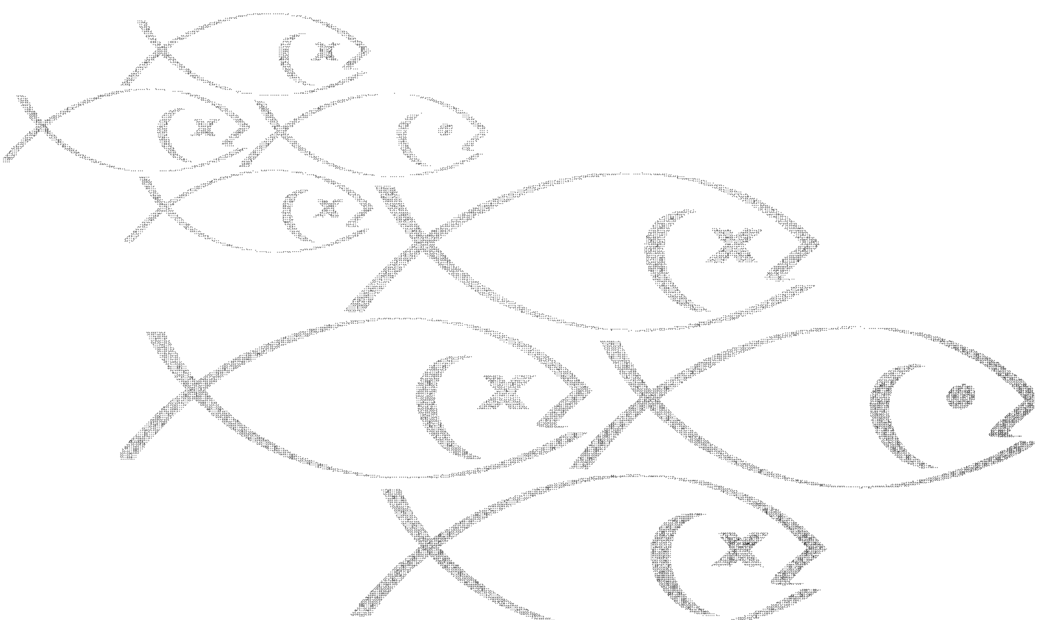
We also do not know if the elimination of fishing would reverse the trend. It didn't for Atlantic cod or Atlantic salmon. Scientists believe that in southwest Vancouver Island, 14 of 65 chinook stocks (that is 36 per cent) could be at a high risk of extinction, and an estimate of 46 stocks in total could be in a state that is close to extinction. Authors of this report believe that 17 chinook stocks—or about 2 per cent of all BC stocks—are extinct. As with coho, the issue is that the inevitable errors in management or extreme environmental conditions or both will cause additional extinctions when natural abundance of some stocks are at their current levels.

The long term concern is the virtual absence of direct studies on biodiversity. We avoid studying ecosystems and we do not support taxonomic studies. The reasons vary. But the high priority on assessing the impacts of fishing on single species and providing annual fishing quotas leave little time and resources for other research. The paradox is that it is unlikely that we will improve our assessment capabilities until we understand more about the mechanisms that regulate abundance. The concern of the Convention on Biological Diversity is that human activities around the world are causing irreversible harm to coastal marine ecosystems. There is some evidence of such harm in British Columbia. However, there is no integrated and co-ordinated process to study and protect our coastal marine ecosystem. It is important to look around the world and be concerned and it is equally important to get started on our own ecosystem."

"One of the things that we have to cope with in the real world is that those who draw our attention to problems are seldom thanked for it."

Now, ladies and gentlemen, that is not something that was dreamed up by eco-freaks or extremists. That comes from a respected senior scientist at the Canadian Department of Fisheries and Oceans. And what he said relates only to this coast, and to anadromous fish. What you are doing today is extraordinarily important. It needs to be repeated all over the world. The situation is by no means hopeless, and there is much that we can do. One of the things that we have to cope with in the real world is that those who draw our attention to problems are seldom thanked for it. And one of the reasons for that, of course, is that suggestions are seldom made as to what it is we ought to do about it. But the first thing we have to do is to find out the facts, and we have got to transmit those facts in intelligible and legible terms to the peoples of our different countries and to those who are elected to office. There is no use having a mass of scientific information which is not getting out to the public. It is going to require people of scientific training to assist others to put it out in a form that maintains its accuracy and its integrity but at the same time has some punch.

I was in politics for many years. One of the things that you try to learn is that there is no use spending all your time preaching to the converted. I am going to make the assumption that most of you really don't need lectures. What you do need is encouragement from someone who speaks for a lot of other people who are not here. You need to be told that what you are doing is of intense importance; you need to be encouraged to make sure that what you are considering, what you know, and what you don't know is made clear to a lot of other people who ultimately have to make the decisions, in other words to act on the facts. I want to say to all of you how much I appreciate the fact that you are here, at this Conference. I commend you for your work and I wish you good luck in your deliberations.



Gene Banking for Fish and Other Aquatic Organisms: ICLARM's Perspectives and Experiences*

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Introduction

Aquatic organisms, including freshwater macrophytes (Edwards 1980), marine algae (e.g., Trono and Ganzon-Fortes 1988) and a wide range of invertebrates and finfish (Bardach et al. 1972; Hancock et al. 1997) provide substantial food, livelihood and various other goods and services to humans. About 85 per cent of aquatic products are harvested from the wild rather than farmed. Such harvests have rarely been managed for sustainability and have usually followed boom-and-bust patterns. This can only be remedied if fisheries management includes ecosystem management: i.e., managing the wide range of biota and the habitats that support the targeted stocks, not just the species that comprise those stocks. A greater understanding of this need and of the limits to growth of capture fisheries is now emerging (e.g., FAO 1995, 1997a; Pauly and Christensen 1995). Some aquaculture operations have also followed unsustainable goldrush-like expansions, with adverse social and environmental impacts—e.g., milkfish pens (Pullin 1981), and shrimp

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(D'Abramo and Hargreaves 1997). This has tarnished the image of aquaculture as the world's fastest-growing food production sector. A more sustainable development path for aquaculture and fisheries enhancement will depend upon their coexistence with other sectors (especially agriculture and forestry), sound management of ecosystems and minimizing adverse environmental impacts through responsible development of farms and enhancement of stocks (Pullin et al. 1993; Pullin 1995a; Blankenship and Leber 1995; Munro and Bell 1997; FAO 1997a, 1997b).

Can gene banking of fish and other aquatic organisms contribute to a more stable and equitable future for fisheries and aquaculture? Gene banking contributes significantly to agriculture but is seldom mentioned in fisheries and aquaculture overviews. This paper considers first the broad picture—what is 'gene banking' in this context and what materials could be gene banked in order to ensure the continuation and, where possible, expansion of the contributions of aquatic organisms to world food security and environmental health? This is followed by examples from the experience of ICLARM and its partners in developing an *ex situ* collection of Nile tilapia *(Oreochromis niloticus)* germplasm and *in situ* conservation of aquatic organisms in aquatic protected areas. Finally, likely future developments are discussed. ICLARM's earlier publications in this area of work (Pullin, 1990, 1993, 1994, 1995a, 1995b and 1997a; Pullin and Casal 1996; Pullin et al. 1997; Lincoln Smith et al. 1997) provide further information on the Center's work and perspectives.

What is Gene Banking?

It is not easy to find a general definition of a gene bank that is applicable to all groups of organisms. A recent review of gene banks in the Consultative Group on International Agricultural Research (CGIAR) omits any such definition (SGRP 1996). The term has been used most widely for *ex situ* collections of plant germplasm. For example, *Elsevier's Dictionary of Plant Genetic Resources* (IBPGR 1991) defines a gene bank as:

> "1. A *genetic resources* centre where *genotypes* (as *seeds*, *pollen*, or *tissue culture)* are stored. 2. A *collection* of cloned *DNA* fragments from a single *genome* and, ideally representing the whole of the genome."

The same dictionary defines a field gene bank as:

> "A *collection of genotypes* of a *species*, kept as plants in the field. They should never serve as a *base collection.* The term is used to replace clonal repository, plantation, orchard, and living collection. See gene bank."

The International Plant Genetic Resources Institute, for its serial publication "Geneflow", has simpler working definitions as follows:

> "Gene bank: Facility where germplasm is stored in the form of seeds, pollen or *in vitro* culture, or in the case of a field gene bank, as plants growing in the field."

Such definitions, with minor modification, can be applied to aquatic plants (macroalgae and macrophytes) and to collections of microalgae, but they do not capture the full range of possibilities for aquatic animals: from *in situ* conservation in protected areas to *ex situ* sperm banks and captive broodstock.

Given the increasingly complex ethical and legal issues surrounding ownership of and access to genetic resources (e.g., IPGRI 1997; Pullin 1998), a more robust and widely applicable definition of the term 'gene bank' will likely be required, especially for legal documents. Within the CGIAR, crop centres undertake custodial, 'holding in trust' roles for genetic resources that are banked *ex situ* in their facilities and they designate much of the germplasm that they hold as falling under the auspices of FAO's Commission on Genetic Resources for Food and Agriculture. This Commission currently has plant and livestock genetic resources under its mandate, with aquatic genetic resources on its future agenda. Hence, it will be important to define for aquatic genetic resources just what is a 'gene bank'. For the purposes of this paper, a gene bank is defined broadly as:

> "Any collection of genetic material kept to ensure the future availability of that material for conservation, study, or production purposes."

This means that aquatic protected areas providing genetic material for conservation, reintroduction and breeding programs are also considered here as gene banks.

The following definitions from the Convention on Biological Diversity are also relevant here:

> **Genetic material** means any material of plant, animal, microbial or other origin containing functional units of heredity.
>
> **Genetic resources** means genetic material of actual or potential value.
>
> ***Ex-situ* conservation** means the conservation of components of biological diversity outside their natural habitats.
>
> ***In-situ* conservation** means the conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties.
>
> **Protected area** means a geographically defined area which is designated or regulated and managed to achieve specific conservation objectives.
>
> **Biological resources** includes genetic resources, organisms or parts thereof, populations, or any other biotic component of ecosystems with actual or potential use or value to humanity.
>
> **Country of origin of genetic resources** means the country which possesses those genetic resources in *in situ* conditions.
>
> **Country providing genetic resources** means the country supplying genetic resources collected from *in situ* sources, including populations of both wild and domesticated species, or taken from *ex situ* sources, which may or may not have originated in that country (CBD 1994).

Gene Banking for Agriculture and Aquaculture: A Comparison

Gene banking for agriculture involves mainly *ex situ* collections of gametes or embryos and populations of whole organisms, reproducing sexually or vegetatively. In addition to the target species that are farmed, associated essential or beneficial species, such as rhizobia for leguminous plants and fodder crops for livestock, are also gene banked *ex situ.* The wild relatives of crops and of many other plants used by humans are also conserved *in situ* in collections on-farm, on-station and in natural habitats. However, the overall picture for agriculture is one of highly domesticated species, with their genetic resources banked mainly *ex situ* (SINGER 1997). Some of the world's museum collections of plants (such as herbaria) and animals might also be considered as potential gene banks, given that they contain DNA, though they were not established with this in mind. The application of molecular genetics to plant and livestock breeding will probably consolidate further this centralized, *ex situ* approach, with complementary *in situ* genetic resources remaining important but used only occasionally in breeding programs. However, Tansley and McCouch (1997) have advocated placing more emphasis on scanning the genomes of wild plant species; i.e., looking for genes, rather than banking more germplasm accessions.

The plant gene banks of the CGIAR comprise: *core collections,* established to represent as much as possible of the genetic diversity of a species; *base collections,* for long-term storage and for distribution; and *working collections,* for ongoing use by breeders, onsite and elsewhere through partnerships and networks. Plant gene banks are usually centralized collections of many varieties for a given species and are maintained for long periods of time. Gene bank standards are being developed for plant genetic resources (e.g., FAO/IPGRI 1994; Sackville Hamilton and Charlton 1997) and a Global Plan of Action has been drawn up (FAO 1996). Livestock gene banks are also well developed in the form of cryopreserved semen and as populations of pedigree herds. Plant and livestock gene banks are maintained by the private and public sectors. Indeed, much of the wealth of the planet's genetic resources for agriculture is held on farms and by private corporations that develop and supply 'seed'.

Most farmers, in developed and developing countries, no longer raise their own seed but rely on seed suppliers in the private and public sectors. Likewise, for aquaculture, hatchery, nursery and growout operations are usually in different hands. The overall situation for gene banking of aquatic organisms is, however, very different to that for agriculture, at this point in the history of aquaculture development. The domestication of most farmed aquatic species has a very short history or has yet to begin. Science-based fish breeding programs are rare in developing countries, where most breeders are forced to rely on whatever broodstock are available from wild and farmed populations. Gene banks can help to improve this situation and to ensure the future availability of genetic material of endangered species and strains, for conservation and breeding purposes. Captive broodstocks can undergo rapid domestication selection, by natural selection on their different life-history stages, especially on the very large numbers of eggs and larvae produced by most aquatic species. Genetic management (including sex reversal, ploidy and gene transfer) can now be applied to some widely farmed species (such as carps, catfishes, salmon, tilapias and trout) and will become applicable to many more farmed and potentially farmable species. Sperm banks (and egg or embryo banks, if technology for their cryopreservation becomes available) and gene 'libraries' will probably be increasingly used for such applications.

In capture fisheries, the overexploitation of wild stocks might have genetic consequences (Martinez i Prat 1995) but this has been little investigated (Smith 1996). Re-establishment or enhancement of fisheries from wild-collected seed and from captive breeding is an emerging trend, e.g., for giant clams *(Tridacnidae)* (Munro 1993). The genetics of such operations is an important consideration and the broodstocks upon which they depend, whether located *in situ* (open waters) or *ex situ* (captive support breeding) are also gene banks in the broad sense.

What Can be Gene Banked for Aquatic Species?

Garibaldi (1996) listed 262 species used in aquaculture: 39 crustaceans, 72 molluscs and 151 finfish; with 95 per cent of global production derived from 31 species (6 crustaceans, 8 molluscs and 17 finfish). Wildtypes of these and other species with potential for aquaculture can be conserved *in situ* (i.e., gene banked according to the broad definition used here) in aquatic protected areas (e.g., Pullin 1990). *Ex situ* gene banking for aquatic species has limitations, which have been summarized as follows:

> "Could *ex situ* conservation of marine and coastal biodiversity, in gene banks and aquaria, complement *in situ* conservation? Yes, but probably much less so than in the huge *ex situ* gene banks established for crop plants and the extensive holdings of botanical gardens. Given the difficulties and costs of simulating natural aquatic environments *ex situ* (for example, the deep sea, the intertidal zone and coral reefs), the huge diversity of aquatic species and communities, and the inevitability of domestication selection in captive breeding over successive generations, it is hard to envisage how even a 1 per cent coverage of the world's wild aquatic biota could be achieved as live *ex situ* collections. However, to complement some *in situ* conservation efforts and to bank genetic resources for aquaculture and breeding programs, some *ex situ* conservation is possible and will become more important; for example, as live collections in public aquaria and in commercial aquaculture, and gene banks for cryopreserved fish sperm." (ICLARM 1997).

This recognition of the limitations of *ex situ* gene banking does not, however, diminish the importance of the role that *ex situ* gene banks can play both for conservation and breeding purposes, as described by Harvey (1996). McAndrew et al. (1993) have provided a thorough review of the potentials and constraints to *ex situ* gene banking of aquatic organisms, especially cryopreservation (Tave 1986). Smitherman and Tave (1987) and Tave and

Table 1

Cryopreserved sperm of Nile tilapia (*Oreochromis niloticus*)*

STRAIN	# OF FISH SAMPLES	STRAWS PER FISH	DEWAR NOS.	CANISTER NO.	STRAW COLOR	BEAD COLOR
FOUNDER STOCKS						
Israel	35	1-7	1A & 1B	1	Clear	Silver & Clear
Singapore	52	1-10	1A & 1B	6 & 9	Green	Silver & Clear
Taiwan	49	1-7	1A & 1B	2	Yellow	Silver & Clear
Thailand	28	1-7	1A & 1B	7	Orange	Silver & Clear
Egypt	22	1-7	1A & 1B	4	Pink	Silver & Clear
Ghana	41	1-7	1A	8	Purple	Silver & Clear
Kenya	33	1-6	1A & 1B	3	Blue	Silver & Clear
Sénégal	21	2-7	1A & 1B	5	Purple	Silver & Clear
SELECTED STOCKS						
Crosses	52	1-6	XC	3	Clear	Red Blue
Base Pop.	68	1-7	2A & 2B	4 & 5	Pink	Black
1st Selection	54	1-7	XC & 5XR	7 & 9	Cream	Red
2nd Selection	54	2-7	XC	2 & 8	Red	Black
3rd Selection	72	1-7	XC	9 & 10	Orange	Orange
4th Selection	53	1-7	2B	1	Green	Green
5th Selection	44	1-6	3A	1 & 2	Yellow	Blue
FAC Selected Lines	83	2-6	1B & 2B	10	Pink	Clear

* Maintained by the GIFT Foundation International Incorporated, Philippines. FAC Selected Lines are fish produced by the Freshwater Aquaculture Center of Central Luzon University, using within family selection, in a project funded by the International Development Research Council (IDRC) of Canada.

Smitherman (1988) have reviewed the genetic considerations for management of hatchery brookstocks.

An Example of an *Ex Situ* Fish Germplasm Collection: The GIFT Experience

Nile tilapia *(Oreochromis niloticus)* germplasm was collected for the Genetic Improvement of Farmed Tilapias (GIFT) Project and held in Philippine national facilities at Central State University (CLSU) Munoz, Nueva Ecija, about 150 km. north of Manila. The founder populations comprised the live fish and cryopreserved spermatozoa of eight strains of Nile tilapia *(Oreochromis niloticus)*: four African wild strains imported during 1988, 1989 and 1992 into the Philippines from Egypt, Ghana, Kenya and Sénégal; and four Asian farmed strains popularly known in the Philippines as 'Israel', 'Singapore', 'Taiwan' and 'Thailand' (Eknath et al. 1993). Note that all of these fish were acquired by the project before the entry into force of the Convention on Biological Diversity (29 December, 1993). The GIFT Project finished in December 1997 and the resulting collections of tilapia germplasm are now kept under the auspices of the National Freshwater Fisheries Technology Research Center of the Philippine Bureau of Fisheries and Aquatic Resources (NFFTRC/BFAR) and by the Genetic Improvement of Farmed Tilapia (GIFT) Foundation International Incorporated: a non-stock corporation, incorporated in the Philippines.

The live fish collections consist of: (a) the original founder stocks and replacement (backups); (b) a synthetic strain (the base population) built by interbreeding the eight strains and their crossbreeds); and (c) lines from a breeding program based on selection, spanning seven generations to date and continuing. These collections are kept on Central Luzon State University (CLSU) land in the facilities of the NFFTRC/BFAR. The facilities are part of a newly established Center for Biological Fish Breeding and Genetic Research (CBF-BGR). Live fish are held in earthen ponds and in tanks, designated as Reference Collection Tanks. The Reference Collection Tanks are located in a completely isolated facility (land area, 3,000 m^2). All tanks are covered with nets to prevent entry by birds. All fish are individually tagged with Passive Integrated Transponder (PIT) tags. A 24-hour water supply is maintained by the use of a heavy duty water pump. The effluents from the tanks pass to a sump pond. Two grow-out (800 m^2), two nursery (600 m^2) and ten (200 m^2) ponds are used for the maintenance and replacement of live fish collections.

A protocol for the replacement of GIFT reference strain was devised by Bentsen (1995) but has not been fully implemented. Replacement stocks are currently produced by breeding from four breeding pairs per strain (Longalong and Danting, in preparation).

Spermatozoa of the founder stocks, their replacements and the selected lines are cryopreserved (Table 1). The cryopreservation procedures and facilities for the GIFT Project were set up through advice and visits from Dr. Krishen Rana, then with the Institute of Aquaculture, University of Stirling, UK, now at FAO Headquarters, Rome. The cryopreservation procedures used during the project are published in a manual (Danting et al. in press). The Cryopreservation Laboratory is housed in a one-story concrete building with a floor area of about 60 m^2. This laboratory comprises an air-conditioned cryopreservation room, a recirculating incubation facility and an aquarium area. The laboratory houses the following equipment: One Kryo 10 programmable cryochamber; inverted microscope for checking pre-freezing and post-freezing motility of sperm; and storage Dewars. There are six storage Dewars (each with a 15,000 straw capacity) and two 35-l capacity liquid nitrogen transport containers.

The GIFT Project spent about US$150,000 (UNDP funds) to establish the facilities for maintaining these live fish collections and cryopreserved spermatozoa. This included capital costs, equipment, labour and other recurring expenses to date. The annual costs of maintaining these collections are approximately PhP 835,050 (US$32,120) for the live fish and PhP 744,460 (US$28,640) for the cryopreserved sperm. Three technical staff and two field assistants are involved (Table 2).

Table 2

Annual recurrent costs for collections of Nile tilapia (*Oreochromis niloticus*)*

ITEM		PhP	US$
A. Cryopreserved spermatozoa			
Liquid nitrogen		96,000	3,692
Supplies (cryo materials, office and chemicals)		120,000	4,615
Electricity		24,000	923
Depreciation cost of facilities/equipment		250,000	9,615
Transportation (purchasing LN2)		24,000	923
Purchase of new dewar		97,000	3,731
Miscellaneous (10 per cent)		52,460	2,019
	Sub-Total	**663,460**	**25,520**
B. Live fish in ponds and tanks			
Feeds		480,000	18,460
Supplies (cleaning materials)		12,000	462
Diesel fuel for pump		36,000	1,385
Purchase of PIT tags (250 pcs. at US$3.00)		19,500	750
Electricity		18,000	692
Depreciation cost (equipment, tag reader, etc.)		120,000	4,616
Miscellaneous (10 per cent)		68,550	2,637
	Sub-Total	**754,050**	**29,000**
C. Staff Costs			
(A+B combined: staff time is split equally between these)			
1 Senior staff (half-time honorarium)		28,000	1,077
1 Full-time seconded (full-time honorarium)		43,200	1,662
1 Technical Staff (half-time)		54,000	2,077
1 Field Assistant (half-time)		36,000	1,385
	Sub-Total	**162,000**	**6,230**
D. Total Costs			
For cryopreserved spermatozoa		744,460	28,640
For live fish		835,040	32,120
For all		1,579,510	60,760

* Germplasm kept at the National Freshwater Fisheries Technology Research Center of the Philippine Bureau of Fisheries and Aquatic Resources (NFFTRC/BFAR), Muñoz, Nueva Ecija, Philippines and by the GIFT Foundation International Incorporated; these data derive mainly from a period in which the exchange rate of the Philippine Peso (PhP) was about 26.00 to US$1.00. Totals are rounded to the nearest US$10.

Over its span of nine years, the GIFT Project has generated much data on its collections of tilapia germplasm. These data are lodged in a computerized database. A card catalogue is maintained as a back-up for cryopreserved sperm collections. The documentation of genetic resources for aquaculture is also part of the coverage of FishBase (Froese and Pauly 1997) though, as indicated by earlier attempts to accommodate genetic data in this database (Agustin et al. 1993) there is still much to be done, especially for molecular genetics.

Aquatic Protected Areas

The absence of technology for the routine cryopreservation of gametes and embryos of many aquatic species means that it is currently most practical to maintain gene banks of aquatic animals *in situ*. The limits to the number of individuals of each species that can be maintained in aquaria, the difficulties in propagating individuals in a way that maintains genetic diversity (effective breeding numbers are often low in captive broodstocks of aquatic species; see references in Munro and Bell 1997), and the high cost of holding facilities are the main problems with technology presently available for *ex situ* gene banking of aquatic species.

The maintenance of aquatic species in aquatic protected areas can help to overcome these problems. In well-located aquatic protected areas, whole communities of species can be "banked" together for the cost of effective enforcement of the protected area. Another advantage is that individuals are not removed from their roles in production on-site. Rather, aquatic protected areas allow the number of individuals of exploited species to increase, and to attain a greater mean body size (Bohnsack 1990, 1993; Roberts and Polunin 1991). In the case of most marine fisheries species, this results in an exponential increase in egg production, dispersal of greater numbers of 'seed' to areas outside the protected area, and replenishment of the proportion of the population vulnerable to fishing (Bohnsack 1990, 1993).

The benefits of establishing aquatic protected areas as gene banks are not limited to the populations on-site. Representative groups from populations that are at risk elsewhere can be transferred to aquatic protected areas provided that health and biosafety requirements are met. Such transfer measures are most suitable for sedentary species that feed low in the food chain, as implemented for abalone (*Haliotis* spp.) in California (Tegner 1993) and suggested for giant clams (Munro 1993; Bell 1998).

The metapopulation structure of many marine species (Sinclair 1988; Man et al. 1995; Planes et al. 1996), and the differences in gene frequencies among populations, especially those of freshwater species among catchments (see references in Casal and Agustin 1997), dictate that care must be taken to establish aquatic protected areas that provide representative *in situ* gene banking. In the case of marine species with pelagic larval dispersal, the protected areas must match the location of substantial spawning stocks (sources) and the locations where the larvae settle (sinks). For migratory species (for example, diadromous species), these locations will usually be widely separated.

To provide effective gene banks, protected areas must also be large enough to support adequate breeding numbers. Otherwise, removal of animals for spawning *ex situ* or their relocation to other areas might affect the ability of the remnant stocks to spawn effectively.

More detailed analyses of the effects of location and size of protected areas, and the total number of reserves, on the maintenance of metapopulations of aquatic species for conservation and fisheries have been given by Carr and Reed (1993), Quinn et al. (1993) and Man et al. (1995).

ICLARM is collaborating with The Nature Conservancy, the Government of Solomon Islands and the Great Barrier Reef Marine Park Authority to monitor a marine conservation area of 83 km^2 in the Arnavon Islands, Solomon Islands. The monitoring program has been described by Lincoln Smith et al. (1997) and is designed to provide unequivocal evidence for changes in populations of exploited tropical marine invertebrates in the protected area relative to nearby islands and reefs that remain open to fishing. Preliminary results from this study, and those of Russ and Alcala (1996) on coral reef fishes in the Philippines, indicate that the amount of time needed to establish reasonable densities of animals in aquatic protected areas where fishing has been heavy, and replenishment erratic, may run into decades. If aquatic protected areas are to be used as *in situ* gene banks, as we believe they should be, then there is a need to establish many more, at appropriate spatial scales and sooner rather than later, while such options remain.

The Future

The main question for the development of gene banking to support aquaculture, fisheries and conservation is whether, as domestication, breed development and conservation efforts proceed more rapidly, aided by biotechnology, this gene banking will come to resemble the situation for agriculture or be different. For *ex situ* operations, it will undoubtedly resemble gene banking for agriculture in focusing largely on the target species, without much diversification into banking of the food and other beneficial organisms. However, there could be more gene banking of, for example, larval food organisms, such as *Artemia* strains, and microorganisms for managing detrital foodwebs. Both *ex situ* and *in situ* gene banking for aquatic organisms will probably continue to differ from agriculture by being less centralized, because of the high costs and environmental requirements of captive populations of aquatic organisms. Moreover, there will probably be much more reliance on *in situ* banking of the genetic resources of whole communities of plants and animals, in aquatic protected areas. At this early stage in the development of fish gene banking, one clear priority is the provision and accessibility of accurate information on what material is banked where. FishBase is a means to address this for finfish and ICLARM would be pleased to receive data on the holdings of fish gene banks for this purpose.

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Ex Situ Conservation, Gene Banks, and Responsible Fisheries

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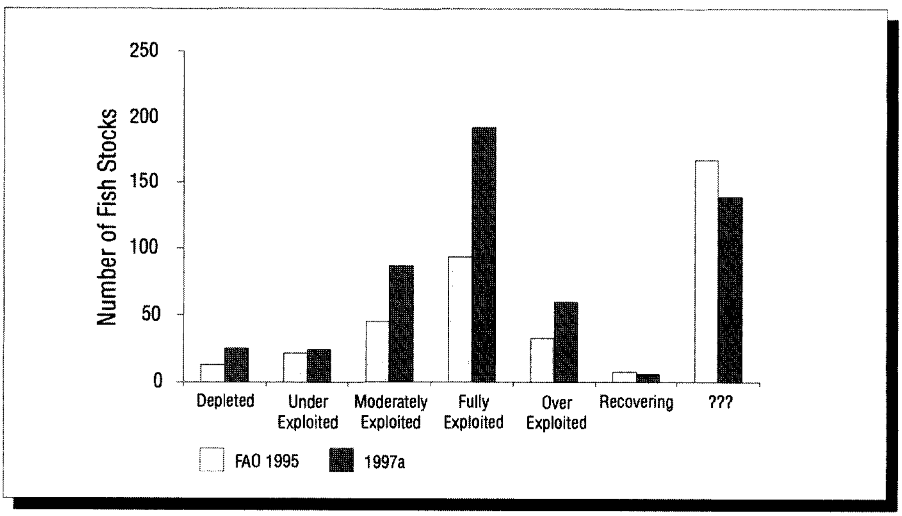
Introduction

In the face of declining resources in many of the world's fisheries (Figure 1; Nehlsen et al. 1991; FAO 1997b), the international community is developing fora to frame policies and guidelines concerning the conservation and sustainable use of biological diversity, including aquatic genetic resources. In 1995, the Commission on Genetic Resources for

Figure 1

Status of the world's major fisheries



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Food and Agriculture, formerly concerned only with plant genetic resources, decided to incorporate aquatic genetic resources into its mandate. In October 1995, the FAO Conference unanimously adopted the Code of Conduct for Responsible Fisheries (CCRF) to provide a framework for national and international efforts to use sustainably and conserve aquatic living resources. This Code was the result of international deliberations among FAO member countries, intergovernmental organizations and non-governmental organizations and complements other significant international mechanisms such as the Convention on Biological Diversity (CBD) and Agenda 21. Cooperation and collaboration among international organizations involved in the conservation and sustainable use of biological diversity is essential, and efforts at implementing the CCRF will also help implement the articles of the CBD and vice versa.

In this report I will review the two most significant international conventions, the CCRF and the CBD, and examine how *ex situ* conservation fits into their philosophy towards implementation.

Definitions and Nomenclature

Because of the broad scope of these international mechanisms—the Code of Conduct for Responsible Fisheries—covers both capture fisheries and aquaculture, and the CBD and Agenda 21 cover all biological diversity—a standard nomenclature is necessary when discussing use, sustainable use, conservation, *in situ* and *ex situ* conservation and gene banks.

Use: means not only extractive harvest, as in fishing, but also non-extractive use such as sport diving, boating or hiking.

Sustainable use: is the "use of the components of biological diversity in a way and at a rate that does not lead to the long-term decline of biological diversity, thereby maintaining its potential to meet the needs and aspirations of present and future generations" (UNEP 1994).

Conservation: is a dynamic process where resources are maintained or managed so that they can be utilized at some future point. We should acknowledge that the "diversity of... basic...resources is under threat and the genetic composition of these resources will be increasingly reliant on human practice and manipulation" (Meryl Williams, in Pullin and Casal 1996).

***In situ* conservation:** involves conserving the resource in the setting that has given it its special characteristics. Therefore *in situ* conservation of anadromous fish would require the fish to be in an environment that had marine and freshwater habitats and a connecting water body, whereas *in situ* conservation of a genetically improved Nile tilapia (Eknath 1993) would require maintaining the animal in aquaculture facilities. Maintaining this tilapia in the natural environment would not conserve the special characteristics of this domesticated fish. The conservation of resources in farm settings is what the crop and livestock sectors usually refer to as *in situ*.

Ex situ **conservation:** refers to keeping the resource in a facility different from that area where it achieved its special characteristic—e.g., zoos, aquaria, live gene banks and freezers.

Gene banks: are a type of *ex situ* conservation and can consist of both frozen and live material. Live gene banks are extremely important; however, they are costly to maintain unless they are associated with viable business enterprises (e.g., supported by private industry as a source of aquaculture stock) or are actively supported by private or public funds (e.g., zoos and aquaria) for research and public entertainment/education. See Pullin et al. (this volume) for an expanded definition of gene banks.

This paper will focus on cryopreserved gene banks and how they can facilitate implementation of the CCRF and the CBD and Agenda 21. For aquatic organisms this means, in practice, the storage of semen (haploid gamete) in low temperature or cryopreserved gene banks. Very limited success has been achieved at freezing eggs or embryos except in some invertebrates (Diwan and Kandasami 1997). Freezing of diploid cells from tissue culture and the cryopreservation of embryonic cells combined with nuclear transfer may have potential for *ex situ* conservation (Harvey 1983; McAndrew et al. 1993), but will not be included in this discussion.

The analogy of a "bank" is extremely important in *ex situ* conservation, as it should imply active management of the asset (genetic resource) and the investor (States, fish farmers, humanity, etc.) should have an idea of the time-frame of the investment. Gene banking may be long-term, as in the case of archive or reference collections of germplasm, or short-term, as in the case of low temperature storage or cryopreservation to facilitate breeding in aquaculture or species recovery programs.

UNCED: The Convention on Biological Diversity and Agenda 21

The Convention on Biological Diversity and its companion framework for action, Agenda 21, call for the conservation and sustainable use of biological diversity and the fair and equitable sharing of the benefits derived from such use (UNEP 1994). The Convention recognizes that *ex situ* conservation has a role to play in the management, protection and recovery of aquatic species and ecosystems.

Ex situ conservation is explicitly mentioned in Article 9 of the Convention on Biological Diversity (UNEP 1994), which states that *ex situ* conservation is "predominantly for the purpose of complementing *in situ* measures". Article 9(c) requires that measures be adopted for the recovery and rehabilitation of threatened species for their reintroduction into the wild. Thus, I take the view that *ex situ* conservation is not an end in itself but a means to help ensure the continued viability of natural and farmed populations of aquatic organisms. Due to limited resources available for gene bank maintenance and collection of biological material and the seemingly unlimited assaults on aquatic ecosystems, decisions will need to be made on what threatened resources will be included in gene banks and for how long.

Articles 9(a) and 9(b) call for the establishment and maintenance of *ex situ* facilities for the components of biological diversity. These articles further state that research should be a component of *ex situ* conservation and that the collections should be in the country where the resource originated or achieved its special character. Except in extreme situations, such as immediate danger of extinction or extirpation, the collection of material for *ex situ* conservation should not threaten ecosystems or species.

In situ conservation is the preferred mode of operation in the CBD (Article 8). *Ex situ* storage can facilitate several requirements of Article 8 that require the recovery and rehabilitation of ecosystems. Management strategies to restore populations may involve the short-term storage of semen to be used at an appropriate time when semen may be in short supply. Recovery efforts for natural populations must maximize effective population size, which is often difficult in endangered or rare species. Often reproductively active males and females are not available at the same time for reproduction, and short-term gene banking may facilitate breeding. For example, in Iran, spawning runs of mahi sephid *(Rutilus frisii kutum)* are intercepted by Iranian fishery officers at the mouths of rivers entering the Caspian Sea for river-side stripping and spawning. This is time- and labour-intensive work, and males and females migrate at different times. Due to the difficulty in getting gametes at the correct time, only a small portion of the spawning run is collected and therefore spawned (Shilat, Iranian Fisheries Company, personal comm.). Short-term low temperature storage of semen would allow a wider range of the spawning run to be collected, and therefore effective population size and genetic variation would be increased. Natural reproduction in the rivers is negligible due to dams and other fishermen that prevent upstream migration.

In situ conservation also involves minimizing threats to natural populations, and Article 8(g) requires the regulation, management or control of the risks from living modified organisms. Thus, it should be recognized that frozen semen can be considered living, and it can certainly be modified by genetic engineering, polyploidization, sex reversal and hybridization (McAndrew et al. 1993). Semen from natural tetraploid individuals that have been found among diploid populations (Pandey and Lakra 1997) would be capable of fertilizing normal haploid eggs, thus creating triploids without the need for traumatic pressure, heat or chemical shocks. Stable lines of transgenic coho salmon and Atlantic salmon have been produced that are capable of passing the transgene in their germ line (sperm) (Devlin et al. 1994). In this regard, the codes of practice (e.g., ICES 1995) or biosafety protocols (as called for by Article 19(3)) could also apply to frozen or low temperature stored gametes.

Additionally, the Convention calls for access to and transfer of technology that promotes conservation and sustainable use. Article 10(d) requires support for local populations in the development and implementation of remedial action in areas where biological diversity has been reduced. In order to facilitate this transfer, Article 18 calls for technical and scientific cooperation and Article 19 calls for (i) the creation of legislative, administrative and policy measures to participate in biotechnology research, especially in developing countries, (ii) fair access to results and benefits, and (iii) safe handling, transfer and use of living modified organisms (which can include frozen semen). Transfer of technologies or biological material should also include relevant information on its use and safety regulations as well as information on potential adverse effects.

Access to biological diversity, and in this case the genetic diversity contained in gene banks, is an important issue internationally. Article 15 attempts to promote fair access by recognizing the sovereign rights of States over their resources, by promoting an enabling environment and by advancing prior informed consent and mutually agreed terms for such access. The provisions of the CBD apply to resources that are maintained in the country of origin or those that have been acquired in accordance with the CBD; collections made prior to the entry into force of the CBD are not covered.

The government of Australia is trying to forge a national policy on access to genetic resources and has established principles and goals for such a policy. Access should promote conservation and sustainable use and be fair, with transparent operating mechanisms (Australia 1996). Two views are presented in Australia that are probably common elsewhere: (i) that Australia is not getting adequate returns from the export of biological resources (including genetic resources not limited to *ex situ* collections) and that there is no mechanism to ensure such returns and (ii) that the potential returns are not very high and more benefit could be realized by allowing for greater low-cost access to Australia's resource.

Agenda 21 is a blueprint for action and international collaboration and cooperation in order for the world to use sustainably and conserve biological diversity. Chapter 14, on sustainable agriculture, sounds the warning that "Many existing gene banks provide inadequate security, and, in some instances, the loss of plant genetic diversity in gene banks is as great as it is in the field." Section 14.69(a) recommends that appropriate United Nations and other international and regional agencies should: "Promote the establishment of regional gene banks to the extent that they are justified, based on principles of technical cooperation among developing countries."

Chapter 15, on the conservation of biological diversity, requires that cooperation should "Promote national efforts with respect to surveys, data collection, sampling and evaluation, and the maintenance of gene banks." Technical means of implementing the recommendation call for "improved and diversified methods for *ex situ* conservation with a view to the long-term conservation of genetic resources of importance for research and development."

Chapter 16, on environmentally safe use of biotechnology, points out that biotechnology also offers new opportunities for global partnerships, especially between the countries rich in biological resources (which include genetic resources) but lacking the expertise and investments needed to apply such resources through biotechnology and the countries that have developed the technological expertise to transform biological resources so that they serve the needs of sustainable development. Biotechnology can assist in the conservation of those resources through, for example, *ex situ* techniques.

Code of Conduct for Responsible Fisheries

The Code of Conduct for Responsible Fisheries (CCRF) lays out general principles in the areas of Fisheries Management, Fishing Operations, Aquaculture Development, Integration of Fisheries in Coastal Area Management, Post Harvest Practices and Trade and Fishery Research. The essence of the Code is that States and users of living aquatic resources should conserve the diversity of aquatic ecosystems for present and future generations to use sustainably in order to promote food security and alleviate poverty and that degraded ecosystems or stocks should be rehabilitated as far as possible and when appropriate.

Article 7 on Fisheries Management calls for the maintenance or restoration of stocks to at least the level of Maximum Sustainable Yield (MSY) (as qualified by environmental and economic factors) by such measures as conservation and protection of ecosystems and endangered species and by allowing depleted stocks to recover or by actively restoring them and their habitats.

Regarding responsible international trade in Article 11.2, the World Trade Organization (WTO) currently sets international standards, and the provisions of the CCRF should be interpreted and implemented in accordance with this agreement. It is recognized that trade is an economic necessity and will probably increase as aquaculture increases. Trade policy in aquatic species and products should be consistent with the Sanitary and Phytosanitary Measures and Agreements on Technical Barriers to Trade of the WTO. One stipulation of this agreement concerns the movement of aquatic pathogens and hosts and states that countries may establish trade barriers in order to protect disease-free zones. Thus, specific species may be barred from entering certain zones because of the risk of transferring specific pathogens. The International Office on Epizootics in Paris (OIE) is the official competent authority to determine disease status of zones and fish and maintains a database on "notifiable"diseases that must be regulated and reported when encountered (Dr. B. Hill, University of Weymouth, UK, personal communication). The trade or movement of frozen gametes or embryos is now being addressed in fish health and trade. In a similar manner to regarding gametes as a form of genetically modified, living modified or potential alien "species", it should be recognized that semen can be a vector for disease transmission.

Article 9 on Aquaculture Development stresses in many sections the importance of the conservation of genetic resources in aquaculture and in culture-based fisheries. Technologies for *ex situ* conservation and information systems on such conservation are recognized as important in implementing Article 9.3.5 concerning endangered species and maintaining the genetic diversity in species recovery programs that are supported by artificial or assisted breeding programs. Section 4 of Article 9 calls for the documentation and periodic assessment of genetic resources in aquaculture development. McAndrew et al. (1993) suggested that baseline collections of germplasm that are subsequently to be used in genetic improvement programs should be placed in gene banks so that in the future whole organisms could be regenerated and changes to the resource could be judged accurately.

Obviously another alternative approach to cryopreserving material in advance of some development activity so that it can be analyzed later to determine the effects of the activity is simply to do the genetic analyses with tissues acquired before the activity. This has many advantages, and if extra tissue is collected, this tissue could be stored so that genetic material could be analyzed later if advanced analytical techniques become available. The advantage of regenerating whole fish from a gene bank is that it makes it possible to compare growth and other physiological parameters using live organisms, rather than just comparing gel banding patterns.

Similarly, Article 12 on Research states that fish stocks that have been previously unfished or only lightly fished should be examined to provide baseline data that could be used to determine actual effects of fishing. An added component to this research could be the preservation of germplasm from the unfished stock, so that if fishing effects did compromise the genetic resources of the stock, fishing could be curtailed and the conserved germplasm could be re-inserted into the population.

Precautionary Approach

The precautionary approach to development has received a great deal of attention recently in international and national fora. However, many of its advocates appear to be unclear about what precisely this approach involves. The FAO and the government of Sweden convened a technical consultation to address this ambiguity and to provide scientific rigour to its application in fisheries and species introductions (FAO 1995).

One of the components identified in this approach is that reference points should be established in relation to desirable stock biomass or population size. The reference points may be either target or limit points or both. When a development activity approaches a limit reference point, pre-agreed contingency plans are implemented. One of the contingency plans could be the collection of semen for gene banking.

Determining the reference point that triggers the pre-agreed action will not be easy. The World Conservation Union (International Union for the Conservation of Nature, IUCN) has established criteria for listing species in their Red Book of endangered species.A taxon is Critically Endangered "when it is facing an extremely high risk of extinction in the wild, in the near future, as defined by any of the criteria (A to E)". Criterion E states that a species is Critically Endangered when a quantitative analysis indicates that the probability of extinction in the wild is at least 50 per cent in 10 years, or three generations (whichever is longer).

It should be noted that other higher priority contingency plans should also be implemented and that complacency should not be instilled simply because material has been "banked". The point I wish to make is that the precautionary approach does not demand the freezing of all semen from all stocks of aquatic species, only those at a specified level of risk. Species of sturgeon in the Caspian Sea are being decimated by illegal fishing, overfishing and habitat degradation from oil exploration, pollution and obstruction of tributary rivers. In light of the diversified threats, the political situation in the newly formed littoral States following the break up of the USSR and the tremendous financial return from oil exploration and extraction, prospects for ameliorating the situation in the Caspian Sea are not great; the most practical chance for improving the probability of survival for critically endangered species such as the ships sturgeon, *A. nudiventris*, is for immediate gene banking of gametes and hatchery propagation (Bartley and Rana 1998), as efforts to ameliorate the situation in the Caspian Sea continue.

International Cooperation and Coordination and Access

Agenda 21, the Convention on Biological Diversity and the FAO Code of Conduct for Responsible Fisheries all call for international cooperation in areas of information gathering and exchange Agenda, research, management and development of aquatic resources. The international community has been debating issues associated with *ex situ* collections since the early 1980s. These discussions focused on plant genetic material generally collected in centres of diversity of cultivated species and usually in developing countries. Questions arose as to whether this material belonged to the country where it was collected, to the country or institution where it is maintained, or to humanity in general. To deal with these issues, the International Undertaking on Plant Genetic Resources was adopted by FAO and an inter-governmental forum was established, the Commission on Genetic Resources for Food and Agriculture (CGRFA—formerly the Commission on Plant Genetic Resources). General conclusions were that germplasm held in government gene banks or public institutions was owned by the State in which such banks were located. However, the status of germplasm held in International Agriculture Research Centres (IARCs) and the Consultative Group on International Agriculture Research (CGIAR) could not be adequately defined in terms of legal ownership and access. The IARCs have developed a policy that essentially states that these collections are held "in trust" for the international community; however, such agreements were informal and unsatisfactory. To refine the status, an international Network of *Ex Situ* Collections under the auspices of FAO was planned. In 1992, the IARCs offered to place collections of plant genetic material into the Network, and in 1994 an agreement was signed between FAO and 12 Centres for "designated" germplasm. Thus, collections of designated germplasm held by the Consultative Group on International Agriculture Research are now under the auspices of FAO.

FAO and the CGRFA have the responsibility to set policy for these resources and have agreed not to claim legal ownership or seek intellectual property rights on the germplasm or related information, and that those groups receiving designated germplasm agree to abide by these terms as well. These agreements may be integrated into a revised International Undertaking on Plant Genetic Resources which may also become integrated into the Convention on Biological Diversity. Currently, access to *ex situ* collections, especially those made prior to the CBD and those maintained outside their countries of origin, is an unresolved issue within the CBD.

The International Centre for Living Aquatic Resources Management (ICLARM) is the CGIAR centre that deals with aquatic animal genetic resources and maintains a small living gene bank of Nile tilapia, *Oreochromis niloticus* (Pullin et al. this volume). The precise relation between the FAO and ICLARM concerning this resource has not been elaborated. However, in an external review of the CG system it was recommended that *ex situ* collections of animal and fish germplasm of the CG system not be placed "in trust"—i.e., under the auspices of the FAO—but rather be maintained as research collections (TAC 1994).

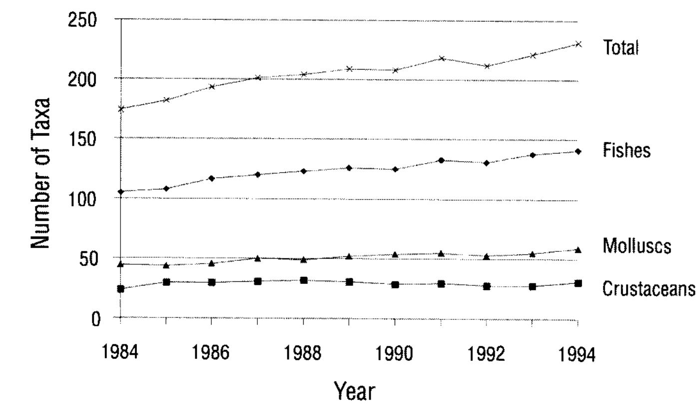
Conclusion

Clearly, many of the articles of both the CCRF and the CBD could benefit from the use of *ex situ* gene banks. However, long-term gene banks may be expensive and require maintenance of the collection, and there are other management activities that may take precedence in certain situations. The value of long-term archive collections for, *inter alia*, research is acknowledged to be important. A framework is needed to help decide what germplasm to include in the gene bank and the best management or recovery strategy given the specific details of the ecological system and the threats it faces. Goals and objectives of a gene bank should be clearly established, as should the expected time-frame for the deposit in the bank. The gene banks should be seen as active conservation and not simply as freezers or museums. The appropriate use of *ex situ* conservation will be in support of *in situ* measures, in support of research and education, and should strive to reward countries of origin and developing areas with any benefits derived from collections of germplasm.

Several groups, both national and international, have stressed that *ex situ* conservation should also be tied to sustainable use (Ponniah this volume). As an international organization mandated to alleviate poverty and increase food security, this is in line with the goals of FAO. Thus, efforts may be made to link aquaculture development to gene banks. Aquaculture is a rapidly growing industry in many parts of the world (FAO 1997a), and the number of species for which breeding technology is becoming available is increasing (Figure 2; Garibaldi 1996). As aquaculturists realize the benefits they may derive from cryopreservation and low-temperature biology, they may be valuable partners in developing *ex situ* conservation in ways that benefit both the industry and natural aquatic genetic resources.

Figure 2

Number of taxa of farmed aquatic animals (after Garibaldi 1996)



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Development of Policy Frameworks for Plant Genetic Resources: Possible Lessons for the Fish World

Ruth D. Raymond [1]

Introduction

The conservation and sustainable use of aquatic genetic resources are critical to food security and to improving the quality of peoples' lives. In this sense, aquatic genetic resources share important common ground with another vital component of the world's biodiversity (plant genetic resources).

The diversity that exists in both the world of plants and that of aquatic creatures is a critical characteristic that they share. It is such an important and distinguishing characteristic that many of the broad policy and institutional questions—as distinct from technical issues—that relate to plant diversity will apply equally to fish diversity (and to domestic livestock diversity for that matter).

The fact that there is a Convention on Biological Diversity (rather than a Fish Convention and Plant and Animal Conventions) reflects the recognition that the various sectors share important commonalities and thus warrant, even require, common international legal and policy frameworks. Accordingly, in 1995 the mandate of the FAO Commission on Plant Genetic Resources was broadened to encompass all components of genetic resources of importance to food and agriculture, including fish and livestock. The Commission is the principal intergovernmental forum for the discussion of policy matters related to genetic resources.

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Likewise, the System-wide Genetic Resources Programme (SGRP), which links the genetic resources activities of the Centres of the Consultative Group on International Agricultural Research (CGIAR), relates to fish, animal and microbial genetic resources as well as to plants. An important objective of SGRP is to ensure consistency in policies and strategies both among the Centres and across the sectors it encompasses.

Differences Between the Fish and Plant Sectors

Despite certain commonalities, important differences between the fish and plant sectors have arisen as a result of historical and technical factors. The dispersal and exchange of crop plants have gone on since the early spread of agriculture. Important food crops are widely distributed and may be major staples in regions far from their origin. This has led to the development of important secondary centres of diversity over thousands of years that can be critically important to a breeding effort.

The reliance of countries on introduced crops means that no country is self-sufficient in plant genetic resources. Even though many nations harbour significant genetic diversity in gene banks, on farmers' fields and in the wild, they still require access to the diversity available elsewhere.

The movement by people of aquatic species around the world is very recent compared with that of plants—mostly due to the relative difficulty of transporting fish over long distances—and thus has not been nearly so extensive. As a result, the diversity of a particular fish species tends to be limited to a country or a group of neighbouring countries. As breeding programs evolve, and as *ex situ* conservation of fish genetic resources becomes more common, the number of countries providing material for a particular breed is likely to grow.

Countries collaborate in the exchange and use of genetic resources to increase their access to the resources and to improved material, technologies and information. But while the most compelling argument for collaboration in the plant world relates to the interdependence of all countries with regard to plant genetic resources, this does not yet seem to have a real parallel in the fish world. Interdependence is the fundamental justification for the germplasm collections of the CGIAR, which are held in trust for the world community, and for the current efforts by countries to forge a multilateral system governing access to genetic resources and the sharing of benefits arising from their use.

In brief, plant and fish genetic resources are linked by the fact of their importance to human nutrition and by the necessity of ensuring the conservation and use of their genetic diversity. This linkage suggests that policy, legal and institutional frameworks established to ensure their conservation and use might share common features. At the same time, clear differences exist between the plant and fish genetic resources sectors, mostly due to historical and/or technical reasons. The improvement through selection by humans of fish genetic resources has a far shorter history, and global exchanges have been limited. The *ex situ* management of crop genetic resources is far more prevalent than *in situ* conservation, while the opposite is true with fish. These differences will have an effect on the impact of access and benefit-sharing conditions that might be applied in each sector, although they are likely to recede in the longer term as further progress is made in fish-breeding efforts and exchange.

Approaches to International Collaboration

The next section of this paper describes the characteristics of the two basic approaches to collaboration in the conservation, use and exchange of genetic resources and how these approaches might apply in the case of the fish and plant genetic resources sectors.

- **Bilateral** approaches to collaboration involve partnerships negotiated between two parties for their mutual benefit and are generally formalized through a contract or memorandum of understanding. Bilateral exchanges can be highly specific (e.g., to cover a single transfer between two institutions) or fairly broad (e.g., general exchange agreements between two governments or to cover all transfers of a range of genetic resources).
- **Multilateral** arrangements involve a number of parties sharing the costs and benefits of collaboration and making decisions collectively. These arrangements can be very broad, even global in scope (e.g., exchanges conducted within the context of the CGIAR) or limited to a region (e.g., networks, such as that which includes the ECP/GR program) or to a gene pool (e.g., crop genetic resources networks).

Each approach to collaboration has its distinguishing and positive characteristics. For example, a bilateral agreement can be tailored to the needs and circumstances of the parties and can deliver targeted and highly focused results. Partners collaborate because of their shared objectives without risk of diluting their efforts through a need to work with partners that have less in common.

A multilateral approach, on the other hand, offers participants access to a far greater range of genetic resources than is generally possible under bilateral arrangements. It is likely to provide greater opportunities than bilateral arrangements for exchanging and screening genetic resources. It also provides access to a wider range of information than is available bilaterally and offers opportunities to use information cost-effectively, by sharing databases, for example.

These different characteristics make each approach preferable in particular situations and less suitable in others. For example, bilateral approaches may be preferable when a small number of countries have, or need access to, the genetic diversity of a particular species or group of species, or when expensive and specialized research gives a competitive advantage to a single or limited number of institutions. Such conditions may occur in the case of some industrial crops, for example rubber, and in certain sectors, for example pharmaceuticals. In the case of fish, bilateral arrangements could be appropriate for aquatic resources that have a limited distribution or where there is limited interest in them.

On the other hand, exclusively bilateral arrangements are likely to be very complicated in the case of staple food crops, given the large number of potential actors (and thus individual agreements) involved, the complex pedigrees of crop lines (and consequent difficulty of assessing and apportioning benefits) and the limited capacity of some partners to be able to negotiate favourable terms. Nor are such arrangements likely to yield significant financial rewards given the difficulty—or impossibility—of capturing benefits where the material is in the public domain and seed is largely produced on-farm and disseminated among farmers, as in the case of most staple food crops in developing countries. The same could presumably be said of fish species such as tilapias which are distributed widely and where material from a number of countries is combined in breeding programs. The more complex the pedigree line and the more countries that contribute material to a breeding program, the more complicated and cumbersome bilateral agreements will become.

Multilateral approaches may be most appropriate in situations where individual countries harbour only part of the genetic variability of interest, or when farmers and professional breeders in many countries need access to a wide range of genetic resources. They may also be preferable when there is a high social stake in successful species improvement and when the pooled efforts of many are likely to be more effective in promoting improvement than the efforts of a few. These conditions prevail for the majority of staple food crops.

The Traditional Approach to Exchange

Recent trends and policy shifts with regard to the exchange of plant genetic resources may hold some lessons for the fish world. The traditional approach to the exchange of plant genetic resources cannot be characterized as either strictly bilateral or as purely multilateral; it includes elements of both models. It comprises a multiplicity of simple and complex relationships between partners. These relationships have resulted in the development of a web of alliances between and among individual national programs, within regions, and with and between NGOs, international agricultural research centres and the private sector. This approach is flexible, dynamic and adaptable. It has been responsible for much of the food crop germplasm collected and exchanged internationally over the past two decades.

To date, most international exchanges of germplasm between breeders and researchers have been carried out on an informal basis. Countries have generally permitted collecting missions on the conditions that their own scientists participate and that duplicate samples of collected material (and related information) are provided for storage at a local facility.

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[2] In 1995, the Brazilian Agriculture Research Corporation (EMBRAPA) and the Rubber Research Institute of Malaysia (RRIM) signed an agreement whereby EMBRAPA would exchange Brazilian wild materials of the genus *Hevea* for elite clones of *Hevea braziliensis* developed by RRIM. A collecting expedition was carried out in the Brazilian Amazon under the terms of national legislation. These require that at least 50 per cent of collected materials must remain in Brazil and that a Brazilian institution must participate in field explorations. Improved clones selected by RRIM from these materials can be transferred to third parties only after EMBRAPA's authorization. In exchange for the wild materials collected in the Amazon, RRIM has sent nearly 80 elite clones to EMBRAPA. The materials negotiated under this agreement can only be used for scientific and technological development. Information provided by *Brazilian National Research Center for Genetic Resources and Biotechnology (CENARGEN).*

Bilateral arrangements have long been common in industry, typically through the use of formal contracts such as material transfer agreements (MTAs). There are also bilateral exchange agreements between governments—for example the agreement between Brazil and Malaysia to exchange rubber.[2] Nonetheless, the use of MTAs in public sector exchanges is a recent phenomenon and still relatively rare.

The Consultative Group on International Agricultural Research (CGIAR)

The CGIAR is a multilateral system that operates within the context of this web of exchange alliances. The plant germplasm collections housed by the CGIAR Centres—which represent the world's largest international holdings of basic food crops—fall under the auspices of the FAO Commission on Genetic Resources for Food and Agriculture. CGIAR policy, as confirmed by Agreements signed in 1994 with FAO, is that the collections are not Centre property but are held in trust for the world community. As such, that intergovernmental body sets the broad basis for exchange and benefit sharing with regard to the CGIAR collections. The FAO Agreements, it should be said, were largely prompted by the fact that, since the CGIAR cannot be party to the Convention on Biological Diversity, the collections are not legally bound by its terms. Even if the CGIAR were bound by the Convention, the bulk of the material in the Centre gene banks was collected prior to the coming into force of the Convention and thus falls outside of its scope.

The CGIAR Centres have followed a policy of allowing unrestricted access to the plant genetic resources in the collections. The FAO Agreements essentially re-affirm and give legal weight to the CGIAR's commitment to hold its collections in trust for the international community. Accordingly, Article 3(b) of the Agreements state that centres "shall not claim legal ownership over the designated germplasm" or "seek any intellectual property rights over that germplasm or related information".

The operational policy of the system is set by the CGIAR members, which currently include 57 countries (of which 30 per cent are from the southern hemisphere), and by the co-sponsors, themselves intergovernmental organizations (FAO, UNDP, UNEP and the World Bank). The collective decision-making that characterizes the CGIAR system has resulted in the formulation of standard rules and procedures that govern the germplasm exchanges between the Centres and their partners, for example the standard agreements that accompany the release of materials from CGIAR gene banks.

The Drift Towards Bilateralism

The CGIAR collections represent about 10 per cent of the world's *ex situ* holdings of plant genetic resources. Currently, approximately six million germplasm accessions are housed in more than 1,300 gene banks around the world.[3] Eighty-three percent of this material is held by national institutions. With the entry into force of the Convention on

[3] There is considerable duplication within and between collections and the number of unique accessions in collections intended for conservation is believed to range between one and two million. It is estimated that the CGIAR holds up to 40 per cent of these unique accessions in its collections, thus underlining their importance.

Biological Diversity, the national conservation imperative received formal recognition, as did the sovereign rights of countries to control access to their biological diversity and to make it available under mutually agreed terms and conditions between providers and recipients. Among other things, these conditions support the right of providers of original material to negotiate a fair and equitable share of the benefits arising from its use by others.

Concern is growing that a restrictive enforcement of the principle of sovereign rights might lead to fewer exchanges of genetic resources. Indeed, this appears to be the case; in response to the Convention's recognition of national authority to determine access to genetic resources, a number of countries have started to regulate germplasm transfers. For example, the South African Development Community (SADC) countries have imposed a temporary ban on the transfer of any biological resources not covered by existing conventions and where prior informed consent is not in effect. Some countries, such as the Philippines, have introduced specific control mechanisms or have negotiated regional exchange arrangements that control the release of genetic material from member States to outsiders (e.g., the Andean Pact countries). Others are in the process of developing and adopting national access legislation.

It is important to stress that the access legislation under development generally relates to the exchange of all biological resources, including aquatic resources. Ironically, at a time when efforts are being made to enhance international collaboration in the conservation of fish genetic diversity, many countries are imposing restrictive exchange arrangements that could make such collaboration problematic.

Coupled with the policy shifts towards greater restrictions on access to genetic resources has been a trend towards greater privatization of breeding and research, and increasing pressures to enact stricter intellectual property legislation in conformity with the GATT/TRIPS legislation.

Thus we have started to see a steady "drift" towards bilateralism. This is cause for concern, as a strictly bilateral approach to the exchange of major food crops would be likely to impede the flow of germplasm overall, with serious implications for food security.

Policy Frameworks and the FAO Commission

The time would appear to be ripe to consider the development of internationally agreed policies governing access and benefit-sharing arising from the use of fish resources, perhaps along the lines of those being negotiated for plant genetic resources. This process might be undertaken within the context of the FAO Commission on Genetic Resources for Food and Agriculture, which, as noted, has recently taken on responsibility for aquatic and livestock genetic resources.

The FAO Commission is currently renegotiating the International Undertaking on Plant Genetic Resources in harmony with the Convention on Biological Diversity. Adopted in 1983 by the FAO Conference, the International Undertaking is a non-legally binding agreement to cooperate in the conservation and use of plant genetic resources. The revised Undertaking may become a legally binding agreement and perhaps a protocol to the Convention. One task of the Commission is to frame an agreement on the range of biological materials to be covered by the Undertaking (known as the scope of the agreement) and terms of access to genetic resources within the context of the International Undertaking. The re-negotiated Undertaking is likely to provide the framework for a global exchange system.

At the most recent meeting of the FAO Commission on Genetic Resources in December 1997, the access issue was debated at length. Delegates generally agreed with the option for a multilateral system for major food crops. Access to material in the system would be facilitated without imposing restrictions that run counter to the Convention on Biological Diversity.

Most of the discussions in the Commission to date have focused on the six million germplasm accessions currently held *ex situ*. The issue of material held *in situ* has been skirted but raises a number of difficult and complicated questions which are unlikely to be resolved any time soon. For example, countries differ in the degree to which they recognize ownership rights over genetic resources by the individuals, groups or institutions that are the actual holders or guardians of the material. A country's accession to the International Undertaking would not guarantee that material held *in situ* by farmers and indigenous communities would be available for exchange within the system since the prior informed consent of these private groups would be needed. The terms of that consent and the rights—including farmers' rights—of local communities and of other non-government holders of genetic resources *in situ* most likely need to be established, or at least administered, at the national level. Similar questions have been raised relative to the *in situ* conservation of aquatic resources.

A tentative list of crops for inclusion in the system was developed, using the criteria of their importance for food security and the interdependence of countries with regard to particular plant genetic resources for food and agriculture (PGRFA). All PGRFA not included on the list would be exchanged on the basis of bilateral or other separately negotiated arrangements. Non-PGRFA uses of listed material might be subject to different conditions of access. There is considerable support for enhancing the list through periodic reviews with the idea of eventually increasing scope to include a more complete coverage of PGRFA.

Generally, there is support for placing the CGIAR collections in the multilateral system for facilitated access. Most delegates also want the inclusion of national collections, although some noted that this might be difficult in cases where they are under private rather than national jurisdiction.

With regard to benefit-sharing, it was proposed that participants be entitled to the benefits arising from the exchange and use of the material (facilitated mutual access to PGRFA, information, cooperation in collection, research, training, technology transfer, etc.). This would be realized through the development of integrated strategies, networks, joint programs and the improved coordination of existing mechanisms and systems. Other proposals also envisaged the creation of a fund, which could be supported, for example, by the payment by countries of a percentage value of the crops listed in their territory and/or a share of royalties on materials protected by intellectual property rights. The fund could be used in a number of ways, e.g., to cover the costs of supporting the system or to finance the implementation of the Global Plan of Action for the Conservation and Use of Plant Genetic Resources,[4] or it could be divided among members.

Conclusions

The eventual results of the Commission's negotiations are still unclear; however, one thing is certain. The future of sustainable agriculture requires the development of systems to facilitate efficient conservation, promote access and ensure an equitable sharing of the benefits arising from the use of plant genetic resources for food and agriculture. While the history and extent of international collaboration on fish genetic resources is far less advanced than it is on plants, policy trends currently affecting the plant sector could have significant implications for future collaboration in the fish sector. Without internationally agreed policies governing the terms of such collaboration, the genetic diversity of aquatic species could fall prey to the "tragedy of the commons", threatened with destruction by uncontrolled use because no one feels any ownership or responsibility for its protection. Thus any effort to advance the conservation of fish genetic resources on an international scale would do well to consider the lessons learned in years of negotiating policy frameworks to govern the exchange of plant genetic resources.

A system that allows complete and unrestricted access by all countries to all categories of genetic resources, including those with potentially high value, could jeopardize the ability of source countries to claim a fair and equal share of the benefits arising from the use of these materials. On the other hand, an overly restricted system could drastically reduce the flow of material worldwide—to the detriment of all— and would be complex and expensive to administer. In terms of efficiency and equity, the logical approach would be broad enough to accommodate the best characteristics of both.

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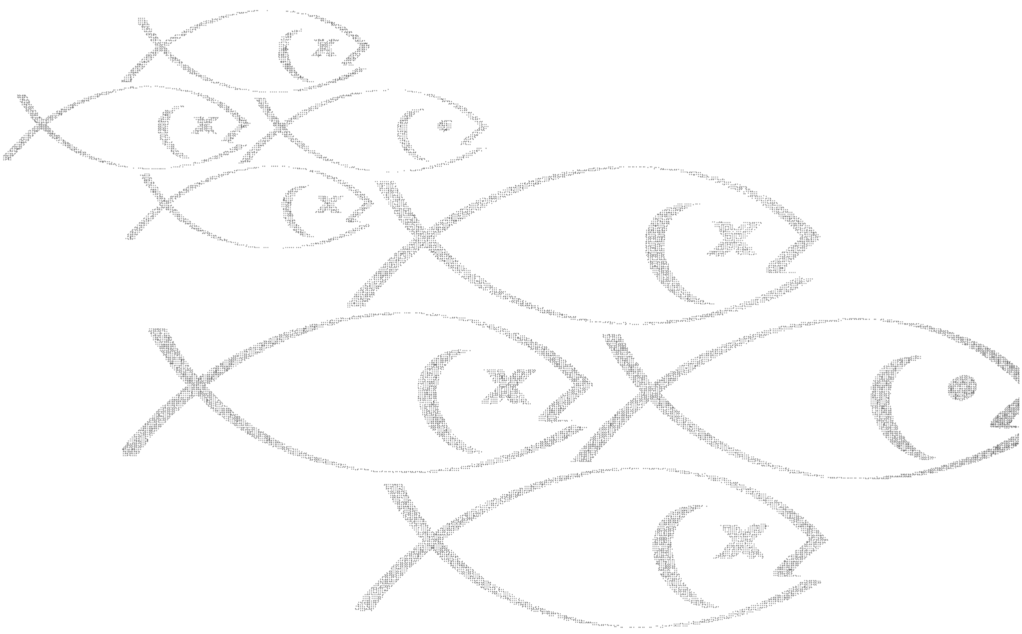
[4] The Global Plan of Action describes the 20 most urgent actions needed to protect the world's shrinking supply of plant genetic resources for food and agriculture. It was negotiated and approved by 150 countries at the International Technical Conference on Plant Genetic Resources, in Leipzig, Germany in June 1996.



World Bank Experience and Initiatives Related to Conservation of Freshwater Biodiversity*

Maria Isabel J. Braga [1]

Introduction

This paper provides an overview of some World Bank experiences and initiatives related to the conservation of freshwater biodiversity in various countries around the world. These initiatives follow and build upon the recommendations from the Bank's *Water Resources Management Policy Paper* (World Bank 1993) and from *Mainstreaming Biodiversity in Development: A World Bank Assistance Strategy for Implementing the Convention on Biological Diversity* (World Bank 1995). As the demand for fresh water and protein continues to increase, it is the Bank's mission to help client countries meet these needs through sustainable use of all resources from freshwater ecosystems, including freshwater and the aquatic organisms within these ecosystems.

From fiscal year 1988 to 1997, the Bank's total water resource portfolio, including irrigation and drainage, flood control, water supply and sanitation, industrial effluent control, hydropower, and natural resources management accounted for more than 400 projects with Bank loans accounting for more than US$33 billion. Within that portfolio, loans and grants dedicated specifically to sustainable use and conservation of freshwater biodiversity conservation total approximately US$250 million or about 0.75 per cent of the total investment (World Bank in press). Although freshwater biodiversity still has little representation in the overall biodiversity portfolio, there are many opportunities for investing in its conservation and restoration due to the extensive nature of the overall water resources development portfolio.

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* The findings, interpretations, and conclusions are the author's own and should not be attributed to the World Bank, its Executive Board of Directors, or any of its member countries.

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The World Bank's Experiences and Initiatives Related to Freshwater Biodiversity

Support for the overall sustainable use of freshwater ecosystems within World Bank activities can be grouped in one of four basic categories:

1. Direct investments in projects that target the conservation of natural resources and biodiversity, such as the establishment of national parks and other conservation areas, restoration of degraded freshwater ecosystems, etc. Some examples of projects that fit this category are:
   - **Lake Victoria and Lake Malawi Projects**—These East African lakes are evolutionary centres for freshwater fish and contain many endemic species. However, this biodiversity is increasingly threatened by the effects of a growing human population and resulting economic activities in their watersheds. Under the tri-national Lake Malawi/Nyasa project, the first comprehensive surveys of the lake's biodiversity are being conducted along the shorelines of Malawi, Tanzania and Mozambique to map the lake's biodiversity. This information will be used to develop a biodiversity management plan that will provide recommendations regarding the establishment of protected areas in the lake, and for devising guidelines for sustainable harvest of fish.
   - **Danube Delta Program**—This program focuses on conservation and management of the Danube Delta ecosystem in Romania and Ukraine. Most of the activities, including ecosystem and species surveys, aim to strengthen monitoring and database management and to contribute to the development of a scientific basis for planning and managing the use of those resources. Also included is a pilot restoration project to re-establish some of the original hydrological circulation to an important wetland in the area.
   - **Management of Natural Resources in Amazonian Floodplains**—This project is part of the Pilot Program to Conserve the Brazilian Rain Forest (PPG7), and aims to establish the policy, technical and management foundations for the sound and sustainable management and conservation of Amazonian flood plains. The seasonally inundated flood plain to the Amazon River contains several fragile habitats, most of which are vital for the growth and reproduction of the region's freshwater fishes, but which are highly threatened by recent demographic and economic changes in the Amazon region. Current changes in land use involve clearing of forest and farming in seasonally inundated flood plains, thus affecting many fish species which feed on, and depend upon, the bounty of the flooded forest (fruits, nuts, detritus and insects).
2. Engaging in policy dialogue with client countries to institute or strengthen policies and legislation conducive to the sustainable use of freshwater ecosystems, and to support initiatives that foster capacity building within the country.

- **Workshops**—The Bank supports and organizes workshops to bring together a variety of stakeholders to discuss issues relevant to the conservation of inland aquatic biodiversity. EDI's[2] Water Policy Reform Program promotes workshops and other activities where participants exchange lessons from successes and setbacks, and from other worthwhile experiences in water resources management around the world. Examples include national seminars on integrated water resources management in Yemen, Senegal, Kenya and Tanzania, and a regional workshop in the Caribbean. A major follow-up of the Tanzania seminar was the development of an innovative River Basin Management and Smallholder Irrigation Improvement Project. The project addresses institutional issues (water resources policy, legislation, river basin boards, etc.) as a way to deal with emerging water use conflicts between hydropower interests and farmers, between upstream and downstream farmers, between farmers and pastoralists, and between farmers and environmental interests; one project component will study the hydrology and ecology of the Utengule swamps, upstream from the main reservoir (Mtera Dam) regulating flows for hydropower generation (providing 85 per cent of the national power needs).
- **Argentina Flood Protection Project**—To cope with increased floods in the Parana River basin, the government of Argentina is implementing a new flood protection strategy that recognizes the ecological importance of floods and the buffering effects of natural wetlands and floodplains. The strategy calls for (a) improved management of the Basin's major natural resources through improved coordination of flood-related actions within and among the provinces; (b) structural measures consisting of well-defined, long-term investments in defending the area's most important assets, such as raising of bridges and improvement of existing as well as construction of new essential drainage channels; and (c) non-structural measures consisting of a combination of actions for "living-with-floods", such as implementation of flood warning and civil defense measures, and development of plans and regulations that discourage urban expansion in high risk areas.
- **Giving Priority to Integrated River Basin Management**—The Bank assisted Nepal in an exercise to identify possible sites for future hydropower projects that could supply the country's projected medium- and longer-term power needs in the most sustainable way. The process involved extensive stakeholder consultation and information-sharing and, out of the 138 potential sites identified, seven were chosen for further feasibility and detailed Environmental Impact Assessment (EIA) studies. A similar exercise is being conducted in Vietnam (Vietnam National Hydropower Study), and in this case information from field surveys of freshwater biodiversity will be added to the comprehensive studies of economic, environmental and social implications of each potential site.

3. Inclusion of freshwater biodiversity conservation, impact mitigation and freshwater habitat restoration components in lending activities to the traditional water sector,

[2] The World Bank's Economic Development Institute

i.e., irrigation, hydropower, transportation, flood control, water supply and sanitation. This category has been slower than expected in fully integrating the ecological needs of freshwater ecosystems, and this may be partly due to factors such as shortage of overall baseline data on freshwater organisms in tropical areas, and the usual fragmentation of institutions with jurisdiction over aquatic ecosystems—usually the Ministries of Environment, Energy, Agriculture, Urban Issues and Housing, Industry, Transportation, etc. The following are examples of some of the projects that did consider conservation or restoration of freshwater biodiversity.

- **Orissa (India) Water Resources Consolidation Project—Naraj Barrage**—This new barrage on the delta of the Mahanadi River replaces the aging Naraj Weir that diverts water from the Kathjori branch into the Mahanadi and Birupa branches of the Mahanadi delta, providing water to an important irrigation scheme. The new barrage will have gates for flow regulation, a feature that opens the possibility to manage to a certain extent the fresh water and sediment inflow into the different branches of the Mahanadi delta. A fish pass of 2 m width is included. Apart from its irrigation function, the new Naraj Barrage will be useful to increase early monsoon floods in the Mahanadi branch to enhance fish spawning in the river, and to maintain certain minimum flows in the Kathjori branch the first three months after the monsoon in view of upstream prawn migration.
- **The Manantali Energy Project**—The Manantali Dam, in Mali, was completed in 1988 without World Bank financing and aimed to respond to the drought and famine of the 1970s and 1980s by providing a steady supply of water for agriculture. However, the construction and management of the Manantali Dam, combined with changing rainfall patterns, has resulted in the curtailment of natural flooding and diminished or threatened the aquatic and floodplain production systems. The Manantali Energy Project, which finances the installation of a 200MW hydropower facility at the Manantali Dam and a transmission system to distribute power to Mali, Senegal and Mauritania, is an infrastructure project that attempts to address ecosystem function issues by restoring some of the original characteristics of the natural ecosystem. The project includes assistance with the implementation of an environmental mitigation and monitoring plan along with studies aimed at the optimization of reservoir management and mitigation measures for environment, traditional agriculture and health. A Reservoir Management Manual has been prepared, and artificial flooding is proposed to enhance aquatic and floodplain habitats, and increase overall production. Except in the driest years, artificial flooding will re-establish many of the traditional floodplain functions impacted by the Manantali Dam, and considerable increases in flood recession agriculture, dry season pastures, and fish production are expected.
- **Yacyretá II Hydropower Project**—The Yacyretá Dam, on the Parana River between Argentina and Paraguay, poses a significant barrier to fish migrations. In an experimental attempt to facilitate passage of migratory fish upriver, the dam design includes two fish transfer stations, the first of its kind in the tropics, each

with two elevators to lift migratory species including those that lack the jumping ability to utilize a fish ladder. Two elevators are already installed and functioning properly, and although fish survival within the elevators exceeds 99 per cent, scientific estimates indicate that only about 7 per cent of the fish seeking to move upriver past the dam succeed in finding the elevators and using them. While the elevators cannot help restore pre-dam fish migration patterns, they are nonetheless useful for ensuring that the reservoir and upstream river areas have a genetically diverse breeding stock of native fish.

4. Activities that foster the dissemination of best practices and current knowledge that could contribute to the conservation of freshwater ecosystems and biodiversity.
   - **Publications**—Recent initiatives in this area include the publication of the World Bank Technical Paper No. 343 *Freshwater Biodiversity in Asia, With Special Reference to Fish* (Kottelat and Whitten 1996) and of *Mainstream*, a newsletter distributed three times a year both inside and outside the Bank. The goal of this newsletter is to foster the dialogue among water managers and planners, engineers, and biologists, thus contributing towards environmentally sustainable water-related development projects.
   - **Support for Global Initiatives and Strategic Partnerships**—Examples of freshwater-related partnerships where the Bank is heavily involved include the Global Water Partnership (GWP), the World Water Council (WWC), and the World Commission on Dams. The Bank also works closely with the Secretariat for the Convention on Biological Diversity, the Secretariat for the Wetlands Convention, IUCN, Wetlands International, World Wildlife Fund (WWF), and other organizations during project identification, preparation, implementation, and supervision. On a different note, in early 1997 the Bank organized and co-sponsored the international workshop **Mainstreaming Freshwater Biodiversity in Water Development Projects,** at the White Oak Conservation Centre, Florida. The event brought together water resources managers, hydro engineers, and biologists to discuss both tried and innovative measures to prevent or mitigate negative impacts of water development projects on freshwater biodiversity and ecosystems. The information presented and discussed during the workshop is being organized in a publication, under the sponsorship of the Inter-American Development Bank, and will soon be available for public distribution.

The Recommended Approach

The World Bank's approach is mostly one of conserving freshwater biodiversity *in situ* through conservation of freshwater ecosystems and mitigation of the impacts from land-based activities on those ecosystems. Projects that directly target conservation of freshwater biodiversity are showing up in increasing numbers in the Bank's portfolio, but the biggest opportunity for interventions that contribute to environmentally sustainable use of freshwater ecosystems still lies with the traditional water resources portfolio. There are numerous opportunities, within the various water resources management projects, to contribute positively to the long-term sustainability of freshwater ecosystems and their biodiversity. It is important that the Bank takes full advantage of those opportunities, but it is more important still that client countries take the initiative to conserve these important natural resources not only in the context of World Bank-assisted projects, but in all of their development activities.

Most impacts from water development projects stem from changes in freshwater habitat conditions, or availability and access to those habitats. Preservation of natural freshwater habitats is essential for conservation of freshwater biodiversity. A successful plan to achieve this goal must include four major elements (NOAA, 1996):

- **Protect and Conserve**—assess human-induced impacts at levels ranging from sites to ecosystems, provide scientifically based advice to reduce or eliminate those impacts, and form partnerships to protect and conserve habitats of living aquatic resources. Track natural habitat trends for perspective and to assess progress.
- **Restore and Create**—restore and create habitat, thereby reversing the net loss occurring from continued growth and development or resulting from natural events.
- **Understand**—obtain, interpret, and share scientific information needed to manage important habitats, increase awareness of habitat values, and enhance the role of relevant government agencies.
- **Operate**—support those actions by developing government policies, pursuing partnership agreements, leveraging funds, sharing staff, and other creative solutions that improve effectiveness and efficiency.

A basic principle for adoption by countries and private sector partners is that species or genetic diversity should not decline as a result of a water development project. This means the project does not cause the extinction or endangerment of any species, during either its construction or long-term operation. The specific means to achieve this goal may vary from one project or region to another depending on project characteristics and the existing conditions of local biodiversity.

The likely effects of a project on biodiversity must be predicted more accurately *prior* to the final commitment of funds to the project. Project design and operation can often be adjusted to minimize project effects on biodiversity, and this is *especially true when biodiversity considerations are incorporated into the process from the very initial stages of project preparation and design.* Only on very rare instances would this process lead to cancellation of a project.

The Role of Environmental Assessments (EAs)

In many water development projects more than three years is spent on finding the optimum dam site for maximum power production and maximum dam safety. Finding the optimum dam site is important to engineers and they *expect* to have enough time and money to do it right. So why is it that, once the best site and configuration has been painstakingly identified, the environmental evaluation is almost an afterthought? Why should first-rate engineering be followed by second-rate understanding of the environmental implications of a project? *The notion that an environmental impact analysis coming at the end of the project design is adequate, and that after-the-fact mitigation will compensate for biodiversity losses, is a totally false one!*

Intelligent and useful biological input to the development process is absolutely dependent upon having reliable data and information on the resources that will be affected by the development project. Biological systems do not conform to project development time-frames, and as long as a "point-in-time" understanding of biological systems is perceived as sufficient information for decision-making, freshwater biodiversity will continue to decline.

The first step for accurate prediction of possible project impacts is adequate understanding of the biological resources and processes in the areas directly and indirectly affected by the project. For that purpose, a thorough inventory of the freshwater ecosystem must be conducted. A resource inventory is not simply the counting of individual species, it must be *habitat*-based to have meaning in terms of biodiversity, cultural diversity and sustainable development.

The inventory must be hierarchically organized to maintain ecological integration, scale, content and context for all resources at all levels of investigation and planning. The first step in the inventory of the freshwater biodiversity resources within a river basin is an ecological classification that differentiates landform down to river channel types while maintaining scale, content, and ecological context. Once classification, preferably using GIS techniques, of the river basin is complete the real inventory begins on the basis of priority habitats.

The following assessments must take place within each habitat targeted for investigation:

- Structure of biological community, including riparian vegetation;
- Major feeding, migratory and reproductive patterns of freshwater species;
- Structure and quantity of microhabitats;
- Temporal and spatial variation in water flow, microhabitat availability, physical and chemical characteristics of the aquatic environment;
- Degree of connectivity among different microhabitat patches across seasons.

Five Steps for Successful Integration at the Project Level

The Bank's Water Policy Paper recommends that more rigorous attention be given to maintaining biodiversity and protecting ecosystems in the design and implementation of water projects. Water and energy supplies gained through conservation and improved efficiency can be used instead of developing new supplies to extend service to communities and to maintain water-dependent ecosystems. The water supply needs of rivers, wetlands and fisheries must be considered in decisions concerning the operation of reservoirs and allocation of water.

There has been a large increase in the amount and quality of information, as well as in the number of tried techniques, regarding mitigation of negative effects of water development projects on the health and ecological integrity of freshwater ecosystems. However, although discussions in Europe and North America currently focus on the best approaches to determine minimum instream flows required for maintaining freshwater biodiversity, in many other countries discussions related to hydropower and irrigation dams still centre on whether any water at all should be "wasted" downstream to support the ecological needs of freshwater ecosystems.

Aiming to contribute to the effective integration of conservation of freshwater biodiversity and water development projects, Hill (1997) developed the following guidelines regarding the steps that should occur during the five main stages of project planning and implementation:

Stage 1—Initial Resource Inventory

Water development projects begin with the identification of problems to be fixed (e.g., flooding) or benefits to accrue (e.g., irrigation, hydropower) and the region or area where the project is needed. This is generally recognized as the *identification* stage where the host government and the donor/lender evaluate different project strategies. Project goals and general geographic area are established at this stage, and this is when an initial biological resource inventory should be initiated, in conjunction with initial hydrometeorological, geological and socioeconomic surveys. *The resource inventory must be initiated as early as possible to maximize the time available to collect long-term data and to minimize design and construction delays at a later stage.* Early involvement of aquatic biologists is essential to ensure that the right kinds of questions are asked and the proper aquatic habitats are sampled.

The level of intensity and detail for this initial inventory of freshwater biodiversity resources should match the predicted level of potential impacts (local vs. basin-wide), economic importance of existing resources, quality of the existing baseline information (if any), presence of known endangered species or habitats, etc. When the inventory is complete, it is the responsibility of the investigative team to inform decision-makers by presenting the information in such a way that intrinsic values of biodiversity, cultural diversity and related environmental conditions are clear and understandable. The presentation of data should include not only the identification of threatened and/or endangered species, but must also illustrate cause and effect relationships between biological communities and structural diversity at the habitat and ecosystem level.

Stage 2—Feasibility Study Input

The principal actions at the feasibility stage involve site selection, project sizing, and economic analysis. Sustainable development should be a shared goal at this stage. The project team comes together at this stage to exchange knowledge and to jointly prepare a feasibility report. *Biologists must be an integral part of the team also at this stage; they must use the data and information gathered in the initial inventory to assist with site selection and project sizing.* The analysis of project alternatives will illuminate more discrete data gaps that become the focus of the next stage.

Stage 3—Preliminary Design Input

This is the stage at which the basic project has been decided upon and the site, size and magnitude of the project is fixed. This establishes a more discrete geographical area within which to focus the more detailed inventories of biodiversity and other resources that will be directly affected by the project. *At this stage, a thorough evaluation of cumulative impacts (e.g., the synergistic interaction of multiple water projects in a watershed or river basin) can be made.*

The more detailed inventory to be conducted at this time should include information on:

- Ecological indicators;
- Keystone, umbrella and flagship species;
- Endangered or threatened species;
- Species particularly vulnerable to predicted project impacts;
- Critical habitats and processes such as migration corridors, floodplain depth and habitat connectivity.

Stage 4—Design Modification

The final design and project alternatives are decided upon prior to full project funding and implementation, and include the selection of the preferred alternative. The objective at this stage of project development is to provide decision-makers with enough information that the consequences of their decisions are clear. *It is important that decision-makers make informed decisions and are made well aware of the consequences of their decisions and actions.*

At this stage the project team makes final modifications to the design with these priorities in mind:

- Interventions needed to conserve, protect, and/or enhance biodiversity thresholds;
- Avoidance of environmental impacts;
- Minimization of environmental impacts;
- Mitigation of unavoidable and irretrievable impacts.

Stage 5—Mitigation Planning and Project Cost Integration

Mitigation plans must be formulated early in project planning and the cost must be included in final project financing. Mitigation costs should not be viewed any differently than the cost of steel and concrete—both are necessary project expenses and must be financed accordingly. Early planning should result in enough time for the purchase of land or other structural elements that normally require some time for selection and negotiation.

Design criteria for pilot scale studies, such as experimental fish ladders, must also be addressed early in the process.

Long-term monitoring plans are an important budget consideration in the project financing. Early development and implementation of monitoring plans will result in an early supply of data to guide and support adaptive project management during both construction and early operation phases.

Conclusion

The rapidly increasing number of freshwater species and whole ecosystems that are on the brink of extinction, and many more which are entering the threatened category, point to the urgent need to be proactive and coordinated in our efforts to conserve and restore freshwater ecosystems and species. There is still no conclusion to this unfolding story, and how the conclusion will read in a few years' time depends on the direction we choose to go from here. The responsibility to protect and restore freshwater ecosystems around the world lies not only with development banks and agencies, such as the World Bank, but also with governments, NGOs, international organizations, research institutions, and civil society as a whole.

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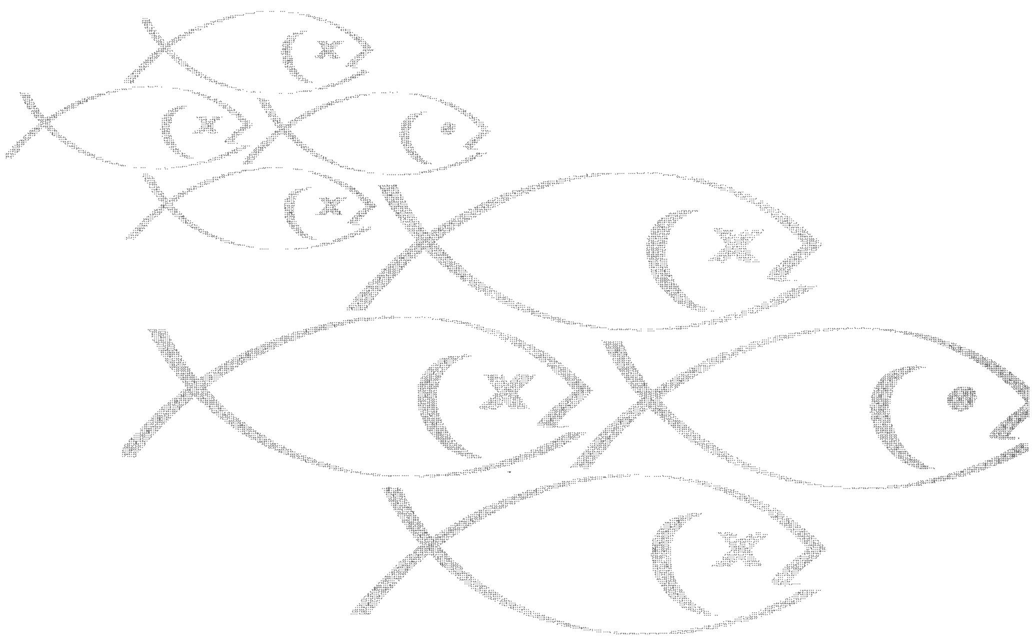

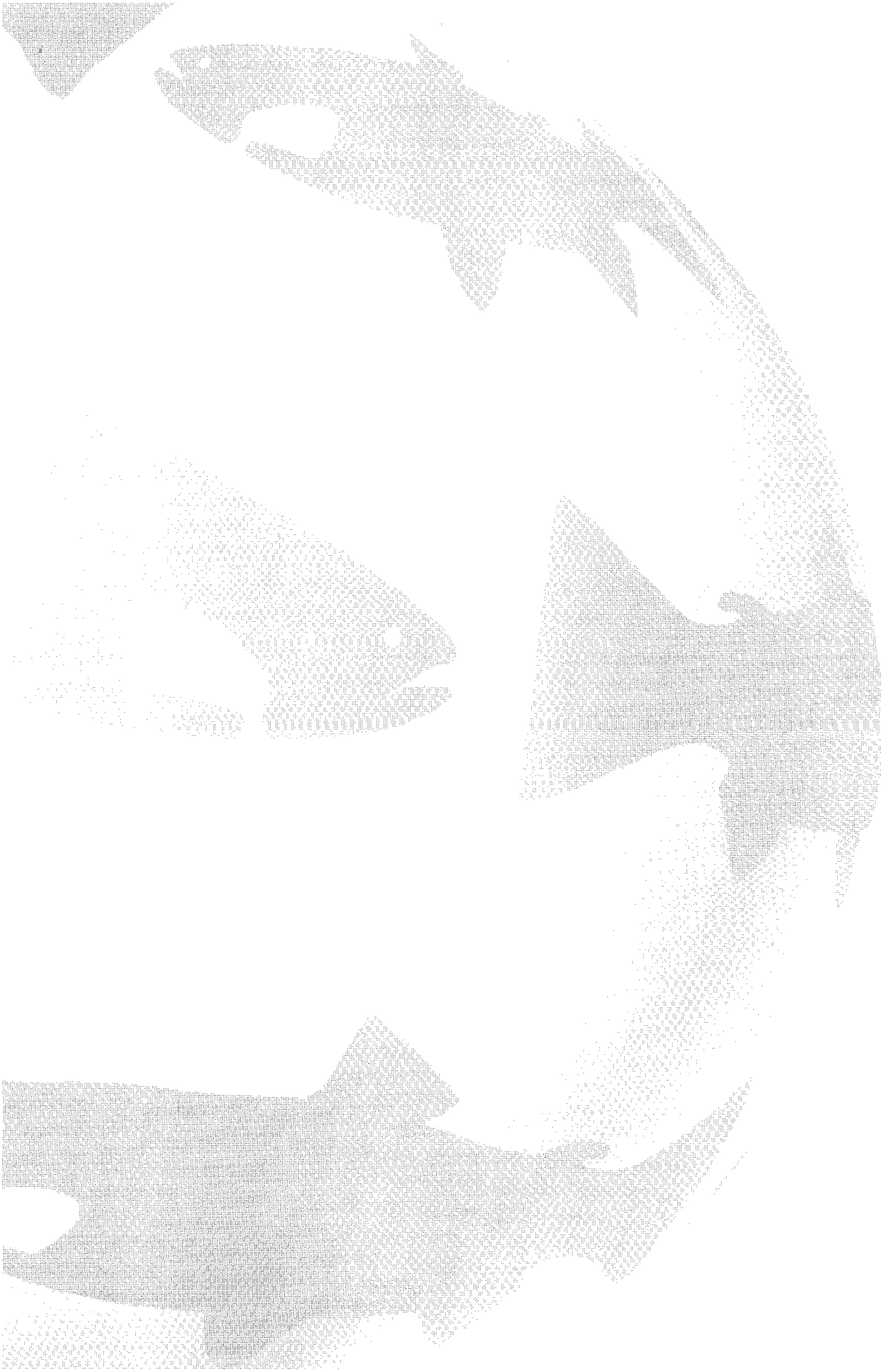

Initiatives Related to Conservation of Freshwater Biodiversity

M.L. Windsor and P. Hutchinson [1]

Introduction

The North Atlantic Salmon Conservation Organization (NASCO) was established in 1984 to contribute, through consultation and cooperation, to the conservation, restoration, enhancement and rational management of salmon stocks, taking into account the best available scientific information. The members of NASCO are Canada, Denmark (in respect of the Faroe Islands and Greenland), the European Union, Iceland, Norway, the Russian Federation and the United States of America.

Because of limitations on production in the freshwater environment, the Atlantic salmon is a small resource in comparison with many marine fish species and some Pacific salmon species. Although annual catches of Atlantic salmon in the period 1960-1996 averaged approximately 8,500 metric tons, in recent years catches have been less than 4,000 metric tons. Despite the small harvest, the Atlantic salmon is a valuable and highly prized resource which brings employment and prosperity to many regions where other occupations are hard to find.

Atlantic salmon occur in approximately 2,000 river systems in the North Atlantic area. It is clear that genetic differences exist between different populations, even in locations close to one another. In general, comparisons of more distant geographical populations show greater genetic differences. This genetic diversity, which is maintained by accurate homing, even within catchments, is believed to determine the range of characteristics shown by populations and to underpin their productivity and viability. Evidence from recent studies is highly indicative that adaptively significant genetic variation is a determinant of performance. Protection of this genetic diversity is, therefore, a very important objective of salmon fisheries management. In this paper we will examine some of the

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threats to this diversity and measures which have been developed through international cooperation to conserve the genetic resources of the Atlantic salmon.

Pressures on the Resource

The anadromous life-cycle of the Atlantic salmon means that it is exposed to a wide range of pressures in both the freshwater and marine environments. In recent years, in response to falling abundance, a wide range of measures have been introduced to reduce exploitation. These measures comprise regulatory measures adopted by NASCO for the distant water mixed stock fisheries and measures taken at a national or regional level partly as a result of obligations under the NASCO Convention and partly for domestic reasons. Despite these measures stocks remain at a low level and vulnerable to other pressures. It is estimated that about 250 rivers in the North Atlantic region are considered to be threatened with loss. A further 120 rivers are already known to have been lost to salmon production.

When NASCO was established, salmon farming was in its infancy. The industry now produces in excess of 400,000 metric tons annually in the North Atlantic region alone. Hatchery production, especially when accompanied by intense selection for commercially important traits, is known to alter the salmon genome. Escapes from salmon farms are inevitable and, in some areas, have been estimated to be about 1 per cent of production. In some North Atlantic rivers up to 90 per cent of the salmon are known to be fish farm escapees. There is conclusive genetic evidence that escaped farmed salmon breed in the wild and that they interbreed with wild salmon. However, the practical significance of interbreeding remains uncertain and a matter of controversy. There is, however, concern that in Europe the use of only a few strains in farming may lead to genetic homogenization of the wild populations. Managers of the wild stocks are, therefore, facing the possibility of irreversible genetic changes in these stocks. If the views being expressed by many authoritative scientists on this subject prove to be well founded, such changes could lead to the demise of the wild salmon in their present diverse forms.

There is also concern about the damage that can be caused by poorly planned introductions and transfers in support of aquaculture and in order to 'improve' the natural stocks. In North America, introduced species have been implicated in 68 per cent of extinctions of native fish species. The risks to the wild stocks of Atlantic salmon have been highlighted by the damage caused by the parasite *Gyrodactylus salaris* following its introduction to Norway from the Baltic region. This parasite, to which the Baltic salmon appear tolerant, has threatened the North Atlantic salmon populations with extinction in nearly 40 rivers and drastic and very expensive intervention measures have been necessary to prevent their loss.

Conservation of Genetic Resources

It is clear that there are real threats to the genetic diversity of the wild Atlantic salmon. Protection of this diversity is important not only for those charged with conserving the wild stocks but also for the salmon farming industry, which, at least in its infancy, benefited greatly from the variety present in the wild stocks.

A conservative approach to the conservation of genetic resources would require that all genetic diversity should be retained so that the probability of inflicting irreversible harm upon systems which are not fully understood would be minimized. Such an approach is consistent with the precautionary principle and with the UNEP Convention on Biological Diversity, the objective of which is the conservation of biological diversity, including the diversity within species, between species and of ecosystems.

A number of international conferences have adopted this "conservative approach" in their recommendations. For example, the Nordic Symposium on Gene Banks held in 1978 recommended the establishment of gene banks in each of the Nordic countries and research into techniques of storing eggs and milt. A symposium on "Fish Gene Pools—Preservation of Genetic Resources in Relation to Wild Fish Stocks", arranged by the Swedish Commission for Research on Natural Resources and held in 1980, recommended that "Management programs should be initiated for the conservation of as much genetic variability within and between populations as possible..." and advised that "Techniques need to be developed for the long-term storage of sperm, eggs and embryos." An Expert Consultation of the Food and Agriculture Organization of the United Nations (FAO), held in 1982 on the Genetic Resources of Fish, formulated a number of recommendations including:

- UNESCO and perhaps other international organizations should consider establishment of a program of education and training on genetic resource conservation/preservation in fish. The program should assemble baseline information (a) on the diversity and vulnerability of aquatic genetic resources (b) on procedures for identifying vulnerable species and populations and (c) on appropriate methods of assuring that information regarding vulnerability and direct threats comes to the attention of agencies competent to act.
- the many international organizations for the regulation of fish stock exploitation are encouraged in their efforts to prevent the extinction and genetic deterioration of valuable stocks.

The Convention on Biological Diversity recognizes that *ex situ* measures have an important role to play in protecting genetic resources.

The Norwegian gene bank for Atlantic salmon, established by the Ministry of the Environment through its Directorate for Nature Management, demonstrates that the techniques for the successful cryopreservation of salmon spermatozoa now exist and that fertilization rates of 60 per cent can be achieved with material stored in this way. Living gene banks have also been established such as those for threatened and endangered fish species at the Dexter National Fish Hatchery, New Mexico and for salmon in Norway. These living gene banks are expensive to run and do not offer absolute protection, but they may be beneficial in some circumstances.

The way to prevent further extinctions of Atlantic salmon populations, with the consequent loss of genetic diversity, is by rigorous protection and, where there is scientific uncertainty about the impacts of activities on the resource, implementation of a Precautionary Approach. In some circumstances, gene banks may have a role to play in conserving genetic diversity. However, they should not be regarded as an alternative to *in situ* conservation measures.

NASCO's Actions

In 1990, in response to concerns about the genetic impacts of fish farm escapees, NASCO adopted guidelines for the establishment and operation of salmon gene banks, for use as appropriate by the Parties. The advantage of having internationally agreed guidelines in place is that where a Party decides to proceed with the establishment of a gene bank, internationally agreed techniques are available which are designed to prevent the introduction of unacceptable levels of genetic selection into the recovered population. These guidelines are annexed to this paper.

NASCO has also developed measures designed to improve the containment of farmed salmon through attention to cage design, deployment and operation. However, physical containment measures cannot be 100 per cent effective and the cost of increasing containment can be prohibitively high. The use of sterile salmon (all female, triploids) in farming may offer a way forward to protect the genetic integrity of the wild stocks, but there may be drawbacks in terms of yield, fish health, ecological impacts, consumer resistance and other marketing factors. These disadvantages will need to be balanced against the risks to the wild stocks from existing practices. Efficient methods of containment will be even more important if transgenic salmon are adopted by the industry in the future.

Conclusions

The Atlantic salmon is a species with many different populations which are thought to be genetically distinct and adapted to the local environment. Through damage to habitat, some of these populations have already been lost. There is now a threat of irreversible genetic change to these wild populations because of interbreeding with escaped farmed fish. This threat, which is real, could lead to genetic homogenization.

Such changes to the diversity of wild salmon populations would not be consistent with the NASCO Convention or the Convention on Biological Diversity. It would not be in the interests of the salmon farming industry either. All concerned with the Atlantic salmon have an interest in maintaining the diversity and abundance of the wild populations. Gene banks, though expensive, are a valuable measure to protect the genetic diversity of the wild stocks and could play an important role given the difficulties of ensuring 100 per cent containment of farmed fish. NASCO is attempting to address the problems on three fronts—by taking measures to allow the wild stocks to re-build through controls on exploitation; by developing, in consultation with the salmon farming industry, measures to improve containment; and through the adoption of guidelines on the use of gene banks.

Annex 1

Guidelines for the Establishment and Operation of Salmon Gene Banks

1. *Sampling strategy*

1.1 In establishing a gene bank, priority should be given to those stocks which are considered to be particularly valuable or vulnerable to loss in order to preserve those genotypes.

1.2 Representative sampling of population gene pools is a major undertaking, requiring the maintenance of very large collections with consequent cost implications. Excessive sampling of any one population reduces the total number of populations that may be sampled with given resources. The objective of the sampling program should, therefore, be to obtain as representative a sample of the genetic diversity in the population as possible with samples being collected from all components of the spawning stock. The level of sampling will be determined by the losses in genetic variation that can be tolerated.

1.3 In the absence of genetic mapping, samples from 50-100 individuals from each river system should be collected. In rivers where the populations are threatened with loss, repeat sampling over a number of years may be necessary in order to obtain an adequate number of samples. In addition to the genetic aspects of the sampling strategy, the number of individuals sampled will influence the number of first generation progeny than can be produced for a given river system from the gene bank.

1.4 In the event that the results of genetic mapping are available, the optimal sampling strategy should be determined on the basis of this information. In particular, the occurrence of sympatric genetically isolated populations may require additional sampling.

2. *Sampling techniques*

2.1 Donor males should be collected and anaesthetized and excess water should be removed before stripping begins. Care should be taken in handling the fish since in rivers threatened with loss these fish may be able to participate in natural spawning after release.

2.2 Precautions should be taken to ensure that reared fish are not included with males selected for stripping.

2.3 All equipment coming into contact with the semen should be sterilized and dry.

2.4 Donor males yielding watery or bloody semen should be discarded. Care should be taken to avoid contamination of the semen by excess water, urine and excretions from the gut. A number of techniques such as catheterization and removal of semen by syringe can be used to avoid contamination. Only semen that is creamy white in colour should be collected.

2.5 The same volume of semen should be collected from each individual.

2.6 Precautions should be taken to prevent the spread of diseases and parasites between sampling locations.

2.7 Samples may either be cryopreserved in the field or stored chilled for up to one month and transported back to the laboratory for cryopreservation in conditions permitting precise control of freezing rate.

3. Cryopreservation techniques

3.1 The easiest and most practical cryogen for long-term storage of salmon semen is liquid nitrogen (-196°C). During storage the samples should be maintained under liquid nitrogen since storage in the vapour phase can give rise to variations in temperature.

3.2 Prior to cryopreservation it is necessary to add a cryoprotectant and an extender solution to the semen. A number of cryoprotectants and extender solutions have been successfully used with Atlantic salmon semen. Dimethyl sulphoxide (DMSO), glycerol and methanol have all proved to be successful cryoprotectants for freezing Atlantic salmon milt. The most successful extender solutions have generally been the simpler solutions that most closely resemble the major constituents of seminal plasma. High fertilization rates have been achieved with a freezing medium consisting of 0.3 per cent glucose and 10 per cent DMSO. The use of this medium at a dilution rate of 1:3 has resulted in high fertilization rates although higher dilution rates (up to 1:8) have also been used. The most suitable technique for gene banking will be that which minimizes the sperm to egg ratio.

3.3 A number of techniques for storing the extended semen are available including "French" straws or pellets stored in vials. There is little to choose between the techniques since both have been successfully used. The choice of technique will determine the equipment needed.

3.4 Freezing rates should be in the range 30-160°C per minute. Slower freezing rates have generally been unsuccessful. Fine control of freezing rate is most easily achieved using the straw technique.

3.5 The viability of a sub-sample from each fish should be tested following cryopreservation. In addition, genetic screening may also be undertaken in order to try to eliminate any fish farm escapees which may have been inadvertently sampled.

4. Documentation and security

4.1 Careful attention should be paid to a system of documentation which will guarantee identification of samples such as a computer database providing identifying information. In addition to a unique reference number this database should include information on date and place of capture, results of any viability testing, genetic screening or disease certification. Consideration could be given to the establishment of a central data bank of salmon populations in the North Atlantic from which samples have been cryopreserved.

4.2 Ease and speed of access to samples can be facilitated by colour coding storage containers and straws or vials. Care should be taken when accessing material since samples warm rapidly (400°C per minute) when removed from the liquid nitrogen.

4.3 In addition to identifying information, biological data on the donor male could be included such as length, weight and age data.

4.4 Storage units for cryopreserved samples should be fitted with alarms to warn when low levels of liquid nitrogen occur. Consideration could also be given to a duplicate gene bank to guard against catastrophic loss.

5. *Using the gene bank*

5.1 Although a great deal of research has been carried out into the methods of freezing, more research is needed into thawing rates and subsequent fertilization procedures in order to formulate protocols for the optimum use of gene banks. A number of techniques for thawing cryopreserved semen have been used, including thawing at ambient temperatures, addition of water and use of heated water baths (40-50°C).

5.2 In general, thawing rates should be rapid enough to prevent recrystallization. Since cryopreserved sperm remain active for less time than fresh sperm, partly thawed sperm should be added to the eggs.

5.3 In the event of loss of a natural population, the gene bank samples from that population could be used for re-establishment of the population. In the case of total loss, female gametes could be obtained from the nearest neighboring river with similar ecological conditions. Alternatively, the recently developed techniques of androgenesis in which the nuclear DNA in the egg is inactivated by irradiation could provide a method of producing progeny with paternal genes only. Either tetraploid males could be used or the diploid condition could be re-established by hydrostatic pressure shock. Since male Atlantic salmon are heterogametic, both male and female progeny would result. These techniques are still experimental, however, and will not be applicable to gene banks established to protect the wild stocks until high survival rates are possible.

The Cost of Pacific Salmon Conservation: A Shuswap Perspective on Fisheries Conservation and Local and Indigenous Communities

Fred Fortier [1]

History of Salmon Declines in Shuswap Territory

The Secwepemc or Shuswap people of south-central British Columbia have always understood conservation of Pacific salmon. It is said by the Shuswap that the Coyote of legend reappears every few thousand years to smash the "dams" that threaten the migration and survival of the salmon.

Salmon runs numbering greater than 1.3 million fish first disappeared in the upper Columbia River above some 15 mainstem hydroelectric dams in the 1950s, while salmon stocks once said to number tens of millions showed perilous declines in the Fraser and Thompson Rivers in the latter half of the 1900s. The federal Department of Fisheries and Oceans (DFO) was forced to issue conservation closures on Shuswap fisheries on numerous occasions to protect escapements, and permitted the bands to fish on the mainstem Fraser, where mixed-stock migrations of salmon created a higher abundance, often in other First Nations territories. This practice changed in 1990 with the Supreme Court of Canada's Sparrow decision, which established aboriginal allocation priorities and articulated the scope of the communal aboriginal fishing right as one restricted to traditional territories.

Salmon harvesting by the Shuswap has also declined in the last 80 to 100 years, because unsuccessful fishermen were forced to seek food elsewhere. The first effects on their salmon

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fishery were documented in a Memorial to Sir Wilfrid Laurier in 1910 by the region's Chiefs, referring then to "over-fishing by the whites" and "severe restrictions put on us lately by the government re: hunting and fishing".

Preventive Measures Including Gene Banking

In the 1980s the Shuswap began collaborative stock assessment and management work with DFO in the hope of reversing declines in escapements in many local streams. By 1993 the Shuswap began gene banking salmon stocks in the Thompson watershed that were threatened by declining stock productivity and over-fishing. Other measures appeared insufficient to save stocks from extinction.

Since 1993 and with technical assistance from World Fisheries Trust, the Shuswap have collected, cryopreserved and stored sperm samples from steelhead, coho and chinook salmon in six watersheds in their traditional territory. These samples are presently held in trust pending decisions on their use in rehabilitating stocks.

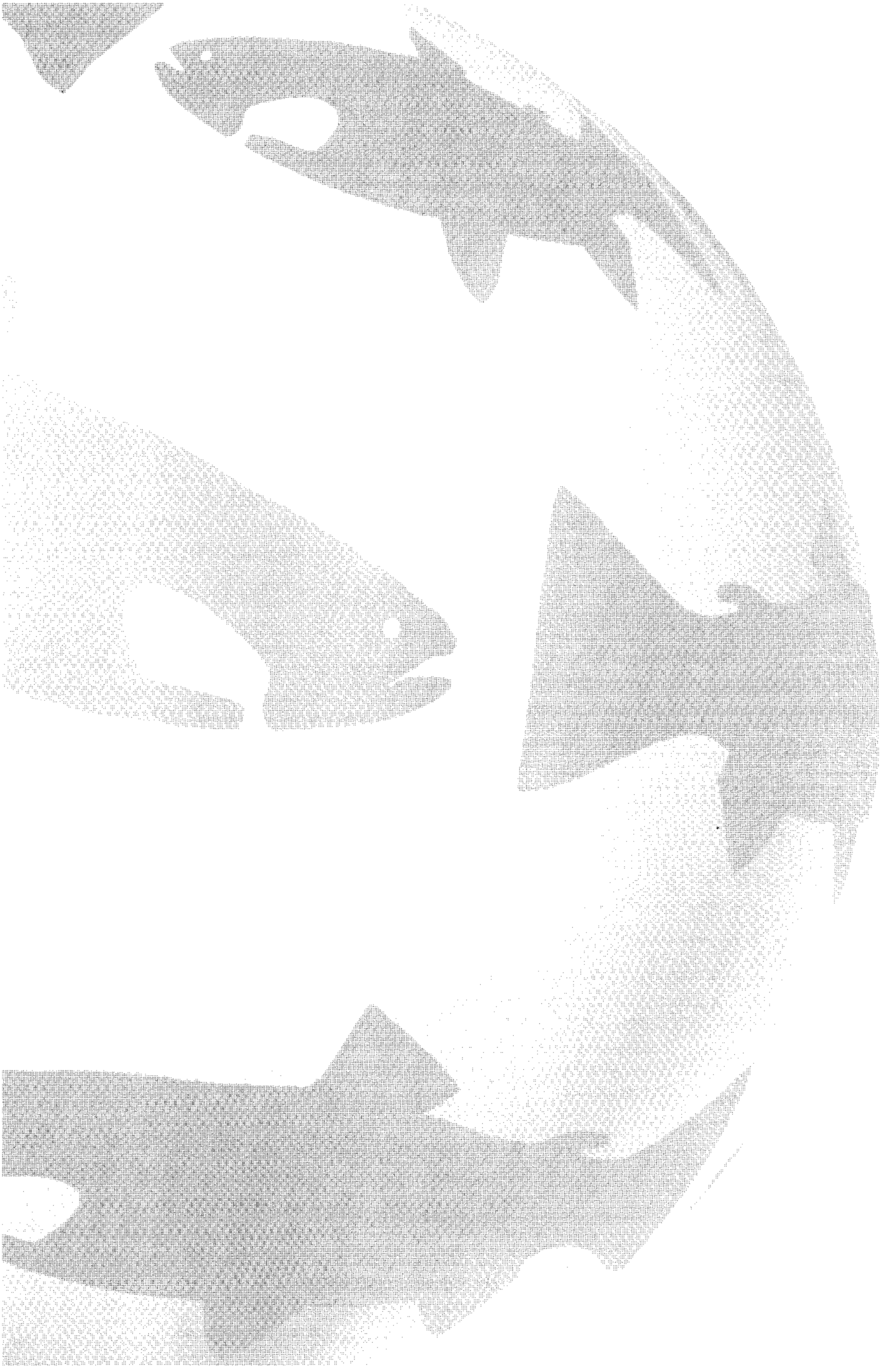
The cost of conservation has amounted to a century of lost fishing opportunities, and DFO has said that the cost of recent salmon stock assessment, monitoring and rehabilitation work, funded through the Federal Aboriginal Fisheries Strategy, may be accounted for, along with buy-back programs from industry, in any future treaty settlements. Bands also continue to fund the maintenance costs for gene banks.

The decline in numerous salmon populations in the Shuswap Territory amounts to a loss of the genetic currency necessary for BC's salmon populations to survive environmental impacts such as recent trends of decreased ocean productivity. Today the Shuswap recognise that the beleaguered salmon stocks in the Thompson and upper Fraser and Columbia rivers affect coastal mixed-stock salmon fisheries, and are working to develop partnerships with those that also depend on the survival of these fish. While the cost of conservation is significant, the incentive for the Secwepemc is the survival of their fishing rights. The cost of conservation is escalating, and the question about who pays for conservation is becoming more significant as each generation of salmon returns to Secwepemc country, one of the most important bedrooms of the Pacific fishery.

Recommendations for Policy Development

- *Wild salmon population reconstruction should be the foundation of stock recovery work,* rather than sustainable development planning based on the existing beleaguered Pacific salmon fishery;
- A move toward more selective and terminal fisheries is necessary to allow optimum productivity from all salmon stocks;
- Industry diversification and value-added fisheries should be encouraged to maximise the value of the fishery;

- *Community-based management of the salmon fisheries will be the cornerstone of future salmon recovery efforts,* thus expanding the number of contributing stocks for a stable salmon fishery in the future, and the benefits from wise use and management to more people;
- *An endowed trust fund is required to support conservation efforts* and to defray the costs to local communities of wild salmon rehabilitation efforts.



IBAMA's Role in Conservation of Fisheries Resources in Brazil: Aquaculture and Fisheries Management by Hydrographic Basin

Carlos Fischer [1]

Introduction

The Brazilian Institute of Environment and Natural Renewable Resources (IBAMA) acts in several ways that are relevant to the genetic conservation of fisheries. In this paper I present an overview of two of these areas: aquaculture and freshwater fisheries management.

Aquaculture

Aquaculture has been used as an important tool for the conservation of a great number of aquatic species throughout the world. IBAMA has sponsored several aquaculture projects aimed at developing culture technology of native aquatic species. These have included projects on the Brazilian oyster *Crassostrea brasiliana,* the marine shrimp *Penaeus brasiliensis, P. schimitti, P. subtilis,* and *P. pulensis,* the freshwater fish pacu *Piaractus mesopotamicus,* tambaqui *Colossoma macropomum,* piracanjuba *Brycon orbygnianus,* matrinxã *Brycon cephalus,* surubim *Pseudoplatystoma coruscans,* pirarucu *Arapaima gigas,* and

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others. Most of these projects have been carried out at the National Research Center of Tropical Fishes—CEPTA, located in Pirassununga, State of São Paulo. Five other aquaculture stations of IBAMA are currently also being linked to CEPTA to act as living "gene banks" of native fish species that are farmed in Brazil. The aims of these stations will be to develop improved strains and breeds in disease-free environments and act as a source of broodstock for commercial operations. In related activities, fry are also produced to restock environments that have been impoverished by overfishing.

Fisheries Regulation and Basin-oriented Fisheries Management

IBAMA, in collaboration with various other governmental agencies, research institutes, fisheries representatives, non-governmental organizations, and hydroelectric companies has recently launched a new initiative for continental fisheries management in Brazil (Fischer et al. 1992). Prior to 1989, no overall management model existed for continental fisheries in Brazil, with management taking the form of various isolated legislative acts responding to immediate local political or ecological problems. To correct ongoing problems resulting from a lack of an overall management scheme, the Department of Fisheries and Aquaculture (DEPAQ) of IBAMA launched a new hydrographic basin-based fisheries management plan in 1990, through its Division of Continental Fisheries (DIPEC). This management plan considers continental fisheries within an integrated context of the fishing industry, ecology, human culture, economy and society to permit the step-by-step development of specific legislation which will allow the organization and planning of long-term sustainable fisheries activities. The plan thus is a unified political action that provides within the scope of its legislation a broad range of options which can be used in developing specific management plans for each hydrographic basin.

Principal strategic directions of this new program are:

- Organize continental fisheries for sustainable development;
- Organize continental fishery activity using hydrographic basins as the basic management unit;
- Organize continental fishery activity to integrate with other environmental uses that have a direct or indirect impact on the fisheries;
- Organize continental fisheries so that stocks are not compromised over time;
- Support fisheries management that is based on sound scientific and technical data and procedures and involves the participation of all user groups;
- Support research projects that guarantee the development of knowledge necessary for fisheries management;
- Support the decentralization of fisheries management by developing partnerships between and within institutions and strengthening communication between the various fisheries user groups, users of other natural resources, and regulatory agencies;
- Support the organization of the various user groups of the fishery resources to allow the representation of communal interests of the group;

- Develop mechanisms to ensure that the negotiation forums between user groups are politically balanced.

Activities to be executed in each hydrographic basin to support these strategic directions are:

- Adapt current regulations to become compatible with technical management needs and to fit the different regional realities;
- Identify and support lines of priority research that support the management process;
- Develop the administrative tools which allow the zoning of fisheries activities;
- Participate in various forums on the use of water resources, basin management, etc., aiming to integrate fishery activities with other activities that use the environment, especially forestry, agriculture, and mining.

Operational Implementation of the Management Plan

Considering that the main strategy of the program is fisheries management by hydrographic basin, priorities and necessities are being developed separately for each of these. As such, the first step is to adapt current legislation to the local fisheries situation. Together with this process, other activities that are necessary to make this fisheries management viable include integration between and within regulatory agencies, decentralization of control, strengthening scientific and technical capacity, participation of the various user groups in decision making processes, etc.

In operational terms, the adaptation of legislation in each basin has taken the form of technical meetings between central IBAMA, its State superintendencies, and research institutions active in the area. As the first step, the current problems of the fisheries, their regulation and current conflicts are discussed, as well as means of addressing weaknesses of existing management policies. A technical proposal is developed by consensus out of these meetings for the regulation of fisheries in the particular basin. As the second step, this proposal is discussed with all parties involved in the system (technicians, researchers, and users of the resource), combining technical, scientific, and empirical local knowledge to make any necessary changes. To legitimize any decisions arising from these meetings and to make the process adequately transparent for public scrutiny, the participation of formal representatives of all the user groups is fundamental, including representatives of the Fishing Colonies, Fishing Associations, Non-Governmental Organizations, State Environmental organizations, etc.

Progress in Implementing the Plan

To date, results have been achieved in this program in the Amazon, Araguaia/ Tocantins, São Francisco, and Parana River basins. Consultative meetings have been held in all of these basins, and technical reports on the current status of fisheries management in each are in preparation (Anon 1998 a-d). In the Amazon and Araguaia/ Tocantins basin, governmental decrees have been issued by IBAMA to normalize fisheries regulations to regional realities. In addition, the State agencies of IBAMA in these areas have been given the authority to develop local fisheries regulations as dictated by local requirements. In the São Francisco basin, the discussions have developed into a more inclusive "Program for the Integrated Use of Natural Resources of the São Francisco River Basin" with fisheries resources as a focal point. This program aims to involve all stakeholders in its development, and is targeting the summary of information, research, and technical development on fish biology and ecology and cultural and socioeconomic aspects of fisheries within the basin. The objective of this program is to develop a sustainable fishery that is integrated and compatible with all other economic activities in the river basin.

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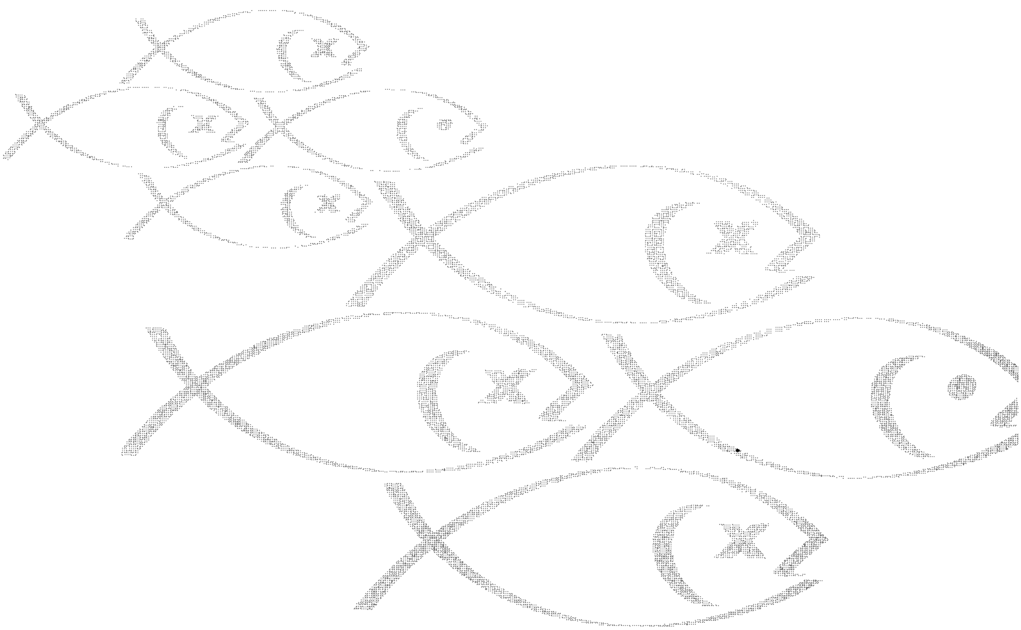

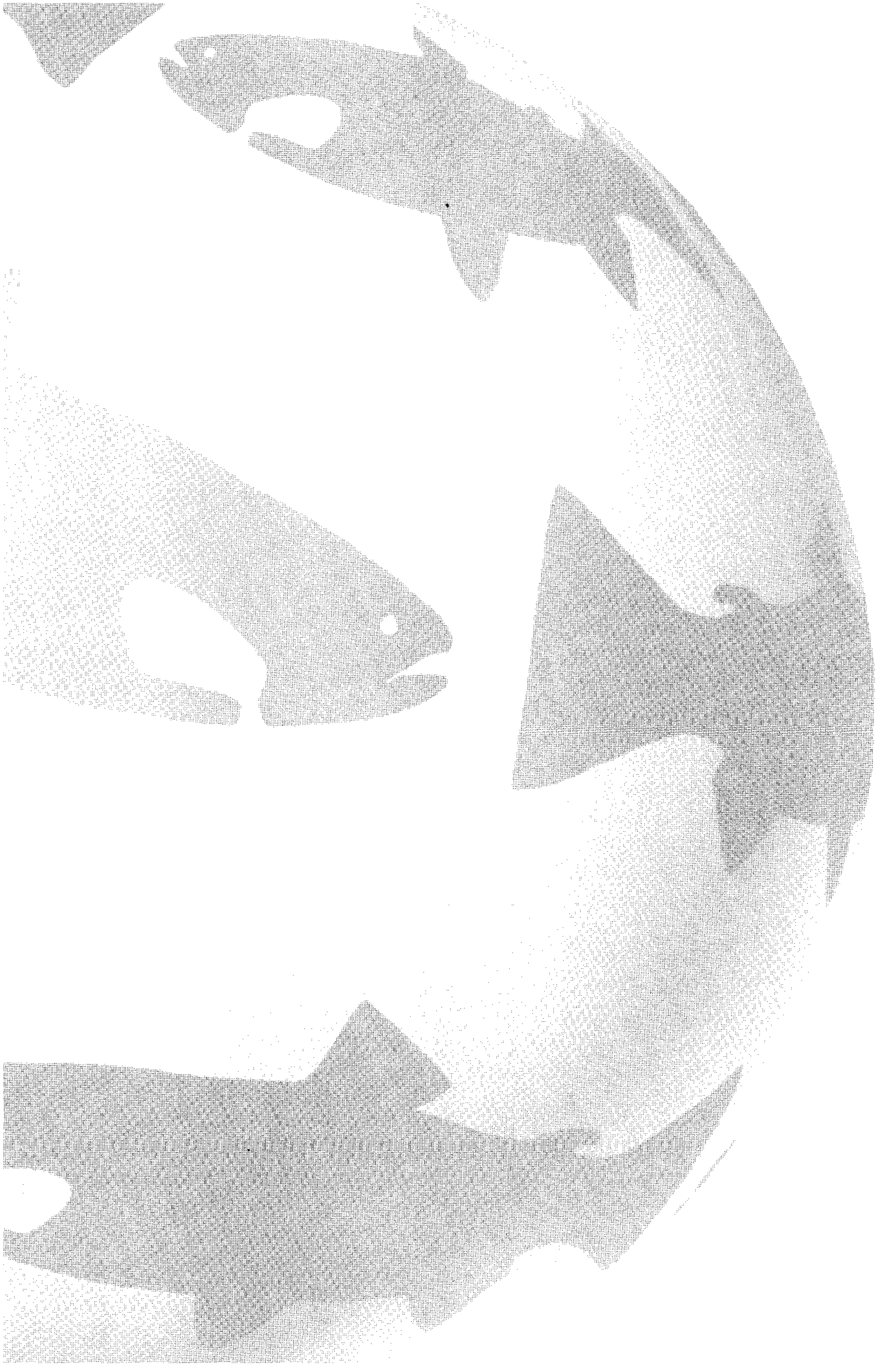

The Norwegian Gene Bank Program for Atlantic Salmon *(Salmo salar)*

Øyvind Walsø [1]

Introduction

The national gene bank for salmon was established by the Directorate for Nature Management in 1986 because of the difficult situation for Norwegian wild salmon. A survey of Norwegian rivers containing salmon as of January 1st, 1998 showed that 41 salmon stocks have become extinct and another 54 are severely threatened (Table 1). The main reasons are acid precipitation, hydroelectric development, the salmon parasite *Gyrodactylus salaris* and the spread of salmon escaping from fish farms. Characteristics with a genetic basis have been found in Norwegian salmon stocks and have been described as genetic adaptations to local environmental conditions. Interbreeding with escaped farmed fish can lead to a change in these adaptations. It is therefore important to secure the genetic material from the individual stocks.

Objectives and Strategies

The main purpose of the gene bank is to contribute to nation-wide preservation of the genetic diversity and characteristics of natural salmon stocks.

Milt bank

During the initial developmental phases, the gene bank was based exclusively on deep freezing milt. The goal was to preserve genetic material from more than 100 stocks and from at least 50 individuals from each stock. The resultant frozen milt is expected to be used exclusively for broodstock production because of limited volume of milt. Addi-

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Table 1

Categories of Norwegian stocks of Atlantic salmon *(Salmo salar)*

Category	Number of stocks	Causes of threats
Extinct	41	Acidic precipitation Water power development *Gyrodactylus salaris*
Threatened by extinction	54	*Gyrodactylus salaris* Acidic precipitation Over exploitation Farmed salmon
Vulnerable and reduced	147	*Gyrodactylus salaris* Acidic precipitation Water power development Pollution Fish diseases Farmed salmon Over exploitation
Small stocks (no human impact)	241	E.g. small coastal rivers
Large production	98	
Reintroduced	9	
Unknown	79	

tionally, some of the frozen milt will be stored for future use. Emphasis is put on collecting sperm from salmon stocks from different parts of the country as well as different kinds of environments. Stocks that are threatened by extinction, of special scientific value or valuable for fisheries are given priority. Salmon from a single river or from large tributaries are considered a single stock in this cotext.

The following sampling strategy has been employed by the milt bank project:

- Milt from at least 50 individuals from each stock is frozen. Since the sampling cannot be carried out on identifiable stocks, each river is considered a sampling unit. Big tributaries are sampled separately;
- Sampling is carried out for a period of at least three years in each river to reduce the chances of gross overrepresentation of a single year-class;
- Emphasis is placed on sampling from stocks representing a wide geographical and ecological range;
- Stocks which are threatened by extinction are given priority over other stocks;
- Stocks which are of particular scientific value or valuable for fishing purposes are also given priority.

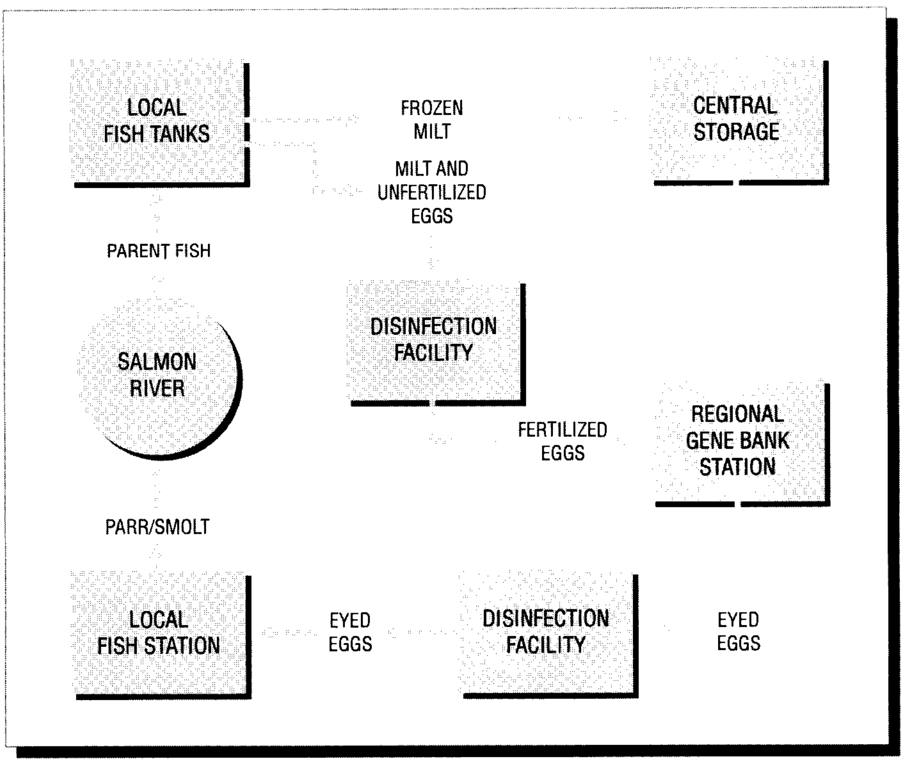
Living gene bank

In 1988 it was recommended to establish broodstock stations, so-called "living gene banks", for Atlantic salmon. The main purpose was to establish a living reservoir of genetic material which could be used for the re-establishment or enhancement of threatened stocks. The preservation of live fish in living gene banks is a measure used for the most seriously threatened salmon stocks. Stocks that are no longer capable of surviving in their river are saved until the danger is over. The stations facilitate the accumulation of genetic diversity through the use of frozen milt and the acquisition of several year-classes of parent fish from individual rivers. The stations supply eggs to local hatcheries that produce fish for release.

The gene bank strategy for Atlantic salmon in Norway is shown in Figure 1. Fish that are caught in the rivers are kept in a tank in the vicinity until they are sexually mature and can be milked. Deep-frozen sperm go to a central store, and fresh sperm and unfertilized eggs are taken to a gene bank centre. Eggs from each female fish are fertilized with sperm

Figure 1

Schematic presentation of the gene bank strategy



from one male fish, disinfected and placed in hatching cylinders. The eggs from every individual female are kept separate throughout the process.

After hatching, each family group is kept in separate tanks until the fish are sufficiently large (about 7 cm) to have their fins clipped. Families from the same stock are then put together in a tank. During the second year of their life, when they reach smolt size, about 30 individuals from each family group are given individual tags. For the next two years, each generation is kept isolated in separate rooms. This means that each year-class is kept isolated for four years, making it possible to combat any fish diseases which might break out in the facility and simplifying the job of monitoring the family groups, year-classes and stocks. After four years, the fish from the oldest year-classes are put together, but the various stocks are still kept apart.

Each of the captive stocks will spend two generations in the station. The production is based on the following guidelines aimed at retaining their genetic diversity:

- Maximum survival;
- Long generation time;
- Identification of family groups;
- Equal size of family groups;
- A minimum effective population size of 50 for each generation;
- Surplus fish-production for safety;
- Mating schemes including the use of frozen milt.

A great deal of effort goes into keeping the fish free of disease. The health of all the breeding fish is checked and strict routines have been put into place for how work with the fish will be carried out, for instance with regard to handling the fish and timing of transportation of eggs and sperm. All the eggs that are going to be placed in the gene bank are sterilized beforehand. Fresh water is used in the gene bank centres, because sea water is frequently infected with furunculosis bacteria and other organisms that cause diseases in fish. To minimize the risk of spreading fish diseases to the rivers, only disinfected eggs are exported to the hatcheries.

Organization of the Gene Bank Program

Authorities, organizations and the business sector are all actively involved in the gene bank for salmon. The Directorate for Nature Management is responsible for implementing nature management policy in Norway. It was established in 1985, and is responsible to the Ministry of Environment. Its authority derives from various acts and regulations. In addition to its statuary tasks, the Directorate is also responsible for identifying, preventing and resolving environmental problems through cooperation with other authorities and user groups and providing advice and information. The Directorate administers and has scientific responsibility for the gene bank work, which is funded over its operating budget.

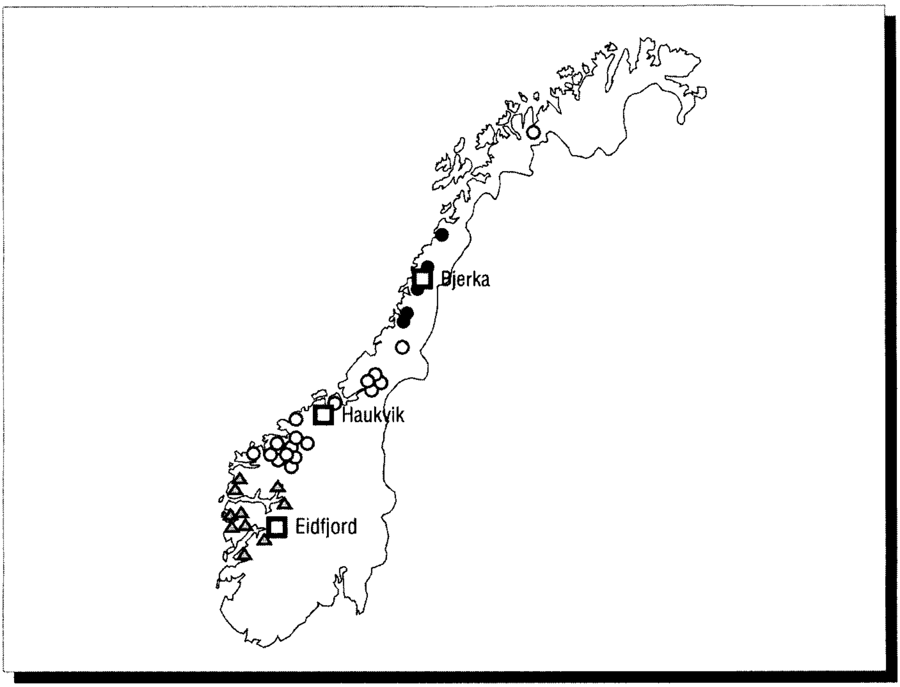
The State Veterinary Institute in Trondheim is responsible for the everyday running and practical implementation of the gene bank work on contract from the Directorate. It coordinates cooperation between the various participants. The milt bank material is stored at the breeding centre for Norwegian Red Cattle in Trondheim.

The building of a live gene bank for West Norway (Eidfjord) was a joint project between the Directorate, the Norwegian Farmers' Union, Eidfjord Borough Council and the State Power Systems. The gene bank for North Norway (Bjerka) is run by the State Power Systems, and that for Central Norway (Haukvik) is run by a private firm, Haukvik Kraftsmolt A/S, on contract from the Directorate.

The selection of watercourses to be represented in the live gene bank and the sperm bank is made in cooperation with the various county environmental departments, along with local fishery associations and bodies carrying out cultivation work on the rivers.

Figure 2

The three gene bank centres (squares) are preserving 33 stocks: 5 in Bjerka (closed circles), 18 in Haukvik (open circles) and 10 in Eidfjord (triangles)



Results and Experiences

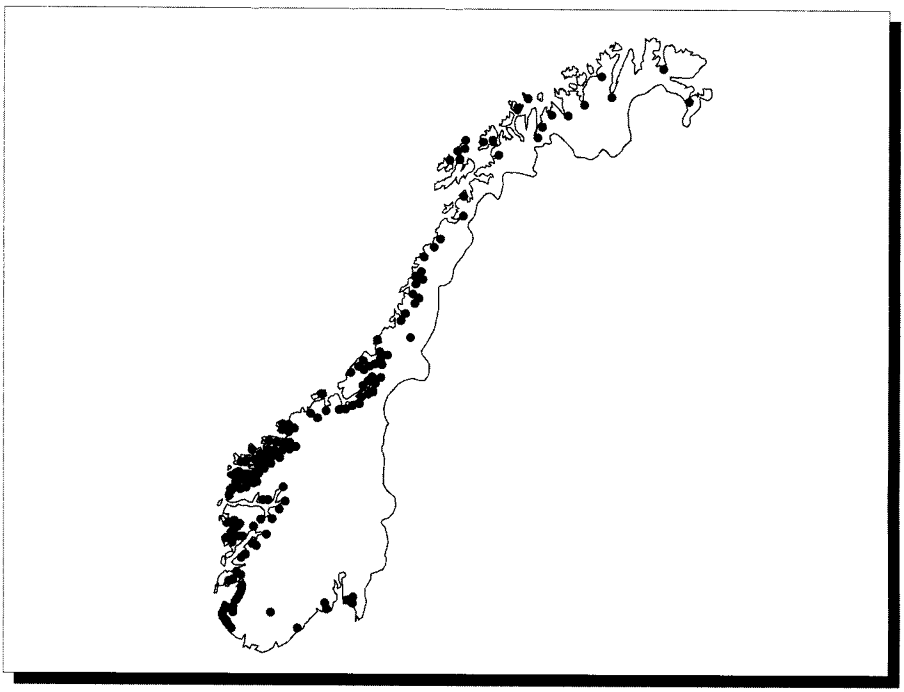
Status of the program

Gene bank centres for live fish have been established at Eidfjord in western Norway, Haukvik in the middle of Norway and Bjerka in northern Norway (Figure 2). The three gene bank centres are now preserving 33 stocks: 5 in Bjerka, 18 in Haukvik and 10 in Eidfjord.

Deep freezing of milt enables the preservation of genes for a virtually unlimited period. Two milliliters of sperm are taken from each fish, enough to fertilize about 1,000 eggs. The milt bank now contains material from 6,163 individuals from 155 salmon stocks (Figure 3). There is also material from four trout stocks. The aim is to collect sperm from at least 50 individuals from each stock. Collecting takes place over at least two years to reduce the chance of overrepresentation of a single year-class. The milt bank is at the breeding centre for Norwegian Red Cattle in Trondheim.

Figure 3

Locations of the 155 rivers included in the milt bank

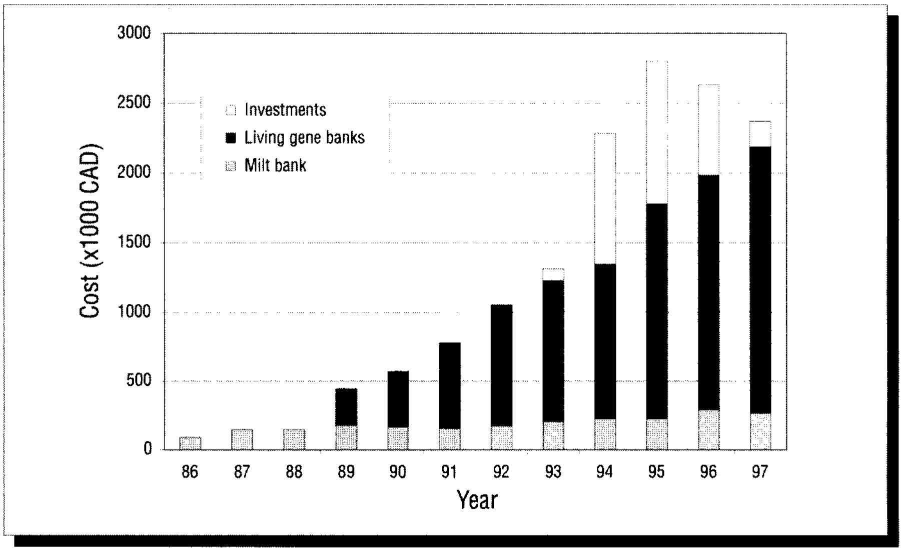


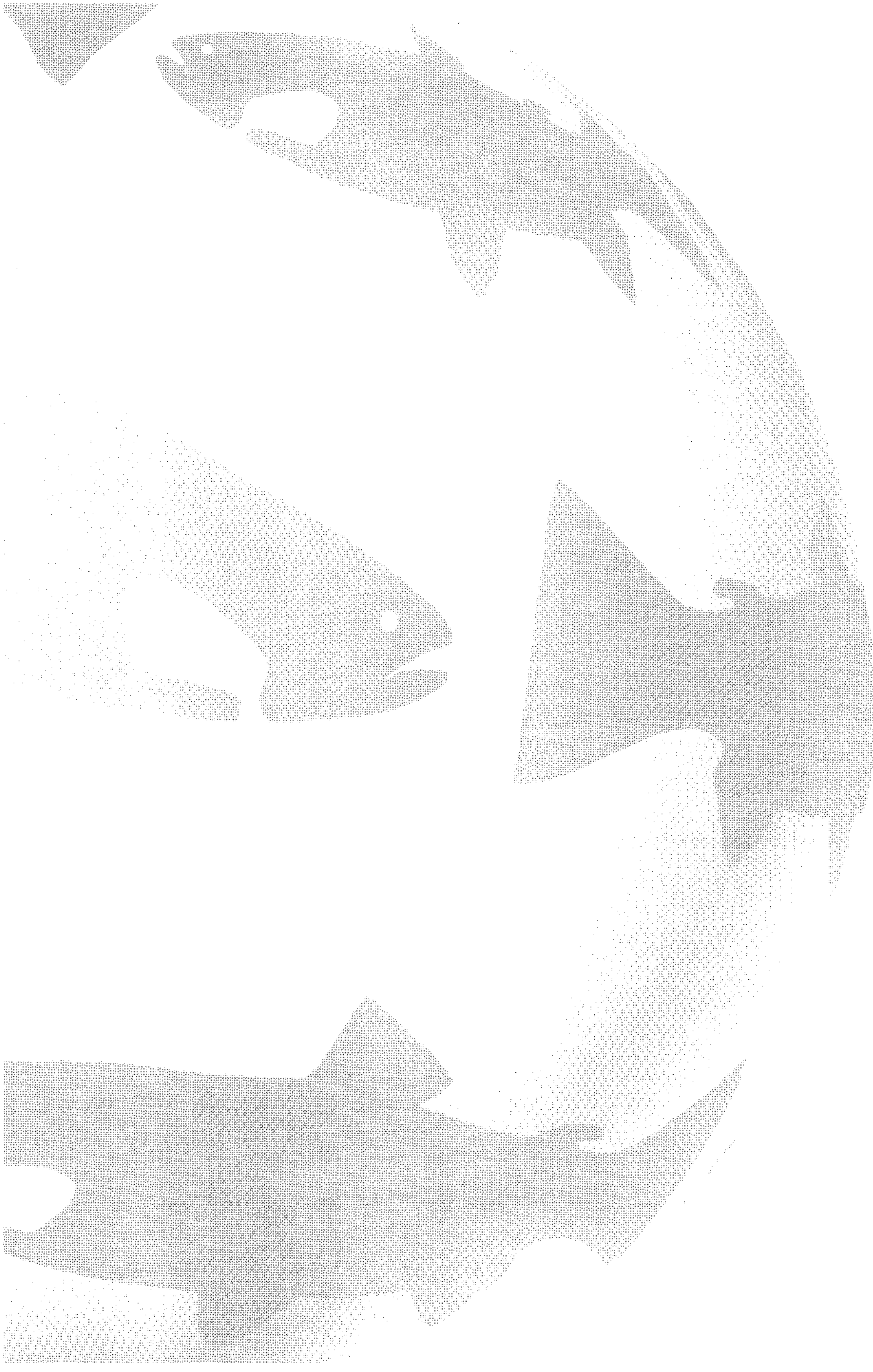
Economy

The total cost of running the gene bank program so far has been about $14.6 million CAD (Figure 4). Living gene banking, including the cost of building the centre in Eidfjord, has accounted for 83 per cent of the total cost throughout the period. With investments now complete, under the present strategy we expect the cost to be kept at today's level in the years ahead.

Figure 4

Costs connected to the investments and the everyday operation of the Norwegian Bank Program





Preservation Programs for Endangered Fish Stocks in Finland

Jorma Piironen [1]
Petri Heinimaa [2]

General Background

The commercially most important segment of Finland's fish stocks—the species and strains that spawn in flowing water—has been disturbed by human activities for about a century, and has suffered in particular from river damming for hydroelectric power stations. However, other forms of water level and stream flow regulation, including channelization, dredging, flood control and timber floating, have also been deleterious. Some 6,000 km of our waterways have been dredged and reconstructed for water transport purposes, while some 40,000 km have been modified for timber floating. There are about 150 hydroelectric power plants and about 1,000-1,500 other obstacles to migratory fish. These figures mean that very few waterways and their fish populations have remained untouched.

Migratory salmonids, especially Atlantic salmon, various races of brown trout and whitefish have suffered the greatest losses. Of 18 indigenous Baltic salmon *(Salmo salar)* stocks, only two naturally spawning populations, namely those in the Tornionjoki and Simojoki rivers, remain. Similarly, only five sea trout *(Salmo trutta trutta)* populations have survived out of an original total of 47. Similar trends have been detected among a number of freshwater fish stocks.

The accelerating disappearance of migratory fish following the hydroelectric development programs of the 1940s led both to obligatory stocking and to the introduction of breeding programs to conserve endangered fish. Here in Finland, the latter has been seen as a task for the State because of its importance to society as a whole and the long-term and

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extensive nature of the work involved. Current international commitments, including the Rio Convention and various European Community (EC) directives, have further emphasized the obligation of States to preserve the genetic diversity of original fish species and their stocks.

The Finnish Game and Fisheries Research Institute (FGFRI), a government-funded research organization having separate units for game and fish research, is also responsible for State aquaculture activities. The institute's Aquaculture Unit has 11 technically well-equipped fish farms and two field stations located all over the country to undertake the tasks required. The view taken by Finnish fisheries policy is that aquaculture is best practiced by the State so as to safeguard the continuing availability of the genetic material (eggs and fry originating from known differentiated local populations) needed to rear fish for stocking purposes.

The importance of building up brood stocks to ensure supplies of eggs and fry has been accentuated as wild populations dwindle. As the rate of reproduction of migratory species declines and fishing pressure on them increases, extensive stocking has become an essential requirement for the preservation of many species and populations. Since this is nowadays mainly based on eggs produced at the FGFRI's stations, the aquaculture programs maintained by the State also have an important role in replenishing stocks of migratory species and ensuring that they remain at levels that permit them to be fished.

This report summarizes the overall activities of State aquaculture in Finland and describes the main principles and programs aimed at maintaining endangered fish species and their stocks.

Goals and Operational Philosophy of State Aquaculture

Tasks

The present tasks and goals of the Game and Fisheries Research Institute have largely taken shape on the basis of several reports produced by committees and working groups appointed by the Ministry of Agriculture and Forestry and the Council of State (Government) in the 1970s and 1980s. The private sector's fear of competition was one of the main factors that caused a need for a division of labour between private-sector and public-sector aquaculture and stocking operations. The demarcation lines between the sections in which the State and the private sector operate are nowadays quite clear and undisputed. The preservation of endangered species and stocks and of their biodiversity, as well as the production of eggs from these and other species, is seen quite generally as a State task in Finland. That is because such activities do not harmonize well with commercial operations—e.g., the work has a social dimension, there are international obligations, special expertise is required, genetic purity is important, programs are long-term and interdisciplinary, and rewards are rarely immediate. The provision of highly trained personnel, engaging in international cooperation, breeding programs that require special premises and expensive equipment, and experimental and development programs have also been regarded as tasks best looked after by the State.

The principal goals of the State Aquaculture Unit have recently been defined as follows:

- to maintain original fish stocks and their biodiversity and to produce high-quality fish and crayfish eggs and juveniles both for the State's own needs and to serve as seed material for private aquaculture;
- to safeguard opportunities for fishing and the preservation of endangered stocks and populations by means of contract rearing;
- to practice breeding programs which produce high-quality material for use in aquaculture for purposes of food production;
- to develop aquaculture in collaboration with research bodies in the field.

To implement these goals, the State's Aquaculture Unit:

- maintains endangered and regressing fish stocks and their biodiversity by means of broodstock cultivation, stocking and milt banks;
- produces eggs and juveniles of fish and crayfish that are of high quality, disease-free and of known background to meet the requirements of conservation, research, State obligations and private demand;
- produces high-quality material for use in public and private breeding and cultivation operations by means of selective breeding research programs;
- supervises the State's contract breeding so as to ensure fishing opportunities and guarantee the preservation of endangered stocks;
- develops and markets better and more economical products, methods, equipment and consultancy services.

There has been a deliberate policy of State aquaculture programs to only include activities that are not commercially profitable and which for that reason would not interest the private sector. Of course, such activities are vitally important for ensuring that both aquaculture and fisheries management in general can continue to develop. A good example is the so-called M74 phenomenon, which caused high mortality in salmon fry hatched from eggs that had been gathered from the wild. If stocking programs had been based solely on these eggs, the populations would have collapsed, but the fact that eggs were also available from our Institute's breeding programs made it possible to maintain stocking at the level considered desirable. Thus salmon fishing has been able to continue in several rivers, including the Tornio-, Simo- and Iijoki, and stocks there have been maintained at a good level.

The scope and volume of the Institute's work are guided by the funding made available to it in the budget submitted to and approved by the Parliament each year and in the program of goals appended to the appropriation request. This program contains specific sub-goals for aquaculture and details the amount of funding requested for this purpose. In addition to this, the Ministry of Agriculture and Forestry specifies a set of targets for the Institute each year, including some specifically for aquaculture.

Goals with respect to use of products

There are three main goals in relation to the use of aquaculture products, services and expertise:

1. Preservation of fish stocks and their biodiversity
- preservation of endangered and specialized stocks in aquaculture conditions;
- revival of natural populations through stocking;
- preservation of genetic material in milt banks.

2. Promotion of aquaculture as a livelihood
- production of seed (eggs and fry) for various purposes of fish farming and research;
- production of breeding material for use in aquaculture;
- development of aquaculture products and technology.

3. Improvement of fishery conditions
- management of valuable fish stocks in the Baltic;
- maintaining the State's stocking obligations;
- development of stocking projects for fisheries.

Fish and crayfish eggs and juveniles are sold to private fish farms as seed material. In 1996, 33 per cent of the Institute's own production of juveniles was sold and 50 per cent (in numbers of individuals) used to preserve and revive endangered stocks. The most important objects were salmon and sea trout stockings in the Gulf of Bothnia and the stocking programs that the State is required to implement in the Lake Inari area. The remaining juveniles were used to replace breeding stocks, for stocking to compensate for eggs taken from natural populations and for studying the management of fisheries by means of catch studies.

The breakdown of expenditure by main sector of operation is a good reflection of the division of operations between the various sub-fields of aquaculture. In 1996 this expenditure (excluding contract juvenile-rearing) totaled 36.4 million marks (roughly US $7.2 million). Of this amount, 7 million marks (19 per cent) was spent on preserving the biodiversity of fish populations, 11 million (30 per cent) on improving conditions for fisheries and 18.4 million (51 per cent) on promoting aquaculture as a livelihood.

Preservation of Fish Stocks and Their Biodiversity

The primary goal is to preserve Finland's original fish species and their locally specified genetic populations, naturally reproducing in their natural habitats. Over the long term, the genetic representativeness of a species and variation within it is possible only in a natural habitat, where different environments and changes in them ensure that there is also genetic variation. Unfortunately, Finnish fish and their biodiversity have long been suffering either directly or indirectly from the effects of habitat change (e.g., construction projects, pollution, eutrophication, acidification), excessive and/or selective fishing and unplanned stocking (new or competing species, mixing of stocks). These factors, combined with diseases and, for example, the M74 phenomenon in salmon, still pose the greatest current threat to existing fish stocks.

Habitat alterations have been particularly harmful to the species that are most important economically, including river-spawning fish and many populations of Atlantic and landlocked salmon, sea and brown trout, anadromous whitefish and lampreys. Many of the populations that still remain have declined, and their natural reproduction has been jeopardized either as a result of dwindling numbers or because regulation and/or damming of waterways has made spawning impossible. Without measures to manage and assist these populations, many would not now exist.

These tasks are also very much in line with the goals enshrined in articles 9 (a), (b), (c) and (d) of the Rio Convention, which Finland ratified in 1994. Likewise in alignment with the Rio Convention are most of the key points in the Finnish national program of action to preserve biological diversity in the period 1997-2005. This provides for protection to be arranged for certain endangered species and populations by means of *ex situ* measures and the use of these species and populations for restorative stocking. It must additionally be safeguarded so that adequate selections of fish are available to ensure, through aquaculture, the preservation of populations that have declined and the availability of high-quality, disease-free eggs. Where these tasks are concerned, implementation has been entrusted to the Institute both in the national action program mentioned above and in the "Fish Protection Group Report" issued jointly by the Ministries of Agriculture and Forestry and of the Environment in 1996 and in the 1997 "Natural Resources Strategy of the Ministry of Agriculture and Forestry".

Of all the tasks entrusted to Aquaculture, the most important, most comprehensive and most obligating both nationally and internationally is that of ensuring that the original species and populations that have become endangered in Finland are kept, with the aid of aquaculture, as genetically diverse as possible. This includes keeping populations at stations (living gene banks), conducting measures to revive populations that have declined and transplanting them to new habitats, preserving genetic material in milt banks and producing high-quality eggs of original species and populations as well as juveniles, also for the use of private fish farms. The Institute's facilities were largely designed and built specifically for these purposes.

Preservation of endangered and specialized populations by means of aquaculture

All of Finland's economically most important fish stocks are preserved at our Institute's aquaculture stations. These include all of the remaining salmon *(Salmo salar)* populations (Torniojoki, Simojoki, Iijoki and Tenojoki), the Saimaa lake salmon *(Salmo salar m. sebago)* and char *(Salvelinus alpinus)*, 23 sea and brown trout populations (including the three morphs: *Salmo trutta m. trutta, S. trutta m. lacustris and S. trutta f. fario)*, 15 whitefish *(Coregonus sp.)* populations, 9 grayling *(Thymallus thymallus)* populations, the asp *(Aspius aspius)* and several other populations. All in all, 64 different populations representing 13 original fish species and morphs in addition to the crayfish *(Astacus astacus)* are preserved. In addition to indigenous species, several introduced ones are likewise included in the aquaculture program. Among them are the lake trout *(Salvelinus namaycush)*, the carp *(Cyprinus carpio)*, the rainbow trout *(Oncorhynchus mykiss)*, the peled whitefish *(Coregonus* *peled)*, the brook trout *(Salvelinus fontinalis)*, the Swedish Hornava char *(Salvelinus alpinus)* and the signal crayfish *(Pacifastacus leniusculus)*, 11 stocks in all, one of which, the River Neva salmon *(Salmo salar)*, is disappearing from its original habitat.

Indicative of the comprehensiveness of the Institute's programs is the fact that all of the species and morphs listed as very endangered by a committee and a working group in 1991 and 1996—Atlantic and landlocked salmon, sea and brown trout, plankton-eating whitefish and asp as well as the Baltic salmon and the Saimaa char—are now maintained at aquaculture stations. Numerous locally differentiated stocks of these fish are likewise kept. At the moment, we are not aware of a need to include new commercial fish species or populations in our programs. With respect to certain trout populations, breeding needs are currently being studied.

Non-commercial fish are not being bred, but if ongoing studies (including one relating to *Abramis vimba*) indicate a need for this, the Institute has the capacity to expand the scope of its programs. The general expectation is that a growing emphasis on environmental values, fish diseases, the Rio Convention and EC regulations will increase demands for the preservation of fish and their biodiversity as well as for supportive stocking.

Maintaining fish by means of aquaculture is not an end in itself. If the level of protection called for in the EC directive—i.e., the species has an independent capacity to remain viable over the long term—can be achieved, artificial culture of this species could be discontinued. However, despite increased management measures like regulation of fishing and habitat rehabilitation, the EC protection level has not been achieved for any of the species or stocks in the aquaculture programs.

The brood fish at our Institute's stations totaled about 109,000 at the end of 1996 and had a biomass of approximately 111 metric tons. In the case of several stocks, the number of brood fish is greater than the preservation of biodiversity would actually require, because it has been determined on the basis of the amount of eggs and juveniles that need to be produced for purposes of sale or stocking. Using farmed fish as a source of eggs ensures greater dependability of supply than taking eggs from wild fish. A reduction in the difficulties arising from fishing selection are among its other benefits.

The brood fish stocks at the stations are renewed using eggs stripped from wild fish. In the cases of some stocks in harnessed rivers and of other rare natural populations, however, this is not always possible (the River Iijoki salmon, the Saimaa char and some trout populations are examples of this). As needs and opportunities dictate, juveniles caught in the wild and transferred to stations are also used to rear new brood fish. This is done in an effort to preserve biodiversity and prevent artificial selection. As a guide to this activity, the backgrounds of the fish have been explored and their genetic diversity studied using enzyme electrophoresis and other methods. Continuous monitoring of the state of the species and stocks maintained at the stations and genetic comparison with wild fish have begun in collaboration with researchers. The aim is to achieve breeding stocks in which all species and populations have genetic make-ups as close as possible to what is found in those fish remaining in the wild. The need to renew brood stocks so as to maintain biodiversity in populations is currently being studied in collaboration with the Research Units.

To increase the role played by natural selection in the cases of populations that have already disappeared from the wild, stocking programs have been initiated to relocate, for example, the Iijoki salmon in the nearby, unharnessed River Kiiminkijoki and the Saimaa lake salmon in the River Ivalojoki. The aim is to subject also the juvenile stage to natural selection and thereby ensure that the stock remains viable, but also to make it possible to obtain eggs from fish returning to the river. The best way to ensure the greatest possible degree of biodiversity in stocks is to use eggs and juveniles of fish both at breeding stations and in the wild as well as captured wild juveniles to breed new generations of fish and eventually using also frozen milt taken from a large number of males.

The fish stocks maintained at our Institute's stations represent a unique living gene bank even in a European context. Finland is undeniably in the forefront of nations that use aquaculture to preserve fish.

Reviving natural populations by means of stocking

To revive fish populations that have declined and help restore them to their natural habitats, our Institute has long been releasing salmon, sea trout, lake salmon, Saimaa char and anadromous and planktivorous whitefish juveniles of various ages into natural water bodies, including abandoned spawning areas. The most extensive stocking operations have been carried out to revive reduced populations of salmon in the Tornionjoki and Simojoki, the only unharnessed rivers that are home to this species. These operations are carried out on the basis of the annual targets set by the Ministry of Agriculture and Forestry. The juveniles used are bred both at our Institute's own stations and at private ones under contract.

In 1997, in accordance with the Salmon Action Plan adopted by the International Baltic Fisheries Commission in 1996, our Institute began an extensive resettlement program by releasing 106,000 migratory and 99,000 river juveniles of the Tornionjoki salmon into the Pyhäjoki. The intention is to establish this stock in a river flowing into the Gulf of Bothnia and help salmon populations to revive with the aid of natural reproduction. This work follows a stocking and resettlement plan for salmon, sea trout and migratory whitefish recently completed by researchers.

In the cases of many populations, stocking with farmed juveniles will be a permanent task, because harnessing and other use of the water bodies in question have extensively destroyed natural opportunities for reproduction, especially for anadromous fish. In addition to that, hydroelectric dams prevent anadromous fish from reaching any spawning beds that may remain intact upstream. Overfishing is likewise a threat to many of our populations, in addition to which it detracts from the effectiveness of stocking programs. Without stocking, many of our original fish populations, such as the Saimaa lake salmon and char, the Iijoki and Simojoki salmon and the Tornionjoki and Lestijoki sea trout would have already disappeared and many other populations would be on the brink of extinction.

Preserving populations in milt banks

Fish genetic material can also be preserved in deep-frozen form in milt banks. Our Institute has preserved the milt of the River Tenojoki salmon, the Lake Saimaa salmon and char, three populations of brown trout, two populations of planktivorous whitefish and seven populations of anadromous whitefish in liquid nitrogen. Freezing techniques suitable for different species are under development and the intention is to have stores of milt of all endangered species and populations in banks within a few years. In collaboration with Norwegian authorities, the River Tenojoki milt bank is being expanded. The use of frozen milt will also enable the genetic diversity of fish at our Institute's stations to be broadened.

Milt banking of the River Tenojoki salmon

The Tenojoki River is unique, at least in Europe, for having such a wealthy and productive wild Atlantic salmon population and for being unregulated. The Tenojoki or Tana River is a border river between Norway and Finland for over 238 km. Below Nuorgam, the Tana River runs about 77 km through Norway before flowing into the Tana fjord in the Arctic Ocean. The drainage area of the river is 15,690 km^2, of which 5,029 km^2 is located on the Finnish side.

The great importance of this salmon stock and possible present threats including the spreading of *Gyrodactylus salaris*, a lethal salmon parasite, and other serious fish diseases as well as possible genetic introgression through the escapees from net pen cultured salmon stocks were the main motives for starting the gene banking project for this salmon population. In 1989, the Norwegian and Finnish governments ratified an agreement for protection of the salmon and other fish and their biodiversity in the Tana River. The biodiversity of salmon in the Tana River is very high as they inhabit the main river and about 30 tributaries with a total length of about 1,000 km. Genetic analyses support the existence of several locally specified sub-populations (e.g., Elo et al. 1994; Elo et al. 1995).

The milt banking project was started in Finland in 1993. The plans were drawn on the basis of the genetic information as well as the structuring of the Tana River. The original goal was to collect sperm from a total of 1,450 males originating from the main stream or certain tributaries. The task was subdivided further for multi-seawinter males (MSW), grilse (one-seawinter males) and precocious parrs. The minimum number of individuals per location was set at 50. The specific goals and the actual situation are described in Table 1.

Table 1

Goals for collecting milt and the number of collected samples from Tana River salmon, 1993-1997

Location	Precocious parr		Grilse (1-SW)		MSW-males		Total	
	Goal	Collected	Goal	Collected	Goal	Collected	Goal	Collected
Main stream	50	**74**	50	**76**	100	**49**	200	**199**
Main tributaries (9)	450	**221**	500	**5**	100 (2 rivers)	-	1050	**226**
Minor tributaries (4)	200	**19**	-		-		200	**19**
Total	700	**314**	550	**81**	200	**49**	1450	**444**

Close collaboration at the practical level was begun with Norwegian authorities in autumn 1997 to exchange the collected material, experience and information. The purpose is to add milt from 300 males from tributaries on the Norwegian side to the Finnish milt bank during a three-year period.

Milt from all males is frozen in two straws, which will be stored in separate containers in order to minimize risks during the storage. One part of milt is added to three parts of the extender of Lahnsteiner et al. (1995) and the mixture is frozen for 5 minutes in the vapour about 1-1.5 cm above the level of liquid nitrogen. Large 2.5 ml straws (0.6 ml of milt) are used for grilse and MSW-males and 0.125 ml straws for precocious males. All the fish collected from the river are sampled for several purposes, including veterinary examinations for bacterial and viral diseases as well as for genetic, physiological and other research.

In addition to the milt freezing, a small number of eggs from wild female salmon have been fertilized to establish living gene banks which are maintained at Inari Aquaculture, about 200 km south of the Tana River. These stocks consist of the progeny from three-year classes at the moment.

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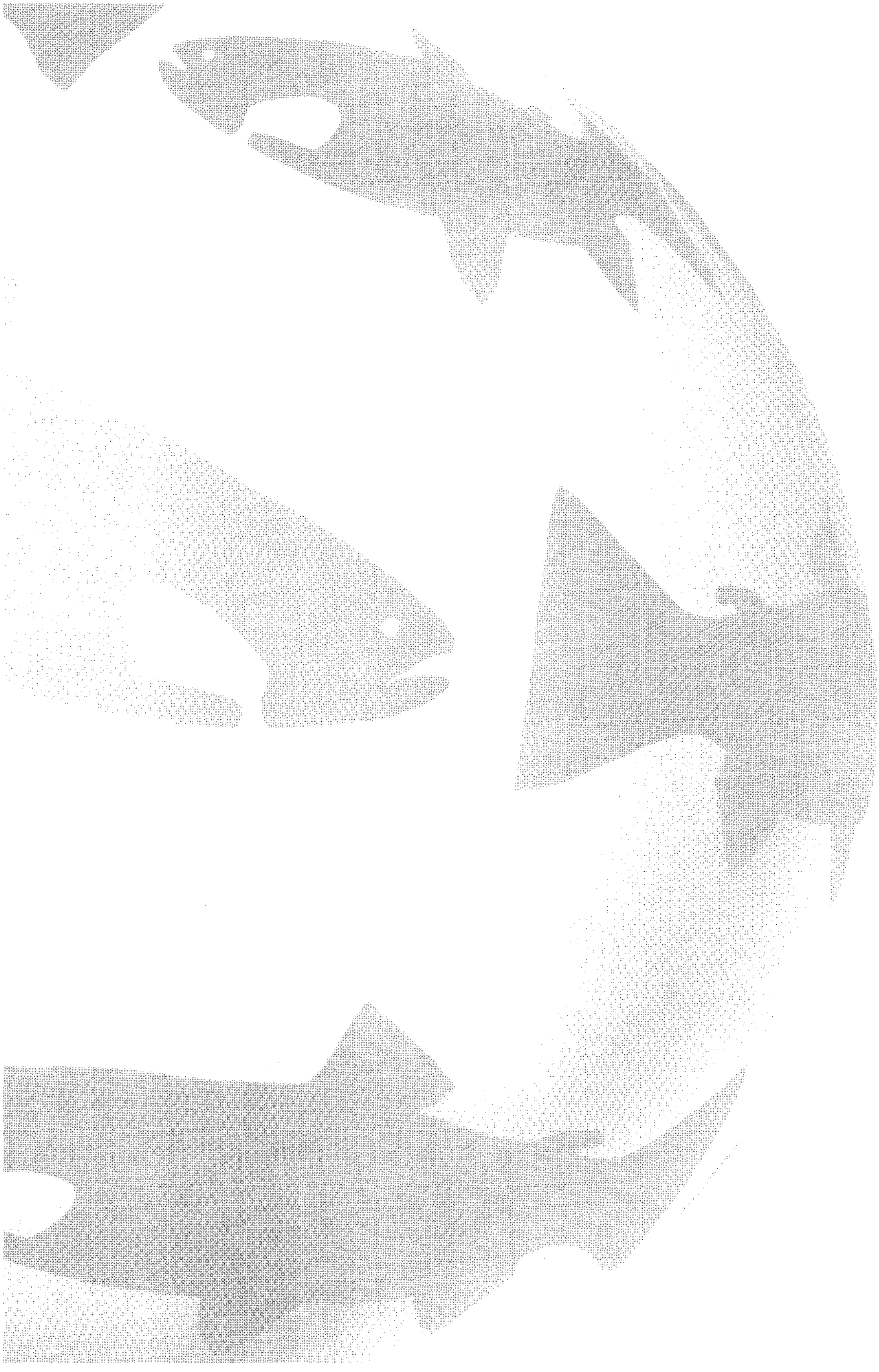

The Status of Icelandic Salmonid Resources, With Special Reference to Genetic Conservation Policy

Árni Ísaksson [1]

Introduction

Iceland is located on the mid-Atlantic ridge between 63° and 66° N and 13° and 24° W with an overall area of 103,000 km^2 (Thorarinsson 1986). It is almost exclusively composed of volcanic rocks, primarily basalts, and volcanic activity is common. Geothermal resources are abundant in certain parts and most Icelandic houses are heated with thermal water.

The Icelandic climate is oceanic with relatively mild winters and cool summers. The climate is very much affected by the warm Gulf stream, which bathes its southern and western shores and gets partly deflected to the North coast. The North coast, on the other hand, is affected by the cold East Greenland polar current, which greatly influences the climate and marine as well as freshwater resources in that area.

Rivers and lakes

There are about 250 large and small rivers in Iceland, and they have been classified according to origin into glacial, direct run-off and spring fed rivers. The productivity of rivers varies greatly according to their location, water source, volume of flow, topography and temperature, especially during the summer months. Only 80 rivers produce Atlantic salmon in any quantity, but minor runs of salmon are found in many sea trout and char rivers.

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There are about 1,800 lakes in Iceland with a total surface area of 1200 km^2, slightly more than 1 per cent of the country's surface (Gudjonsson 1986). Lake productivity also varies greatly and is influenced by many factors, including geology, water temperature, depth and altitude. Due to the cool, temperate climate and short summers, the growing season for fish in both rivers and lakes is short.

Freshwater species

Five species of freshwater fish are native to Iceland, including Atlantic salmon *(Salmo salar)*, brown trout *(Salmo trutta)*, Arctic char *(Salvelinus alpinus)*, European eel *(Anguilla anguilla)* and stickleback *(Gasterosteus aculeatus)*. Only the salmonid species are of economic importance in net and sport fisheries. Additionally, rainbow trout *(Oncorhynchus mykiss)* were imported to Iceland in the 1950s for fish farming purposes. They also have some importance in put-and-take fisheries in some areas, but have not been proven to propagate to any extent naturally.

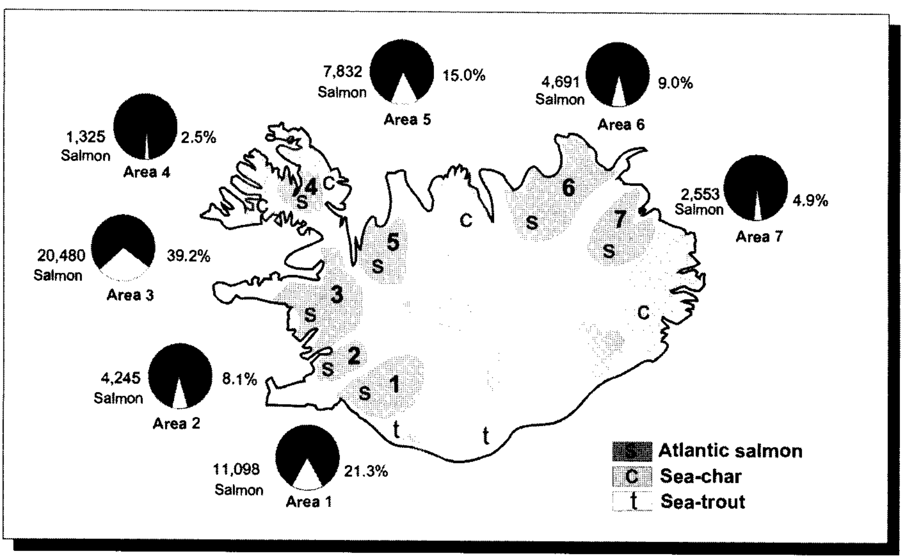
Status of the Salmon Resource

Management and development

Salmon are of greatest economic value with respect to sports fishing. They ascend about 80 rivers, most of them located in the western half of the country (Figure 1). Other

Figure 1

The average yearly catch of wild Atlantic salmon 1974-1993 and the proportion caught in each of seven districts in Iceland. Also shown are the main sea-char and sea-trout areas in Iceland



major sports fishing areas are located in lowland areas of the northwestern and northeastern coasts as well as the productive agricultural area of the south coast.

The best salmon rivers originate in lakes, which ensure stable water flow and favourable temperature during the summer. A river suitable for salmon usually maintains a water temperature of 10°C for a period of three months during the summer. Salmon are thus primarily found in lake-fed and run-off rivers, but some glacial streams have harboured salmon, where they were traditionally harvested with set nets.

Management

The management system in Iceland is based on the fact that fishing rights in rivers and lakes are privately owned and go with the land that adjoins the river. They cannot be separated from the ownership of the land. Fishable sections of rivers are usually in agricultural areas, where the land is most often owned by the farmers themselves.

River owners are obliged by law to form a fishing association, which makes all major decisions regarding fisheries and enhancement. These associations are responsible for management of the local fishery under the Freshwater Fisheries Act, dating back to 1932, but they often also attend to local conservation, hire bailiffs, build and run fishing lodges, build fish ladders and undertake various enhancement activities in order to improve fishing.

Harvesting methods

Icelandic salmon were traditionally caught in fixed set nets, but the importance of sport fishing has been increasing since early in this century. In the 1990s the sports catch has been close to 85 per cent of the river harvest. This change reflects both the increased value of the sports fishery with increased demand and the lowered price of commercially caught salmon as a result of the great supply of salmon from aquaculture. In some glacial rivers this has led to leasing of nets by the owners of upriver tributaries.

There is great enthusiasm for salmon angling among Icelanders, and a number of foreign anglers visit Iceland each summer. The fishing season starts in early June and extends through September, but each stream can only be fished for 3.5 months.

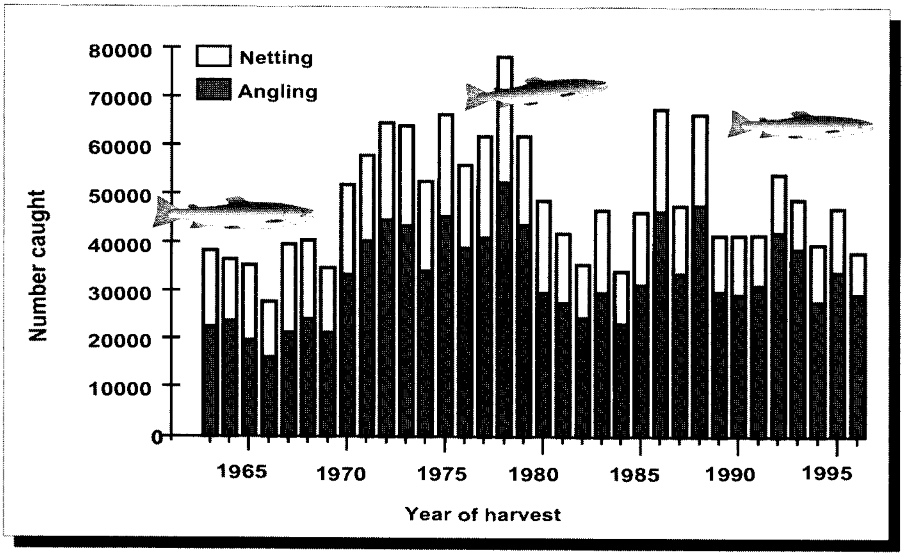
Salmon catch statistics

The collection of official salmon catch statistics started in 1897. Since then their accuracy has been increasing, and Icelandic angling statistics are today among the most comprehensive in the world, documenting size and sex of each fish as well as bait and approximate location of capture.

The total in-river rod and net catch of salmon from 1959 to 1997 is shown in Figure 2. In order to reflect changes in wild salmon catches, it excludes some catches in legal coastal nets, which primarily catch salmon from West coast ranching facilities. These catch figures demonstrate fluctuations in abundance between years, although a general increase has been recorded, especially in the 1970s. Fluctuations tend to be much greater on the North and East coasts, which represent only a small part of the total catch. Low periods in the North coast rivers occurred in the 1965-69 period as well as in the early and late 1980s,

Figure 2

Icelandic salmon catches 1963-1996 broken down by method of exploitation



which corresponded to cold environmental conditions and low productivity in the arctic water off northern Iceland.

Enhancement

As Icelandic rivers are privately owned, the cost of enhancement is entirely financed by the owners of the resource—in this case the owners of the adjoining land. The State acts mainly in an advisory capacity but provides some financial assistance through the Salmonid Enhancement Fund, which is financed through a small fixed percentage of the net profit of Fisheries Associations and the sale of electricity by hydroelectric companies.

Salmon enhancement has been carried out both by indirect methods such as river improvement and by direct methods such as artificial propagation. The indirect methods have primarily included construction of fish passes over waterfalls, improvement and creation of fishing pools and river-flow regulation. During the last 40 years about 50 fish passes have been constructed, opening up about 500 km of river bed to anadromous fish, primarily salmon (Gudjonsson 1988).

Enhancement with smolts is of course based on the same principles as salmon ranching from sea ranching stations. This principle has been applied in one large river, the Rangá, which does not produce salmon naturally, for the sole purpose of providing salmon for angling. This principle has been called "ranching for the rod", meaning that all the returning salmon will be harvested through a sports fishery.

Since 1989 releases into this river have ranged from 50,000-100,000 smolts, and returns have in some instances been highly successful. For example, in 1990 the Rangá had a total catch of 1,600 salmon, the highest sports catch in Iceland that year. In 1994 the same river was one of the top three rivers and in 1997 it had by far the highest catch, showing the potential of this method of enhancement.

Sea ranching

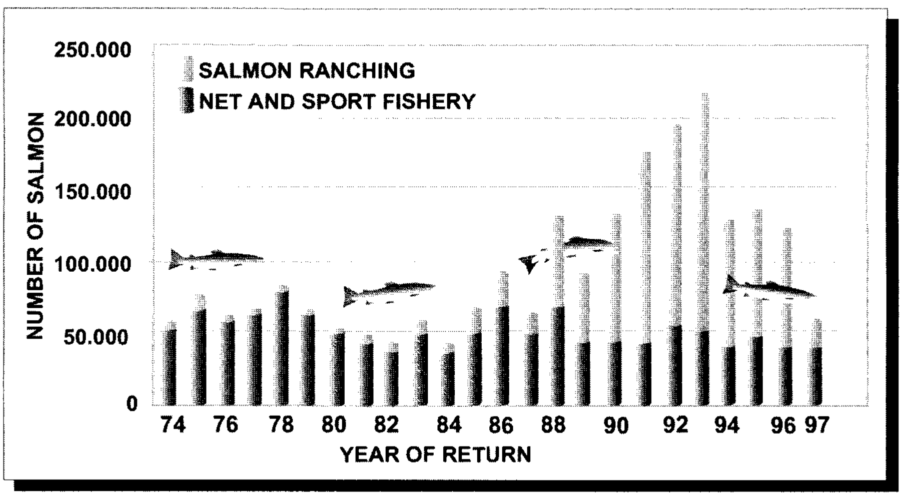
In the early 1960s the Institute of Freshwater Fisheries established Kollafjörõur Experimental Fish Farm, which was instrumental in promoting fish cultural activity and started experimenting with salmon ranching in 1965 (Gudjonsson 1973). This activity and the fact that harvesting Atlantic salmon in the sea has been prohibited by law since the 1930s laid the foundation for private ranching in Iceland.

Although experimental releases have been performed since the mid 1960s, commercial ranching only started in the mid 1980s and peaked at six million smolts released in 1991. This activity was mostly confined to Iceland's west coast. The proportion of ranched salmon in the Icelandic salmon catch subsequently increased from less than 20 per cent in 1980 to more than 80 per cent in the early 1990s (Figure 3). Due to the closure of the largest facilities, the proportion of ranched salmon has greatly declined in recent years.

At the present time private ranching of Atlantic salmon is not a profitable venture, and the large-scale ranching stations operating in Iceland in the late 1980s and early 1990s have closed down. Most of the stations were in fact long-term developmental ventures as there was no previous experience of scaling up the releases in a single ranching station from a few hundred thousand smolts to releases in excess of three million smolts.

Figure 3

Contribution of salmon ranching to the total Icelandic salmon catch during the last 24 years



Salmon farming

Although Iceland has ample ground water resources and thus relatively favourable conditions to produce smolts, it does not have a suitable environment for net-pen rearing of salmon in the sea. Sheltered areas are limited, winter storms frequent and undercooling of seawater common in some areas. Many net-pens started operation on the Southwest coast of Iceland in mid 1980s, but all have either gone bankrupt or stopped operation.

Many land-based farms were built in the 1980s, primarily in the Southwest, where many stations harness thermal energy to enhance growth (Ísaksson 1991). A number of these farms have gone bankrupt, but the remaining operations have had a considerable increase in turnover as a result of great progress in controlling diseases through vaccinations and improved rearing conditions. Most of these farms are using Norwegian rearing stocks, which have proven to be more favourable for rearing than the local rearing stocks, which tend to mature too early. In spite of many obstacles, salmon farming in land-based units will probably play a significant part in the Icelandic economy in the future.

Environmental Concerns

In general one can say that Icelandic salmon stocks are in a good state. This has been confirmed through electrophoretic studies (Daníelsdóttir et al. 1997) and through spawner recruit analysis. Salmon stocks mostly escape mixed stock fisheries due to the ban on fishing for salmon in the sea, in force since 1932. Other environmental threats are of minor importance, with the possible exception of disease and genetic effects from ranching and rearing activity.

Pollution

Pollution has not affected major salmon rivers, except for some municipal sewage flushed into some large rivers on the south coast. The extent of this pollution does not seem to be detrimental to salmon and trout populations, which mostly spawn in upstream tributaries. Acid rain has not affected Icelandic rivers, probably due to the nature of the climate and the great distance from continents with significant industrial pollution.

Threats from farming and ranching

The greatest concern regarding wild freshwater species in Iceland has been the increase in salmon farming and ranching in recent years. Every effort, however, has been made to safeguard wild salmon stocks. Many biologists fear that continuous straying of reared and ranched fish into rivers may be detrimental to wild stocks, which have adapted to a specific environment for thousands of years. Ranched and reared salmon, in contrast, have adapted to the rearing environment at least through a part of the life cycle and might thus be unsuited for life in the wild.

Icelandic enhancement and ranching operations have yielded a great deal of practical information on the straying of ranched salmon into rivers and between ranching stations as well as the straying of wild fish into ranching stations and between rivers. Some of the findings are summarized in the following sections.

Straying of salmon

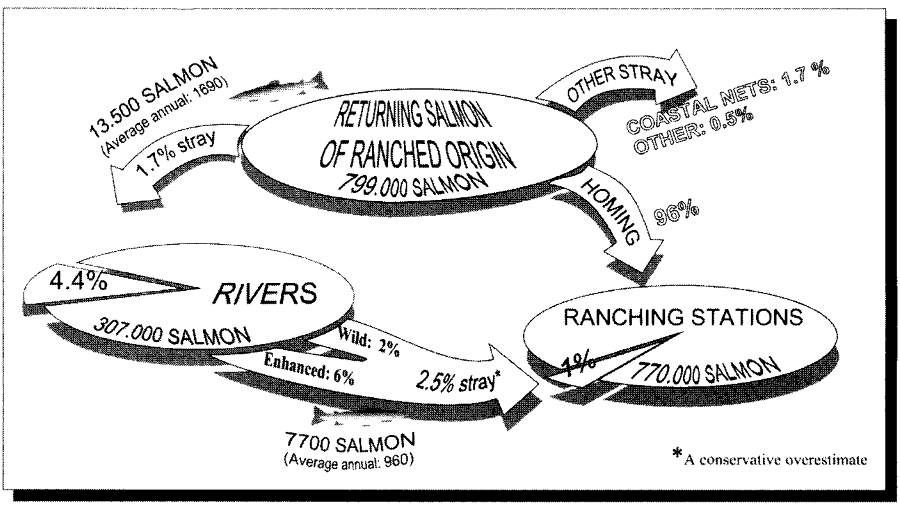
Escape and straying of salmon into nearby rivers from sea-water pens was fairly common in southwestern Iceland between 1987 and 1991 due to a fairly substantial production of salmon in sea-cages (Gudjonsson 1991). The strays were most pronounced in rivers in the vicinity of Reykjavík, the capital city. This problem is currently of minor concern, as all of the cage farms have closed or gone bankrupt.

There is considerable information available in Iceland on the straying of ranched salmon, both to other ranching stations and into rivers (Ísaksson et al. 1997). Salmon of wild and enhanced origin have also been observed at ranching stations, compounding the problem.

Figure 4 summarizes reciprocal straying between rivers and ranching stations on the West coast, showing the estimated number and proportions of salmon straying and the approximate corresponding component in river stocks and ranching station catches during an eight-year period (Ísaksson et al. 1997). The figure shows that twice as many salmon are straying from ranching stations into rivers as are straying from rivers into ranching stations. The strays from rivers into ranching stations are probably overestimated and strays of ranched salmon into rivers underestimated due to non-reporting of tags in some rivers. According to these figures, wild and enhanced components in the harvests at ranching sta-

Figure 4

Overview of reciprocal straying into ranching stations and rivers on Iceland's west coast 1988-1995 expressed as numbers and percent. Also shown is the ranched component in rivers and wild/enhanced component in ranching stations
(From Isaksson et al. 1997)



tions have been close to 1 per cent but the ranched component in rivers has been close to 4.4 per cent.

The occurrence of wild salmon at ranching stations has been a major concern as there is reason to suspect that this phenomenon is related to harvest strategy as well as the location of the ranching station in relation to major salmon rivers. Estuarine traps might be catching more strays from wild salmon rivers than would occur in a freshwater trap, and a ranching station located in the migration path of wild salmon would catch more wild salmon than a station located at a bottom of a long fjord. Ranching experience, on the other hand, has shown that ranched salmon are reluctant to enter freshwater except during freshets, which can be an infrequent occurrence. The resulting salmon are coloured due to maturation and practically unfit for export markets. Such delays often result in greater numbers of strays from ranching sites to neighbouring rivers.

Estuarine traps are thus of great importance for the salmon rancher. They procure a steady supply of bright salmon throughout the season and have the added benefit of reducing late-summer straying to salmon rivers, preventing genetic effects (Ísaksson 1994). A negative effect is the catch of some strays from wild salmon populations in the ranching stations, which has caused considerable controversy between ranchers and river owners in some areas.

Genetic concerns

Danielsdóttir et al. (1997) concluded that genetic distance between wild Icelandic salmon populations within the same region was lower than that between populations in different regions. They also concluded that genetic mixing of ranched stocks with wild populations should be prevented to avoid breakdown of stock integrity.

Ísaksson (1992) reviewed genetic resource management related to salmon ranching in various parts of the world with the intent of formulating such a policy for Iceland. He found no clear guidelines to follow on this issue, but many countries have adopted conservative policies in accordance with the precautionary principle. He stressed that ranched salmon should be harvested in a terminal fishery and local stocks should be used to improve homing and reduce straying. A workshop on biological interactions of natural and enhanced stocks of salmon in Alaska concluded that it was of utmost importance to maintain genetic diversity within and between natural populations of salmon to sustain productivity of both wild and enhanced stocks (Thomas and Mathisen 1993). Ecological concerns are linked closely to genetic issues.

Put-and-take fishery for Atlantic salmon

Put-and-take fisheries for trout are very common in many parts of the world, both by planting catchable fish into lakes or streams on a large scale as an enhancement measure or through sale of sports fishing licences into smaller fish farming ponds. In Iceland this practice has mostly been operating in some fish farms and in a few small rivers containing little or no natural populations.

It is well known that salmon caught in angling are 10-15 times more valuable than ranched salmon sold on the market. It was thus soon apparent that release of ranched salmon into under-utilized rivers could create considerable income and benefits for the local farmers through sale of fishing licences and accommodation.

The first put-and-take fishery for ranched salmon was operated by the Ármenn angling-club in the Kálfá River, a tributary of the Þjórsá river on Iceland's south coast in 1976, when 140 salmon were transported to the area for angling purposes (Isaksson 1980). The experiment demonstrated that the salmon could tolerate considerable transport and a high proportion could be angled. Some of the salmon, however, were injured by a fence crossing the river, and the method was no longer pursued.

Attempts to transport ranched salmon for angling purposes were renewed in 1988, when salmon were transported into several rivers for angling purposes. This development was related to a great increase in ranching activity and the availability of ranched salmon throughout the summer.

Prior to 1988 the only government permits regarding this activity were related to fish diseases and were granted by the Fish Disease Committee. In that year a regulatory measure was set which prohibited such transports, but the Ministry of Agriculture could grant exemptions from the ban after consultation with river associations in the area and with the Fish Disease Committee.

Although all the rivers used for this purpose have had little or no fish populations, there have been genuine disease and genetic concerns regarding these transports, especially into complex river systems such as the Hvítá system in western Iceland. In order to minimize all risks, the transports have only been permitted with the conditions that the salmon are prevented from leaving the river by a fence, most of the salmon have been tagged to detect straying, and spawners have been netted out of the river at the end of the fishing season. More recent conditions include testing of all salmon for major diseases.

Disease concerns increased in 1995, when furunculosis was diagnosed for the first time in Iceland, and no transports were permitted in 1996. Since no further outbreaks of furunculosis were observed in Icelandic salmon in 1996, there has been great pressure to grant exemptions from the ban in 1997 and some permissions will be granted with strict conditions, including a total stoppage of transport at any time during the season if serious disease outbreaks occur.

It seems likely that put-and-take fisheries for ranched salmon in small streams flowing directly into the sea could create considerable income for the river owners and benefit the local economy and the tourist trade in some rural areas. Releases of ranched salmon into complex river systems, however, will always be debated and create conflicts between different interest groups as well as experts. The practicality of such practices is thus questionable and a risky business for the entrepreneurs, which will be subject to various interventions by the authorities if disease conditions worsen.

Figure 5

Main regulations controlling the interactions between ranched, farmed and wild salmon

1. Sea cages and ranching stations must be:
   - 15 Km away from streams with an average catch of more than 500 salmon per annum.
   - 5 Km away from streams with an average catch of more than 100 salmon per annum.

2. Sea cages and ranching stations must be at least 2 Km away from each other.

3. Foreign stocks must not be used for enhancement or ranching.

4. Ranching stations must tag 10% of their release up to a 10 thousand smolt minimum.

5. Indigenous wild stocks must be used for local enhancement.

Measures Enacted

Regulatory measures

In 1988 a regulatory measure was set to minimize the effects of ranching and rearing on wild salmonid stocks. It specified a minimum distance between major salmon rivers and the rearing and ranching operations as well as between ranching operations (Figure 5). It further prohibited the rearing of imported stocks in sea-cages and specified that ranching stations should microtag at least 10 per cent of their releases up to a 10,000 smolt minimum. Most stations have microtagged many times that number annually.

Establishment of a gene bank

As reported by Guõjónsson (1991), a fairly large proportion of strays from cage rearing were observed in salmon rivers in the Faxaflói area, in particular in the river Elliõaár. This high straying rate was of great concern to managers as well as the Reykjavík Angling Club, which leased the river.

In 1989 the Reykjavík Angling Club, to commemorate its fiftieth anniversary, donated US $10,000 for the establishment of a salmonid gene bank, based on liquid nitrogen deep-freezing principles, at Hvanneyri in Borgarfjörõur, linked to a cattle-breeding facility. The first milt from the Elliõaár stock was stored in the bank in the fall of 1989.

Since that time milt from three additional wild salmon stocks in western Iceland and one wild sea-trout stock from southern Iceland has been stored in the bank (Figure 6). In addition the bank has been extensively used to store milt from Norwegian salmon stocks used for land-based rearing and several families from the Kollafjörõur ranching stock in connection with a selective breeding program (Jonasson 1994).

Figure 6

Number of salmonid milt samples preserved in the Icelandic Gene Bank

Origin	Stock	1990	1991	1992	1993	1994	1995	1996	1997	Total
Wild salmon	Elliðaár	46	12							
	Leirvogsá	8								
	Norðurá	6	17							
	Laxá í Dölum	20								**109**
Ranched salmon	Kollafjörður	56	8	30		12				**106**
Reared salmon	Silfurgen	13								
	ISNO	8	4							
	Stofnfiskur								27	**52**
Sea trout	Grenlækur						32			**32**
Brown trout	Þingvallavatn		1							
	Kollafjörður	1								**2**
Char	Kollafjörður		1							**1**
Rainbow trout					4					**4**

It seems quite clear that interest in freezing milt from Icelandic wild salmon stocks, which is entirely voluntary and funded by individual river associations, has dwindled since its establishment in 1989. This is probably primarily related to the liquidation and subsequent removal of all cage farms in the Faxaflói area. Substantial straying of ranched salmon into those rivers in subsequent years did not rekindle any interest or attract new customers to the bank.

The principle, however, has been well received and extensively used by the aquaculture industry, which sees numerous possibilities in parental crossings, especially in selective breeding programs.

Status of Other Salmonid Stocks

Brown trout (Salmo trutta)

Anadromous brown trout are found in various Icelandic rivers but are most abundant in rivers on the south coast (Figure 1). They ascend rivers in the September-October period and overwinter in the streams. Their abundance varies depending on environmental conditions in fresh- and salt-water but has not been threatened by human activity. Harvest is mostly by angling, which takes place both during seaward migration in April and during the fall migration.

Landlocked brown trout are found in various lakes and some rivers. The best-known angling areas with large trout have been Lake Thingvallavatn, the Veiõivötn lake complex and the upper areas of Laxá í Thing (outlet of Lake Myvatn). Brown trout abundance is limited by the availability of suitable spawning in inlet and outlet streams.

A prime stock of brown trout in Lake Thingvallavatn was heavily affected by the damming of the lake's main outlet in the late 1950s. This curtailed spawning and subsquent recruitment of the trout, which was followed by a decrease in abundance. Restoration is under consideration.

The Veiõivötn lake complex is a prime angling area for landlocked brown trout. Being in a volcanic area, the spawning areas are limited and the fishery has been greatly enhanced through fry releases, making the area one of the best areas for lake fishing in Iceland.

The landlocked brown trout below the outlet of Lake Myvatn is a unique stock, which is in great demand for angling. There are great interannual variations in abundance depending on environmental and limnological conditions in Lake Myvatn (Ólafsson 1979). Mining of diatomaceous deposits in the lake may have influenced these conditions.

Arctic char (Salvelinus alpinus)

Sea-run arctic char are primarily found in cold artesian rivers and steep run-off rivers on Iceland's north and east coasts (Figure 1). The land-locked variety is very abundant in numerous lakes, and stunting due to overcrowding is common. Angling in lakes is common, but angling of sea-char is of greater economic value. The effects of human activity on char populations are minimal. Reduced netting in lakes, however, may be a major reason for overcrowding and stunting of char populations. The importation of mink to Iceland in the 1930s has also introduced a new predator into the Icelandic freshwater ecosystem, which has affected sea-run populations of brown trout and char in smaller rivers.

Conclusions

In this paper I have reviewed the status of Icelandic salmonid resources as they are reflected in the catches and monitoring of adults and juveniles in rivers and lakes. On the whole salmonid stocks seem to be in a satisfactory state, especially with respect to the freshwater part of their life-cycle. This means that land-erosion, pollution and acid rain have not yet affected the resource significantly. It seems unlikely that Icelandic activities would have increased effects in the near future, but drastic changes on a global scale, such as global warming, oceanographic changes and disease transmission from aquaculture in neighbouring countries, could have serious effects.

There are indications that the ecosystem in some parts of the North Atlantic has not been favourable for salmon stocks in recent years. This is partly related to normal oceanographic fluctuations, but could also be partly related to predation by or competition with other marine fish or marine mammals, whose numbers have soared in recent years. Additional effects could be related to a general increase in fishing effort and gear efficiency in many fisheries for marine species. It is thus clear that additional research into the Atlantic salmon's marine phase is sorely needed.

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An Integrated Approach to Gene Banking of India's Fish Germplasm Resources

A.G. Ponniah [1]

Introduction

Aquatic resources are the only food resource of major significance that still remain primarily non-domesticated. They sustain the economic livelihood of low-income groups and contribute significantly to the GNP of countries like India. To safeguard these valuable resources against dangers of species extinction and loss of genetic diversity, there is an urgent need to conserve them. *Ex situ* conservation of genetic resources has become increasingly important since the Convention on Biological Diversity was adopted.

Ex situ conservation, as defined by the Convention, is 'the conservation of components of biological diversity, outside their natural habitats'. This could be in the form of captive brood stock held in 'live banks' like aquaria or farms or cryopreserved gametes/embryos in 'gene banks'. With regard to aquatic genetic resources, the role of *ex situ* conservation will be to supplement *in situ* conservation, unlike the case of plant genetic resources where *ex situ* conservation has played a key role in the conservation and breeding of new varieties. This is mainly because a greater proportion of aquatic biodiversity is still provided by wild species. The aquaculture potential of many fishes has not been evaluated, and even in cultured species the level of domestication is low, especially in developing countries like India (Ponniah 1998). It is nevertheless important to plan *ex situ* conservation programs so that they are integrated in the overall program of conservation and sustainable use of aquatic resources. This paper describes approaches to India's fish genetic resource conservation as well as details of work carried out under different research programs.

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The Need for *Ex Situ* Conservation

Many people are of the opinion that *ex situ* conservation should not be encouraged as it might be taken as an admission that *in situ* conservation has failed and divert attention and funds from *in situ* conservation programs. However, it cannot be denied that at times *ex situ* conservation is the only way out when habitat has deteriorated and a species is faced with extinction. *Ex situ* conservation can also be considered as an additional insurance for prioritized species.

Another argument made against gene banking, especially of cryopreserved material, is that it represents a narrow static view of a species at a particular point in time while the dynamic evolutionary process by which a species adapts to a changing environment can only be conserved by an *in situ* program. But every species contains the genetic blueprint by which it can adapt to varying conditions, and cryopreserved material can be used in restocking programs at a later date, provided that the original habitat has not completely changed.

One other major argument against gene banking is that it requires substantial funding and skilled manpower. Though the initial expense is high, recurring costs can be judged to be reasonable, considering the potential benefits, especially for cryopreserved gene banks. Also, the training requirement for the field-based method of milt cryopreservation is minimal (Harvey 1996).

Live Banks

The 'live bank' approach of holding captive breeding populations in *ex situ* conservation requires more funding and manpower for maintenance than a cryopreserved gene bank. Also, it is difficult to avoid domestication, inadvertent selection, inbreeding, genetic drift and unexpected genetic bottlenecks in hatchery rearing. Controlled experimental studies in a hatchery-based enhancement program (Bartley et al. 1995) have indicated that it is difficult to avoid genetic change.

Live banks should be a short-term program in order to avoid the pitfalls of hatchery rearing. They are worth the cost and effort involved if they are linked to active genetic upgrading programs like that of GIFT tilapia (Eknath et al. 1993) or are being actively used in species recovery programs (Gausen 1993). For endangered species, holding a captive population should be used only if there is reason to believe that the endangered species will become extinct in the wild if not held in captivity (Johnson and Jenson 1991). Otherwise it is better to opt for either aquatic sanctuaries and marine protected areas where self-sustaining populations of endangered species can thrive (Roberts and Polunin 1991) or species recovery programs with components of ranching and habitat restoration.

Gene Banks

Presently, many stock enhancement programs are carried out with hatchery seed which have originated from brood stocks differing considerably from local wild populations in their genetic makeup. The use of local wild brood stock in ranching programs will ensure retention of the natural genetic identity and avoid introgression of hatchery stock. In the absence of facilities for breeding local wild stocks, milt collected and cryopreserved from a sufficient number of wild males can be used in ranching programs.

One of the emerging requirements for undertaking gene banking of aquatic resources is the need to build a genetic base collection that can be used by breeders for evolving new strains. Most of the plant varieties that have been produced are based on gene bank collections. Strain development in aquatic organisms, especially in developing countries like India, is only now being carried out and is based on populations collected directly from the wild and maintained in a live gene bank (Reddy et al. 1998). In the meantime, the natural genetic base of aquatic organisms is being eroded at a faster rate. In addition, the input of accidental and intentional release of hatchery stocks on natural stocks is alarming (Hindar and Johnsson 1995; Unwin and Glova 1997). Therefore it is essential that gene banking of cultivated and cultivable aquatic species be undertaken expeditiously.

Cryopreservation gene banks, however, suffer from the fact that, at present, it is possible to cryopreserve only the male gametes of finfishes and there is no viable technique for finfish eggs and embryos. Certain approaches to fish embryo cryopreservation like blastomere cryopreservation (Harvey 1983; Nilson and Cloud 1993) and nuclear transplants (Lu and Chen 1993) look promising. However, they are yet to be translated into viable techniques. Success with rotifer embryo cryopreservation (Toledo and Kurokura 1990) and vitrification of multicellular *Drosophila* embryo (Mazur et al. 1992) are indicators that finfish embryo cryopreservation can become a reality in the near future. The use of the androgenesis technique, by which the whole genome can be reconstituted only from cryopreserved milt, is promising (Thorgaard and Cloud 1993). However, the low hatching percentage in androgenesis curtails the wider use of this technique. With cryopreserved milt it is possible to transfer only half of the genetic make-up of a selected population. However, through repeated backcrossing or through androgenesis, it might be possible to arrive at a genetic make-up similar to that of the original stock.

Other uses of cryopreserved fish milt, outlined below, can be applied in a gene banking program. For many species, wild brood stock are still being used for culturing, and milt cryopreservation can play an active role in seed production. It can also help in species where there is asynchronization in the maturity of sexes. In hatchery stocks, it can be used for the introduction of wild genome (Cloud et al. 1990) and for increasing the effective population size without the cost associated with increased brood stock transport and maintenance. Also, breeding techniques such as rotational line crossing, used to avoid inbreeding, can easily be carried out with cryopreserved milt. It can be used to maintain a reference population of founder/base stock used in a genetic upgrading program and for wider use of superior selected stocks. It can be an effective tool for enhancing output in chromosome and genetic engineering programs and for marketing monosex sperm. Gene banks can also effectively preserve Genetically Modified Organisms (GMOs) which are not propagated commercially but still have potential future research applications. Cryopreserved milt can serve as an excellent tool in basic reproductive development and hybridization studies since it can be used to overcome geographic, spatial and behavioural barriers in reproduction.

What to Cryopreserve

In view of the cost and effort involved, what should be targeted for conservation through gene banking of cryopreserved gametes needs careful consideration. At the species level, priorities should be determined by economic importance or endangered status. Economically important species include those that are presently cultured as well as those with potential for use in aquaculture and ornamental fish trade or as value-added pharmaceutical product. Among threatened species, priority should be based on the conservation status assigned.

Below the species level, for aquatic resources, unlike plant and animal resources, the genetic subdivisions are neither well defined nor uniformly adopted (Pullin 1995). The evolutionary significant unit (ESU) proposed by Waples (1991) can be used as the genetic unit within a species that needs to be conserved. There is also a need to conserve ecologically significant units exhibiting the adaptive life history trait complexes of different populations of a species that has evolved over thousands of years. Ecologically significant units can be defined as geographic populations exhibiting distinctive biological features that differ from other populations of the same species and have a strong genetic basis. These features could include tolerance to environmental parameters, reproductive features, growth patterns and body characteristics. The difficulties in establishing the link between ESUs and ecologically significant adaptation could be mainly due to limited differentiating power of isozymes as genetic markers for identification of ESUs. With the advent of DNA markers, it is hoped that better correlation might emerge between identified evolutionary and ecologically significant units (EESUs).

Few studies on genetic characterization of natural populations are adequately linked with investigations of life history traits (Wood et al. 1975; Wood 1995). If such studies are carried out, it would help to identify adaptations that are ecologically significant and of potential use in genetic breeding programs. These studies can finally be translated into screening of economic significant units (EcSU) that can be prioritized for gene banking and used as a founder population for genetic upgrading programs targeted at specific farming systems or agro-climatic regions. Within a threatened species, EESUs exhibiting higher heterogeneity and ecological adaptability should be prioritized to ensure the success of an enhancement program.

Lastly, the unit of conservation should also be targeted at the gene. The proportion of genetic variation in cultivated crops is only a fraction of that found in wild relatives (Tanskley and McCouh 1997). It has been proven that phenotypic evaluation to determine breeding values for quantitative traits is misleading and genes of considerable economic importance are locked in wild populations. It is in this context that DNA fingerprinting of all cryopreserved collections is essential since it will help to determine the uniqueness of each collection and determine the probability of identifying a large number of useful alleles. These studies, coupled with genetic linkage maps based on molecular markers, will help screen for specific genes and for undertaking DNA marker assisted selection programs using cryopreserved milt.

India's Conservation Programs

India's gene banking work has to be considered in the context of its total conservation strategy to understand how the various aspects outlined above have been incorporated. The main thread that links it with various components of the strategy is the prioritization of species and areas with the objective not only of conservation but also of sustainable use of the resources (Ponniah 1998). The prioritized species are of economic importance or endangered.

Among the economic species, the first to be prioritized are the three Indian major carps catla *(Catla catla)*, rohu *(Labeo rohita)* and mrigala *(Cirrhinus mrigala)*, which account for more than 90 per cent of India's freshwater aquaculture production. Also included are the exotic fishes, rainbow trout and common carp, which are important culture species in the upland areas. Under the mission mode program of fish germplasm conservation under the National Agricultural Technology Project (NATP), EESUs of presently cultured as well as potentially cultivable and aquarium fishes will be identified. The proposed NATP program to be initiated in the coming year will be carried out by establishing linkages with local organizations in the species-rich northeast and western ghat regions of India.

To identify the endangered status of Indian fishes, the National Bureau of Fish Genetic Resources conducted an exercise in 1992, and a list of 79 endangered species has been compiled (Mahanta et al. 1994). This list has been further expanded recently based on IUCN criteria in a conservation assessment and management plan (CAMP) exercise and is presently in the process of validation. Among the endangered fishes, initial priority has been given to golden mahseer *(Tor putitora)*, hilsa *(Tenualosa ilisha)* and a peninsular carp *(Labeo dussumeri)*. Golden mahseer and hilsa have been selected from the 'flagship' concept to focus attention on conservation of fish germplasm. All three are of economic importance and are endangered in parts of their earlier distribution.

For species specific recovery programs, it is essential to identify habitat variables that influence the survival of different life stages and the unique adaptive characteristics of local populations. For the endangered golden mahseer, habitat inventory (Srivastava et al. 1996) and life history studies have been initiated. Also a pilot scale mahseer *in situ* conservation program with mass awareness, public participation and ranching has been undertaken by NBFGR in Uttar Pradesh hill areas (Mahanta and Joshi 1996). For all the prioritized species, genetic characterization work has been undertaken using isozyme, eyelens and haemoglobin isoelectric focussing and cytogenetic markers (Barat et al. 1996; Barat and Ponniah 1998; Barat et al. 1997; Gopalakrishnan et al. 1997; John et al. 1993a, 1993b; Ponniah 1996a). The detection of genetic introgression in Indian major carp hatchery stocks (Padhi and Mandal 1997; NBFGR 1998c) raises the urgent need to gene bank wild stock of these species in view of ongoing programs of many State governments to stock reservoirs and natural rivers with hatchery seed of Indian major carp. The six rohu founder stocks used by the Central Institute of Freshwater Aquaculture to develop the improved rohu variety 'Jayanthi' were found to be free of genetic introgression and had adequate heterozygosity levels when screened with species-specific and polymorphic isozyme markers (NBFGR 1998c). Recently, a Fish DNA Fingerprinting Centre has been set up at NBFGR and in future it would help in DNA fingerprinting of all accessions in the fish gene bank.

Work on carp DNA markers has been initiated in India (Padhi and Mandal 1995a; Majumdar et al. 1997; Mohindra and Ponniah 1998). A database list of Indian fishes, along with details of ecosystems and macro distribution, has been compiled (NBFGR 1998). Cytogenetic information along with karyotype figures of 128 Indian fishes has been published (NBFGR 1998a). These databases will be combined with others and updated with synonyms, photos of fishes, micro distribution details and biological and genetic information on prioritized species in a GIS platform, so that they can be used for the conservation and sustainable use of India's genetic resources.

India's Gene Banking Program

Sperm cryopreservation protocols have been developed for a number of Indian species. At NBFGR the work has concentrated basically on three endangered species and six commercial species (Ponniah 1996b). Initially milt cryopreservation work was started on the Indian major carp rohu, and a hatching rate of 10 per cent was achieved using cryopreserved milt. Recent trials with three Indian major carp, common carp and trout, incorporating modifications in protocol, have provided a hatching rate similar to control. Also viable larvae from five-year old rohu cryopreserved milt have been produced. Successful production of viable hatchlings from cryopreserved milt of endangered deccan mahseer *(Tor khudree)* (Ponniah et al. 1992; Ponniah et al. 1998d) and golden mahseer *(Tor putitora)* (Ponniah et al. 1998c) have been achieved. For the prioritized exotic species like rainbow and brown trout as well as common carp, field-based cryopreservation protocols have been developed (Gopalakrishnan et al. 1998; Thakur et al. 1997). Based on protocols developed it has been possible to successfully evaluate the use of cryopreserved sperm as a tool for transfer of genome into common carp stock of Himachal Pradesh State and rainbow trout stock of Tamilnadu State of India (NBFGR 1997 a, b).

Though studies have been carried out with programmed freezing of fish spermatozoa (Ponniah et al. 1998a), all the protocols developed have been based on the field adaptable technique of freezing extended milt in 0.5 cc straws over liquid nitrogen vapour and stored under liquid nitrogen (Bye and Ponniah 1983; Ponniah 1996b). Though during initial trials

DMSO, glycerol and methanol were evaluated, presently for most of the species 10 per cent DMSO has been found to be an ideal cryoprotectant. Extended equilibration time and dilution ratio of milt with extender 1:9 followed in initial trials has been replaced with 15 min equilibration time and a dilution ratio of 1:4. Utilization of activating solutions and altered fertilization protocols have resulted in an enhanced hatching percentage (Ponniah et al. 1998b, d). Electron microscopy studies have indicated that they can be utilized for further refinement of cryopreservation technique (K.K Lal personal communication). NBFGR's experience in developing cryopreservation protocols has indicated that the modifications required have been basically on selection of extenders. However, in the endangered hilsa *(Tenualosa ilisha),* different extenders successfully used for cryopreservation of eight species were found to activate the spermatozoa, requiring the development of extender with high KCl concentration of 1500 mg/100 ml with 2 per cent egg yolk as additive (Lal et al. 1998).

Besides the work at NBFGR, in other Indian laboratories cryopreservation protocols have been developed for rohu (Gupta and Rath 1993), *Heteropneustes fossilis* (Padhi and Mandal 1995b), *Liza macrolepis* (Sultana et al. 1998) and *Cyprinus carpio* (Ravinder et al. 1997). Some of these studies and work on many species in other countries are based on motility observations. The trials carried out at NBFGR indicate that motility can be taken as an indicator for initial screening of extenders, activators, etc. and hatching percentage is the only criterion that could be depended upon.

An experimental mini gene bank, in operation at NBFGR since 1989, presently contains cryopreserved milt of three endangered and three commercially important species (Ponniah and Lal 1996). These collections (Table 1), based on hatchery and wild stock, have not been split into core, base and working accessions. All of these are the outcome of experimental work to develop cryopreservation protocols. One of the obstacles to developing a fish gene bank at NBFGR has been the irregular and erratic supply of liquid nitrogen from local commercial units.

With the establishment of new infrastructure in 1998, NBFGR will be able to set up a fish gene bank with collections of cryopreserved milt of presently cultured, potentially cultivatable and aquarium species under the NATP project involving local collaborating institutions. This facility will be linked with the DNA Fingerprinting Centre, and all important

Table 1

Gene banking at NBFGR

	Species	Year of collection
Endangered	*Tor khudree*	1990,1991
	Tor putitora	1991,1992
	Tenualosa ilasha	1994,1995
Commercial	*Labeo rohita*	1989,1990,1991,1992,1993,1996
	Cirrhinus mrigala	1990,1993,1996
	Catla catla	1990,1993,1996

accessions will be genetically catalogued. DNA extracted from sperm (Cummins and Thorgaard 1994) will help identification of individual accessions. In the coming years the gene bank will be upgraded into a national facility for gene banking of fish gametes and DNA materials. By linking the accessions to the central data base, it will be possible to use cryopreserved milt in ranching programs as well as in developing new strains.

Cryopreservation of fish embryos has met with only limited success (John et al. 1993c, 1998), and as in other countries, there is no viable technique for fish embryo cryopreservation. In the penaeid prawn, survival of thawed larvae was observed up to a freezing temperature of -10°C (Diwan and Kandasami 1997), -30°C (NBFGR, 1997) and -40°C (Subramoniam and Newton 1993). The barnacle *Balanus amphitrite* nauplius has been successfully cryopreserved (Anil et al. 1997). Trials on development of androgenetic techniques for construction of whole genome only from sperm has given encouraging results (Ponniah et al. 1995) and requires further standardization to be developed into techniques that can be applied to cryopreserved milt.

Linkages and Access Issues

Most gene banking programs in other parts of the world have concentrated on one or two species. Also, many of these programs are carried out by organizations that are not involved in other aspects of conservation like genetic characterization, including DNA fingerprinting, endangered life history and habitat studies. India's conservation programs carried out through NBFGR are targeted at many species with the concurrently running objectives of conservation and sustainable use. These targets can only be met through strong linkages. India has a strong research institutional capacity for undertaking gene banking efforts. Besides NBFGR, there are separate fishery institutions for capture and culture fishery of freshwater (Central Inland Capture Fisheries Research Institute, Central Institute of Freshwater Aquaculture, National Research Centre on Coldwater Fisheries), brackish water (Central Institute of Brackishwater Aquaculture) and marine ecosystems (Central Marine Fisheries Research Institute). Besides these, fishery related research programs are taken up in many colleges and agricultural universities throughout the country. This vast network makes it possible to collect and preserve the fish biodiversity of a big country like India with a rich fish biodiversity. Due to the early start of fish gene banking efforts in India, it is possible for other countries, especially those belonging to the South Asian Association for Region Cooperation (SAARC), to establish linkages with India's program and benefit from it.

With the advent of community-based biodiversity registers in parts of India and the proposed biodiversity legislation, access to biodiversity, especially genetic material held in gene banks, is an open question and guidelines are yet to crystallize. However, ongoing consultation indicates that, at least for research programs aimed at conservation and development of new strains within the country, *ex situ* collection protocols would not be restrictive. By developing linkages with local communities in the development of gene banks, transparency and all around participation can be ensured. International linkages and exchange of germplasm might initially suffer due to varying restrictive conditions imposed by some countries. However, in the long run, agreements benefiting all the partners might emerge.

Conclusion

The approach to conservation of fish genetic resources in India is based on integration of *ex situ* programs with work from different areas like habitat inventory, life history studies, genetic characterization and data base management. Utilizing its strong research institutional capabilities, the gene banking program on conservation can be linked to the genetic upgrading programs to ensure long-term funding and support. It is hoped that the convergence of different interests, including those of local communities, will result in a gene banking program that will facilitate conservation and sustainable use of India's fish biodiversity.

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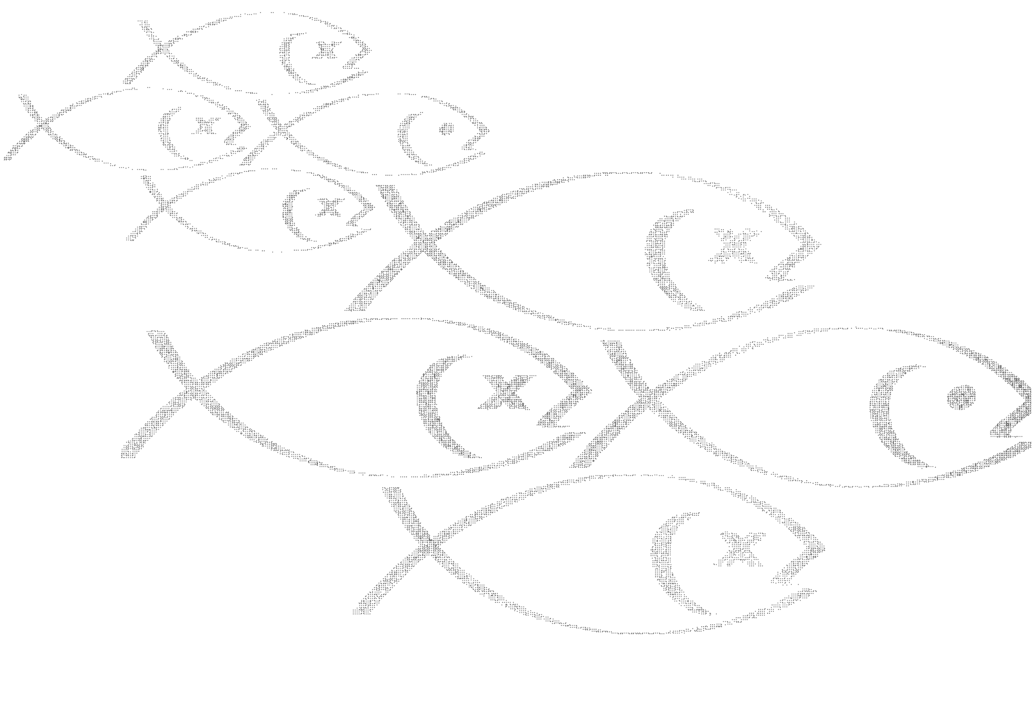

Cryopreservation of Fish Gametes and Embryos in the Ukraine: Yesterday, Today and Tomorrow

Eugeny F. Kopeika [1]

Introduction

In 1972 the Institute for Problems of Cryobiology and Cryomedicine was established in the Ukraine (Kharkov) and since 1973 has been studying mechanisms for cryodamage and cryoprotection of fish sperm. The study of the role of ice-formation and osmotic effects in spermatozoa cryodamage has led to a proposal for standardized freezing of fish spermatozoa.

Attempts to Establish a Gene Bank in Ukraine

In 1976 we developed a method of sturgeon sperm cryopreservation which makes possible a rate of production of about 55 per cent fertilized eggs from thawed sperm. In 1979 a method for cryopreservation of carp sperm was developed, and the laboratory of fish sperm cryopreservation in the Institute was established. Two years later, in 1981, the three-year old carp produced from thawed sperm were presented at the All-Union Exhibition of Achievements in Moscow.

In the same year the Council of Ministers of the Ukraine issued a decree for the creation of a fish sperm bank in the Ukraine, but it was not opened because no funds were provided for its establishment. Instead we had to seek other organizations which would be more interested in our research and in the organization of such a bank. Since that time we

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have worked with scientists at the Selection and Genetical Centre in Rybnoye village in the region of Moscow. In 1985 we finished our research into improvements in carp sperm cryopreservation, and the following year we introduced a collection of carp sperm into the low temperature bank of our Institute. Part of this material was later transferred into the Moscow bank.

Activities Until 1992

In 1987, in the warm water of a power station in the Primorsky region, we got the first semi-industrial scale batch of fish (7 metric tons) produced from insemination of common carp eggs by thawed sperm of German and Parsky carp. In 1989-90 we developed a method for salmon sperm cryopreservation in the Kamchatka and Sakhalin regions. In the same year, under the leadership of V.P. Veprentsev, the "Low Temperature Genetic Bank of Industrial and Rare Fish species and Aquatic Invertebrates" program was organized.

Apart from our laboratory, the participants of this program had no experience in fish sperm cryopreservation, and all the work on the creation of a carp sperm collection, its cryopreservation and the organization of a fish sperm bank was accomplished by us and colleagues from the Centre. We have also organized a school to train experts from the former USSR in methods of cryopreservation and estimation of the quality of sexual cells. After the dissolution of the USSR, the Ukraine continued funding these projects. After all expeditions became impossible, due to huge customs taxes, our work on fish sperm collection for the Moscow region bank that started in 1986 was interrupted in 1992.

Cryopreservation Research and its Application in Other Countries

Studying spermatozoa from different habitats has revealed a high cryolability of cells between and within species. Investigation of biochemical characteristics of spermatozoa membranes, cellular volume dynamics, their energetics at different stages of cryopreservation and the physiology of males permitted us to identify the causes of high cryolability and to propose ways of cryoprotection. Our methods were also transferred abroad; in France, for example, with Dr. Patrick Williot in 1996, we cryopreserved the sperm of Siberian sturgeon *(Acipenser baeri)* and also froze the sperm of freshly caught Atlantic sturgeon *(Acipenser sturio)*. Due to a lack of eggs, we tested the fertilizing capability of thawed sperm in the eggs of sterlet. It did not practically differ from the control (control—42 per cent, experimental—38 per cent).

Our studies on the cryopreservation of eggs, embryos and embryonic cells have been less successful. After cryopreservation of carp embryos at the late blastula stage, only single embryonic cells survived. Intensive studies in this field have not yet been carried out. Our cryopreservation of embryonic cells of loaches *(Misgurnus fosilis)* was more successful, resulting in a cell viability rate of 70 per cent.

Outlook for the Future

These studies have resulted in significant potential for further research. We have worked with more than 30 fish species from different habitats, developed methods for gamete cryopreservation and provided the basis for two cryobanks of fish sperm. However, we have not been able to capitalize on the results of this research in the Ukraine. Our experience in the theory and practice has been used by the scientists in other countries, including Dr. Patrick Williot in France in 1994-1996 and Prof. Herbert Stein in Germany in 1996-1997. In 1995 funding on fish gamete cryopreservation in the Ukraine was terminated due to financial difficulties and the laboratory was closed. Because gene banking projects were funded by the State, there is little option for further research. Therefore, if anybody is interested in our experience we are prepared, for mutual profit, to undertake work on cryopreservation or the creation of a fish gametes bank.

Research Results and Perspectives of the Program: "Low Temperature Gene Bank of Marketable, Rare and Endangered Fish and Aquatic Invertebrate Species"

V. I. Ananiev [1]

Introduction

Twenty years ago, at the Fourteenth General Assembly of the International Nature Conservation Union, Dr. Boris Veprintsev of the Institute of Biophysics of the Cell at the Russian Academy of Sciences described the need to conserve rare and endangered animal and plant species by deep freezing sexual cells and whole organisms. Since that time scientists in many countries have been working actively to meet that need through the development of cryopreservation technologies.

Dr. Veprintsev described the low temperature gene bank (LTGB) as a modern-day Noah's Ark with the potential to save species endangered by natural or human-caused disasters. Cryopreservation of a genome in such cases may indeed be the essential last resort both for preservation of a species and for its future restoration when live representatives no longer exist in nature.

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Strategic approaches to conservation of biodiversity in aquatic organisms were developed by Dr. Veprintsev and his successors at the Interdepartmental Ichthyological Commission's program, "Low Temperature Gene Bank of Marketable, Rare and Endangered Fish and Aquatic Invertebrate Species" (1990-1996). Regrettably, Dr. Veprintsev died at an early stage in the development of the program and was unable to put all his ideas into practice.

The program was set up to address the need to conserve fish species that may disappear forever if urgent measures are not taken. The Red Book of Russia lists 51 fish and fish-like taxons pertaining to 42 species, some of which are represented in Table 1. The real number of endangered and threatened species is much greater.

Cryotechnologies are needed not only for species conservation but also to re-establish genetic heterogeneity of marketable fish stocks and thereby for the purpose of maintaining stable fish harvests and increasing the efficiency of aquacultural technologies.

Allow me, as a representative of the Interdepartmental Ichthyological Commission, a scientific body coordinating fish farming research in our country, and as one of the direc-

Table 1

List of certain species (populations) of Russian fishes requiring protection

Species (population)	Status
1. *Acipenser medirostris mikadoi*	1 (E)
2. *Acipenser baeri baicalensis*	II (V)
3. *Acipenser schrencki* (population of the Zeya and Bureya rivers)	I (E)
4. *Huso dauricus* (populations of the Zeya and Bureya rivers)	I (E)
5. *Acipenser sturio* (populations of the Baltic Sea basin)	I (E)
6. *Acipenser nudiventris* (population of the Black and the Azov Seas basin)	I (E)
7. *Huso huso*	II (V)
8. *Salmo mikis* (a diadromous species)	II (V)
9. *Salmo trutta caspius*	I (E)
10. *Salmo trutta labrax*	I (E)
11. *Coregonus lavaretus baeri*	I (E)
12. *Coregonus lavaretus baeri natio swerensis*	I (E)
13. *Salmo salar*	II (V)
14. *Coregonus lavaretus pallasi natio asptus*	I (E)
15. *Coregonus albula pereslavicus*	II (V)

For the evaluation of a species' condition (status) categories in the Red Book of the International Union for conservation of Nature (IUCN) letter symbols are used and in that of Russia figure symbols are employed.

I (E) - species endangered, i.e. those which are disappearing or are about to disappear. This category includes species whose numbers are reduced to such a dangerous level that their conservation is impossible unless urgent measures are taken.

II (V) - species whose numbers are falling (vulnerable ones). This category includes species which are numerous enough so far, but their numbers are reducing rapidly. Therefore, protection measures are indispensable to prevent their passage into category I.

The table only includes some species, which are important for harvesting and aquaculture, but at present included in the Red Books of the International Union of Nature Conservation and of Russia as requiring urgent measures for their protection and rescue. Urgent measures should be taken in order to avoid irreparable loss of genetic resources of many populations and even fish species. This status also refers to aquatic invertebrates.

tors of the program, to describe the scope of problems we have tried to solve, summarize some results, and outline further directions of our research and practical work.

The Program

The first stage of research was undertaken by teams from 25 scientific research bodies of the former USSR, mainly Russia and the Ukraine. These interdisciplinary teams were drawn from institutions such as the Research Institute of Pond Fish Farming, the Institute of Sea Biology (Vladivostok), the Institute of Biophysics (Pushchino), the Biological Institute of the State University of Saint Petersburg, the Institute of Fish Farming and Oceanography of the Pacific (TINRO) and the Special Design Board of the Institute of Cryobiology and Cryomedicine, which was researching cryopreservation of genomes of sea urchins, crustaceans and bivalves.

The programís primary task was to create low temperature gene banks and to store genetically representative collections of fish genomes taken from marketable as well as rare and endangered species. In addition, the program was designed to develop cryotechnologies to facilitate wild species restoration, as well as the delivery of elite planting material (larvae, embryos) and sperm taken from high-class producers, to aquaculture operations.

Limitations in cryobiological methods dictated the need to include exploration of cryodamage and cryoprotection mechanisms and of cellular and embryonic engineering, as well as perfection of methods for taking sexual products from fishes and aquatic invertebrates. For these reasons, the program employed a combination of applied and academic research.

Research and practical work planned for the program (see Table 2) include the following:

- Refinement of methods and technologies for cryopreservation of genetic material from fishes and aquatic invertebrates;
- Investigation of the impact of cryopreservation on genetic, physiological, biochemical, developmental and other characteristics of fishes;
- Refinement of methods of taking sexual products from rare and endangered fish species;
- Refinement of methods for low-temperature collection of fish genomes;
- Collection and cryopreservation of fish sperm and storage of genetic collections in low-temperature gene banks;
- Refinement of methods for preserving and accessing genetic information contained in deep frozen cells and tissues;
- Refinement of cryogenic equipment for research work undertaken by the program;
- Scientific systematization of data on fish and aquatic invertebrate species represented in LTGBs, including creation of a data base on the composition of cryocollections, the biology, ecology, population and genetic structure of species preserved by cryopreservation, etc.;
- Construction and use of low temperature gene banks for fishes, aquatic invertebrates, etc.

Table 2

The main research and practical work envisaged by the Program

Scientific subdivision of the National Program
1. Elaboration of methods and technologies for cryopreservation of fishes' and aquatic invertebrates' genetic material
2. Investigation of cryopreservation's influence on the genetic apparatus, physiological and biochemical, developmental and other characteristics of fishes
3. Elaboration of methods for taking sexual products from rare and endangered fish species
4. Elaboration of scientific grounds for low-temperature fish genoms' collectioning
5. Collection and cryopreservation of fish sperm and storage of genetic collections in low temperature fish gene banks (LTGB)
6. Refinement of methods for preserving and accessing genetic information contained in deep frozen cells and tissues
7. Elaboration of cryogenic equipment
8. Scientific systematization of data on fish and aquatic invertebrate species represented in LTGB, creation of an information data base on the composition of cryocollections, the biology, ecology, population and genetic structure of species preserved by cryopreservation, etc.
9. Construction and exploitation of low temperature gene banks of fishes and aquatic invertebrates, etc.

Results

The most important work carried out during the six-year period of the program, from 1990 to 1996, relates to the practical conservation of biodiversity of fishes and, in part, that of invertebrates. Like researchers in other countries, we have been most successful in refining methods for the cryopreservation of fish sperm, including that of sturgeon, salmon and carp. Our research focused on up to 50 taxonomic groups (species, populations, and breeds). In addition, research was conducted on the cryopreservation of several species of Baikal Lake white fish and Baunt Lakes white fish, Davatchan loaches *(Salvelinus alpinus erythrinus)*, Far East pink salmon *(0. gorbusha)*, chinook salmon *(O. tschawytscha)*, coho salmon *(O. kisutch)*, and sockeye salmon *(O. nerkas)*. Data on the motility and fertility of the thawed sperm are shown in Table 3.

Good results were obtained with *O. nerka* salmon and other species of Pacific salmon (Zheltonozhko 1998).

The All-Russia Research Institute of Fresh Water Fish Farming has accumulated considerable experience in the cryopreservation of carp, the primary farmed species in Russia and the Ukraine. Succeeding generations produced by cryopreserved sperm from two, three-, four-year and older fishes were found not to differ in fish farming and biological data from fishes in control groups. Moreover, according to Dr. Savushkina and Dr. L. Tsvetkova, succeeding generations demonstrated accelerated growth and ability to survive (Savushkina et al. 1994; Savushkina et al. 1996). The experimental facilities of the Institute contain sexually mature fishes from the experimental stock which have produced new generations.

Table 3

The quality of cryopreserved sperm*

Fish Species	Fresh Sperm %			Cryopreserved Sperm %		
	Motility	Fertility	Hatch	Motility	Fertility	Hatch
Cyprinus carpio	70-100	50-100	40-80	30-60	15-60	15-45
Rainbow trout	38-60	50-80	15-40	10-40	12-29	8-16
Steelhead trout	40-70	50-70	12-38	10-40	-	-
Acipenser baeri	90-100	76-97	40-60	20-40	36-64	0.8-12.7
A. ruthenus	40-90	79-95	47-68	10-40	40-62	-
Huso huso	90-100	77-98	45-87	20-40	2.9-12.5	0.9-12.7
Huso huso x A. ruthenus	90	83.5	83.3	20-40	12.2	10.3
Polyodon spathula	80	67.5	27.1	10-20	6.8-9.4	1.2-6.1

*Data - Dr. L Tsvetkova, Dr. V. Ananiev and Dr. A. Bagrov (personal communication)

Methods used by Drs. E.F. Kopeika and L.I. Tsvetkova for cryopreservation of sturgeon sperm have demonstrated acceptable fertility results (Kopeika et al. 1983; Kopeika et al. 1992; Tsvetkova et al. 1997; Ananiev et al. 1998; Billard et al. 1997; Tsvetkova et al. 1996). However, attempts to monitor the development of cryopreservation-produced young sturgeon through to survivable stages have been unsuccessful to date. Nor are good results consistently obtained with certain sig fishes (whitefish, lake sigs), although fertilization has been observed to occur with sperm showing zero motility.

Our data and the analysis of research materials from other countries enable us to conclude that, for the majority of fish species, existing sperm cryopreservation methods are sufficiently developed for practical application. Nevertheless, they require further refinement for use with sturgeon and salmon and for consistent cryopreservation results.

Research

Productive approaches to the program's research on cryopreservation of fish eggs and embryos, including embryo-engineering, are still being evaluated. While working with genetic material of marine invertebrates, we succeeded in developing cryopreservation methods for sea urchin *(Strongylocentrotus)* sperm and late blastulae, trepang sperm and larvae, young (before the start of feeding) veligers of mollusc *(Mytilus edulis edulis)*, oysters and scallops (Gakhova et al. 1990; Gakhova et al. 1996; Bregman et al. 1992; Manokhina 1996; Manokhina and Ananiev 1996; Manokhina 1998).

Our efforts to monitor and control low temperature gene banking for gametes and embryos are limited by the fact that less is known about fishes than about invertebrates. At present, a group of researchers working at the VNIIPRH Central Cryobank have succeeded, for the first time, in producing physiologically strong young sturgeon of a weight of 2-3 grams from cryopreserved sperm (Dr. Savushkina personal communication).

Despite difficulties in the refinement of cryopreservation methods, research to date has shown considerable success in developing ways to establish gene banks to store fish sperm and larvae, thus providing the potential to preserve genomic diversity and to perfect selection and breeding processes at aquaculture farms.

In order to save fish species from extinction, it is essential to store in cryobanks genetic material other than sperm—for example, embryos isolated from yolks, embryonic cells at blastula stages or somatic cells. Theoretical opportunities for the employment of the genetic material of a protected or salvaged species to obtain bisexually spawning populations are shown in Figure 1, compiled by Dr. Rott, of the Institute of Biophysics of the Cell.

Androgenesis

The most practical way to conserve the heredity of a preserved species is through absorptive cross-breeding. This, however, requires too much time to obtain maternal genetic input. To address this problem, Drs. Neyfakh, A. Grunina and A. Rekubratsky established methods of obtaining androgenetic hybrids of carp using cryopreserved sperm (Neyfakh et al. 1995; Neyfakh et al. 1997; Grunina and Neyfakh 1997; Grunina et al. 1997).

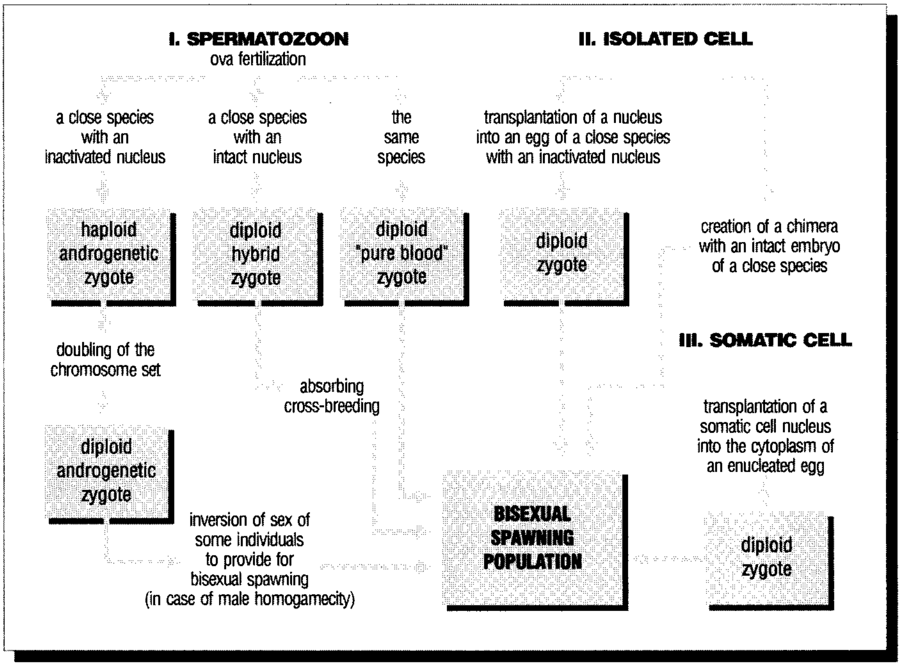
In employing induced androgenesis for the re-establishment of rare species (populations, breeds), it is necessary to use not their own eggs, but those of closely related species or breeds in order to obtain androgenetic nuclear cytoplasmic diploid hybrids. However, as those hybrids have been shown not to be capable of surviving, the same group of researchers developed a means of obtaining diploid androgenesis with the use of roe from ordinary hybrids. The research necessary to achieve this used carp, silver crucian carp and their hybrids and included the following steps:

- Obtaining hybrids of closely related species by the usual method;
- Inactivation of female chromosomes in oocytes of hybrids through ionizing radiation, ultraviolet radiation, etc.;
- Insemination of a re-established species of genetically inactivated oocytes of hybrids by cryopreserved sperm and obtaining androgenetic haploids;
- Re-establishment of diploidy in haploid androgenetic embryos through heat shock or other methods, and obtaining survivable diploids.
- Androgenetic carp obtained during the experiments demonstrated acceptable survival.

The same researchers worked out a method to obtain androgynes in sturgeon. With the help of insemination of irradiated ovules and subsequent heat shock, providing for a convergence of chromosomic sperm sets, they obtained for the first time androgenetic nucleo-cytoplasmatic hybrids of female stellate sturgeon *(Acipenser stellatus)* and male great sturgeon *(Huso huso)*. The resulting young fishes demonstrated acceptable survival. This method, once perfected, will provide significant opportunities for the re-establishment of genotypes of sturgeon from cryopreserved sperm.

Figure 1

Possible ways to employ cryopreserved sperm, isolated eggs and somatic cells



Research on obtaining diploid androgenesis of pelad, by Dr. M. Andriyasheva from the GOSNIORH, and induced androgenesis of Brook lamprey *(Lampetra fluviatilis)* has not yet been completed (Andriyasheva et al.; Saat and Tombets 1990; Saat 1990).

Other Research

Other types of research, being carried out on model fish such as loach and danio, have not yet proceeded beyond the experimental stage. When a species (population) near extinction is represented by females only, conservation may be achieved through the transplantation of the nucleus of a somatic cell into a non-fertilized egg of the same species. Research on the feasibility of this method has been conducted for several years in the USA, Great Britain and China, and in Russia is being done at the Department of Embryology at Moscow State University, by Drs. L. Sleptsova and D. Popov (Sleptsova et al. 1991a; Sleptsova et al. 1991d; Sleptsova and Popov 1997). In the future it will be necessary to study and identify specific properties of the recipients' eggs and donor forms, in order to obtain a sufficient number of developing nuclear cytoplasmic hybrids.

As mentioned above, cryopreservation of whole fish embryos or fish embryos isolated from yolk has not yet been achieved, but promising cryopreservation results for separate cells at the blastula stage have been obtained. The acquisition of information contained in them and its transference to succeeding generations are being achieved in two ways: by transplantation of those cells to an egg of a closely related species and by obtaining chimeras out of those cells and the intact embryo. Researchers have attempted intra- and inter-species transplantation of nuclei of loach blastula cells: to the egg of loach of the same species or to the danio egg. In the first case, nuclei of cryopreserved cells were used. In both cases embryos were obtained which developed until the gastrula stage (the intra-species transplantation of nuclei of cryopreserved cells) or before the beginning of organogenesis (inter-species transplantation). The problem of obtaining mature fishes after such experiments remains to be solved.

Low Temperature Gene Banks

In addition to the research work, organizational measures were carried out in order to create low temperature gene banks and provide them with equipment and chemicals, transportation, mobile laboratories, etc.

As a result of the accomplishments of the Fish Cryobank Program (1990–1996), several cryobanks have been established or modernized. These include the cryobanks at the All-Russia Research Institute of Fresh Water Fish Farming (Rybnoye township, the Moscow Region), the Institute of Biophysics of the Cell (Pushchino township, the Moscow Region), and the Kamchatka Cryobank (Paratunka township, the Kamchatka Region). In addition, technical and economic viability studies were carried out for the construction of a cryobank for mariculture species in Vladivostok. Finally, research work under the program provided impetus for the establishment of a cryobank for the reproduction of fish resources by the Glavrybvod.

A concept for the conservation and stable usage of "biodiversity with the employment of cryopreservation methods" has been worked out whereby tasks for low temperature collection and storing are determined, and advantages and limitations in the employment of cryobanks are presented (see Table 4).

The inclusion of cryobanks in the system of nature conservation enlarges the opportunities of the traditional set of measures, including the re-establishment of degraded ecosystems, the establishment of nature reserves and ichthyoparks, the farming of protected species, and the wider establishment of fish farms.

Cryopreservation of genomes, as distinct from other approaches to the conservation of biodiversity, enables us to protect rare populations from complete disappearance without waiting for the implementation of expensive and long-term measures designed for the re-establishment of degraded aquatic ecosystems. It is the cheapest and most practical means for the preservation of genetic diversity of species and populations, because LTGBs can store an unlimited quantity of genetic information in the form of deep-frozen sexual and somatic cells, embryos, etc.

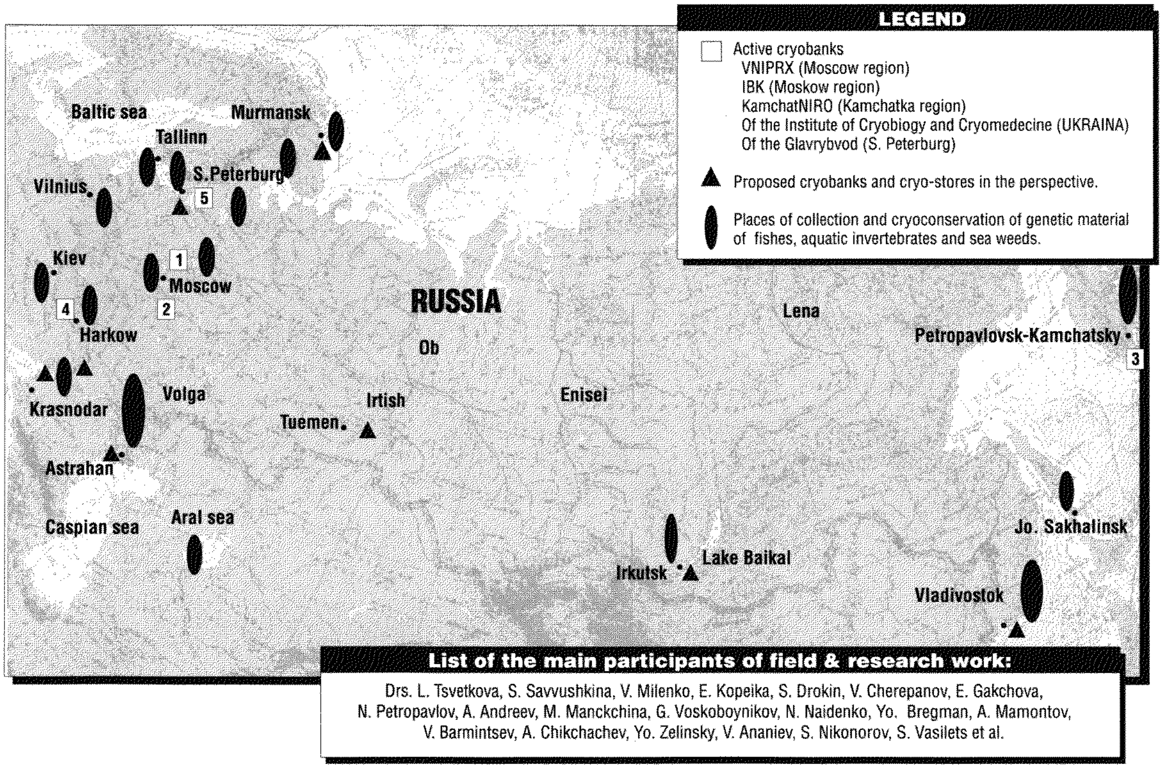
Table 4

Strategy and practical measures for the protection of aquatic biodiversity

Directions and measures for the conservation of biodiversity	Advantages of cryobanks in traditional nature conservation	Limiting factors (disadvantages)
I. Protection and re-establishment of stable populations in their natural environment (*in situ* conservation)		
1. State control of the use of Nature including restriction or prohibition of economic activities:		
2. Re-establishment of degraded ecological systems:		
■ facilitates the tasks of conservation and re-establishment of biodiversity ■ requires great financial investments and much time for the re-establishment or the reconstruction of new aquatic ecological systems	■ preventive (before an ecological crisis emerges) creation of LTGBs facilities and the protection of key species during the re-establishment period of hydroecosystems	■ the existing methods of cryoconservation are limiting the capabilities of modern cryobanks for storing representative genetic collections
3. Creation of protected areas (reserve zones, national parks, ichthyoparks, etc.):		
■ is primarily employed for the protection of endemic fishes inhabiting limited areas ■ requires removing considerable territories from economic activity	■ improves the potentialities of protected territories in the conservation of biodiversity ■ reduces the need for vast areas apportioned for their establishment	■ additional expenditure is required to build and exploit LTGBs including personnel training
II. Conservation of components of ecological biodiversity outside their natural environment (*ex situ* conservation)		
1. Introduction of conserved species into aquaculture:		
■ provides for the conservation of diadromous species ■ the number of species introduced into aquaculture is limited by economic conditions ■ limited number of spawners used for breeding purposes	■ employment of the whole of the genetic potential of a species cultured; avoidance of in-breeding consequences	■ construction and exploitation of the LTGB will require additional expenditure
2. Creation of natural (live) collections of protected species and aquatic invertebrates:		
■ provides for the conservation of one or various animal groups (population, species) determined by economic reasons —does not provide for the conservation of the whole of the genetic resources of a fish species	■ extends the opportunities for the collection of a broader range of genetic resources. Reduces the expenditure for the maintenance male broodstock	■ the efficiency of LTGBs' work will depend on the solution of theoretical and experimental problems on reproduction of genotypes of deep-frozen sperm, elaboration of methods of cultivation and re-introduction of species into nature
3. Creation of cryobanks:		
■ provides for the conservation of the genetic diversity of species protected ■ facilitates the avoidance of genetic funds' losses in case of ecological disasters	■ extends the opportunities of nature protection measures, reduces expenditure	■ the use of genetic funds of aquatic organisms preserved in cryobanks is impossible without the employment of traditional approaches to the conservation of biodiversity

Figure 2

Map of collection and cryopreservation of genetic materials of fishes, aquatic invertebrates and sea weed (Fish Cryobank Program 1990-1991)



If we want to protect species, we need a means of re-establishing species that at present are on the verge of extinction, and therefore should start establishing cryobanks to stock genetic collections of those species. This has been the ultimate purpose of the Fish Cryobank Program.

To ensure effective management of cryobanks, we developed the necessary regulations to control their operations and methods of collecting and storing genetic material, created scientific criteria for low temperature collection and storage, and began monitoring the condition of fish populations in the former USSR.

In 1990-91, large-scale work on the collection of cryopreserved genetic material of rare and endangered species and populations was undertaken. This included the collection of cryopreserved sperm of eight fish species listed in the Red Book of the former USSR and its republics and of four rare species listed in regional catalogues of protected fish species. Expeditions to meet this need were undertaken in different parts of Russia (the Central Non-Black Soil Zone, the Far East, the Baikal Lake Basin, Karelia, the Azov and Black Sea Basin), the Ukraine, Usbekistan (the Aral Sea Basin), Lithuania, and Estonia. A map showing the regions where genetic material was collected is attached (Figure 2). A collection of cryopreserved genetic material of marketable fishes, such as carp and trout, including rare species (populations) of the species listed, was compiled. For the first time the sperm of transgenic trout and carp was also cryopreserved and stored for the long term.

Regrettably, the dismemberment of the Soviet Union and a sharp decline in funding influenced our work very negatively. Part of the collection, containing genomes of rare species, was lost. This included collections stored in temporary cryobanks on the Baikal, in Karelia, and the Ukraine. Large collections of genomes of Russian fishes are stored in the low temperature gene bank of the Institute of Cryobiology and Cryomedicine. At present the cryobanks of the All-Russia Research Institute of Fresh Water Fish Farming (VNIIPRH), the Institute of Biophysics of the Cell and the Kamchatka Research Institute of Fishing and Oceanography store a very representative sperm collection taken from Russian fishes, including species that are rare, endangered or reduced in numbers (see Table 5). The cryobank of the VNIIPRH stores sperms of ship or spiny sturgeon *(Acipenser nudiventris)* [status I], Sakhalin sturgeon [status I], Baikal sturgeon [status II], black amur *(Mylopharingodon piceus)* [status I], *Pereslav ryapushka'* [status I], and lake salmon [status II]. The species mentioned are listed in the Red Book of Russia.

The banks also store species that, though not listed in the Red Book, show a sharp decline in numbers or are considered to be rare. These include the Volga population of great sturgeon *(Huso huso)* and other sturgeons, as well as the Volga population of whitefish *(Stenodus leucichthys)*, Shuya salmon, Arctic char, etc.

In the Kamchatka Cryobank the sperm of a representative of the Pacific salmon (*Salmo mykiss*, listed in the Red Book of Russia as status 3) is preserved.

Sperm of a number of sturgeon and salmon species of Russia are stored in the low temperature gene bank of the Institute of Cryobiology and Cryomedicine in the Ukraine. That cryobank stores the biggest number of sperm samples taken from the Azov population of great sturgeon, listed in the Red Book of Russia (see paper by Kopeika, this volume).

Table 5

Collection of fish sperm in the cryobank of the Research Institute of Pond Fish Farming (Rybnoe, Moscow region)*

	Species, breed	Number of samples	Volume of sperm, ml	Number of spermdoses
ACIPENSERIDS	*Acipenser baeri* (population of Baikal)	26	545.0	363
	Acipenser baeri (population of Lena)	44	722.5	481
	Acipenser medirostris	4	151.0	101
	Acipenser ruthenus	35	313.35	208
	Acipenser steelatus	76	504.0	336
	Acipenser nudiventris	2	34.5	23
	Huso huso	19	1263.5	842
	Acipenser queldenstaedti	17	372.8	249
	Poliodon spathula	20	367.5	245
	Total	**243**	**4274.15**	**2849**
CYPRINIDAE	*Cyprinus carpio haematopterus*	117	1014.0	10140
	Cyprinus carpio Para	150	1873.25	18732
	Cyprinus carpio ZNK	19	170.25	1702
	Cyprinus carpio F-cryo	27	276.25	2763
	Cyprinus carpio Zagorsk	14	235.5	2355
	Cyprinus carpio Boreless	30	362.0	3620
	Cyprinus carpio Japan	81	843.5	8435
	C. carpio Middle-Russian	260	2035.0	20350
	C. carpio Ropsha	20	256.5	2565
	C. carpio Sarboyan	11	255.5	2555
	C. carpio Lithuania	6	183.5	1835
	C. carpio Roumania fresinet	30	215.0	2150
	C. carpio Inversia	45	390.45	3905
	C. carpio Mutagenized	30	285.5	2855
	C. carpio Transplantant	2	13.5	135
	C. carpio Transgenic	1	28.5	285
	Hypophthalmichthys molitrix	76	342.85	3428
	Aristichthys nobilis	16	74.5	745
	Ctenopharyngodon idella	33	321.0	3210
	Mylopharyngodon piceus	9	30.0	300
	Ictiobus cyprinellus	10	23.4	234
	Total	**987**	**9229.95**	**92300**
ONCORHYNCHUS MYKISS	*O. mykiss* (River)	70	263.13	526
	O. mykiss (Lake)	14	63.0	126
	O. mykiss (Kamloops)	12	51.5	103
	O. mykiss (Donaldson)	76	233.4	467
	O. mykiss (Gibrid River)	14	39.0	78
	O. mykiss (Gibrid River + Donaldson)	12	42.5	85
	O. mykiss (Steel head)	20	47.5	95
	Salmo salar (Baltic)	20	19.0	38
	S. salar (Lake)	13	101.5	203
	S. salar (Shuya)	68	505.56	1011
	Coregonus lavaretus (Lake Yanisyarve)	17	17.5	35
	C. lavaretus (Lake Syamoserc)	77	33.25	66
	Coregonus albula (Lake Yanisyarve)	2	1.0	2
	C. albula (Lake Urasosero)	61	22.875	46
	C. albula (Lake Plesheevo)	5	6.3	13
	Oncorhynchus keta	82	183.5	367
	Mugil soiuy	26	43.25	87
	Coregonus peled	53	25,.2	52
	Stenodus leucichthys	88	269.25	538
	Salvelinus lepechini	5	10.0	20
	Salmo mykiss	6	29.5	59
	Total	**740**	**2008.435**	**4017**
TOTAL		1970	15512.535	99166

*Data - Dr. L. Tsvetkova (personal communication)

The LTGB of the Institute of the Biophysics of the Cell also has collections of species considered rare, endangered or reduced in numbers. These include the Volga population of great sturgeon, the Volga population of whitefish *(Stenodus leucichtys)*, brown trout—kamja *(Salmo trutta trutta)*, lake char paliya *(Salvelinus alpinus m. Iepechini)* and Shuya salmon.

Conclusion

In conclusion, our program is operating under the very critical economic conditions that have emerged in Russia. Nevertheless, we are doing all in our power to carry on. A program for 1999-2003 is being developed to continue the 1990-1996 program. However, the main priority of the new program will be the creation of cryobanks in Russia, with the goal of storing and using their cryo-collections. In addition, we shall try to solve problems of a purely academic nature, beginning with the development of methods for the cryopreservation of fish eggs and embryos.

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Conservation of Sturgeon Genetic Diversity: Enhancement and Living Gene Banks

Michail Chebanov[1]

Introduction

Human activities are causing a sharp fall in the abundance in many regions of sturgeon, Acipenseridae, both in distinct populations and in the species as a whole.

In addition to a decline in the catch of sturgeon, which once supported a commercial fishery, there is a decline in, and in some cases a disappearance of, local populations that earlier did not appear to be affected by human activity. There is also a severe decrease in species areas, which were widespread not long ago. There is a sharp decline in the abundance of giant sturgeon *(Huso huso)* and stellate sturgeon *(Acipenser stellatus)* in the north of the Adriatic Sea. Atlantic sturgeon *(A. oxyrhynchus)* and lake sturgeon *(A. fulvescens)* are now rarely found in North America.

These declines pose a dangerous threat to sturgeon genetic diversity in general. *Acipenseriformes*, like other ancient species in the *Latimeridae* and *Polypteridae*, have less genetic diversity and a smaller number of low-range taxons than fish groups of more recent origin. Moreover, the potential of sturgeon for adaptive radiation is smaller than that of evolutionarily advanced fish, and they have used a great number of ecological niches.

While *Cypriniformes* and *Perciformes* demonstrate a great number of spawning behavior models under very different conditions, *Acipenseriform* species can spawn only in running fresh water on dense substrate, and on the whole their relationship to habitat is more restricted than in the case of teleost fish. In spite of a large individual fecundity (100,000 to 500,000 eggs), sturgeon have an extremely low population fecundity that greatly increases

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the difficulty of restoring their abundance. This is the result of several factors, including a complicated population structure with multiple ages, very late sexual maturation of breeders, intervals of several years between spawning, and human pressures.

Sturgeon are characterized by a complicated population organization which is formed both by differentiation of population systems (local stock) and by differences between numerous generations, making up spawning broodstock and seasonal races which are more substantial in sturgeons than in salmonids.

Current Situation

The survival of rare sturgeon and conservation of their genetic diversity is a matter of great significance. Because the potential for the management and protection of wild populations is limited, it is important to develop and implement measures for the conservation of a wide diversity of world sturgeon populations in artificial conditions.

Existing measures focus on holding live breeders in captivity. Aquaculture has demonstrated the possibility of holding and rearing a wide variety of aquatic animal species, and shows the potential for conserving near-extinct species and populations of sturgeon through management under controlled conditions.

All sturgeon species were placed in Appendix II of CITES in June 1997, which means that all international trade in sturgeon products will come under CITES control as of April 1998. Additionally, these species require urgent conservation measures under the terms of the Biodiversity Convention and under Article IV of the Bonn Convention on the Conservation of Migratory Species of Wild Animals, under which agreements should be concluded to benefit migratory species which have an unfavourable conservation status and which require international efforts for their conservation.

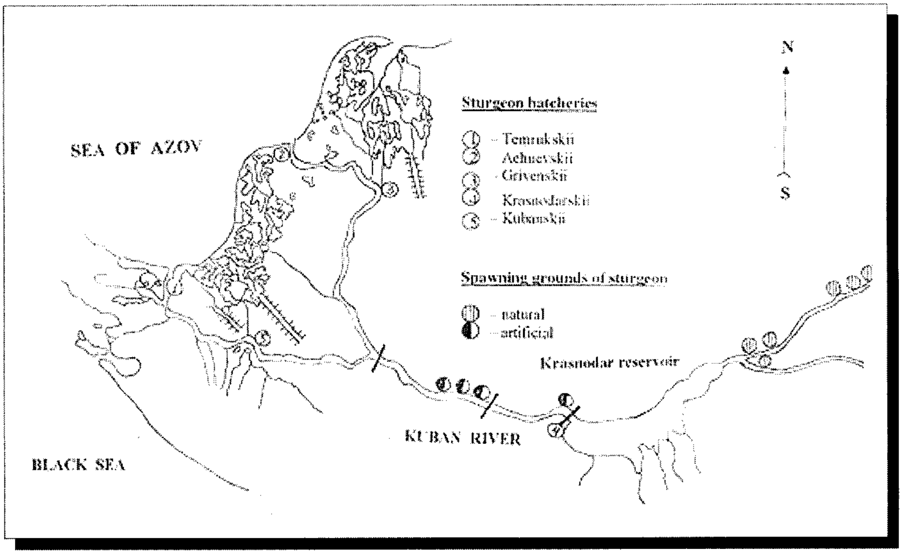
Example–Sea of Azov

The importance of genetic conservation is illustrated by considering the Sea of Azov, a suitable model for genetic monitoring of artificially formed sturgeon populations. The sturgeon catch in the Sea of Azov has sharply declined, though not as much as in the Caspian Sea. In 1996 the catch was 690 metric tons, comprising 480 metric tons of Russian sturgeon and 480 metric tons of stellate sturgeon.

More than 90 percent of the sturgeon catch and juvenile release from hatcheries originates from the basin of the Kuban River (Figure 1). For the last ten years, natural propagation of sturgeon has been practically non-existent there, because the irrevocable discharge of water from the river is 60 percent of annual runoff. This is more than in other Russian rivers. One of the effects of decreased freshwater runoff has been the formation of bars which have blocked the river mouth and reduced the influence of brackish water and silt, which is needed for attracting brood fish into the river.

Figure 1

The Sea of Azov and the 5 Kuban River Basin hatcheries



Construction of dams has restricted sturgeon migration to a degree that could have an impact on genetic integrity of species by segregating them into isolated stocks and causing the loss of genetic diversity.

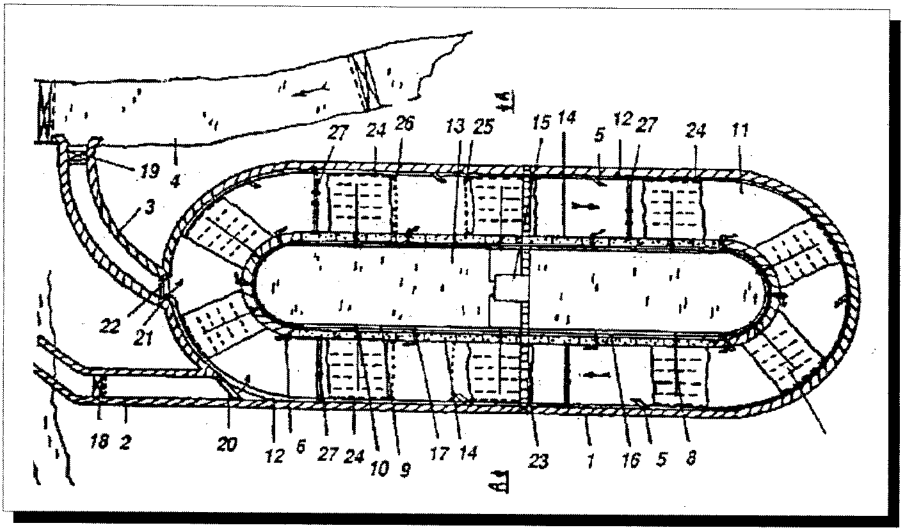
For the last ten years the fish lift of Krasnodar (Upper) Dam permitted the migration of fewer than 1.5 percent of fish moving into the Kuban River, and about 6 percent migrated through the Fedorovskaya (Lower) Dam. Between 1991 and 1995, only 22 breeders were able to pass instead of the 63,000 a year intended by the fish-lift project. Thus, the problem of sturgeon migration for natural propagation in rivers with a regulated flow is not solved yet.

Since 1967, the number of natural sturgeon spawning grounds above the Fedorovskaya Dams has been sharply reduced. Ten-hectare artificial spawning sites, built below the dams, were effective for some years until they were silted over as a result of the change of water regime and erosion of the river bed. This led to the development of artificially constructed spawning sites, built out of the river bed, with a controlled hydrological regime, creating a "pseudomigration" of breeders by an alternate migration route (see Figure 2).

Under natural river spawning conditions, stellate sturgeon were primarily represented by the summer spawning migration group. Flow regulation radically transformed the seasonal dynamics of spawning migration (see Figure 3). As a result, stellate sturgeon, the most numerous species in the sea, with a long-term period of breeding migration into the sea and the largest summer spawning runoff of larvae into the sea, is now primarily represented by an early spring intrapopulation biological group. The mass spawning run does not exceed 20 days.

Figure 2

An artificially-built spawning ground for natural propagation of sturgeon with controlled hydrological regime and ensuring the conditions of "pseudomigration" of breeders by the recycling migration route



1 - Circle spawning channel
2 - Channel for letting broodstock to pass and runoff
3 - Channel for juvenile runoff
4 - Water body for rearing of pre-larvae
5 - Canopies for regulating current velocity
6 - Ejectors
7 - Spawning grounds
8 - Rinsing flute
9,16 - Circle water pipelines
10,17 - Turn off valves
11 - Pool
12 - Larvae collection tray
13 - Internal water body
14 - Drainage filters
15 - Pump station
18,19 - Sluice gates regulators
20,21 - Protective meshes and turn off dampers
22 - Removable large-seized fish protective net
23 - Crossing gangways
24 - Gauze screens
25 - Grooves for removable gauze blocking gratings
26 - Blocking gratings

High sturgeon catches (Figure 4) are explained by a stable release of juveniles (25-27 million pieces) reared at five hatcheries in the region for the last 20 years.

Spawners for hatcheries were captured in the sea near the river mouth. This simplified strategy for artificial reproduction, directed only to the release of a great number of juveniles and using for breeding only the most mature spawners of Russian and stellate sturgeons of an early spring spawning run, resulted in considerable changes in specific and intrapopulation structures of sturgeon in the Sea of Azov and Caspian Sea.

Before the river flow was regulated, stellate sturgeon showed considerable diversity in the state of gonads, size and oocyte nucleus polarization (Figure 5). Females caught during the migration period were found to have gonads from III-IV stages to IV complete stage of gonad maturity where the ratio of the IV stage was less in both the first and second part of

Figure 3

Changes in seasonal dynamics of anadromous migration in periods of different flow regulation in the Kuban River

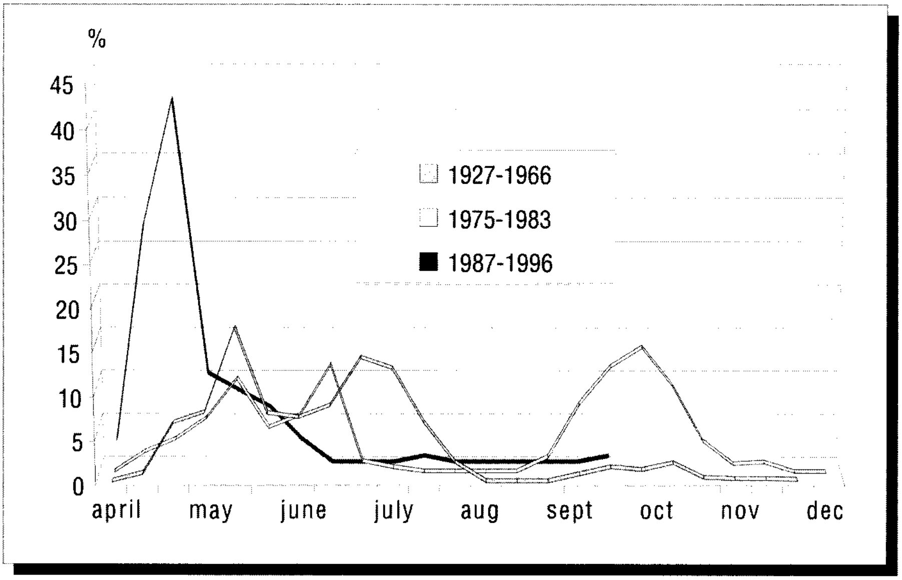


Figure 4

Release of juvenile *A. gueldenstaedti* and *A. stellatus* into the Kuban River basin 1974-1995 (millions)

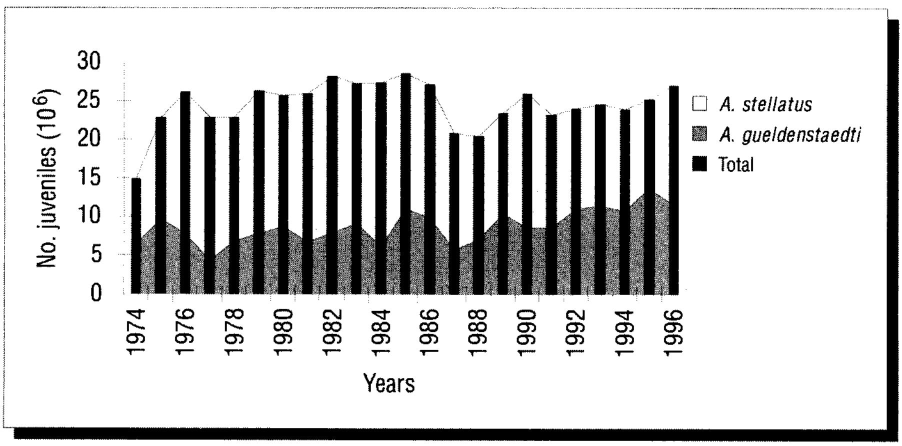
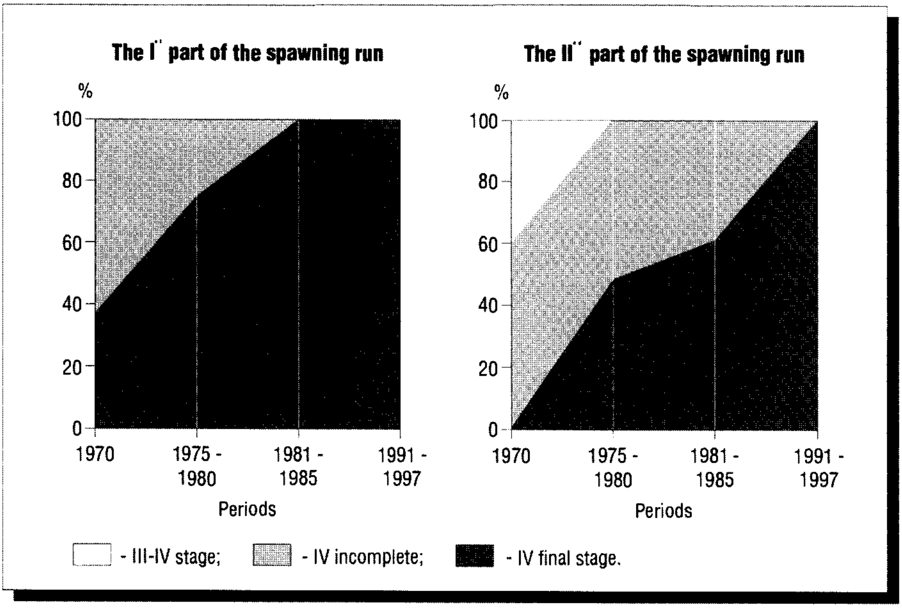


Figure 5

Comparison of stage of gonad maturity in female *A. stellatus* at different stages in the spring spawning migration period



the spawning run. Such an intrapopulation differentiation made possible maximum use of grazing areas and different spawning sites.

In recent years females with gonads even at IV incomplete stage have been absent. Only very ripe individuals have been captured in the period of mass spawning.

Thus the regulation of river flow, combined with long-term artificial selection restricted to early spring migrants, has caused an adaptive sturgeon response of high functional maturity which permits breeders to spawn at the lower dams.

Artificial Breeding and Enhancement

The main problem this has created for artificial reproduction is the long-term preservation of functional maturity in brood fish at hatcheries, especially in view of the decreased duration of the spawning run and an increase in the number of coastal nets catching fish far from the river mouth.

For that reason the total duration of juvenile release (about 25 million pieces) from all the hatcheries into the river is restricted to 12 to 15 days.

Traditional technologies for artificial breeding seem not to be effective in modern ecological conditions. When sturgeon stocks are enhanced by artificial reproduction, technologies should be oriented to the restoration of natural heterogeneity of populations by using not only spring-spawning but also winter forms of brood fish.

It is necessary to maintain each structural unit of the population system as a unique bearer of local adaptations in order to conserve diversity while exploiting natural resources.

The development of environmental and hormonal methods of managing seasonal propagation of sturgeons makes it possible to shift the reproduction cycle of migrants of various species to earlier dates (to five months) and later ones (to six months) and to provide year-round progeny from "wild" broodstock (Figure 6).

Methods for transferring sturgeon spawners from the resting state into the spawning temperature regime for different species depends on the length of time they are kept at low temperatures. As investigations have already shown, it is not possible to move sturgeon into the final stage of the reproduction cycle by a simple linear increase of temperature, as earlier proposed (Kazanskii, 1973).

It has been established that long-term holding of breeders at low temperatures requires an oscillating temperature regime.

Statistical analysis of hatchery experimental results and the physiological state of fish makes it possible to determine optimal conditions for preparing wild breeders from different biological groups for hormonal injection after different durations of low-temperature holding (see Figure 7).

River Survival

One of the goals of effective reproduction is an increase in the survival of reared sturgeon juveniles, which during the first months after release into the river is presently only 1-3 per cent. This is a result of predation, mortality in agricultural water intakes, and changes in estuarine salinity.

Figure 6

Technological scheme for year-round reproduction of different species and seasonal forms of sturgeon

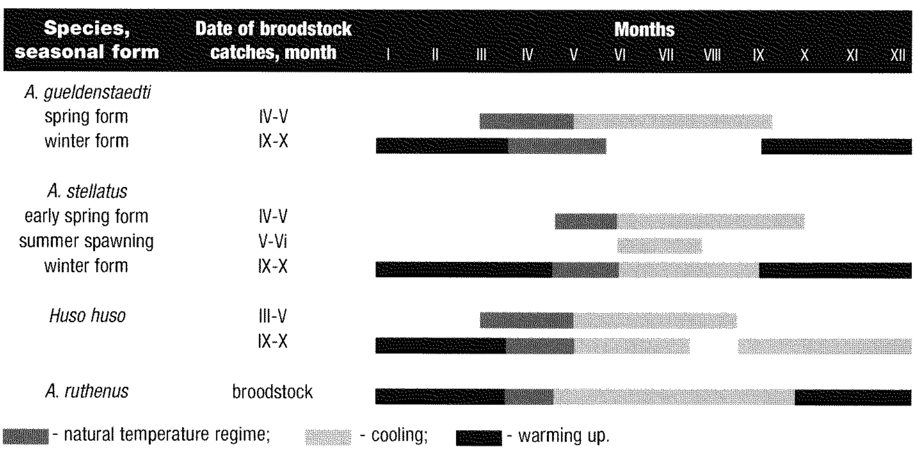
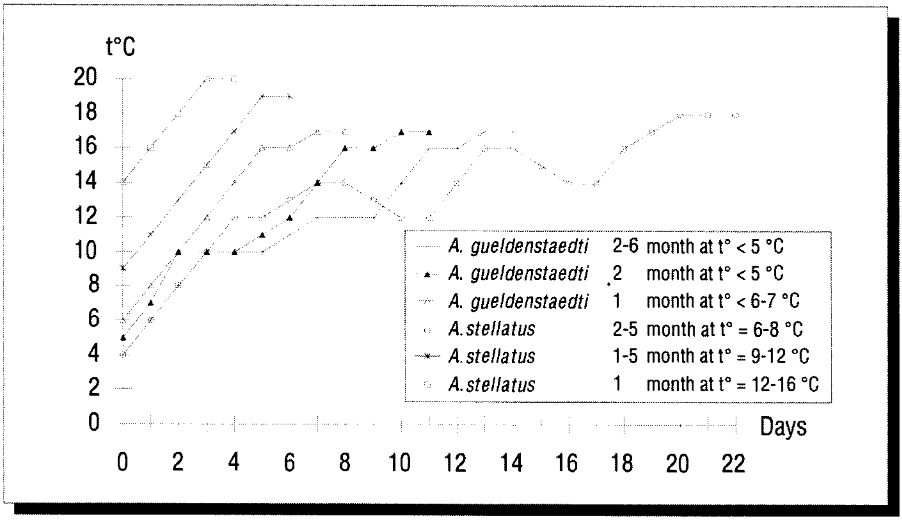


Figure 7

Regime of "wild" sturgeon broodstock transition into the spawning state after long-term holding at low temperatures



Research on seasonal regime dynamics, food sources in brackish lagoons and rearing ponds of sturgeon hatcheries, index of survival and rates of growth of various age-graded Russian and stellate sturgeon juveniles produced by natural and artificial reproduction in different conditions have resulted in a proposal for a new scheme of releasing juveniles to natural water bodies. This consists of three main variants of juvenile location in river estuary areas or coastal brackish lagoons, depending on rearing dates, mean mass, species, and year type (wet or dry).

Different release locations of sturgeon juveniles of various sizes and ages in various conditions would help maintain artificial populations and minimize selective consequences of artificial reproduction. Sturgeon juvenile migration into the sea in natural conditions at different ages has a deep adaptive meaning which confirms the biological importance of intrapopulation differentiation. The conservation of a variety of Russian stellate and giant sturgeons, released to grazing at different dates, permits a gradual and more rational use of the feed base of brackish lagoons and coastal parts of the sea, compared with traditional large-scale and simultaneous standard juvenile release into rivers.

Thus, when hatchery propagation is the main source of sturgeon in the Sea of Azov, its ecological-biotechnological conception should be based on the restoration of heterogeneous sturgeon populations. Maintenance of genetic fitness must not be compromised.

Unfortunately, the use of modern biotechnology for sturgeon reproduction, rational management in fisheries and restriction of water consumption are already insufficient to protect stocks. In the Azov basin, sterlet *(A. ruthenus)* is almost non-existent and ship sturgeon *(A. nudiventris)* has completely disappeared.

Living Gene Banks

One of the most urgently needed measures for conservation of sturgeon biodiversity is living collections. Conservation of complicated population systems requires an assessment of total genetic variability, including intrapopulation variability. This is needed to ensure the adequate conservation of genetic resources according to the population structure of different species.

Since 1994 we have begun the formation of a federal live collection of the Azov and Black Sea species of sturgeon for the conservation of sturgeon biodiversity. The main sturgeon species included in this collection are shown in Table 1.

The main principles for collection of all sturgeon species and groups are:

- to catch spawners of natural populations in order to study genotypes;
- to equalize the genetic contribution of each individual to the following generation;

Table 1

Conservation status of sturgeon held in the Krasnodar living gene bank

Species	English name	Distribution	Status (national listing of latest studies) Status [6]	Reference	IUCN listing 1996 [5]	CITES Appendix1997
Acipenser gueldenstaedtii	Russian sturgeon	Black, Azov, Caspian Seas basins	VU	Lelek 1987	EN	II
		Sea of Azov population	VU, H	Volovik et al. 1993	EN	II
A. nudiventris	Ship sturgeon	Caspian, Black Seas and rivers entering into	EN	Pavlov et al. 1985, 1994	EN	II
		them (Russia, Ukraine)	H	Sokolov & Vasilev 1989	EN	II
A. persicus	Persian sturgeon	Caspian, Black Seas basins	EN	Lelek 1987	EN	II
		Black Sea population	CR	Pavlov et al. 1994	EN	II
A. ruthenus	Sterlet	Caspian, Black Seas basins	EN	Lelek 1987		II
		Azov Sea	CR		CR	II
A. stellatus	Stellate sturgeon or sevruga	Black, Azov, Caspian Seas basins	H	Khodorevskaya et al. 1997	VU	II
		Sea of Azov	VU, H	Volovik et al. 1993	EN	II
Huso huso	Giant sturgeon or beluga	Caspian, Black Seas basins	H, VU	Khodorevskaya et al. 1997	EN	II
		Azov Sea population	EN	Pavlov et al. 1994	CR	II

5. Proposals of the Sturgeon Species Group, IUCN (Birstein 1997)

6. Categories are given in the new IUCN system (IUCN Red List Categories 1994: CR = critically endangered; EN = endangered; VU = vulnerable; R = risk; H = hatcheries) designates species whose natural reproduction is limited; such species are artificially bred and juveniles obtained are released into their natural habitat

- to domesticate different age-graded wild individuals for use as broodstock of species formerly existing in other regions but not observed at present *(A. ruthenus, A. nudiventris)* including breeders of different age, fish with different dates of spawning run and spawning;
- to hold separate groups in different ecological conditions;
- selection, tagging and genetic and physiological monitoring; and
- to identify optimum holding conditions for various interspecific and intrapopulation groups.

It is desirable, while forming a collection, to avoid a prevalence of sibs. To do so, it is necessary to catch fish of different spawning run periods and to take every opportunity to enrich this group with wild juveniles.

Long-term low temperature holding facilities for brood fish and warm water farms are used to ensure optimal breeding conditions of different species and seasonal forms of sturgeons, and optimal schemes for crossing fish in the collection.

To enhance the survival rate of brood fish, a new method (oviduct incision) of obtaining ovulated eggs is used, thus avoiding incision of the abdominal cavity. This is important because the genital anatomy of female sturgeons does not allow stripping of ovulated eggs, as in teleosts.

The Krasnodar Sturgeon Collection Complex, which has about 6,000 fish, is the sturgeon complex closest to the region allowing the highest possible catch of Atlantic sturgeon *(A. sturio)* broodstock—the Rioni River at the Black Sea. Although it is not possible at present to catch a mature male and female simultaneously, a spawner can be kept at low temperature until a male is caught or cryoconserved sperm (e.g., from the centre of CEMAGREF, Bordeaux) is obtained.

A similar successful experiment, with long-term maintenance (more than two months) of an endangered ripe female giant sturgeon, caught in the Sea of Azov and weighing 90 kilograms, was carried out between April and June 1997. Its offspring are being reared at our collection, and 130,000 juveniles have been released into the sea.

Androgenesis

Another means of genetic conservation of sturgeon in the Azov-Black Sea basins is by an improved method of dispermic androgenesis worked out at the Krasnodar Sturgeon Collection Complex (Recoubratsky et al. 1996).

Siberian sturgeon *(A. baeri)* and starred stellate sturgeon *(A. stellatus)* were used in experiments on the induction of dispermic androgenesis. The eggs of these fish were X-irradiated at a dose of 20 kR and inseminated with sperm collected from several males and diluted 1:10 or 1:100. The last dilution of sperm is commonly used for artificial insemination of sturgeon eggs to prevent polyspermic fertilization. To promote fusion of male pronuclei, heat shock was used (37°C, duration 2.5 or 3 min) during the period of the first cleavage. Morphologically normal larvae were obtained from the eggs fertilized with concentrated sperm both in Siberian and ship sturgeons. Karyological analysis confirmed their diploidy (248 chromosomes in *A. baeri* and about 129 chromosomes in *Huso huso*). These larvae proved to be fairly viable; about 50 per cent of them developed into fingerlings.

In 1997 the experimental production of viable androgenetic endangered sturgeon indicates that dispermic androgenesis can be used for reconstructing genotypes of sturgeons using the genetic information in spermatozoa alone.

There is Also a Need:

- to study the effect of different anthropogenic factors on genetic population structures and to create models that make it possible to forecast the population state following different types of anthropogenic influence;
- to develop a genetic basis for artificial reproduction that minimizes the consequences of selective catching by studying allozyme loci; and
- the development of mitochondrial DNA studies by restriction enzymes should be encouraged to obtain a finer definition of population structure and to obtain efficient markers of gene flow and phylogenetic information.

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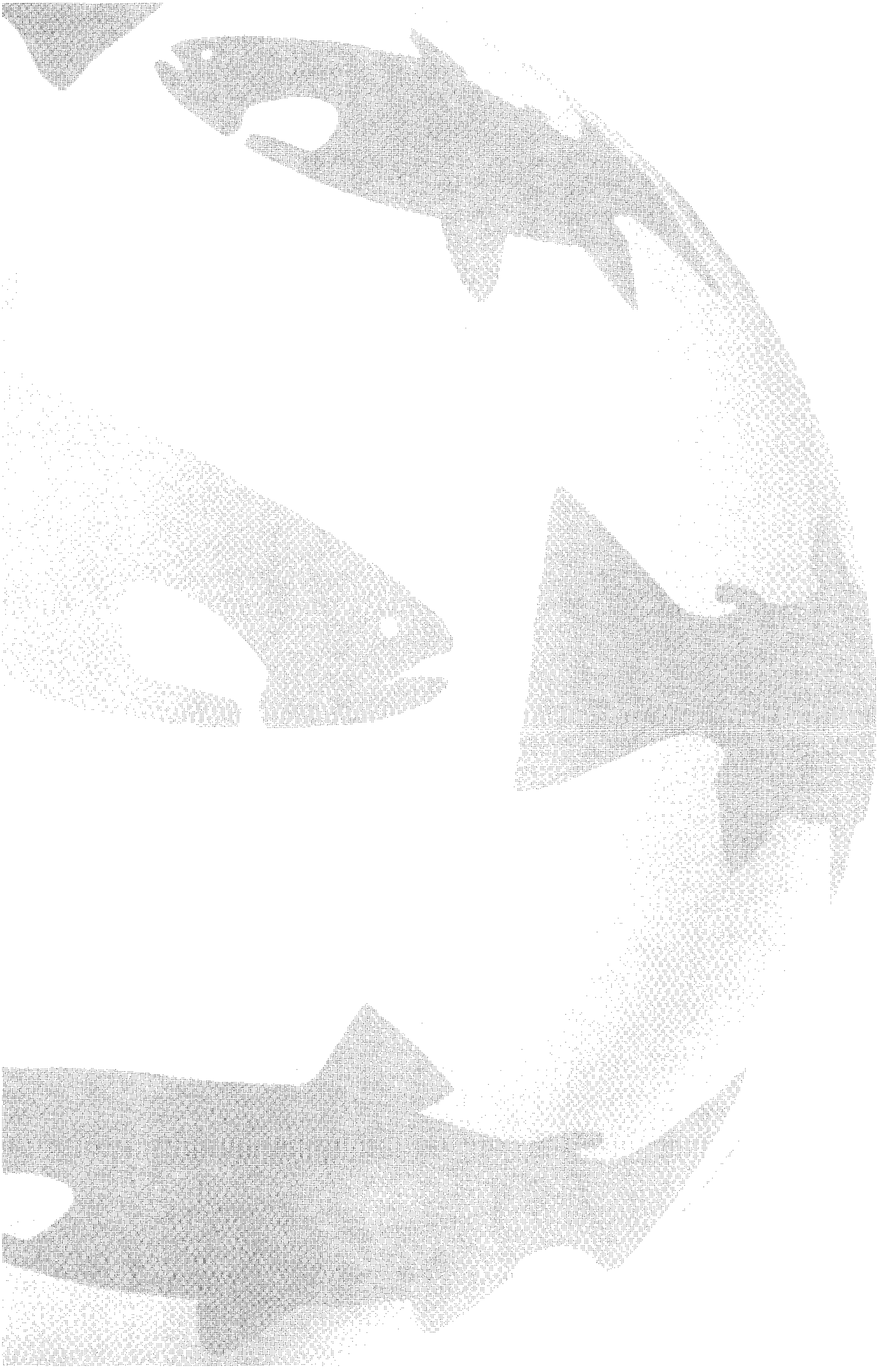

World Fisheries Trust's Experience in Fish Genetic Conservation

Brian Harvey [1]

Introduction

World Fisheries Trust is a science-based non-profit organization that works toward conservation of wild fish stocks through research, training and public awareness. Genetic conservation is one of WFT's program areas, and the organization has been involved both on the practical side (field gene banking and training) and in the development of policy since about 1991, mainly in Canada and South America (ICLARM 1998). In the first few years of WFT's existence, genetic conservation was proportionately more significant (in terms of budget and staff time) than it is today. At present, the bulk of our genetic conservation field work is in South America, and most of the rest of our time is devoted to research (salmon enumeration, DNA fingerprinting studies, enhancement, economic valuation studies, policy development research) and public awareness. Wherever WFT has been involved in fish genetic conservation the rationale has been the same: a broad range of wild genetic material is required for recovery programs aimed at rebuilding wild stocks and for development of sustainable culture of local species, and in many cases that wild genetic material is disappearing before habitat recovery, changes in fishing or other remedial measures can have any effect.

Genetic Conservation Fieldwork and Training in Canada

Canada has anadromous salmon populations on both coasts, most of which are born, die and spend at least half their lives in fresh water. The degree of genetic diversity contained in the many thousands of distinct reproductive populations or stocks makes them an extraordinary example of freshwater biodiversity in their own right, and

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presents as well an enormous challenge in conservation and management of that biodiversity. The depletion and eventual demise of the single-species Atlantic salmon fishery happened years before the world's attention had swung to fisheries collapses and even to freshwater biodiversity as something worth worrying about—the last commercial opening for Atlantics in Canada was in 1986. But Pacific salmon, of which Canada's six species comprise at least 5,000 genetically distinct stocks, are front and centre in the country's collective mind as this is written.

Concerns based on incomplete knowledge

In 1993 the American Fisheries Society undertook what was probably the most ambitious status report on freshwater biodiversity to date, a review and analysis of the number of fish in each of the more than 10,000 genetically distinct populations of six species of salmon in BC and the Yukon. The final report (Slaney et al. 1996) appeared in late 1996, and its main message—that, at the aggregate level salmon stocks other than coho were healthy but that in terms of maintaining the biodiversity represented by small, less economically important stocks there was cause for serious concern—was no surprise for scientists well aware that biodiversity was being lost as weaker stocks felt the pressure of sustained harvest on larger ones.

What was perhaps just as alarming were the study's limitations when it came to assessing the most critical components of salmon biodiversity: lack of information on many stocks dictated basing conclusions on only 57 per cent of the total. For over 4,000 stocks there were insufficient data to draw conclusions; the data do not exist because the stocks have little commercial importance and are infrequently assessed, but, as the authors point out, they are critical to the maintenance of biodiversity. Lack of data on nearly a thousand stocks resulted in estimates of extinctions that were biased low. Limitations in the two major, "official" databases on salmon stocks forced the authors to appeal widely to fisheries professionals and interest groups for verification of status.

The American Fisheries Society study sets the tone for salmon gene banking in Canada: inadequate and out-of-date data leading to conclusions that could alarm or reassure, depending on the audience. World Fisheries Trust's gene banking activities should be viewed in this light. World Fisheries Trust's genetic conservation activities in Canada are primarily in support of conservation of wild genetic diversity, although we have also provided technical advice to a number of aquaculture companies who wish to safeguard particular farmed broodstocks.

Field gene banking started in 1992 in response to a request from First Nations for technical assistance in preserving dwindling genetic variability in several species and stocks. Three years of collection and training of aboriginal workers followed; the Shuswap Nation have so far collected and cryopreserved genetic material from six stocks (three species). Although none of the collected material has yet been used, Fortier (this volume) describes the program and tentative plans for using some of the coho material in 1998. WFT has also trained workers from several other aboriginal groups; one of these, the Carrier-Sekani First Nation, has for the past three years pursued its own gene banking program.

The Canadian Department of Fisheries and Oceans, the agency responsible for management of most of Canada's anadromous fish species, enlisted World Fisheries Trust's technical assistance in a two year pilot gene banking program prompted by concerns for certain Fraser River sockeye stocks. WFT collected and cryopreserved 2,000 samples from 750 fish representing 15 stocks in 1995 and 1996. All of this genetic material is currently held in storage.

Methods

WFT's field gene banking in Canada is done entirely "on the river". Sperm is collected from unanesthetized wild males captured by net or weir, and cryopreserved immediately, on site, in large volume (10 ml) plastic straws. WFT employs a portable cryo kit with a working time in the field of about two weeks; the kit is brought to the scene of collection. We do not perform pre-freeze motility checks, although random samples are checked for post-thaw motility before a shipment enters long term storage. Samples are stored at the BC Artificial Insemination Centre (a livestock semen bank) and accessions are managed using SpermSaver, a software program developed at WFT. In general, the technique allows rapid response to remote areas, portability and the low cost achieved by freezing on site and storing under contract in a large volume semen bank.

Fertility using the frozen-thawed sperm is primarily dependent on the sperm:egg ratio used and is comparable to that reported for other salmonid sperm cryopreservation methods. Apart from fertility testing, none of the stored sperm has yet been employed in recovery programs. This may in part be due to the lack of a policy framework for fish gene banking in Canada. As this is written the Government of Canada is drafting policy on gene banking; without such policy, managers will not develop comprehensive strategies for collecting and using fish genetic resources in conservation.

Genetic Conservation Fieldwork and Training in South America

WFT began to adapt its field cryopreservation technology to South American migratory fish species in 1993 and has since worked in Colombia, Venezuela and most recently Brazil. We are involved as trainers rather than as practitioners of gene banking, and provide a Training Course on Fish Genetic Conservation that includes theoretical, practical and policy aspects of gene banking.

Gene banking migratory species in South America is done using the same field kit as used for salmonids, with modifications for sperm volume. Sperm from the South American species is generally frozen in 0.5 ml plastic straws, and cryoprotectants are modified from the mixtures used with salmon. Collection methods must also take into account the frequently smaller sperm volume delivered by these species. Training courses have been delivered in Venezuela, Colombia and Brazil to a variety of audiences from government, academia and the private sector. Fish for which successful field methods have been developed include *Piaractus, Prochilodus, Salminus, Brycon, Leporinus* and *Pseudoplatystoma.*

Colombian requirements for genetic conservation of migratory species are described by Diaz (this volume).

In 1997, WFT entered into a three-year CIDA-funded project to provide genetic conservation training to a variety of Brazilian partners from government, academia, the hydro-electric power sector and conservation NGOs. The aim of the project (see contributions by Godinho and Zaniboni, this volume) is to preserve migratory fish genetic diversity and utilize it in restocking programs and in the development of culture of indigenous species.

Development of Policies for Fish Gene Banking

World Fisheries Trust's field activities in gene banking and training have at times illuminated a lack of policies for collection and equitable sharing of fish genetic resources. In general, few nations or local governments have equipped themselves with such policies; as Pullin and Raymond point out (this volume), this lag may be due to the relatively early stages of utilization of global fish genetic resources. Whatever the reason, it can be a real impediment to conservation (Pullin et al., 1998). To try and address the need for policies, WFT is carrying out a project to develop model policy for equitable sharing of benefits from fish genetic resources by local and indigenous communities.

Acknowledgments

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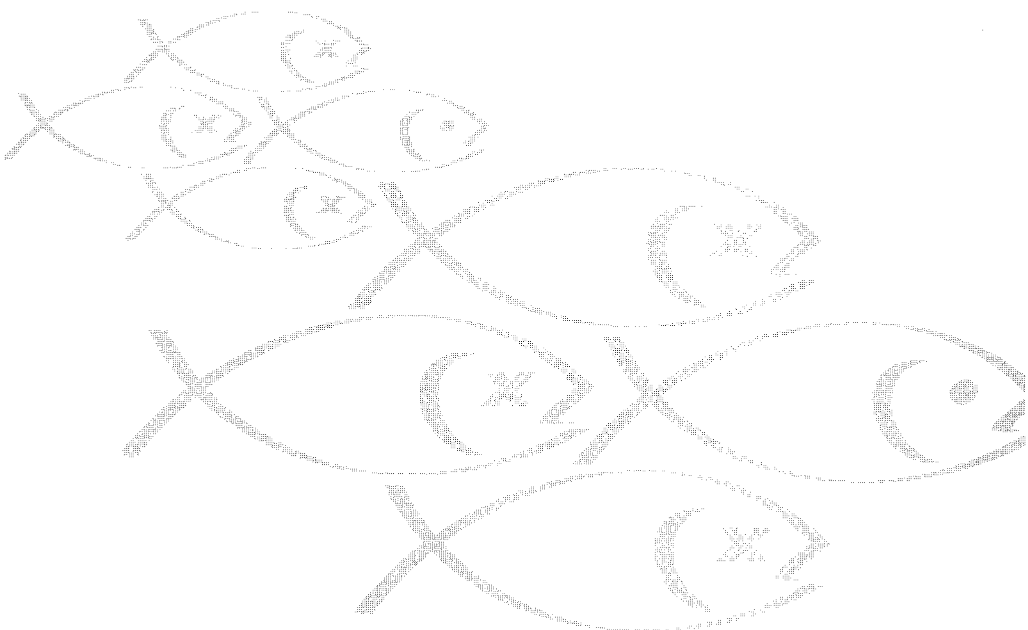

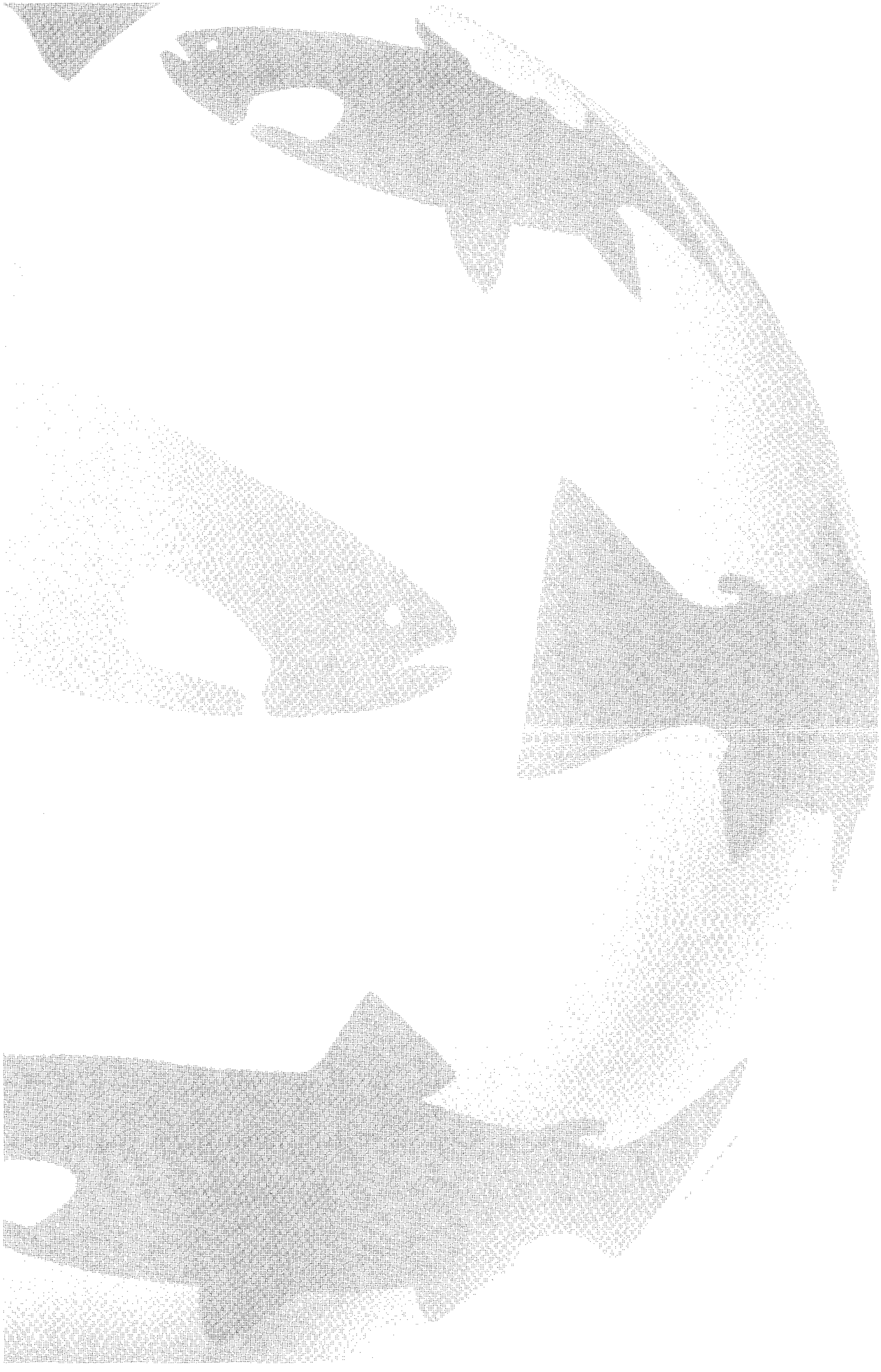

Gene Banking Efforts for Endangered Fishes in the United States

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Introduction

A number of fish species have declined in population size or become extinct in the United States, primarily as a result of habitat changes (Ono et al. 1983; Minckley and Deacon 1991; Cloud and Thorgaard 1993; Magnuson et al. 1996). One approach that has been considered in some cases where drastic declines have occurred is the use of gene banking based on sperm cryopreservation to avoid the loss of valuable genotypes. However, such efforts have been relatively limited to date. This paper reviews some of these efforts and identifies some factors that have limited the application of this conservation approach in the United States.

Government in the United States is relatively decentralized compared to that in many other countries. The national (federal) government has responsibility for anadromous and marine fishes and for enforcement of some national regulations (e.g., the U.S. Endangered Species Act). Relevant federal agencies include the U.S. Fish and Wildlife Service, which is primarily concerned with freshwater fishes, and the National Marine Fisheries Service, concerned with marine and anadromous fishes. The 50 States also have authority over fishes within their borders. Examples of State management agencies in the northwestern United States include the Washington Department of Fish and Wildlife and the Idaho

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Department of Fish and Game. Indian tribes within the United States have their own governmental authority and frequently have their own fishery management agencies and biologists. A given fish population may thus be subject to management by several agencies. Management of the salmon of the Columbia River is a prime example of this overlapping responsibility. This rather complex situation does not always contribute to the most efficient management of the populations.

Given the size of the United States and the complexity of the web of agencies managing fishes in this country, we likely may not have identified the full scope of gene banking efforts for fishes in the United States in this review. However, in discussing this issue with people actively engaged in such efforts we believe that we have identified the major focus areas and programs for endangered fishes. The three major foci of gene banking efforts in the United States appear to be (1) Columbia River salmon, (2) Colorado River fishes, and (3) Midwestern and Eastern US sturgeon. We will review each of these foci in order and then discuss some of the overall issues and constraints common to these efforts.

Columbia River Salmon

The Columbia River was historically one of the largest producers of salmon in the world. Unfortunately, primarily due to problems associated with the passage of juveniles around hydroelectric dams which have been built in this drainage, the salmon runs have declined drastically (Magnuson et al. 1996). This has led to the listing of populations of chinook and sockeye salmon in the Snake River, the principal tributary of the Columbia, as endangered under the U.S. Endangered Species Act. In spite of this listing, the populations have continued to decline.

The Nez Perce tribe, with its ancestral homelands in the Snake River basin, has been particularly sensitive to this decline. They have initiated a program to collect sperm from endangered Snake River chinook salmon (Paul Kucera, Nez Perce Tribal Department of Fisheries Resource Management, personal communication). The sperm is collected and stored at the University of Idaho in the laboratory of Joe Cloud and at Washington State University by Paul Wheeler in the laboratory of Gary Thorgaard (Thorgaard et al. 1998). Sperm from a total of 259 individual chinook salmon has been collected and stored from 12 Snake River tributaries. In addition, these universities are storing sperm from 20 other chinook salmon and from 122 Snake River sockeye salmon for the Idaho Department of Fish and Game. Additionally, the Washington Department of Fish and Wildlife is using cryopreservation in its programs in southeastern Washington for Snake River chinook salmon. Together, these efforts represent the most developed gene banking effort at this time for fishes in the United States.

Sperm cryopreservation is also being used in efforts to propagate the endangered winter-run chinook salmon of the Sacramento River (Kristen Arkush, University of California Bodega Marine Laboratory, personal communication). These efforts are primarily focused around breeding management, although sperm samples have been held for as long as five years and could be used in recovery efforts in the event of future population declines.

Colorado River Fishes

The Colorado River flows through one of the most arid parts of the United States, and there has been considerable development of dams in this basin to allow maximal utilization of the limited available water resources. This impoundment of the river has resulted in dramatic alteration in the habitat of much of the basin and declines in abundance of the native fishes, primarily cyprinids and catostomids (Ono et al. 1983; Minckley and Deacon 1991).

Unlike the salmonids, the technology for cryopreservation of the Colorado River fishes had not been previously developed. Work at Louisiana State University has developed these methods for several Colorado River fish species (e.g., Tiersch et al. 1997; Tiersch et al. 1998). These efforts complement the captive breeding programs which are ongoing for these species at U.S. Fish and Wildlife Service facilities such as the Dexter, New Mexico National Fish Hatchery (Johnson and Jensen 1991).

Although hundreds of sperm samples are being held at Louisiana State University, no comprehensive gene bank has yet been developed for Colorado River fishes. Some biologists in this region have expressed concerns that a gene bank might divert efforts away from habitat protection and enhancement.

Midwestern and Eastern U.S. Sturgeon

A number of sturgeon species have declined in the midwestern and eastern United States, primarily due to habitat changes but also due to overfishing on these long-lived and late-maturing fishes. The technology for sperm cryopreservation is not as well developed for these fishes as it is for the salmonids, cyprinids or catostomids. This may be due to differences in sperm cell structure, including the presence of an acrosome.

Interest in sturgeon sperm cryopreservation comes from both an aquaculture and a conservation standpoint. The efforts by Steve Mims at Kentucky State University and George Brown at Iowa State University have resulted in the improvement of methods for cryopreservation of sperm from paddlefish and shovelnose sturgeon (Steve Mims, Kentucky State University, personal communication). Also in this region, the laboratory of Konrad Dabrowski at Ohio State University is working to develop a Midwest Sperm Bank which is designed to maintain a collection of lake sturgeon (Ciereszko et al. 1996), perch, walleye and muskellunge samples (Konrad Dabrowski, Ohio State University, personal communication). In the Southeast, the U.S. Fish and Wildlife Service's Technology Center at Warm Springs, Georgia, under the directorship of Vincent Mudrak is involved in cryopreservation of sperm from the shortnose sturgeon, as well as the robust redhorse, a catostomid species (Greg Looney, Warm Springs Technology Center, personal communication) in collaboration with Bill Wayman and Terry Tiersch of Louisiana State University.

Common Issues and Constraints

The complexity of governmental structure in the United States has likely been one of the factors that has limited the development of gene banking efforts for fishes in the country. With several agencies often involved in the management of a specific fish population, the question of who is ultimately responsible for preventing extinction of a given population is sometimes unclear. This has been evident in the Pacific Northwest with salmon, where divided responsibility can lead to confusion and inaction.

The ecological, as opposed to agricultural, background of many fishery biologists may also have contributed to inaction in the gene banking arena. While people with an agricultural background are comfortable with the notion of gene banks, having been exposed to such activities for cattle and seed banks for crop plants, many ecologically trained biologists appear to view such efforts as a distraction and possibly even a threat to efforts to protect the habitat, which they correctly view as their top priority. However, when inexpensive efforts to provide an insurance policy for species approaching extinction are blocked under such circumstances, such good intentions may ultimately and ironically lead to the extinction of the very populations requiring protection.

Perceptions that cryopreservation is complicated and expensive have also hindered the application of cryopreservation to gene banking in fishes. The scientific literature on cryopreservation of fish sperm is distributed across numerous journals and fields with an apparent lack of agreement on suitable procedures for even the well-studied fishes such as salmonids. In reality, cryopreservation is relatively straightforward technically and the use of cryopreserved sperm for production is inexpensive. Recent efforts to convey the simplicity and cost-effectiveness of cryopreservation include special symposia held at the 1997 and 1998 annual meetings of the World Aquaculture Society. In addition, a book on cryopreservation of gametes and embryos of aquatic species is scheduled to be published in 1999 by the World Aquaculture Society with financial support from the U. S. Fish and Wildlife Service, Division of Fish Hatcheries.

In the long term, better coordination among agencies and better explanation of the rationale and cost-effectiveness for gene banking efforts, as well as the success of current programs, may lead to the increased use of gene banks to protect endangered fish populations.

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Fisheries Management and Conservation in Southeastern Brazil: Current Status and Needs

Hugo P. Godinho [1]

Introduction

Neotropical freshwater fish fauna

Neotropical freshwater fish fauna comprise more than 2,400 species and is the most diversified and rich in the world (Lowe-McConnell 1987). South American fish include the following major groups: *Otophysi, Cyprinodontiformes, Cichlidae* and marine invaders *(Sciaenidae)* (Britski 1992). Over 90 per cent of Neotropical species are in the *Otophysi* group, and commercial fisheries in the neotropics focus primarily on large migrant *Otophysi* fishes (Table 1).

Characiformes comprise many families and are distinguished by the presence of scales covering the whole body except the head. They have pelvic fins placed ventrally to the abdomen and premaxillary, non-protactile bones fixed to the cranium. *Characidae* is the largest and most complex characiform family, including about 250 South American genuses with highly diversified feeding habits (herbivorous, omnivorous and carnivorous). *Anostomidae* are usually herbivores living in large rivers. They have asymmetrically incisive teeth ('rabbit teeth') and are grouped in about 10 genuses. *Prochilodontidae* are river-bottom (mud) feeders with thick, protractile lips that help them ingest food.

The main feature of *Siluriformes* is a naked body without scales and covered by a thick skin or bony plaques, which may partially or totally cover the body. They have three pairs of barbels, and the first ray of the dorsal and pectoral fins frequently transform into a large and strong spine. *Pimelodidae* are composed of species measuring from a few centimetres to over two metres in length.

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Table 1

Systematics of main commercial migrants in southeastern Brazil

Series Otophysi (fish bearing the Weber apparatus)
Order Characiformes
Characidae
Salminus spp.
Brycon spp.
Piaractus spp.
Anostomidae
Leporinus spp.
Prochilodontidae
Prochilodus spp.
Order Siluriformes
Pimelodidae
Pseudoplatystoma spp.
Paulicea luetkeni

In central-west and southeast Brazil the main commercial migratory fishes belong to one of the families listed above.

Salminus

The characid *Salminus* comprises two very important species: *S. brasiliensis,* native to the São Francisco river basin, and *S. maxillosus,* from the Paraná-Paraguai river system. These two species are similar, differing only in certain meristic parameters. Both are bright yellow—hence the name 'dourado' (golden fish). They inhabit large rivers, are very voracious and piscivorous, and weigh up to 30 kg. Together with *Pseudoplatystoma,* they are the most admired sport fish and are valuable as table fish. Their stocks are low and are considered extinct in many sites, such as the upper São Francisco River. Both species are considered vulnerable to extinction in the State of Minas Gerais (Lins et al. 1997).

Brycon

These characid fish comprise several large and middle-sized species which occur throughout Central and South America. They feed on insects and plants, mainly fruits and seeds (Britski et al. 1984). *Brycon cephalus* ('matrinchã'), *Brycon lundii* ('matrinchã', which may reach 7 kg of body weight) and *Brycon orbignyanus* ('piracanjuba') are the main representatives of the genus in, respectively, the Amazon, São Francisco and Paraná rivers.

These species have recently drawn the attention of fish culturists due to their fast growth, herbivorous feeding habit and highly appreciated meat, as well their value as a sport fish. Natural stocks of both *Brycon lundii* and *Brycon orbignyanus* are in rapid decline as a consequence of the intensive removal of riparian vegetation in their respective river basins (Mendonça and Melo 1994). These species are also in the Red List of the State of Minas Gerais; *B. lundii* is vulnerable, and *B. orbignyanus* is in danger of extinction (Lins et al. 1997).

Piaractus

The 'pacu' *Piaractus mesopotamicus* (formerly *Colossoma mitrei*) and the Amazonian 'tambaqui' *Colossoma macropomum* form a group of important native fish successfully cultivated in most of tropical South America (Hernández 1989). Pacu, a native of the Paraná-Paraguai river basin, reaches several kg in body weight and is still abundant despite being subject to heavy fishing pressure in the Pantanal region (Paraguai river basin).

Leporinus

The anostomid family comprises several species, two of which are important in commercial fisheries in southeastern Brazil—*Leporinus macrocephalus* from the Paraguay river basin and *L. elongatus* from the Paraná and São Francisco river basins. Both are herbivorous and at times insectivores. *L. elongatus,* the largest known anostomid, may reach 7.5 kg in weight. Its stocks are dangerously low in some parts of the São Francisco river basin.

Prochilodus

The mud-eaters (prochilodontid fish) include a group of species of great importance to river and hydroelectric reservoir fisheries. Almost 20 per cent of the freshwater fisheries yield comes from fishes of this family (IBGE 1988). *Prochilodus marggravii,* an endemic species to the São Francisco River, is the largest fish of the family, weighing up to 15 kg. Although it is one of the most important fish in the São Francisco fisheries, its stocks have been steadily declining and are in risk of extinction in various parts of the river basin.

Pseudoplatystoma

The piscivorous catfishes *Pseudoplatystoma* are the largest Pimelodidae found in the Amazon, Paraná-Paraguai and São Francisco rivers. *P. coruscans* ('surubim', 'pintado') is the only species that occurs in the São Francisco River, where it can weigh well over 100 kg. *P. coruscans* and *P. fasciatum* ('cachara') are found in the Paraná-Paraguai. *P. fasciatum* and *P. tigrinum* ('caparari') occur in the Amazon.

Surubim is the most appreciated freshwater table fish in southeastern Brazil, with prices usually higher than those of other well-known fish such as the Atlantic salmon.

In the São Francisco River, most professional fishing efforts are directed towards the surubim, which represented 86.3 per cent of the captures in a section of that river in the State of Minas Gerais in the 1980s (Godinho et al. 1997). Although there is circumstantial evidence that production is still decreasing, no current data is available.

Paulicea luetkeni

This large piscivorous cat-fish, popularly known as 'jau', is found in the Paraná and Amazon river basins where it lives in large water bodies and may weigh up to 120 kg. Due to intensive fishing and hydroelectric dam construction, its stocks are so drastically reduced that it has been classified as vulnerable (Lins et al. 1977).

Table 2

Main commercial fishes in the São Francisco River and their migratory pattern

Species	Migratory pattern
Pseudoplatystoma coruscans ('surubim')	Migrant
Prochilodus affinis ('curimatã-pioa')	Migrant
Prochilodus marggravii ('curimatã-pacu')	Migrant
Conorhynchus conirostris ('pirá')	Migrant
Rhinelepis aspera ('cascudo-preto')	Migrant (?)
Salminus brasiliensis ('dourado')	Migrant
Serrasalmus piraya ('piranha')	Sedentary
Lophiosilurus alexandri ('pacamã')	Sedentary
Hoplias lacerdae ('trairão')	Sedentary
Hypostomus spp ('cascudos')	Unknown
Brycon lundii ('matrinchã')	Migrant
Leporinus elongatus ('piau-verdadeiro')	Migrant
Pterygoplicthys etentaculatus ('cascudo')	Unknown

Source: Lamas (1993); and adapted from Godinho et al. (1997)

Migration of Neotropical fishes

Many Neotropical species are sedentary, showing only slight movement within their habitats. A significant number, however, migrate along the river basin (Petrere 1985). Migrations are cyclic and in shoals, and may occur for various reasons (Bonetto 1963), including reproduction and feeding.

Migratory fish have high commercial value and constitute the main commercial catches in most of the Brazilian river basins, as shown in Table 2.

Despite its biological importance, reproductive migration in Neotropical fishes has received little attention and is consequently poorly understood. Although circumstantial evidence of migratory activity has been registered in almost all Brazilian river basins, data are available for only a few rivers (Mogi-Guaçu: Godoy 1975; Amazon: Godoy 1979, Barthem et al. 1991; Parnaíba: Braga 1981; São Francisco: Paiva and Bastos 1982; Paraná: Borghetti et al. 1987) and the Promissão reservoir (CESP 1990). These studies have disclosed important regional variations in the migratory patterns of local species in relation to reproduction.

In southeast Brazil, reproductive migration (or 'piracema', an indigenous word) is performed towards the headwaters. It usually occurs in the first half of the rainy season, which extends from middle of September to middle of April, when river water levels and temperatures rise. The classical work carried out by Godoy (1975) between 1954 and 1963 revealed the presence of 'home grounds' for the migratory species of the Mogi-Guaçu/Grande River system. The breeding areas in the Mogi-Guaçu River were situated between two waterfalls, the Emas and Salto do Pinhal. After reproduction the fish returned to their feeding grounds in the middle Grande River, and remained there from January to

August, when they again started a new migratory journey of approximately 660 km to the Mogi-Guaçu headwaters.

In 1957-1959, Paiva and Bastos (1982) tagged 2,828 fish in the São Francisco River. Although migratory fish were recaptured, the results did not provide a good understanding of their migratory condition since there were recaptured only close to the releasing site. Tagging experiments with the pacu Colossoma mitrei (*Piaractus mesopotamicus)* at Promissão reservoir on the Tietê River (CESP 1990) enabled the recapture of individuals in non-reproductive stages 110 km upstream. More complex migratory patterns have been observed for the fish of the Plata River basin (Bonetto 1963) and the Amazon (Goulding 1979; Barthem et al. 1991). It is also possible that more complex patterns may be found in fish from the south-eastern Brazilian river basins.

Role of floodplains in the maintenance of migrant diversity and abundance

The importance of floodplain pools and lagoons in the life history of Neotropical fish has been recognized, at least for the São Francisco river basin, since the beginning of the last century (Saint-Hilaire in Sato et al. 1987) and recently by Pompeu (1997). Eggs and larvae of migratory species are carried down river and, depending on the river water level, may reach the floodplains. These are adequate areas for development of the fish larvae despite the possibility of early drought and competition and/or predation. Some pools and lagoons maintain their water levels throughout the year, whereas others may dry out completely before being flooded again. Episodic flooding allows juvenile fish to leave the area and new batches of eggs and larvae to reach it. Fish populations from temporary pools and lagoons tend to gradually disappear as water volumes decrease, especially during the dry season.

Thirty-seven species of fish were present in floodplain areas of the upper São Francisco River above the Três Marias dam (Sato et al. 1987). This corresponded to almost half of the number of species recorded in the region of Três Marias (Britski et al. 1984). Almost one-third of the floodplain fish abundance resulted from migratory species. Various commercial migratory species, abundant elsewhere in the São Francisco River, were not found in this floodplain. Coincidentally their capture in the Três Marias reservoir was low, suggesting that they were severely reduced in number or even extinct in that section of the river (Sato et al. 1987).

A recent survey of three floodplain lakes in the middle São Francisco River (Pompeu 1997) revealed the presence of 50 species (about one-third of the species of the entire river basin), eight of which were of migrants, including juveniles of *S. brasiliensis, P. coruscans, B. lundii, Prochilodus* spp. and *Leporinus* spp.

In a study of the fish fauna of floodplain lagoons of the Mogi-Guaçu River, Galleti et al. (1990) suggested that these environments represent true natural refuges for juveniles of species such as *Prochilodus scrofa*. It should be noted, however, that the piscivores *Serassalmus spilopleura, Hoplias malabaricus, Salminus maxillosus* and *Salminus hilarii* were also present and certainly exercised strong predation pressure on *P. scrofa* juveniles.

Hydroelectric dams and fish passage facilities

It is well accepted that hydroelectric dams have had highly negative impacts on southeastern Brazilian fisheries (Godinho and Godinho 1994). Large dams represent insurmountable obstacles in migratory routes, thus reducing fish reproductive success. Following damming, the flow regimen in the river section below the dam is regulated to meet hydroelectric industry needs, resulting in an absence of large floods. Besides drastic changes in water quality, extensive floodplain areas which used to be cyclically flooded dry up and are unable to fulfill their role as nursery places for larvae and young of migratory fishes. Changes in river flow thus primarily affect spawning habitat, shelter areas and factors that trigger spawning.

Fisheries in the river segment above the dam also suffer dramatic changes, depending upon the location of the dam in the river basin. Permanent flooding of spawning and nursery habitat and reduction of the length of the river stretch available for migration are the main negative environmental changes.

Proposals for the construction of fish passes (especially ladders) at hydroelectric dams always emerge when management and conservation of the impacted fish fauna are under discussion. Although they have little in common, Brazilian experiences with fish ladders began in the 1930s and were based on the American model. Legal requirements to build fish ladders in rivers subjected to impoundment provoked, in the past, heated discussions between Brazilian legislators and scientists. Almost seven decades ago, von Ihering (1929) suggested that fish ladders could contribute to the maintenance of fish stocks in impounded rivers provided that the obstacle to be surpassed did not exceed 12 metres.

According to Godoy (1985), the arbitrary limitation of the maximum height for fish ladders at a low value (von Ihering 1929; Mendes Sobrinho 1969; Machado and Alzuguir 1976; Torloni 1984) has caused serious constraints to the development of a Brazilian fish pass expertise. Despite all the discussions, 30 fish ladders were built in the State of São Paulo alone (Mendes Sobrinho 1969).

It was estimated that one-tenth of the migrating fish population, comprising at least six species, pass over the Emas Falls through its fish ladder (Godoy 1945). I had the opportunity to capture fish in the fish ladder of the Salto do Morais hydroelectric dam in the Tijuco River (Paraná river basin) in order to evaluate their capacity to move up the ladder steps. The ladder has 25 steps (tanks) and is 78.3 metres long, 2.5-3.0 metres wide and 10.8 metres high. Over 41 species were captured in Salto do Morais area, at least 34 of which were present in the ladder. However, there were few individuals of these species, and only 2 per cent reached the upper section of the ladder. This suggests that the fish ladder is selective and obstructs the passage of all migratory species except *Leporelus vittatus* (Table 3).

If all hydroelectric dams proposed in the region were actually constructed, most major southeastern rivers would be transformed into a succession of large artificial lakes. Under such conditions, migratory species would have difficulty maintaining their stocks. Even in cases where affluents may improve conditions for migration and reproduction, floodplain pools and lagoons required for population recruitment would be insufficient. Moreover, the height of modern dams imposes serious constraints on the inclusion of fish ladders due

Table 3

Migratory fish captured at different levels in the fish ladder on the Tijuco River*

Species	N	R.A. (%)	Fish in the ladder %		
			L.T.	M.T.	U.T.
Leporellus vittatus	68	96.2	60.0	32.4	7.6
Salminus hillari	52	0.0	70.0	30.0	-
Leporinus elongatus	9	28.6	88.9	11.1	-
Pimelodus maculatus	105	22.7	100.0	-	-

*Based on Godinho et al. (1991); N = number of fish captured in the ladder; R.A. = per cent of fish in reproductive activity; L.T. = lower third; M.T. = middle third; U.T. = upper third

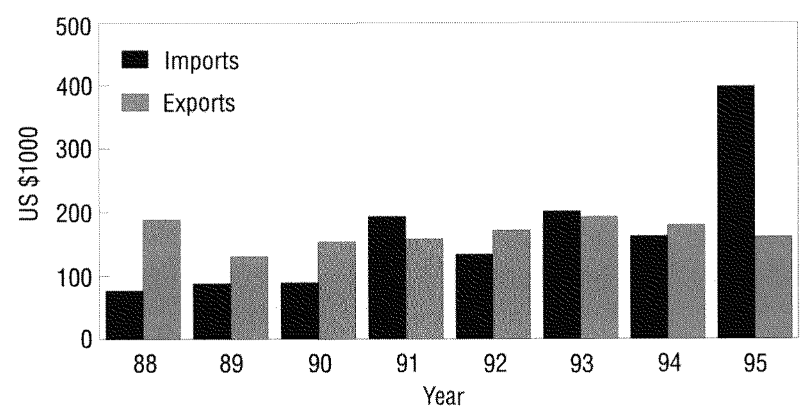
to biological and project design implications. However, due to the lack of substantial scientific knowledge about Neotropical fish ladders, the views expressed here should be taken with care.

The Present Fisheries Situation in Southeastern Brazil

According to recent Food and Agriculture Organization reports (FAO Yearbook 1992, 1995, 1997), Brazil shows the same deficit tendency observed in the international fisheries trade. The negative balance between Brazilian exports and imports has grown significantly in recent years and has been worsened by a decline in fisheries exports (Figure 1).

Figure 1

Brazilian fisheries international trade



Source: FAO (1992, 1995, 1997)

Despite the presence of large river basins, Brazilian freshwater fisheries production is less than 200,000 metric tons per year (Figure 1). Thus, larger deficits may be expected in the future. Although larger in the northern and northeastern region, Brazilian average fish consumption is only about 1 kg per inhabitant per year, which is well below the average world consumption of 19.1 kg per inhabitant per year. The significant growth in fish consumption that is expected to accompany the country's social-economic improvement would in turn provoke a greater international commercial deficit.

River fisheries

The present fisheries situation in the São Francisco River, one of the main large Brazilian river basins, exemplifies what is occurring in all other important river basins of the country. The river basin includes 7.4 per cent of the area of Brazil and is located between latitudes 21° 00' S and 7° 00' S. Historically, the São Francisco basin was one of the most important Brazilian fisheries resources, and in the 1980s about 25,000 fishermen worked in its waters (PLANVASF 1989). There is evidence, however, that its fisheries have consistently declined during the last 20 years. In the 1970s, the fisheries yield was estimated at 25 kg per fisherman per day, whereas in the 1980s it had fallen to about 11 kg per fisherman per day (Sato and Osório 1987; Godinho et al. 1997).

A report ten years ago (Miranda et al. 1988) pointed out that only one-third of the 2,400 fishermen belonging to fisherman associations in the upper-middle São Francisco River were full-time workers, since this activity was not enough to support their minimum living needs. Illiteracy predominated among the fishermen, who employed poor catch, conservation and commercial technologies. Their fishing boats were small wooden canoes with paddles, and the gear consisted primarily of gillnets and lines.

Besides directly affecting fishermen and their families, fisheries debilitation has also affected the tourism industry in the region, since thousands of anglers who used to fish in its waters are now going to other, more productive rivers.

Hydroelectric reservoir fisheries

By the end of 1989, Brazil had 60 hydroelectric power plants in operation, resulting in almost three million ha of reservoirs (almost 0.3 per cent of the Brazilian territory). Most of these plants are located in the southeastern region. The State of Minas Gerais alone now has 650,000 ha of reservoirs, based only on reservoirs larger than 100 ha. By the middle of the next century this figure will almost certainly double. Some of the most important rivers in this area, including the Grande and Araguari, have lost their riverine characteristics and have been transformed into a succession of artificial lakes. The 1990/1999 Brazilian energy program contemplates the construction of 65 new plants. These include 47 hydroelectric plants, some of which are already in operation or under construction. Again, half of these are located either in the southeast or central-west regions.

Reservoirs in southeast Brazil are today an important freshwater ecosystem and should be included in all management and conservation fisheries programs designed for the river basins where they are situated. Data on fishery resources and fish catches are

Table 4

Fisheries yields in some Brazilian reservoirs

Reservoir	River	Area (hax10³)	Age * (years)	Yield ($kg.ha^{-1}.year^{-1}$)
Tucuruí (1)	Tocantins	283	5	5.0
Boa Esperança (2)	Parnaíba	43	5	4.4
Sobradinho (3)	São Francisco	421	6	50.0
Três Marias (4)	São Francisco	80	25	5.0
Promissão (5)	Tietê-Paraná	65	16	4.0
Furnas (6)	Grande-Paraná	145	32	0.7
Itaipu (7)	Paraná	135	5	13.0

* Time between dam closure and year of data acquisition

Source: (1) Formagio, personal communication; (2) Paiva 1976; (3) PROTAM 1987; (4) Sato and Osório 1987; (5) CESP 1991 (6) Formagio 1995; (7) FUEM/NUPELIA 1987

available for several Brazilian reservoirs. However, they usually cover only short time periods, which do not allow for temporal analyses or evaluation of socioeconomic effects. Fisheries yields of some of these reservoirs are summarized in Table 4.

Yields are usually low except in the Sobradinho reservoir. Six years after dam closure, the relatively high fish production in this reservoir was probably due to the primary productivity increment that is characteristic of newly formed reservoirs. This is supported by the fact that three years later the fish yield had decreased to 30 kg per ha per year (PROTAM 1987). The large catchment area of the Sobradinho reservoir could also be responsible for its high yields, particularly for large migratory fishes. The relative newness of the Itaipu reservoir and its large catchment area may play an important role in its fish production.

Migratory and sedentary fishes are among the main commercial species in these reservoirs (Table 5). The presence of migratory fish in the catch lists indicates that recruitment conditions in the respective catchment areas may still be present. *Prochilodus,* which is essentially a mud-eater, represents the main migrant group in reservoir catches. The sedentary *Plagioscion squamosissimus* and *Cichla ocellaris* occur naturally or were introduced.

The number of professional fishermen working in Brazilian reservoirs is fewer than 0.001 fisherman per ha. They work alone or in pairs, with small wooden canoes and paddles and nylon monofilament gillnets as the main gear. At the Tucuruí reservoir, lines and hooks are predominantly used since large areas of the reservoir are covered with floating vegetation, which hampers the use of other fishing gear. Each fisherman catches an average of 2.6 and 3.6 metric tons per year, respectively, at Três Marias reservoir (Sato and Osório 1987) and Boa Esperança (Paiva 1976).

Table 5

Main commercial fish in some Brazilian hydroelectric reservoirs with reference to their reproductive migratory pattern (RMP)

Reservoir	River basin location	Species	RMP
Tucuruí (1)	Tocantins- Amazon	*Cichla ocellaris*	S
		Plagioscion squamosissimus	S
		Prochilodus nigricans	M
Boa Esperança (2)	Parnaíba	*Curimata* spp.	S
		Acuticurimata spp.	?
		Prochilodus lacustris	?
		Plagioscion squamosissimus	S
Sobradinho (3)	São Francisco	*Prochilodus marggravii*	M
		Pseudoplatystoma coruscans	M
		Leporinus elongatus	M
		Schizodon knerii	S
Três Marias (4)	São Francisco	*Schizodon knerii*	S
		Pachyurus spp.	S
		Prochilodus spp.	M
		*Cichla ocellaris**	S
Promissão (5)	Tietê-Paraná	*Plagioscion squamosissimus**	S
		Pimelodus maculatus	M
		Pimelodella spp.	?
		Iheringichtys labrosus	S
		Rinodoras dorbignyi	?
Furnas (6)	Grande-Paraná	*Astyanax fasciatus*	S
		Hoplias malabaricus	S
		Pimelodus maculatus	M
		Oreochromis spp.*	S
		*Hoplias lacerdae**	S
Itaipu (7)	Paraná	*Prochilodus scrofa*	M
		*Plagioscion squamosissimus**	S

? migratory pattern unknown; M = migrant, S = sedentary, * introduced.

Source: (1) Formagio, personal communications; (2) Paiva 1976; (3) PROTAM 1987; (4) Sato and Osório 1987; (5) CESP 1991; (6) Formagio 1995; (7) FUEM/NUPELIA 1987

Fish Communities in Dammed Rivers

Fisheries debilitation in Brazil has various possible causes, which may act simultaneously although individual contributions may vary. The intensive hydroelectric dam construction of the last 40 years, predatory fisheries, lack of information on stock availability, inadequate regulations and enforcement and increasing agricultural, urban and industrial pollution are among the main causes.

Brazilian scientists have only recently begun to evaluate the effects of large hydroelectric dams on fish communities. Changes in fish community structures, especially those related to reproductive restrictions imposed by blocking the route of migratory species, are currently being investigated.

The Grande River example

The Grande is a tropical plateau river that originates in the southeast part of the State of Minas Gerais and extends for 1,300 km to form the Paraná River. The ten large hydroelectric dams built along its course have transformed the river into a succession of artificial lakes.

A recent evaluation of the fish community composition in the area of the future Igarapava hydroelectric dam, presently under construction, has disclosed none of the large migratory species, once abundant in that segment of the Grande River (e.g., surubim, dourado, curimatãs and piapara) (Vono et al. 1995).

The São Francisco River example

Fisheries yields of the São Francisco river below the Três Marias dam in the 1970s and 1980s were mainly sustained by large migratory fish of high commercial value, i.e., *Pseudoplatystoma coruscans, Salminus brasiliensis* and *Prochilodus* spp. Fishing in the Três Marias reservoir, on the other hand, was markedly different. Although the reservoir fishing season is longer than that for the river, its productivity per hectare per year was smaller. Catches of migratory species, except *Prochilodus*, were minimal and fishing relied on small sedentary species of low value. In spite of commercializing larger biomass, the reservoir fishermen's annual income was lower than that of their colleagues fishing the river (Table 6).

A significant decrease in yield has been registered in recent years not only at the Três Marias region but also along the entire 1,150 km segment of the river between the hydroelectric dams of Três Marias and Sobradinho. This is the only stretch where the river flows freely and where its main tributaries enter the river.

Table 6

Fisheries yields at Três Marias reservoir area in the São Francisco River*

Parameter	Reservoir	River
Area (ha)	80,000	2,000
Days of fishing	240	180
Production: $kg.\ ha^{-1}.year^{-1}$	5	117
$kg.fisherman^{-1}.year^{-1}$	2,640	2,000
Price of fish ($Cr\$.kg^{-1}$)	7	16
Annual fisherman income (Cr$)	18,400	32,000
Main species (order of importance)	1. *S. knerii* (s)	1. *Prochilodus* spp (m)
	2. *Pachyurus* spp. (s)	2. *S. brasiliensis* (m)
	3. *Prochilodus* spp (m)	3. *P. coruscans* (m)

s = sedentary, m = migrant

* Adapted from Sato and Osório (1987)

Fisheries Management and Conservation

Brazilian fisheries are basically maintained through stocking and regulation. Despite being in operation for decades, these programs are only now beginning to be evaluated.

Fish stocking and introductions

Brazilian fish stocking programs began in the 1970s with newly constructed hatchery stations owned and operated by State or federal hydroelectric companies. Exotic and native species were included in these programs. Few reports are available about the results of such stocking programs on fisheries yields.

Although several species have been used in stocking programs in various river basins, the few results available in terms of harvesting are disappointing except for *Plagioscion squamosissimus*. This species, a voracious piscivorous Amazonian fish, introduced into hydroelectric reservoirs in the State of São Paulo in 1968, ranked first in biomass in the Promissão reservoir, Tietê River (CESP/UFSCAR 1990), as well as in the Volta Grande reservoir in the Grande River (unpublished observations). At the Itaipú reservoir, it occupied second place in the commercial fisheries (FUEM/NUPELIA 1987). Evaluation of possible damage caused by its introduction to host habitats is not available. Anglers from the Porto Colômbia and Marimbondo reservoirs, both situated in the Grande River below Volta Grande reservoir, complain about the drastic reduction of *Astyanax* spp. after introduction of *P. squamosissimus*.

Cichla ocellaris is another voracious piscivorous Amazonian fish that was introduced in southern Brazilian waters, possibly by anglers willing to have such a nice sport fish closer to their homes. Experimental catches have revealed a great increase in *C. ocellaris* biomass at Três Marias reservoir since it was caught for the first time in 1985. Now it is probably the main species in commercial and sport fisheries in that reservoir.

Impacts similar to those registered in the Gatun Lake, Panamá (Zaret 1982), on the native fish fauna after Cichla introduction may be occurring in Brazilian inland waters (Godinho and Formagio 1992; Pompeu 1997).

Fisheries regulation

Traditionally Brazilian fisheries regulations and controls were under the responsibility of the Brazilian Institute for Environment and Renewable Natural Resources (IBAMA). Recently a few States have established their own regulations. Until a few years ago fishing in most Brazilian rivers was under a single set of regulations, with no major concern about their specific characteristics. This situation has substantially changed; each major river basin is now subject to proper and specific regulations. The following general regulations are common to all Brazilian river basins.

Licenses and permits for professional, sport and scientific fisheries

The revenue obtained from the sale of annual licenses will be directed to pay for specific programs related to fisheries. Scientists have special permits to collect fish for their work.

Season

Fishing is partially closed during the reproductive period of the main commercial species. Lines and hooks may still be permitted during the closed season.

Size and creel limits

Minimum size limits are set on the basis of the length at first maturity. Regulations limiting the amount of harvested fish apply only to sport fishing.

Closed areas

Fishing is prohibited in some special areas such as at distances of less than 200 m below and above dams and waterfalls and the mouth of affluents (commercial fishing is permanently prohibited in two large rivers in the State of Minas Gerais, the Velhas and the Paraopeba).

Fishing gear

The main gear used in professional fisheries is gill nets and cast nets. Restrictions are placed on the mesh size of both types and on the length and height of gill nets.

Cryopreservation of Gametes as a Tool for Management and Conservation

Expertise in cryopreserving Brazilian native fish sperm is limited to a few experimental works (*Prochilodus scrofa* and *Salminus maxillosus:* Cóser et al. 1984; *Rhamdia hilarii:* Silveira et al. 1985; *Leporinus silvestrii:* Cóser et al. 1987; *Prochilodus scrofa:* Kavamoto et al. 1989; *Piaractus mesopotamicus:* Carolsfeld et al. 1990; *Prochilodus marggravii:* Cóser et al. 1992). Only Kavamoto et al. (1989) and Cóser et al. (1992, Table 7) conducted fertility tests with cryopreserved semen. Both used *Prochilodus* with good results, obtaining over 80 per cent fertilization.

Table 7

Fertilization rates of *Prochilodus marggravii* eggs fertilized with cryopreserved semen in various dilutants and DMSO

Time (days)	diluant	DMSO (%)	Fertilization rate (% ± s.d.)
1	5% glucose	7.5	81,0 ± 1.84
720	lecithin-glucose	7.5	41.3 ± 7.28
720	5% glucose	7.5	56.0 ± 1.13
1,080	5% glucose	8.0	83.7 ± 2.76
Control (fresh semen)	-	-	96.4 ±1.20

Source: Cóser et al. (1992)

The first steps towards the establishment of a Brazilian fish gene bank were taken three years ago when pilot studies demonstrated the applicability of Canadian gene banking technology to a few Brazilian species. The work progressed into the organization of an institutional link, with the participation of Brazilian universities, the hydroelectric industry, government, non-governmental organizations and the World Fisheries Trust. As a result, a three-year cooperation agreement between WFT and those Brazilian institutions was signed and funded by CIDA's Brazil Technology Transfer Facility.

As part of this project, field work was conducted at the end of 1997 at the Federal University of Santa Catarina, State of Santa Catarina, and at Furnas Hatchery Station, of the Furnas Electric Company, on the Grande River in the State of Minas Gerais. Its objectives were to refine and adapt Canadian cryopreservation technology to native species. At that time, cryopreservation and fertilization tests were conducted with success with the anostomid *Leporinus elongatus*. Semen of the pimelodid *Pseudoplatystoma coruscans* was also successfully cryopreserved.

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Brazilian Freshwater Fishes: Their Environment and Present Status

Evoy Zaniboni Filho [1]

Evolution of Brazilian Fish Fauna

The geological evolution of South American hydrographic basins allowed the interchange of freshwater fishes among them for a long period of time, resulting in species similarities. The layout of the main basins is a consequence of the Andes Mountain chain formation in the Cenozoic era. The gradual isolation led to the independent evolution of species, allowing a huge diversity, with more than 3,000 species in Brazil alone.

Reproductive Patterns of the Principal Economic Species

In a general way, the biological cycles of the main economic species show a similar pattern. Normal migrations are over 200 km between feeding areas and spawning grounds, in annual cycles starting between two and three years of age. Spawning occurs in spring and summer. Normally the trigger for gonadal maturation is the start of the rainy season, when small changes in water conductivity and turbidity are enough to start up the reproduction process. When water levels rise, schools of adult fishes start migration to the spawning grounds. The reproduction strategy is based on the release of large quantities of pelagic eggs (between 100,000 and 300,000 eggs/kg female) without any parental protection. Eggs are then carried downstream during incubation, and larvae gradually develop swimming ability soon after hatching. With limited energy reserves in the yolk sac, these larvae need a place with food availability in quantity and quality. The marginal lagoons formed in the flood plain, with still water and rich in food and protection, provide ideal habitat for early life stages.

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Effects of Dams

Economic development and human population increases have placed high demands on the environment, directly affecting fish communities. In addition to growing problems related to deforestation and pollution, the growth and development of a country increases demands for electric power. More than 90 per cent of energy in Brazil comes from hydroelectric sources, meaning that economic growth is tied to the construction of new dams.

The effects of dams on fish communities can vary from one region to another and depend on dam characteristics. In a general way, energy demand requires a controlled hydrological cycle, resulting in a reduction in floodplain areas as well as a range of maximum and minimum water levels. In addition, a dam creates a barrier that blocks fish migratory routes and holds nutrients, reducing productivity in reservoirs and downstream. Another problem associated with dam construction is the introduction of exotic species.

Brazilian freshwater fisheries have shown a gradual reduction in biomass. Programs for fry release into reservoirs have yielded poor results, while fish ladders and other bypass mechanisms have been selective and ineffective, resulting in a need for improved design. As a result, natural stocks have been reduced and many species eliminated. In some hydrographic basins, populations of migratory fishes have been confined to smaller and smaller areas, like live genetic banks. Environmental protection areas are not effective in guaranteeing the preservation of migratory fishes species, as these areas can only protect one of the different periods of life in a biological cycle. Dourado *(Salminus maxillosus)*, migrating over 1,200 km, exemplifies the problem very well.

Problems and Projects in the Uruguay Basin

The Uruguay river basin comprises 365,000 km², of which 75,300 km² are located in Brazilian territory and the rest in Uruguay and Argentina. Historically, the river was known for its fish abundance (Godoy 1987), with 145 species (Cordini 1977). Its reduction in stocks is due to deforestation and pollution; there are still no dams blocking the river. In the past decade, there has been a very strong public and governmental demand for the restoration of the river's quality, resulting in some reduction in the input of mainly industrial pollutants. In the 1990s, restoration and protection of vegetation and the treatment of urban effluents has benefited fisheries in a few regions. However, despite concern about the health of the river, the first dam is scheduled to begin operation by the year 2000.

Santa Catarina University, with financial support from Centrais Eletricas do Sul do Brasil (ELETROSUL), Fundo Nacional do Meio Ambiente/Ministerio do Meio Ambiente (FNMA/MMA) and the Canadian International Development Agency (CIDA), has been developing a research program in the Brazilian portion of the Uruguay river basin with the following aims:

- study the biological diversity of the Uruguay River fish and the importance of the river's tributaries;
- maintain a broodstock of the main migratory fish;
- develop reproduction and larviculture techniques for these species;
- apply the techniques of sperm cryopreservation of these species to maintain genetic diversity, and develop a regional gene bank;
- develop environmental education programs;
- stimulate the use of native species for regional aquaculture.

With the development of these studies, we hope to gather knowledge that will enable better management of the river and allow the preservation of the basin's ictiofaunal genetic diversity.

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Conservation Genetics and Living Fish Gene Banks in Brazil

Silvio de Almeida Toledo-Filho [1]
Fausto Foresti [2]

Overview of Research Since 1988

During the last decade, research groups at the Universidade Estadual Paulista (UNESP) and the Universidade de São Paulo (USP) have been developing four programs on Brazilian fish conservation genetics:

Genetic monitoring of artificial hybrids of neotropical Serrasalminae

This first program is on genetic monitoring of artificial hybrids of neotropical *Serrasalminae* used in Brazilian fishculture. Four reciprocal hybrids between species from genus *Piaractus* and genus *Colossoma* have been characterized using cytogenetic C-bands, allozymes and mt DNA haplotypes. The main objectives of this program were:

- To detect diagnostic genetic markers for F1 hybrids;
- To verify the hybrid fertility degree and/or the type of sterility, whether gonadic, gametic or zygotic;
- To make recommendations to aquaculturists and fishery managers about the potential impact of these hybrids on wild and cultivated native stocks.

Four principal electrophoretic markers amongst blood proteins as well electrofocussing of muscle proteins and C-bands were found to distinguish the reciprocal hybrids (Almeida-Toledo et al. 1987; Calcagnotto 1993; Toledo-Filho et al. 1994). Females of both hybrids and 96 per cent of the males of the *Colossoma x Piaractus* hybrid showed gonadic sterility. The remaining 4 per cent of males of this hybrid possessed zygotic sterility or low levels of fertility.

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Detection of living genetic banks of Piaractus

The second line of research is a program of Detection of Living Genetic Banks of Piaractus in the Pantanal wetlands from Brazil using mt DNA. For the genetic analysis of mt DNA haplotypes, 14 restriction enzymes (*Apa* I, *Bam* HI, *Cla* I, *Dra* I, *Eco* RI, *Hind* III, *Kpn* I, *Mlu* I, *Pst* I, *Pvu* II, *Sal* I, *Xba* I, *Xho* I and *Hpa* II) were used. The main objective of this program was to identify *in situ* genetic banks of pacu *(Piaractus mesopotamicus)* inhabiting the Aquidauana, Coxim, Cuiabá, Miranda, Paraguai and Taquari rivers in the State of Mato Grosso. A geographical population structure comparable to level IV as defined by Avise (1989) was detected (Calcagnotto 1998).

Genetic monitoring of a neotropical Prochilodontidae

The third project is a program of Genetic Monitoring of Neotropical *Prochilodontidae* used for fish stocking and/or supplementation in some southeast Brazilian hydroelectric reservoirs. Genetic variability, similarity and introgression, as well as inbreeding levels of wild and cultivated stocks, were monitored with allozymes and mt DNA haplotypes. No naturally occurring inbreeding was found in *Prochilodus scrofa* of the Mogi-Guaçu River (São Paulo State), but fish of this species being stocked into the near-by Paraibuna reservoir possessed an inbreeding index of about 25 per cent (Galhardo 1989; Toledo-Filho et al. 1992; Oliveira et al. 1997). Recommendations were presented to aquaculturists and fishery managers on how to avoid this genetic degradation and preserve the heterogeneity of fish genetic resources used for stocking.

Detection of sibling species of neotropical weak electric fish

The fourth is a program of Detection of Sibling Species of Neotropical Weak Electric Fish *(Gymnotiformes)*, using cytogenetic, mt DNA and DNA microsatellites diagnostic markers. Four different cryptic species of *Eigenmannia* were identified by karyotypic analysis, and an evolutionary tree of the Serrasalminae was proposed based on evidence from mitochondrial DNA (Almeida-Toledo 1996). Evolutionary relationships between *Gymnotus* are being investigated with microsatellites and mitochondrial DNA sequencing (Fernandes-Matioli et al. 1997).

Research Collaborators

The Brazilian researchers presently involved in these four programs and their respective research areas are:

- Fausto Foresti, Professor, UNESP, genetics in fishculture and fish conservation genetics mainly related to stocking, hybridization and use of cytogenetic markers;
- Lurdes Foresti de Almeida-Toledo, Associate Professor, USP, molecular cytogenetics and immunocytogenetics of neotropical fish, order Gymnotiformes;

- Sílvio de Almeida Toledo-Filho, Professor, USP, genetics in fishculture and fish conservation genetics mainly related to stocking, hybridization and use of biochemical-genetic markers;
- Claudio de Oliveira, Assistant Professor, UNESP, molecular cytogenetics and cytotaxonomy of neotropical fish;
- Edson Maistro, Assistant Professor, USP, molecular cytogenetics of genus *Astyanax*;
- Geraldo Bernardino, Researcher at the Centro de Pesquisa de Peixes Tropicais, CEPTA/IBAMA, Pirassununga, Brazil;
- Daniela Calcagnotto, Graduate Student, USP, mt DNA monitoring of native stocks of genus *Piaractus* from the Pantanal basin;
- Flora Fernandes-Matioli, Graduate Student, USP, microsatellites and DNA sequencing of weak electric fish, genus *Gymnotus;*
- Fatima Daniel-Silva, Graduate Student, USP, molecular cytogenetics of neotropical Fish, genus *Astyanax;*
- Soraia Barreto Aguiar Fonteles Santos, Graduate Student, UNESP, genetic monitoring of neotropical artificial hybrids used in Brazilian fishculture;
- Cinthia Moyses, Graduate Student, USP, mt DNA phylogeographic structure of neotropical fish, genus *Astyanax;*
- Fabio Porto-Foresti, Graduate Student, UNESP, molecular cytogenetics of *Characidae;*
- Ruy Alberto Correa, Graduate Student, UNESP/Universidade de Mato Grosso de Sul, genetics in fishculture, fish conservation, stocking and production;
- Maria Carolina Marchetto and Maria Mercedes Okumura, Undergraduate Students;
- Carlos Lopes, Biologist.

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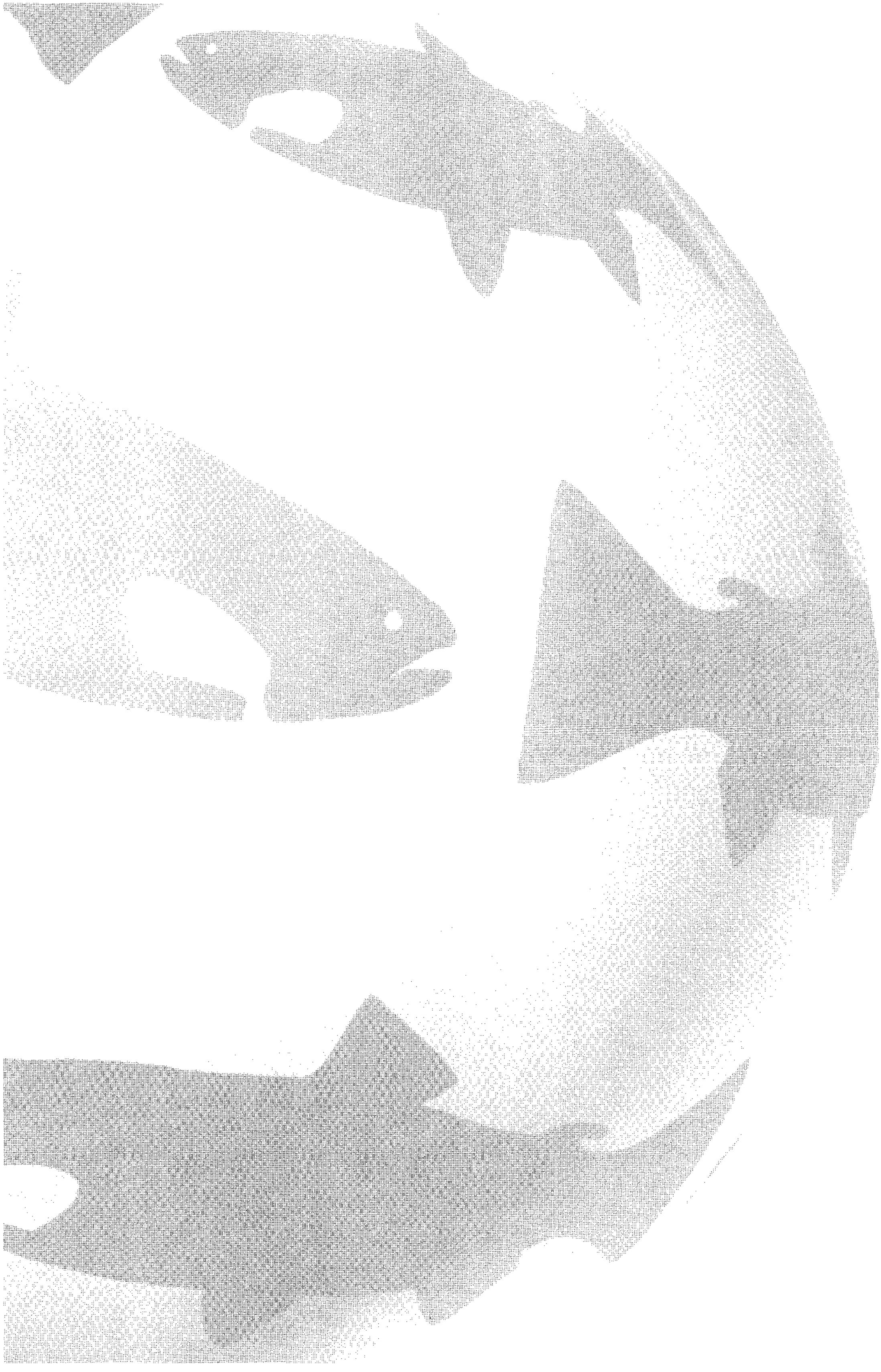

Fish Biodiversity Conservation in Colombia

Jaime Alberto Díaz-Sarmiento [1]
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Colombian Geography and Biodiversity

Although Colombia contains only 0.7 per cent of the continental surface of the planet, it has 10 per cent of global biodiversity. Its tropical position and the complexity of its topography provide the setting for some of the largest river basins of the world, originating in the Andes, the Guyana shield and the Amazonian lowlands. The continental freshwater ecosystems comprise approximately 2.6 million hectares and 25 million cubic metres in the region, making Colombia the fourth richest country in freshwater abundance in the world.

This abundance of water resources supports a great diversity of ecosystems and of fish species and populations. Of the 3,000 to 5,000 species reported for South America, two-thirds (3,050 according to Alvarez-León et al., in preparation) can be found in Colombia, and in some regions of the tropical rain forest more are expected to be discovered (MMA et al. 1997).

Colombia also has a considerable area of marine waters on the Pacific and Caribbean coasts, with nearly 3,000 km of sea shore, 988,000 km^2 of Economic Exclusive Zone, and 3,655 km^3 of water volume. In the Caribbean coral reef ecosystem alone, around 326 species of fishes have been reported, and 700 to 1000 are estimated to occur in the total national areas (MMA et al. 1997).

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Declines in Aquatic Biodiversity

Not withstanding its high biodiversity, Colombia faces several problems related to conservation and sustainable use of the fish resource. As in the rest of the world, the most threatened species occur in freshwater ecosystems. These include *Rhizosomichthys totae* (confirmed extinct following the introduction of the trout, (*Oncorhynchus mykiss*), *Eremophilus mutisii* and *Gambusia aestiputeus* (endangered). Of the 430 species considered by the World Conservation Monitoring Centre (WCMC 1995) to be extinct, endangered, vulnerable or rare, there are only seven reports for Colombia. However, this number may not reflect the real situation, considering the lack of information about the status of most fish populations. Basins like the Magdalena River have shown a 78 per cent decline in catch in the last 20 years, and there has been a continuous declining tendency in the fisheries of the country in the last decade.

In an effort to preserve ichthyological resources, national authorities have developed several laws and decrees: Decree 2811 of 1974 (National Code of the Natural Renewable Resources and Environment), Decree 1681 of 1978 (Regulation of Hydrobiological Resources), Law 10 of 1978 (Territorial Ocean, Exclusive Economic Zone and Continental Shelf), Law 13 of 1990 (General Statute of Fisheries) and Decree 2256 of 1991 (Regulations Pertaining to the Statute of Fisheries). There also exist some specific standards for the Atlantic, Pacific, Magdalena, Orinoco and Amazon basins. These include closed seasons for threatened species and restrictions on fishing methods and gear, culture, transport, sale, and introduction and transplantation of exotic and native species either for food or hobby.

Conservation of Fish Biodiversity

Destruction of habitat, over-exploitation of some species and populations, pollution of watercourses and the introduction of about 58 exotic species (Rodríguez-Gómez 1984; H. Alvarado, personal communications) are directly responsible for the decline of fish diversity in Colombia. Continued declines are expected and are being studied by the Ministry of Environment and other research institutes.

There has also been a transplantation of populations of different species from basins inside Colombia. The two species of Cachama (*Piaractus brachipomus* and *Colossoma macropomum*), originally from the Amazon basin, have been transplanted to cultures in the Magdalena and Cauca basins. Arapaima or Paiche (*Arapaima gigas*) and the Tucunaré (*Cichla ocellaris*), originally from the Amazon basin, have been transplanted to cultures in the Valle del Cauca and Caldas regions, and Bocachico (*Prochilodus magdalenae*), originally from the Magdalena and Cauca basins, have been transplanted to cultures in the Orinoco region. Transplantation of some other native and exotic species like the Arawana (*Osteoglossum* spp.) has yet to be confirmed.

Species like the freshwater Tilapia (*Oreochromis* spp., *Tilapia* spp.) are now cultured in marine environments, and others from marine origins like Lisas (*Mugil* spp.) and Sábalo

(Tarpon atlanticus) are being introduced to freshwater cultures (Alvarez-León 1982; Mercado-Silgado and Alvarez-León, in preparation).

The low level of research and knowledge about native species culture, whether marine or continental, also encourages the spread of well adapted exotic species. Most native species culture (Table 1) is still in the experimental stage.

Lack of funds and infrastructure for research, monitoring and conservation actions, technological deficiencies and poor knowledge at the ecosystem, species and genetic levels

Table 1

Main commercial marine and freshwater fish species of Colombia*

Freshwater Fisheries	Freshwater Aquaculture	Marine Fisheries	Marine Aquaculture
Magdalena Basin	Natives	Caribbean Coast	Caribbean Coast
Pimelodus clarias	*Arapaima gigas*	*Apsilus dentatus*	*Ariopsis bonillai*
P. grasskoffi	*Ageneiosus caucanus*	*Alphestes afer*	*A. proops*
Plagioscion magdalenae	*Brycon fowleri*	*Caranx hippos*	*Bairdiella ronchus*
Prochilodus magdalenae	*B. moorei moorei*	*Elegatis bipinnulata*	*Caranx hippos*
Pseudoplatystoma	*B. moorei sinuensis*	*Coryphaena hippurus*	*Cathorops spixii*
fasciatum	*Caquetaia kraussii*	*Lutjanus griseus*	*Centrpomus eusiferus*
Sorubium lima	*Cichlasoma umbrifera*	*L. synagris*	*C. parallelus*
	Pimelodus clarias	*L. vivanus*	*C. undecimalis*
Amazon Basin	*P. grasskoffi*	*Rhomboplites aurorubens*	*Diapterus rhombeus*
Arapaima gigas	*Prochilodus magdalenae*	*Hemulon aurolineatum*	*Dormitator latifrons*
Colossoma macropomum	*Pseudoplastytoma*	*H. plumieri*	*Elops saurus*
Brachyplatystoma	*fasciatum*	*Lachnulaimus maximus*	*Eugerres plumieri*
filamentosum	*P. tigrinum*	*Cynoscion jamaicensis*	*Lutjanus analis*
B. flavicans	*Plagioscion magdalenae*	*Scomberamorus regalis*	*L. bucanella*
Prochilodus nigricans	*Triportheus magdalenae*	*Euthynnus alleteratus*	*L. griseus*
Cichla ocellaris		*Thunnus atlanticus*	*L. synagris*
	Exotics		*Megalops atlanticus*
Orinoco Basin	*Aristichthys nobilis*	Pacific Coast	*Micropogonias furnieri*
Goslinia platynema	*Ctenopharingodon idella*	*Opisthonema libertate*	*Mugil incilis*
Paulicea luetkeni	*Cyprinus carpio*	*Cetengraulis mysticetus*	*M. liza*
Plagioscion surinamensis	*C.c. var. specularis*	*Bagre panamensis*	*Oreochromis spp*
Prochilodus mariae	*Micropterus salmoides*	*Centropomus robalito*	*Rhomboplites aurorubens*
Brachyplatystoma vaillanti	*Hypopthal michthys*	*Lutjanus argentiventris*	
Hidrolicus scomberoides	*molitrix*	*L. guttatus*	Pacific Coast
	Oreochromis hornorum	*Diapterus peruvianus*	*Centropomus armatus*
Atrato Basin	*O. mossanbicus*	*Cynoscion squamipinnis*	*C. robalito*
Brycon medemi	*O. niloticus*	*C. phoxocephalus*	*Diapterus peruvianus*
Ageneiosus caucanus	*O. urolepis*	*Mugil curena*	*Lutjanus argentiventris*
Cyrthocharax magdalenae	*O. spp.*	*Sphyraena ensis*	*L. guttatus*
Curimata atratoensis	*Onchorhyncus kisutch*	*Sconberomorus sierra*	*Mugil cephalus*
Pimelodella chagresi	*O. mykiss*	*Pneumatophorus peruanus*	*M. curena*
Prochilodus magdalenae	*Salmo salar*	*Coryphaena hippurus*	*Oreochromis spp.*
	S. trutta	*Istiophorus platypterus*	
San Juan Basin	*Salvelinus fontinalis*	*Thunnus alalunga*	
Brachyplatystoma juraensis	*Tilapia rendalli*		
Brycon oligolepis			
Rhamdia wagneri			
Geophagus pellegrini			
Agnostomus monticola			
Roeboides hildebrandi			

* According to Alvarez-León (1982); Rodríquez-Gómez (1984); INPA (1997); Mercado-Silgado and Alvarez-León (in preparation); and Alvarez-León et al. (in preparation).

are also big obstacles for fish diversity conservation. Institutional and legal structures are not well defined and/or implemented, causing failures in the recognition and distribution of exploitation rights and benefits and in the valuation of the natural resources. As a result, the importance of *ex situ* collections is not well appreciated and there are some hindrances to their implementation.

Rights to Genetic Resources

There are several problems related to the conservation and use of the enormous biodiversity of the country and particularly of fish genetic resources. *In situ* conservation of natural resources has been well considered by the Colombian Constitution of 1991; however, the genetic material needed for this conservation was not defined until the directives of Agenda 21 and the Convention of Biological Diversity were adopted by Law 165 of 1994, which described genetic material as "any material containing the functional units of heritage whose property rights belong to the nation".

The national government will provide the necessary conditions to harmonize the current use of the biological diversity and inherent components with its conservation. When an adverse effect is identified, the government must regulate and manage the related processes and activities. It will also adopt the measures required to recover and rehabilitate endangered species and reintroduce them to their natural habitats. In order to achieve this aim, *ex situ* collections must be developed.

The Andean Decision (Law 391 of 1996), known as "The Common Regime of Access to Genetic Resources", as an extension of the Convention on Biological Diversity, differentiated between property rights to biological resources in general and rights to genetic resources. Biological resources as a whole can be either publicly or privately owned, but genetic resources are only publicly owned and are considered an intermediate good between the biological resource and the intellectual property right, derived from the information found in genetic material. Genetic resources cannot be sold, acquired through expiration of a previous agreement or confiscated.

In the past, managers of the country's biodiversity may not always have been familiar with international conventions, agreements and protocols signed by the government of Colombia. However, Colombia takes such international obligations seriously and has moved to develop internal strategies as well. Last year the Colombian Ministry of Environment released general guidelines for a National Policy on Biodiversity and announced the formation of nine groups of specialists in different topics (Restoration of Ecosystems, Biodiversity Compounds, Traditional Practices, Valuation, Sustainable Management, Derivative Benefits from Biodiversity Use), one of which is related to *ex situ* conservation and gene bank development. These groups are developing a document to design an Action Plan which will further divide the main topics into specific subjects like fish genetic diversity banking and *ex situ* collections of fish.

Developing a Strategy for Fish Biodiversity Conservation

The complexity and uncertainty associated with the conservation and use of fish diversity of the magnitude existing in Colombian waters are huge, due to the many aspects involved, some of them unique to the cultural, historic, socio-economic and political situation. The development of a national strategy to preserve and sustainably use fish diversity in Colombia must begin with the establishment of a conceptual framework that considers these factors as an important part of the process.

The first step in achieving this is an understanding of Colombia's marine and fresh water systems as an integral part of an international complex of basins and seas. To accomplish this, it is important to design practical and reliable methods for diagnosing and monitoring their status and identifying areas that have high taxonomic relevance and are susceptible to degradation.

It will also be important to determine the magnitude of the principal causes of loss of fish diversity, including over-fishing, poor aquaculture practices, pollution, habitat degradation and the socio-economic, cultural and political context in which these take place.

Priorities for desired levels of biodiversity conservation and sustainable use need to be established. It must be clear whether methods of doing so are based in genetic, specific, ecosystemic or combined concepts, whether they are based on geographic criteria, and whether they include *in situ* and *ex situ* strategies.

Given the wide distribution of biologically rich areas that are still fairly well preserved but threatened by habitat degradation and destruction, Colombian authorities must place great effort on *in situ* preservation, with special care at the ecosystem level, considering species assemblages and functional interrelationships.

The Colombian government has made clear its willingness to preserve natural environments through the establishment of 45 conservation units in National Natural Parks, Natural Reserves and Flora and Fauna Sanctuaries. Although these protected areas have a combined total area of more than nine million hectares, they appear to have been selected using mainly terrestrial criteria. In order to improve *in situ* conservation of fish diversity, national agencies need to revise the distribution of these areas to protect a connected fluid environment of ecosystems, particularly in the case of plateaus, floodplains and basins.

Management of fish biodiversity is essential since it is the only non-domesticated resource of major significance for human exploitation, and expected human population increases will lead to general over-fishing. In Colombia a management focus is needed on traditional and small-scale fisheries for most fresh and marine water ecosystems. Efforts should be made to introduce mechanisms for collective decision making, conflict resolution and alternative dispute resolution between government and communities that allow national institutions to take advantage of traditional knowledge, despite the need for education, administrative and legal reforms and experimentation.

The primary threat to freshwater ecosystems is overfishing, while in marine ecosystems, small scale coastal fisheries are responsible for the degradation of coral reefs, seagrass beds, mangrove and estuarine habitats. To overcome these problems, Colombian institutes charged with fisheries management responsibilities have set regulations for closed seasons, catch size limits, mesh size, gear types, baits and vessel types among others. However, as these measures are poorly enforced due to the lack of funds and infrastructure, the establishment of alternative models of cooperative management could help.

With the decline of most commercial fisheries in Colombia, aquaculture is becoming a very desirable alternative. However, aquaculture poses several threats to conservation of fish diversity; primarily through the introduction of exotic species which, released into the natural environment, affect native fish communities through predation, competition, spread of disease, and changes in water turbidity and clarity to a degree that, in cases like *Oncorhynchus mykiss,* has caused the extinction of an entire species *(Rhizosomichthys totae).*

The culture and release of native species which interbreed with wild stocks can also seriously affect genetic diversity. To overcome this situation, a more careful approach to fish culture must be enforced, and should include provisions such as those mentioned by the Global Biodiversity Assessment (1996), including closed culture (preventing escapes), sterilization, better location of fish farms, protection of wild populations, restrictions on transport, gene banking, minimization of genetic differences between wild and cultured stocks, and a general improvement in management.

Due to the worldwide interest, most current introductions emphasize ornamental species of *Cichlidae, Poecilidae, Cyprinidae, Anabantidae* and *Characidae,* leading to useful experience in their massive reproduction *(Carassius auratus, Mollinesia latipina, Cichlasoma meeki, Etroplus maculatus, Pterophyllum scalare altum, P.s. scalare, Symphysodon aequitafasciatus axelrodi, S. a. haraldi).*

Since the 1930s and 1940s, when the first introduction of cultured salmonid species *(Oncorhynchus mykiss, Salmo salar, S. trutta, Salvelinus fontinalis)* and perch *(Micropterus salmoides)* occurred in lakes and dams of the Andean region, different institutions have carried out studies to evaluate the potential for native fish culture (Table 1) and management of the above-mentioned species. These studies were later extended with the introduction of Carp *(Aristichthys, Ctenopharigodon, Cyprinus, Hypopthalmichthys)* and Tilapia *(Oreochromis, Tilapia, and their hybrids).*

The Role of *Ex Situ* Conservation

The last ten years have seen increased levels of ichthyological research in Colombia, but knowledge remains severely limited. In spite of high biodiversity, many species and populations are over-exploited and in some cases are being destroyed without awareness of their potential.

In order to improve *in situ* and *ex situ* conservation of fish diversity, Colombian institutions should participate in international networks to develop joint research and access to the most recent information. They also should apply for international funding to complement the limited resources available from national sponsors, and organize training courses to update knowledge of methodological and technological matters.

Ex situ conservation through cryopreservation of genetic material of tropical fish species and the creation of experimental gene banks could be of great importance for the management of fisheries, the improvement of cultured production, and as a preventive measure against possible failures of sustainable use methods. Material preserved *ex situ* can be tested in aquaculture, and used as well in restoration and repopulation projects. These efforts must be done simultaneously with *in situ* conservation of habitats and the identification of the specific population structures, because only by recognizing the importance of distribution, environmental factors and mechanisms that have conditioned the adaptive process can conservation of fish biological diversity be achieved.

Even though the advantages of the use, under clear and well-founded parameters, of *ex situ* collections and cryopreservation of fish genetic material are evident, there is little application of this technique to tropical species. The standardization of cryopreservation techniques will significantly enhance prospects for the autonomy of Colombia in the management of its genetic patrimony while providing economic benefits and preservation of fish resources in the region.

Several techniques for induction, incubation, cryopreservation and culture (tanks, dams, lakes, marshes) have been tried. With the support of national and international institutions, a process including personal capacitation, diagnostic and monitoring of aquatic environments, infrastructure build-up and research station construction has been developed.

The most successful experience with native species has been achieved with Bocachico *(Prochilodus magdalenae)*, Bagre Pintado *(Pseudoplatystoma fasciatum)*, Cachamas *(Colossoma macropomum, Piaractus brachypomum)* and some ornamental species like the Cardenal *(Cheirodon axelrodi)*, Mapurito *(Callophysus macropterus)*, Rojito *(Megaloniphodus swenglesi)* and Tigrito *(Pimelodus* spp.).

Efforts to establish *ex situ* conservation of fish genetic biodiversity in Colombia, especially through cryopreservation techniques, are still at the formative stages. Among the few attempts are the first studies carried out by the National Institute for Fisheries and Aquaculture (INPA), and the Universidad Tecnológica de los Llanos on *Piaractus brachypomum* at the beginning of the decade (Neira 1991). Later, in 1992, a specialist from the International Fisheries Gene Bank gave a training course on methodological aspects of cryopreservation, and since then other investigations on *Prochilodus magdalenae* (Bernal and Uribe, 1993), *Oncorhynchus mykiss* (González 1994), *P. brachypomum* (Caleóo 1995; González and Díaz 1997) and *Pseudoplatystoma faciatum* (Brand 1996) have been done.

At the present moment the results are at the preliminary stage, but the interest shown by some private and public institutions could be the beginning of a more stable research process.

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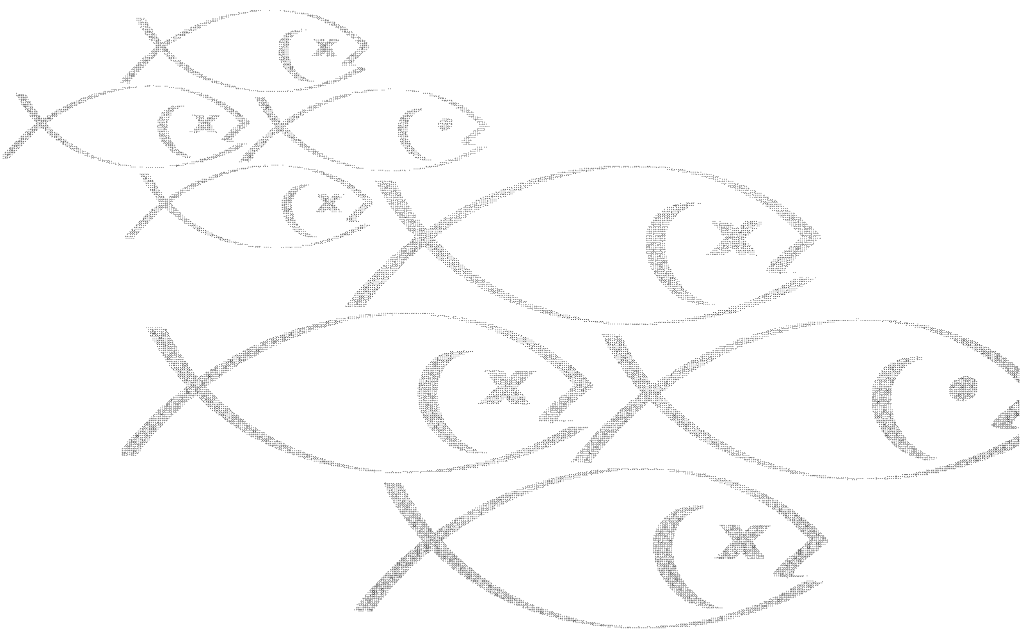

The Present Status of Genetic Conservation of Cultured Aquatic Species in Japan

Katsuhiko T. Wada [1]

Introduction

Japan formerly had the largest fish catch in the world. Since 1991, however, the total production of fish and shellfish has fallen below 10 million metric tons following the withdrawal of Japanese fisheries from foreign 200-mile fishing zones. In addition, the sardine catch has been declining recently.

Efforts to reverse this decline have included resource management geared to sustainable production, stock enhancement in coastal waters through seeding from hatcheries, preserving and refining of nursery grounds, and promotion of aquaculture operations (Kanno 1995). Seed production of hatchery fish and shellfish has increased, as has emphasis on maintaining biological diversity through the genetic conservation of cultured and wild stock of aquatic organisms.

Genetic conservation of fishery resources is supported by two government-assisted programs. These are the research project to develop stable techniques for evaluating and preserving genetic diversity of fish populations, and gene banking of algae through a national agricultural gene bank. This paper examines the present status of, and the effectiveness of support programs for, genetic conservation of cultured fish species in Japan.

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Research Project on Genetic Conservation to Support Domestication and Stock Enhancement of Aquatic Organisms

Genetic manipulation (e.g., artificial selection) of gene or genotype frequencies for the beneficial performance of economic traits in specific environments may interfere with conservation of genetic diversity or preservation of genetic resources in wild populations. The advantage to be gained through improvement or development of artificially propagated populations, as opposed to conservation of wild populations, is always a matter of controversy. In part, this results from the perception that improvement in farmed populations results in short-term changes of gene frequencies, while conservation of wild populations produces long-term benefits. Loss of genetic diversity through plant and animal breeding has often hampered efforts to maintain genetic diversity of stocks, both in agricultural and aquaculture production and in wild populations. Hence the importance of reconciling and learning from past conservation approaches to maintenance of diversity.

Unfortunately, differences in reproductive systems between plants and animals limit the lessons to be learned from agricultural improvements in developing conservation strategies for aquatic organisms. In addition, current knowledge about the genetic diversity of aquatic organisms and effectiveness of methods for preserving wild populations is limited.

The national government in Japan supports research projects to obtain practical information on the effective number of parents needed for fish and shellfish seed production to be released for sea ranching, and on genetic or environmental mechanisms for sex determination of hatchery fish larvae. The program includes the development of techniques for the evaluation of sex ratio at the earliest stage of fish larvae and for ensuring genetic diversity of hatchery-produced seeds for release to the natural habitat.

The program also includes theoretical studies on the genetic impacts of released seeds of fish and shellfish on natural populations. Species studied in the project include ayu *(Plecoglossus altivelis)*, Japanese flounder *(Paralichtys olivaeus)* and other species of fish and shellfish produced in hatcheries for sea-ranching (Iguchi 1997; Fujii and Nishida 1997). The grant supports four laboratories of the fisheries agency, Nippon Saibai Kyoukai (Japan Sea-Farming Association), three prefectural organizations and seven colleges.

Genetic diversity of hatchery-produced seed of ayu and some other marine fish and biological or ecological diversity of fauna in stock enhancement are also studied at the national institutes of fishery and at some colleges in Japan. The object of the program is to improve and to develop more effective techniques for and knowledge on stock enhancement and conservation of fish resources for a sustainable fishery.

Another research project, assigned to the colleges and prefectural institutes, aims to develop techniques for cryopreservation of fish sperm, eggs and embryos and to evaluate the genetic diversity of hatchery-produced populations of fish fry for release by analysis of DNA polymorphism. The species to be studied are rosy bitterling, salmon, flounder and prawn for cryopreservation of gametes, and red sea bream and Japanese flounder for diversity evaluation (Taniguchi et al. 1997).

Genetic Conservation of Cultured Salmonid Fish

Supply of and demand for salmon production in world markets are greatly influenced by the growth of commercially farmed salmon production, which reached a record level of over five million metric tons in 1995. During the same year, record harvests of salmon in both Alaska and Japan yielded less monetary value to fishermen than catches made in 1994. Salmon farming is practiced in roughly 20 countries. While the bulk of production occurs in northern Europe, there is also significant production from North and South America and Japan (Heard 1997). Conservation efforts in Japan have focused on enhancement of wild salmon stocks, and genetic breeding studies have been conducted mainly in the freshwater culture of salmonids for local consumption. As Allendorf and Kanda (1995) have reviewed the conservation of Japanese wild salmonid populations, only cultured populations will be described here.

Some strains of salmon, trout and char have been maintained at the Nikko Branch of the National Research Institute of Aquaculture (Japan's fishery agency) since 1890, when the Imperial Household Agency established a hatchery in Nikko, Tochigi Prefecture (Masaoka et al. 1997). Although these salmonids were bred successively, data describing the stock characteristics are not currently available. Twelve stocks of six species have been maintained and reproduced in freshwater ponds with the exception of the Western Australia rainbow trout, which was recently added. The origin and the time of introduction to Nikko of each stock are as follows:

Oncorhynchus mykiss:
- Nikko strain of rainbow trout (USA, before 1951)
- Donaldson strain of rainbow trout (USA, 1966)
- Steelhead trout (Hokkaido, 1973)
- Albino rainbow trout (Shizuoka, 1964)
- Horai rainbow trout (Aichi, 1970)
- Westalian rainbow trout (Western Australia, 1992)

Oncorhynchus nerka:
- Himemasu (Russia and Canada, 1976)
- Kamchatka kokanee (Russia, 1988)

Oncorhynchus kisutch:
- Coho salmon (USA, 1977-1980)

Oncorhynchus masou masou:
- Masu salmon (Hokkaido, 1979, 1980)

Oncorhynchus masou ishikawae:
- Amago salmon (Gifu, before 1971)

Salvelinus leucomaenis:

- Japanese char (Tochigi, 1965)

In the prefectural, cooperative or private salmon hatcheries, there may be conserved strains of salmonid fish, but published data are incomplete and limited. A recent questionnaire on salmonid culture, provided to organizations in Japan except those in Hokkaido, produced the following results:

- Number of responding organizations: 21 (19 prefectural and 2 cooperative);
- Number of species conserved: 7 (almost the same as in Nikko, although many local strains are preserved at the hatcheries).

Cryopreservation of sperm has been studied for some species in Japan (Kurokura 1983; Ohta et al. 1995a, b), but further studies are needed to develop stable methods for practical gene preservation of salmonid fish in Japanese hatcheries. These studies should focus on methods for obtaining high rates of fertilization, hatching and development of embryos inseminated by cryopreserved sperm based on the reproductive physiology of each species.

Preliminary Studies on Methods to Conserve Genetic Diversity in Wild Populations of Endangered Fish and Shellfish

Studies have been undertaken to estimate the mechanism of loss or reduction in size of small or endangered wild populations of fish or shellfish and to develop a new method of conserving endangered fish by androgenesis. The following is a summary of the results of this preliminary project, funded by the Environment Agency of Japan and conducted at the Japanese colleges and national institutes of fisheries:

- Methods were developed for studying the effect of a reduction in population size on the behaviour of Japanese char in streams and of medaka in tanks. Modified methods for estimating population size were valid for marginal Japanese char. Anal fin cut experiments on male medakas demonstrated that the large anal fin, a secondary sexual characteristic, appears to have an important role in increasing the rate of fertilization of eggs in paired mating.
- A Japanese endemic form of rosy bitterling *(Rhodeus occelatus kuraumeus)*, which is facing extinction, is karyologically replaced through hybridization in open waters by a continental congener, *R. o. celatus*. *Rhodeus occelatus kuraumeus* now inhabits only closed ponds, resulting in increased homogamety in recessive genes and a consequent reduction in genetic diversity and body size (Hosoya 1997).
- A PCR technique was developed to estimate the genetic variability in shellfish populations, and the loss of genetic variability of a freshwater bivalve (Anodonta ogurae) was estimated using TREP-PCR.
- Normal diploid androgenetic amago salmon (Oncorhynchus masou ishikawae) were produced by gamma irradiation of eggs, fertilization with fused sperm and suppression of first cleavage. Eggs were irradiated with an optimal dose of 450 Gy of gamma

radiation prior to fertilization, and optimal timing of the hydrostatic pressure shock was determined to occur at 7.5 hours after insemination at 10°C. The optimal concentration of CaCl solution to fuse the sperm was 85 nM (Araki et al. 1995; Nagoya et al. 1996).

Gene Banking of Algae

Only marine algae are preserved *ex situ* in the National Gene Bank for Fisheries and Aquaculture—one of Japan's national agricultural gene banks. Fish has been proposed as a candidate for gene banking but has not yet been included in the bank. Japan has aquaculture operations for seaweed such as *Porphyra* and *Laminaria*, and other macro algae or sea grasses *(Zostrea)* at the sea coast are important food sources for the nursery grounds of many species of fish and for marine invertebrates such as gastropods, crustaceans and sea urchins. Many species of phytoplankton, such as diatoms, haptophyceae, *Eustigmatophyceae* and *Prasinophyceae* are also propagated in fish and shellfish hatcheries. These species of algae or grass are collected, evaluated and preserved in the gene bank. The project has been conducted at the national research institutes of fisheries of the fishery agency of Japan.

All of these species are conserved by subculturing with the exception of sea grasses, which can be conserved as seeds. The considerable work and time needed for subculturing have limited the development of practical industrial applications. Likewise, the preliminary state of cryopreservation methods limits practical uses of *ex situ* preservation as much as in fish gene banking, where more difficulties exist (Terauchi et al. 1997). Most strains of macroalgae have been bred by artificial selection at culture sites by farmers for growth rate only. Other important traits will be considered in future studies.

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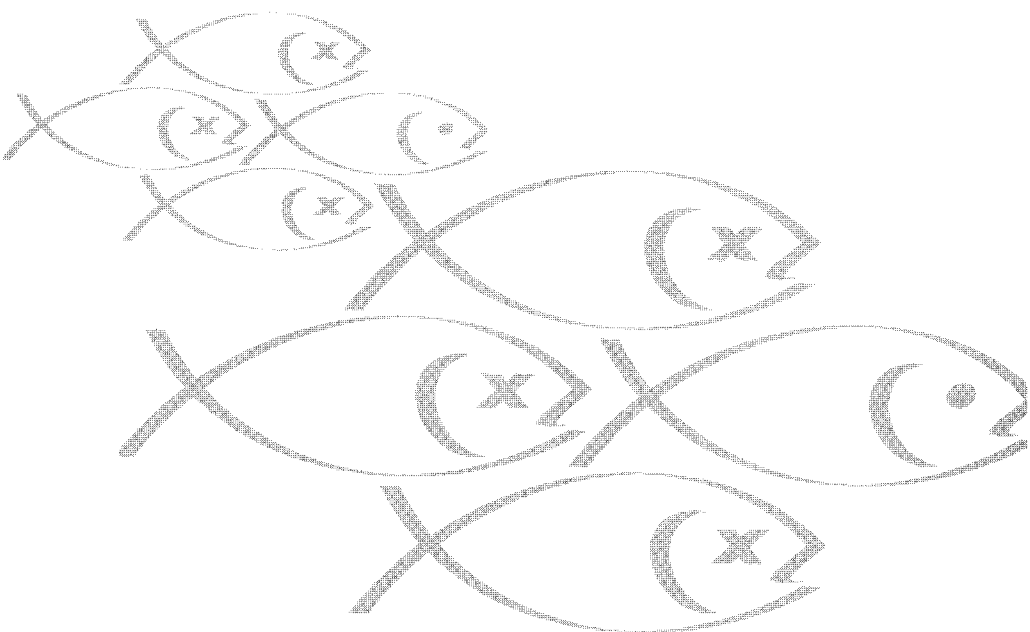

Defining Conservation Units for Pacific Salmon Using Genetic Survey Data

Chris C. Wood and L. Blair Holtby[1]

Introduction

The importance of conserving fish genetic resources is now widely recognised. *Ex situ* conservation techniques such as gene banks and captive broodstocks are increasingly seen as essential elements of a precautionary strategy for conserving threatened fish species (SBSTTA 1997). However, prudent and cost-effective application of these techniques requires a sampling design that is appropriate to both the conservation objectives and the hierarchical organization of genetic diversity within the target species.

In this paper we show how information gained from surveys of neutral genetic variation can help to guide decisions about sampling design for two worthwhile, but very different, objectives for *ex situ* conservation: (1) archiving genetic diversity for the species as a whole; and (2) preserving local adaptations for the restoration of particular wild populations. These correspond to the long-term and short-term objectives discussed in more detail by Bartley (this volume). Although these objectives are different, they are not mutually exclusive, and both could be accommodated by an appropriate sampling design.

We illustrate the importance of considering population structure by contrasting the hierarchical organization of genetic diversity within two species of Pacific salmon (sockeye salmon, *Oncorhynchus nerka* and coho salmon, *O. kisutch*). We also propose a system of nomenclature for defining population structure based on empirical estimates of gene flow among samples from genetic survey data. Finally, we suggest a conceptual framework for delineating the appropriate spatial scale of conservation units to preserve local adaptations when other information on the existence of local adaptations is unavailable.

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Data Sources and Methods

Information on hierarchical organization of genetic diversity in sockeye salmon has been summarised primarily from previous analyses reviewed by Wood (1995). New analyses of population structure and gene flow in Skeena River sockeye salmon are based on allozyme allele frequency data for adult samples from 22 spawning sites, most of which were sampled repeatedly over two or three years; the data (pooled across years) for 20 of these sites have been documented by Wood et al. (1994). Corresponding analyses of Skeena River coho salmon are based on unpublished data for juvenile collections from over 30 sites throughout the Skeena watershed, most replicated over two years, and assayed at eight microsatellite DNA loci; these analyses represent preliminary results of a more comprehensive study involving additional samples and seven additional microsatellite DNA loci (C.C. Wood, L.B. Holtby, J. Nelson, T. Norgard, and B. Finnegan, unpublished data).

Hierarchical analyses of gene diversity (Chakraborty 1980) in the Skeena River were based on allele frequencies at 10 polymorphic protein-coding (allozyme) loci in sockeye salmon, and at eight microsatellite DNA loci in coho salmon. These represent all available loci for which the frequency of the common alleles was less than 95 per cent in at least one sample under consideration. Computations were performed using BIOSYS release 1.7 (Swofford and Selander 1981). Where necessary to accommodate limitations of this program, consecutive microsatellite DNA alleles were binned arbitrarily to create a maximum of nine allele groups prior to the gene diversity analysis. Geographical hierarchies within the Skeena River were structured to be as comparable as possible for the two species, but some differences were unavoidable. Sockeye collections were partitioned into two "basins" defined as the upper and lower Skeena and ten "large tributaries" which correspond to lake systems, of which four were in the lower Skeena basin, and six were in the upper Skeena basin. Coho collections were partitioned into the same upper and lower Skeena basins, but a well sampled neighbouring coastal stream (Lachmach River) was also included as an additional basin. Coho samples were available from four tributaries (a subset of the six available for sockeye) in the upper basin and five tributaries (all four sockeye tributaries plus an additional tributary) in the lower basin.

Allele frequencies within sampling sites are determined by four processes: mutation, random genetic drift which is inversely proportional to the genetically effective population size (N_e), immigration (m), and selection (ϕ). Mutation can reasonably be ignored as a factor affecting allele frequencies in salmonid populations that originated since the last Pleistocene glaciation. Selection can be ignored because we deliberately surveyed variation at loci generally considered to be neutral to selection. Thus, we attribute differences in allele frequencies among sites to the joint effect of parameters N_e and m. Although we could not estimate these parameters separately using our allele frequency data, we estimated their product, gene flow (N_em) expressed as the number of migrants exchanged between sites per generation. Computations were done using Slatkin's (1993) isolation by distance program which uses Weir and Cockerham's (1984) estimator of F_{ST} based on allele frequencies at polymorphic loci, and the equilibrium relationship $F_{ST} = 1/(1+4N_em)$.

Ex Situ **Conservation Objective 1—Archiving Genetic Diversity**

It is desirable to archive as much genic variation as possible to maximise the adaptability of the preserved gene pool, and thus future opportunities for transplants, aquaculture, or other research. A stratified sampling design is critical to the success of this initiative given that genic diversity is partitioned among races or populations within a species. Ideally, by ensuring representation of samples from all of the major partitions, a well-designed stratified sampling scheme would capture as much of the species' genic diversity as possible within the constraints of archive space.

Surveys of neutral genetic variation (e.g., in allozymes or microsatellite DNA) can help to guide decisions about sampling design by revealing how genic diversity is partitioned, both geographically and among life history types (e.g., Chakraborty and Leimar 1987). Of course, only a very small proportion of the species' genome can be examined in genetic surveys. Inferences about population structure will only be reliable if the survey includes a sufficient number of polymorphic loci whose allele frequencies are relatively unaffected by natural selection.

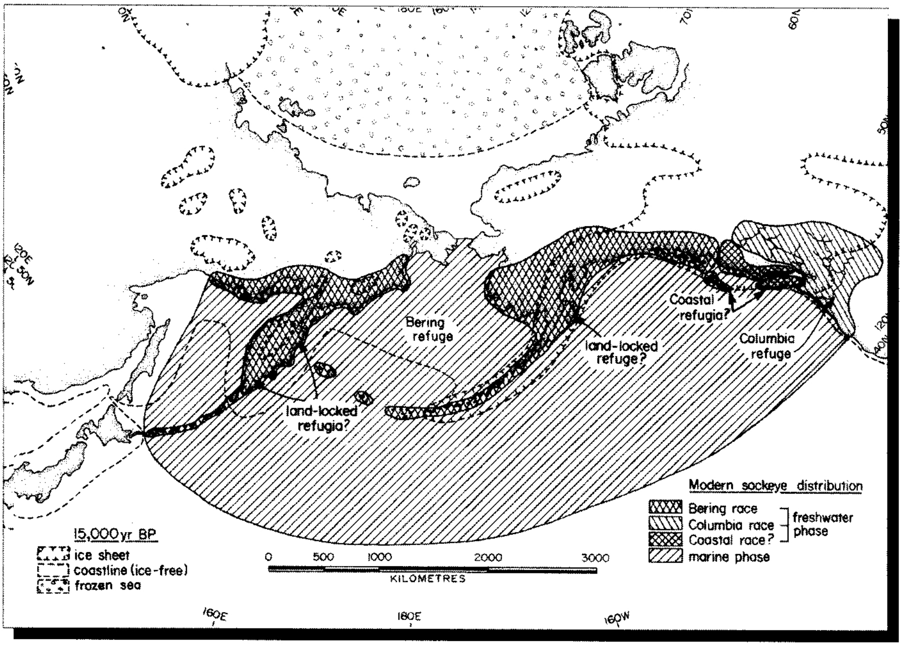
Population structuring occurs in all fish species to some extent, but it is especially prevalent in freshwater fish species that occur in geographically isolated habitats, and in anadromous species that home precisely to their natal freshwater spawning sites (Ryman, Utter, and Laikre 1995). Reproductive isolation typically leads to genetic differentiation among spawning sites through random genetic drift and this may be constrained or augmented by natural selection (Wright 1931). In addition, many temperate species, like salmonids, have evolved in an environment subject to recurrent cycles of glaciation. For example, most extant salmon populations in Canada have originated within the last 15,000 years from ancestors that survived in various refuges beyond the ice sheets (McPhail and Lindsey 1970). Prolonged isolation of refuges during glaciation (typically 60,000 years, Pielou 1994) may have permitted substantial genetic differentiation of potential colonising races. Consequently genic variation in extant populations may be strongly influenced by post-glacial dispersal patterns.

An example—population structure in sockeye salmon

The population structure of sockeye salmon has been studied extensively throughout its present natural range in the Pacific Ocean between 41° and 61° north latitude, and serves to illustrate the degree of differentiation that can exist among populations of anadromous salmonids, despite extensive overlap in their marine distribution (Figure 1). Hierarchical gene diversity analyses of variation at six allozyme loci (from Wood 1995) indicate that 17.1 per cent of the total genetic variation in anadromous sockeye salmon is associated with geographical subdivision—1.9 per cent with differences among political regions, 8.2 per cent among river systems (within regions), and 7.0 per cent with differences among lakes (within river systems). The remaining 82.9 per cent is associated with variation among individuals within lakes. Comparatively little variation is associated with samples from different spawning sites within the same lake system (<1 per cent but this can be highly

Figure 1

Modern distribution of sockeye salmon superimposed on postulated location of ice sheets, coastlines, and refuges at the height of the Wisconsin glaciation, 15,000 years before present (from Wood 1995)



significant both statistically and biologically) or collected from the same sites in different years (Varnavskaya et al. 1994; Wood et al. 1994). When political regions are replaced by three postulated colonising races (Beringian, Cascadian, and coastal British Columbian) as the first hierarchy in the gene diversity analysis, race accounts for 6.2 per cent of total genetic variation within the species, over one third of that attributed to geographic subdivision. Thus modern populations founded by different races are genetically distinct owing to prolonged reproductive isolation during the last glaciation.

Surprisingly, even more genetic variation (7.0 per cent) is associated with differences among sockeye utilising different nursery lakes within the same river system, and presumably colonised by the same race. Allele frequency differences among samples from different lakes are almost always statistically significant. The magnitude of these differences can be used to estimate gene flow between lakes if we assume that the differences arose primarily by random genetic drift and founder effects (i.e., no selection) and reflect an equilibrium between the effects of genetic drift and migration. Averaged over all regions, the number of effective migrants (Nem) exchanged among lakes within the same river system or local area is estimated to be only 3.2 individuals per generation. Such restricted gene flow among lakes underscores the fidelity with which sockeye home to their natal lake system and confirms previous inferences (based on tagging studies, and other genetic studies) that the nursery lake is the primary geographic unit of population structure in sockeye salmon.

Significant population structuring also exists among life history types in sockeye salmon independent of geography. Sympatric anadromous and non-anadromous (kokanee) life history morphs exist as partially or almost completely isolated populations in some lakes (Foote et al. 1989). For example, in Takla Lake, British Columbia, the two morphs spawn in close proximity in the same streams at the same time, yet over 17 per cent of the total gene diversity at polymorphic allozyme loci can be partitioned by morph, and gene flow between the morphs was estimated at less than two migrants per generation (Wood and Foote 1996). In addition, significant allele frequency differences sometimes exist between sea/river-type and lake-type sockeye in the same river system; and between inlet tributary, outlet tributary and beach spawning sockeye, or early and late timing sockeye within the same lake system (reviewed by Wood 1995).

The complicated hierarchical structure of genetic diversity in sockeye (and other salmonid) populations implies that the best way to conserve diversity within the species is to preserve fish in as many different lake systems as possible (MacLean and Evans 1981; Altukhov and Salmenkova 1991; Riddell 1993). Special consideration should also be given to protecting alternative life history types, since these often (but not always) represent reproductively isolated populations. The same logic extends to capturing genic diversity in *ex situ* conservation programs. However, since it is impractical to include samples from all lakes, care should be taken to include samples from regions colonised by ancestors that survived the last glaciation in different refuges. For example, if the objective were to maximise genic diversity in a gene bank of anadromous Fraser River sockeye, samples should be obtained from lakes above and below the Fraser Canyon, a discontinuity that appears to mark the limit of colonisation from refuges in Cascadia and coastal British Columbia, respectively (Wood et al. 1994). Significant additional genic diversity could be captured by including samples from non-anadromous kokanee in the upper Fraser watershed (Foote et al. 1989; Taylor et al. 1996).

Ex Situ Conservation Objective 2—Restoring Wild Populations

The capacity of salmonid populations to support sustainable fisheries, or persist through episodes of unfavourable marine survival, depends directly on local adaptations that improve survival, and hence productivity in particular freshwater habitats (Lannan et al. 1989). Thus, if genetic resources are being conserved *ex situ* with a view to restoring wild salmon abundance in a particular habitat, it will be important to conserve genotypes that evolved by natural selection because they confer adaptive value in that particular habitat.

Salmonids are well known for the variety of local adaptations they exhibit over surprisingly small spatial scales (e.g., Ricker 1972; papers in Billingsley 1981; Taylor 1991; Wood 1995). Adaptation to local conditions is facilitated by reproductive isolation that results from their precise homing. In some cases, homing is sufficiently precise to permit adaptation to water flow and temperature regimes within individual spawning streams. For example, sockeye fry emerging from redds downstream of the nursery lake display a heritable propensity for upstream migration, whereas those emerging from redds upstream of the nursery lake display a propensity for downstream migration (Raleigh 1967; Brannon 1967, 1972). This ability of salmonids to adapt to very localised conditions explains their persistence and ecological success in a wide range of freshwater habitats.

How should proposed restoration programs be prioritised given that resources available for *ex situ* (and other) conservation initiatives are limited? Allendorf et al. (1997) recently proposed scoring guidelines for prioritising salmonid stocks as candidates for conservation action based on three independent criteria: the risk that the stock will become extinct within 5 to 100 years; the consequences of extinction in terms of the genetic and evolutionary legacy of the species; and the consequences of extinction in terms of the ecological legacy for sympatric species. Although the guidelines proposed by Allendorf et al. (1997) represent an important step forward, their scoring procedure can be influenced greatly by how a candidate stock is defined. As yet, the proposed guidelines offer little advice on how to ensure that candidate stocks are defined at appropriate and comparable spatial scales.

Defining population units

In the remainder of this paper, we will consider how surveys of neutral genetic variation can provide insights for defining the appropriate spatial scale for prioritising stock conservation units. First, however, we think it necessary to introduce some explicit terminology based on a conceptual framework for standardizing comparisons. In our experience, ambiguity about the nature of population units causes confusion that can frustrate attempts to reach agreement about conservation priorities. For example, the terms deme, subpopulation, population, and metapopulation are commonly used to describe the hierarchical population structure of salmonids, but they are seldom used consistently. In the following proposed definitions, we distinguish terms used to describe persistent genetic organization from terms used to describe other, non-genetic (e.g., spatial or phenotypic), and perhaps ephemeral grouping schemes. The prefixes used to distinguish various types of demes are taken from Lincoln et al. (1982).

Definitions relating to non-genetic grouping schemes include:

Deme: an interbreeding group of organisms (without genetic connotations).

Topodeme: an interbreeding group occurring in a particular geographic area.

Ecodeme: an interbreeding group occurring in a particular habitat.

Phenodeme: an interbreeding group distinguished by phenotypic characters. The distinctive phenotype may result from environmental effects (a *plastodeme*) or from genetic differences (see *genodeme*)

Definitions relating to persistent genetic organization include:

Genodeme: the smallest differentiated (detectable) unit of genetic population structure. It may comprise non-differentiated topodemes. Gene flow between genodemes is large such that genetic drift and/or migration preclude local adaptation within the genodeme for typical selection coefficients (ϕ).

Subpopulation: a group comprising one or more genodemes that is partially isolated from other such groups ($N_em>10$). Local adaptation may exist if N_e and ϕ are sufficiently high.

Local Population: a group comprising one or more subpopulations that is relatively isolated from other such groups ($N_em<10$) and that is probably locally adapted to its habitat.

Closed population: a group comprising one or more populations that is almost completely isolated from other such groups ($N_em<1$) such that its genetic diversity is at risk through random genetic drift when reduced to low abundance. This is the smallest unit the IUCN considers for Red Book Listing (Baillie and Groombridge 1996), as proposed by Mace and Lande (1991).

Metapopulation: a demographic group comprising smaller groups (usually topodemes or subpopulations) that are interconnected by migration. A metapopulation is demographically distinct from other metapopulations. The concept has no genetic implications if the metapopulation comprises topodemes connected by very high migration rates. However, the concept will have evolutionary implications if the metapopulation comprises populations or subpopulations. A metapopulation that began as a subpopulation comprising genodemes may become a population comprising subpopulations as habitat becomes fragmented and gene flow among the genodemes is disrupted.

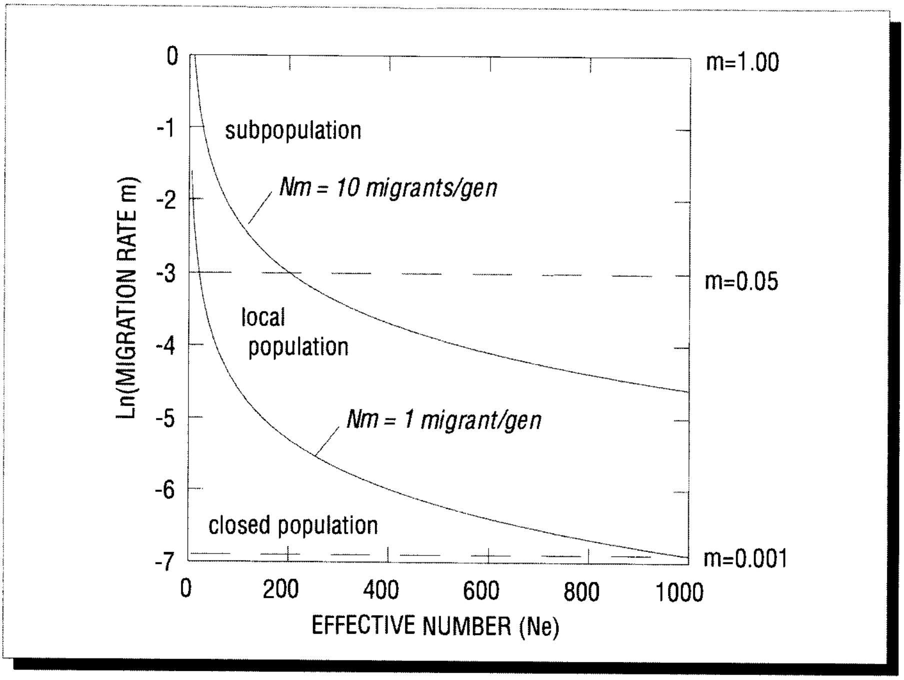
We think these definitions provide a rigorous and consistent framework for discussing salmonid population genetics issues, and we have tried to ensure that they will be useful to other species as well. The values of N_em proposed as criteria for designating subpopulations and populations are illustrated graphically in Figure 2 with respect to both m and N_e; these values are first cuts at the problem and may require revision. At the very least, our approach ensures that population units can be defined consistently in terms of population genetics theory, and this alone should help to standardize comparisons required for prioritization of candidate stocks.

Choosing units for conservation

To effectively restore salmonid runs to a former or desired level of abundance, it will be necessary to conserve local adaptations that are the genetic basis for productivity in their native habitat. From this perspective, any population unit that possesses unique adaptations should be regarded as worthy of conservation. Choosing the appropriate population unit to satisfy this conservation objective is therefore a matter of determining the spatial scale at which local adaptations exist. Utter et al. (1993) suggest a logical process for utilising both ecological and genetic data to accomplish this goal. Although surveys of selec-

Figure 2

Hierarchical units of population structure defined with respect to *N*e, the effective population size and *m*, the proportion of *N*e that has migrated into the group



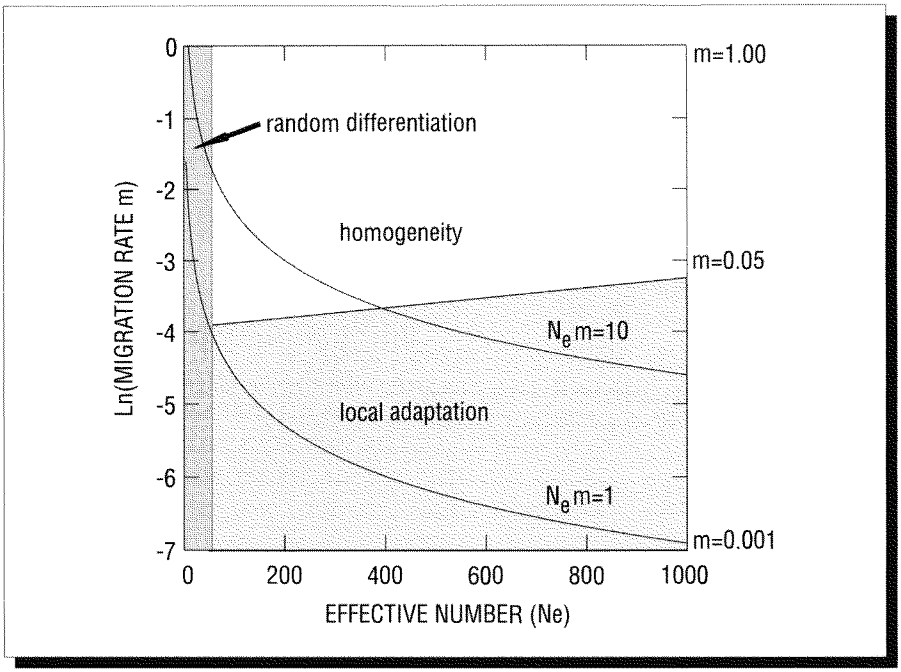
tively-neutral allele frequencies are useful for estimating gene flow, and hence for defining population units, they provide no direct evidence of local adaptation or divergence in evolutionarily significant traits (Clayton 1981). Even so, population genetics theory and simulation studies do provide some insight about the conditions under which local adaptation is possible (reviewed by Adkison 1995).

In closed populations, local adaptation is favoured when effective population size (N_e) is large. The random effect of genetic drift will overwhelm the directional effect of natural selection in a closed population if the selection coefficient, $\phi < 1/2N_e$ (Roughgarden 1979). Conversely, even weak natural selection can lead to local adaptation in a large, closed population. The situation is more complicated where populations are connected by migration. Slatkin (1973) and Nagylaki and Lucier (1980) demonstrated that local adaptation cannot occur if the spatial scale of selection is smaller than a "characteristic length" parameter defined by Slatkin (1973) as $l_c = \sigma_m/\sqrt{\phi}$ where ϕ_m is the standard deviation of the distance individuals migrate (measured in population units). This implies that the spatial scale associated with local adaptations will be larger for species that stray farther or have higher straying rates than for species that home more precisely, other things being equal. Straying between populations that results in a high immigration rate (m, the proportion of immigrants in a population) will lead to homogeneity among populations unless there are correspondingly large differences in selective regimes among the populations. To say that populations are homogeneous with respect to selected traits implies that adaptation has occurred at a larger spatial scale—it is not *local* with respect to the population units being considered.

Simulations by Mork (1994, for Atlantic salmon) and Adkison (1995, for Pacific salmon) demonstrate that for any given selection coefficient, two populations connected by gene flow will evolve local adaptations if m is below some threshold, or become homogeneous if m is above the threshold, provided N_e is large enough to reduce the relative effect of genetic drift. If N_e is too small, the populations will remain randomly differentiated regardless of m. We illustrate this conclusion schematically in Figure 3 for selection coefficients that Mork (1994) and Adkison (1995) considered most realistic for important traits in salmonids. To produce this figure, we superimposed, as best we could, Mork's (1994) and Adkison's (1995) simulation results on the m by N_e plane used previously to define popu-

Figure 3

Approximate conditions favouring local adaptation, homogeneity, or random differentiation of population units with respect to effective population size and immigration rate for a typical selection coefficient (ϕ=0.01) in Pacific salmon (adapted from simulation results of Mork, 1994 and Adkison, 1995)

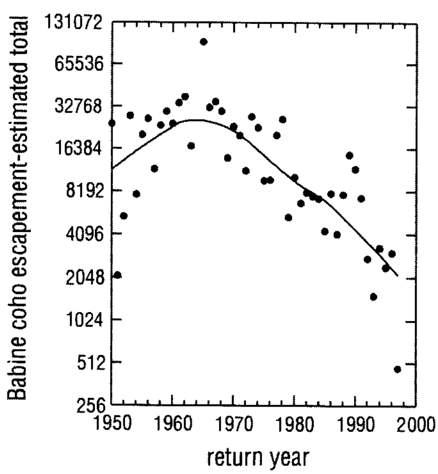


lation units. It should be noted, however, that additional simulations are required to properly delineate the boundaries between outcomes, especially for low values of N_e.

The schematic presentation in Figure 3 suggests that it should be possible to predict the occurrence of local adaptations for any specified selection coefficient, given estimates of N_e and m. In their prioritisation guidelines, Allendorf et al. (1997) suggest approximate methods for estimating N_e from census data (see also Waples 1990; Nunney 1993). Immigration rate is much more difficult to estimate directly, but, as previously described, surveys of neutral genetic variation can be used to estimate the product, $N_e m$, used here to define population units. By combining this information, it should be possible to estimate, at least approximately, the spatial scale at which local adaptation would be expected in the species or stocks under investigation. Note, however, that because the spatial scale of adaptation depends on the selection coefficient as well as the immigration rate, two populations connected by gene flow may exhibit unique local adaptations in traits subject to strong selection, yet be genetically homogeneous or randomly differentiated in traits subject to weaker selection (Gharrett 1994). Clearly, empirical evidence of local adaptation, such as the existence of important phenotypic differences among population units, should receive more weight than theoretical predictions. However, the theoretical predictions may be useful in guiding decisions in situations where no empirical evidence is available. In fact, a similar philosophy is implicit in using obvious differences in habitat (implying different

Figure 4

Trends in coho salmon spawner abundance at the Babine River counting fence*



*Total escapement has been reconstructed from reliable counts for the early part of the run (to 13 September).

selective regimes) as a criterion for defining evolutionarily significant units where more direct evidence does not exist (e.g., Waples 1995; Weitcamp et al. 1995).

Application to Skeena River salmon

To illustrate how this approach might help in prioritising conservation activities, we will examine the situation for coho and sockeye salmon inhabiting the Skeena River in northern British Columbia. A continuing decline in the abundance of coho salmon spawning in tributaries of the upper Skeena has raised concern about viability of these runs in the face of current mixed-stock fishing patterns (Figure 4). Virtually nothing is known about phenotypic variation or the extent of local adaptation in coho within the Skeena watershed. However, between 1995 and 1997, juvenile coho were collected from over 50 rearing locations throughout the Skeena watershed and many of these have been assayed for microsatellite DNA variation (see Methods). Our recommendations for conservation programs were necessarily based on these genetic survey data, combined with inferences about trends from a limited number of indices of adult and juvenile abundance.

We began by comparing the hierarchical organization of gene diversity in sockeye and coho salmon within the Skeena River (Table 1). The two species were surprisingly similar in that about 4 per cent of the total (neutral) genetic variation could be partitioned geographically within the Skeena watershed. In sockeye salmon, virtually all of the "explained" variation was partitioned among lake systems, which represent obvious geographical discontinuities in terms of juvenile sockeye dispersal. In contrast, the genetic variation in coho salmon was distributed as a cline, reflecting their more or less continuous spawning and juvenile distribution within the watershed.

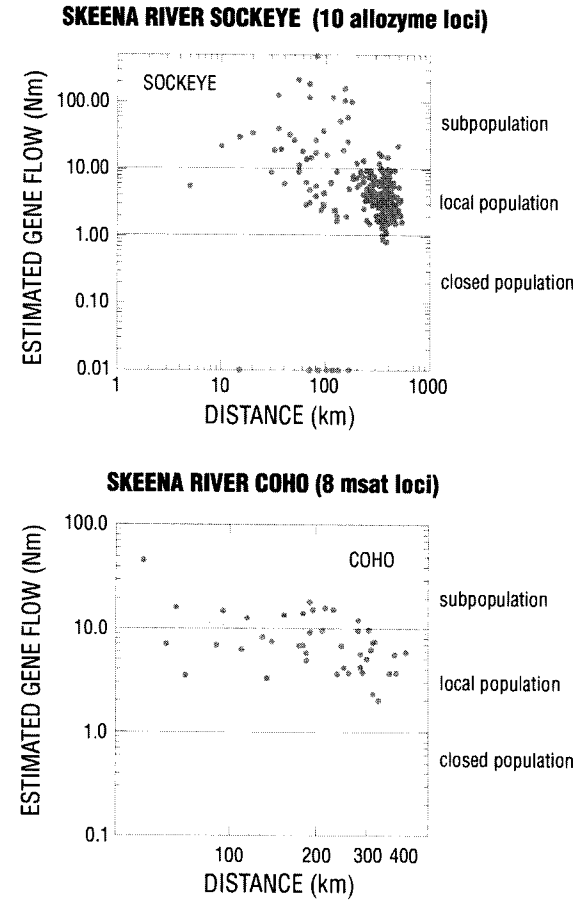
Table 1

Comparison of hierarchical organization of genetic diversity in coho and sockeye salmon in the Skeena River, northern British Columbia

	Coho	Sockeye
River System	Skeena	Skeena
Number of Samples	47	43
Number of Loci	8	11
Type of Loci	msatDNA	allozymes
Source of Variation		
Among Basins	0.010	-0.005
Among Major Tributaries (and Lake Systems)	0.014	0.046
Among Sites	0.015	-0.001
Total Due to Geography	**0.039**	**0.040**
Among Replicate Samples and Years	0.020	0.016
Unexplained Variation (Within Samples)	0.941	0.944

Figure 5

Comparison of estimated gene flow (*Nem*) as a function of distance in sockeye and coho salmon of the Skeena River*



*The fitted line corresponds to the null hypothesis of isolation by distance (Slatkin 1993).

The same pattern is revealed by plotting estimates of gene flow (expressed as the estimated number of migrants, N_em, exchanged between each pair of sampling sites per generation) against river distance separating the two sites (Figure 5). The fitted line is the expected relationship if gene flow were solely a function of distance, and independent of other aspects of geography. The "isolation by distance" model fits the sockeye data very poorly. Some widely separated sites (generally those within Babine Lake, the largest natural lake in British Columbia) appear to be connected by high rates of gene flow, whereas other sites in adjacent lake systems exhibit very low rates of gene flow. The average level of gene flow among sockeye sampling sites was about three migrants per generation. On the other hand, the coho data conform to the isolation by distance model about as well as can be expected given the relatively small number of loci investigated in this preliminary analysis (see Slatkin 1993).

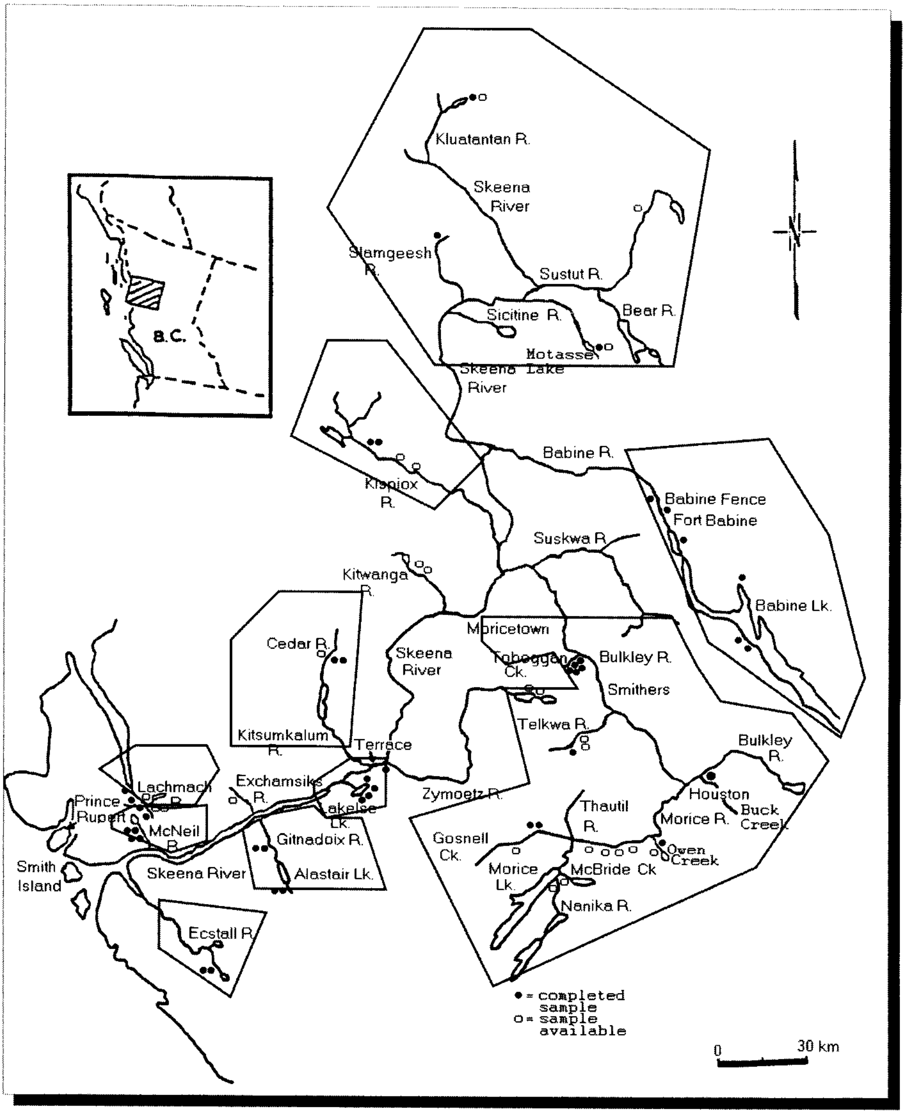
We grouped coho sampling sites together to form local populations according to the empirical estimates of gene flow and our definitions (Figure 6). In general, the isolation by distance model for Skeena coho indicates that sampling locations separated by 100-400 km are connected by gene flow equivalent to 5-10 migrants per generation (Figure 5). Thus, in the absence of more directly relevant survey data, sites separated by >100 km could be considered as local populations using our definitions. This spatial scale corresponds approximately to the major tributary level in our hierarchical analysis (Table 1). However, some tributaries to the lower Skeena exhibited sufficiently low levels of gene flow across smaller spatial scales and were also considered local populations. The average level of gene flow among coho spawning sites in the Skeena River was estimated at six migrants per generation. This is virtually identical to that reported by Wehrhahn and Powell (1987) based on their survey of allozyme variation in coho in southern British Columbia.

Phenotypic variation has been demonstrated in coho salmon for important life history traits such as age and size at maturity (e.g. Holtby and Healey 1986, 1990), egg size and fecundity (studies cited in Sandercock 1991). Most variation in life history traits is apparent only on relatively large spatial scales, but morphological and physiological variation have been demonstrated both on large (e.g. Taylor and McPhail 1985a, b; Taylor 1991; Murray et al. 1993) and small spatial scales (e.g. Rosenau and McPhail 1987; Swain and Holtby 1989). Unfortunately, very little is known about the extent of phenotypic variation in Skeena coho. Traits such as age at smolting, size at maturity, and spawning time are thought to be quite uniform throughout the drainage, although age x.0 males ("jacks") are absent upriver of Terrace (Figure 6). Morphological variation of the types described by Taylor and McPhail (1985a, b) and Swain and Holtby (1989) are known to exist within the Skeena, but there is insufficient information to resolve questions about the spatial scale of local adaptation. We suspect that the morphological differences observed in lake- and stream-rearing coho (Reisenbichler and McIntyre 1977) are heritable (D.P. Swain and L. B. Holtby, unpublished data). Of particular concern is the observation that the census units in which we have observed the most extreme and prolonged declines centre on the several large lakes in the Skeena drainage, like the Babine, the Lakelse, the upper Kispiox lakes, and the Kitwanga (Figure 6).

Because available information is inadequate to determine empirically whether local adaptations or unique phenotypic characteristics occur in any of these Skeena coho population units, we used the conceptual approach illustrated in Figure 3 to predict the spatial scale at which significant local adaptations are expected to occur. We relied upon adult census data to obtain approximate but independent estimates of N_e for some major tributaries. The best census data are available for the Babine coho population (Figure 4) which has averaged 5,500 spawners annually over the last 10 years. This corresponds to an N_e of

Figure 6

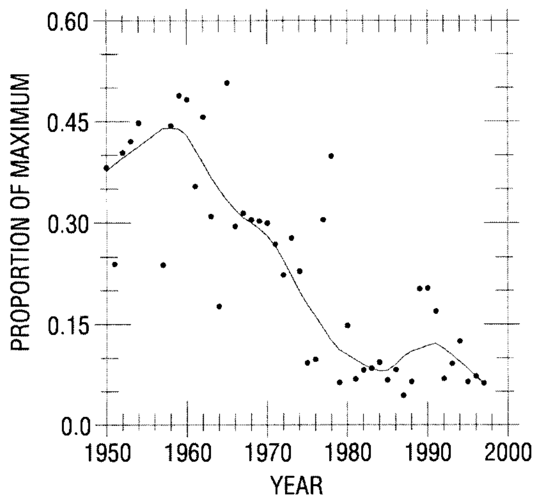
Map of Skeena River showing genetic survey sampling locations for coho salmon and spatial scale of groups defined as populations



about 3600 following the procedures of Allendorf et al. (1997), given that Babine coho spawn once at an average age of 3.3. Populations of this size or larger would be expected to evolve unique adaptations provided that m < 0.05 (Figure 3). From this analysis, we conclude that Babine coho should be considered as a local population possessing unique adaptations to conditions in the Babine River/Babine Lake system. Accordingly, this local

Figure 7

Index of coho salmon spawner abundance for the upper Skeena census unit *excluding* the Babine River (shown in Figure 4)*



*Spawner abundance is estimated visually for a variable number of streams each year throughout the upper Skeena, principally in the Bear, Sustut, and Bulkley-Morice tributaries. The index shown here is based on the proportion of the maximum count recorded for each system, averaged across all the areas surveyed in each year; the fitted line is a LOWESS smoothing function.

population warrants consideration and prioritisation as a candidate for conservation action, particularly given that the prolonged downward trend in abundance suggests an obvious risk of extinction within 100 years (Figure 4). On the other hand, our genetic survey data indicate that the Babine coho population is not so isolated that it should be considered a closed population. Consequently, its genic diversity is not yet threatened by random drift (Mills and Allendorf 1996), and quite appropriately, it does not meet the IUCN's criteria for Red Book listing (Baillie and Groombridge 1996), despite the dramatic decline in its abundance.

Nevertheless, it is important to recognize that the Babine coho population is part of a larger population unit encompassing the entire upper Skeena basin. Although abundance within the upper Skeena is measured inconsistently and with lower precision, an approximate index of abundance suggests there has been an equally severe but far more extensive decline in coho abundance throughout the upper Skeena (Figure 7). We do not know whether this decline warrants an IUCN Red Book listing. Such uncertainty highlights the need to census regularly on a spatial scale appropriate to the population structure of the study species.

An obvious limitation of our analysis (so far) is that we have failed to look for possible reproductive isolation among different coho life history types. As discussed above, it seems possible that local adaptations exist as differences in life history or run timing at a smaller spatial scale than revealed in the present analysis. We hope to address this issue in the near future. However, studies to demonstrate local adaptation are difficult and time consuming because they require that differences in phenotypic or life history traits be observed and documented as being both heritable and adaptive. In contrast, neutral genetic variation can be surveyed quickly, and genetic surveys provide a practical way to gain information in time to develop a systematic and rational response to conservation crises. Even so, it cannot substitute for gumboot biology.

Acknowledgments

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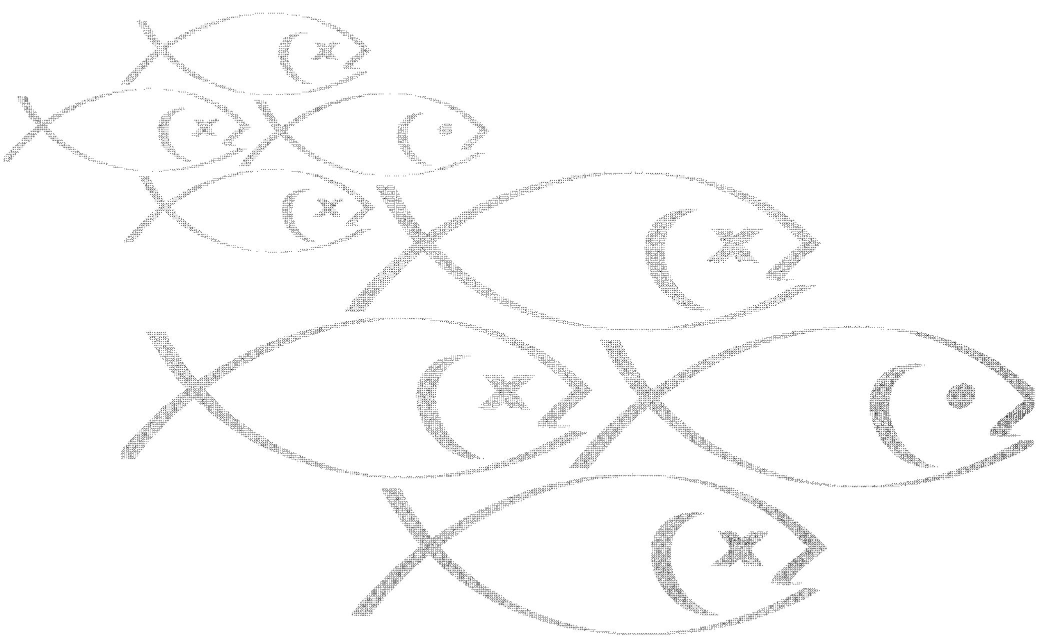

Appendix I - Observers

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Kim Wilby, BC Hydro

Appendix II - List of Acronyms

BFAR	Bureau of Fisheries and Aquatic Resources (Philippines)
CAMP	Conservation Assessment and Management Plan (India)
CBD	Convention on Biological Diversity
CCRF	Code of Conduct for Responsible Fisheries
CG	Consultative Group
CGIAR	Consultative Group on International Agriculture Research
CGRFA	Commission on Genetic Resources for Food and Agriculture
CIDA	Canadian International Development Agency
CITES	Convention on International Trade in Endangered Species
CSD	Commission for Sustainable Development
DFO	Department of Fisheries and Oceans (Canada)
DMSO	Dimethyl Sulfoxide
EC	European Community
EDI	Economic Development Institute
EcSU	Economically Significant Unit
EESU	Evolutionary and Ecologically Significant Unit
EIA	Environmental Impact Assessment
ESU	Evolutionary Significant Unit
FAO	Food and Agriculture Organization of the United Nations
FGFRI	Finnish Game and Fisheries Research Institute
GATT	General Agreement on Trade and Tariffs
GIFT	Genetic Improvement of Farmed Tilapias
GIS	Geographic Information Systems
GMO	Genetically Modified Organism
GNP	Gross National Product
GWP	Global Water Partnership
IARC	International Agriculture Research Centre

Appendix II - List of Acronyms (cont.)

IBAMA	Brazilian Institute for Environment and Renewable Natural Resources
ICAR	Indian Council of Agriculture Research
ICES	International Commerce Exchange Systems
ICLARM	International Center for Living Aquatic Resources Management
IDRC	International Development Research Centre
IOE	International Office of Epizootics
IPGRI	International Plant Genetic Resources Institute
IUCN	International Union for the Conservation of Nature
LTGB	Low-Temperature Gene Bank
MTA	Material Transfer Agreement
MSW	Multi Sea Winter
MSY	Maximum Sustainable Yield
NASCO	North Atlantic Salmon Conservation Organization
NATP	National Agricultural Technology Project (India)
NBFGR	National Bureau of Fish Genetic Resources (India)
NFFTRC	National Freshwater Fisheries Technology Research Centre (Philippines)
NGO	Non-Governmental Organization
PCR	Polymerase Chain Reaction
PGRFA	Plant Genetic Resources for Food and Aquaculture
SAARC	South Asian Association for Regional Cooperation
SADC	South African Development Community
SBSTTA	Subsidiary Body on Scientific, Technical and Technological Advice
SGRP	System-wide Genetic Resources Programme
TRIPS	Trade-Related Aspects of Intellectual Property Rights
UN	United Nations
UNCED	United Nations Conference on Environment and Development
UNDP	United Nations Development Program

Appendix II - List of Acronyms (cont.)

UNEP	United Nations Environment Program
UNESCO	United Nations Educational, Scientific and Cultural Organization
VNIIPRH	All-Russian Research Institute of Fresh Water Fish Farming
WCMC	World Conservation Monitoring Centre
WFT	World Fisheries Trust
WTO	World Trade Organization
WWC	World Water Council
WWF	World Wildlife Fund

Appendix III - Glossary

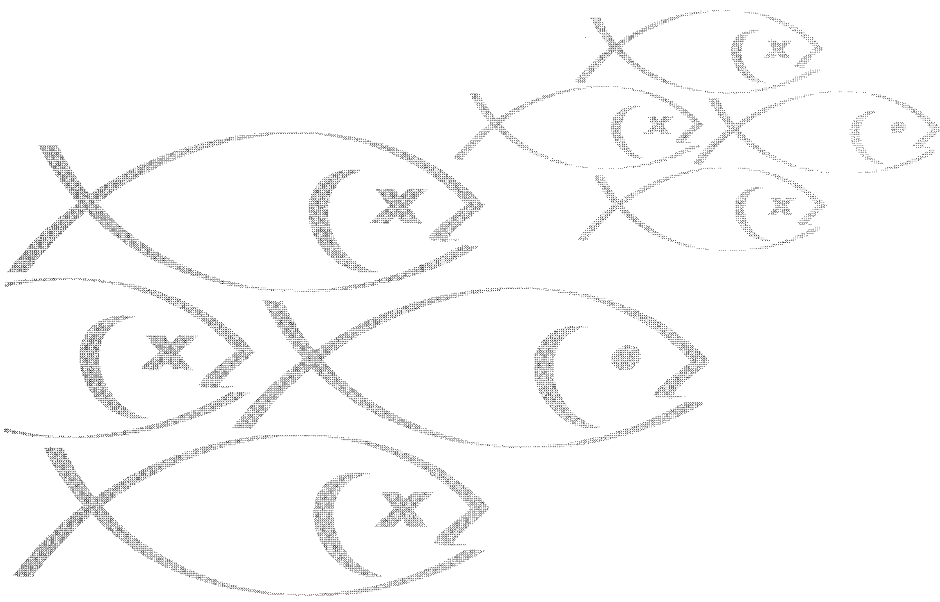
Agenda 21	Plan of action for implementing the Convention on Biological Diversity
allele	One of two or more forms of a gene that can exist at a single gene locus
allozyme	A specific type of isozyme (the form of an enzyme which has the same activity but differs slightly in amino acid sequence), produced by different alleles at a single locus
anadromous	Fish that ascend from the ocean into rivers to spawn
androgenesis	The process of creating a diploid organism using a donor egg from which the genetic information has been obliterated; creates an organism whose genetic makeup is contributed solely by sperm. Produces an androgyne
aquaculture	Farming of aquatic organisms (e.g. fish, shellfish, seaweeds)
biological resources	Includes genetic resources, organisms or parts thereof, populations or any other biotic component of ecosystems with actual or potential use or value to humanity
broodstock	Parents of the next generation of cultured (farmed) organisms
Convention on Biological Diversity	International convention on conservation and sustainable use of genetic resources
cryopreservation	Long term preservation of viable cells and tissue in liquid nitrogen
cytogenetic	The cytological approach to genetics, mainly involving microscopic studies of chromosomes
diadromous fish	Fish that undertake spawning migration from ocean to river or vice versa
diploid	Having two chromosome sets
***ex situ* conservation**	The conservation of components of biological diversity outside their natural habitats
extender	A chemical solution used to dilute fish sperm for use in artificial fertilization or cryopreservation
FishBase	ICLARM's comprehensive database on fish and fisheries

Appendix III - Glossary (cont.)

founder population	First broodstock used to start a fish culture program
gamete	A specialized haploid cell that fuses with a gamete from the opposite sex or mating type to form a zygote.
gene bank	Any collection of genetic material kept to ensure the future availability of that material for conservation, study or protection purposes
gene flow	The movement of genes from one part of a population to another, or from one population to another
gene pool	The sum total of all the genes of all the individuals in a population
genetic drift	A gradual change in allele frequency causing a reproductively isolated population to become homozygous
genetic material	Any material of plant, animal, microbial or other units containing functional units of heredity
genetic resources	Genetic material of actual or potential value
genic variation	The variability in alleles at specific loci without regard to the effects of combining alleles in diploid organisms
genome	The entire complement of genetic material in a chromosome set
genotype	The particular combination of genes present in the cells of an individual
germplasm	Genetic material
grow-out	The stage in aquaculture when young fish are grown to market size
haploid	Having one chromosome set, (cell or organism)
haplotype	A composite genotype defined over multiple loci in single-strand mitochondrial DNA (mtDNA) where all loci are tightly linked
Ichthyology	The study of fish

Appendix III - Glossary (cont.)

***in situ* conservation**	The conservation of ecosystems and natural habitats and the maintenance and recovery of viable populations of species in their natural surroundings and, in the case of domesticated or cultivated species, in the surroundings where they have developed their distinctive properties
karyotype	The entire chromosome complement of an individual or cell, as seen during mitotic metaphase
locus	The specific site on a chromosome where a gene is located
microsatellite	A highly variable section of non-coding nuclear DNA made up of many copies of a simple sequence motif
phenotype	The detectable outward manifestation of a specific genetic trait or genotype
polymorphic	The occurrence in a population (or among populations) of several phenotypic forms associated with alleles of one gene or homologs of one chromosome
polyploid	Having three or more chromosome sets, (cell or organism)
protected area	A geographically defined area that is designated or regulated and managed to achieve specific conservation objectives
stock	A reproductively, and usually geographically, isolated group of fish within a species, that is genetically distinguishable from other groups in the same species
tetraploid	Having four chromosome sets, (cell or organism)
transgenic	Organism whose genetic makeup includes a gene or genes from another genus or species
triploid	Having three chromosome sets, (cell or organism)



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