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**REPORT OF THE STUDY GROUP ON
INTERACTIONS OF WILD, RANCHED (ENHANCED), AND REARED SALMON**

Reykjavik, Iceland, 5 - 6 April 1994

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*General Secretary
ICES
Palægade 2-4
DK-1261 Copenhagen K
DENMARK

TABLE OF CONTENTS

Section		Page
1	INTRODUCTION	1
1.1	Terms of Reference (C.Res.1993/2:62)	1
1.2	Participants of the Study Group	1
2	DEFINING BIOLOGICAL AND MANAGEMENT TERMS	1
2.1	Wild Salmon	1
2.2	Enhancement	1
2.3	Salmon Ranching	2
2.4	Salmon Farming	2
2.5	Local Stock	2
3	REPORTS FROM RELEVANT WORKING GROUPS	2
3.1	Report of the Working Group on Pathology and Diseases of Marine Organisms (see Appendix 3)	2
3.2	Report of the Working Group on Application of Genetics in Fisheries and Mariculture (see Appendix 4)	3
3.2.1	Review of genetic background to Study Group's remit	3
3.2.2	Update on C.M.1991/M:3	3
3.2.3	Research in hand	3
4	STUDY GROUP'S CONSIDERATION OF QUESTION POSED BY NASCO	3
4.1	Genetic Interactions	3
4.2	Diseases and Parasites	3
4.3	Ecological Interactions	4
4.4	Environmental Interactions	5
5	STUDY GROUP'S CONCLUSIONS ON QUESTION POSED BY NASCO	5
5.1	Genetic Interactions	5
5.2	Disease Interactions	5
5.3	Ecological Interactions	5
5.4	Environmental Interactions	5
6	REPORTING TO NASWG	5
7	PROPOSED JOINT SESSION MEETING	6
8	FUTURE OF THE STUDY GROUP	6
9	RECOMMENDATIONS	6
	APPENDIX 1: Occurrence of, and Spawning Interactions between, Wild and Aquaculture Salmon in the Magaguadavic River, NB	8
	APPENDIX 2: UK National Report	9
	APPENDIX 3: Extract from Report of ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO)	12
	APPENDIX 4: Extract from Report of Working Group on Application of Genetics in Fisheries and Mariculture (WGAGFM)	13
	APPENDIX 5: Review of the Report of the Study Group on Genetic Risks to Atlantic Salmon Stocks (C.M.1991/M:3)	16

APPENDIX 6: National Status Report - Summary of Research Relevant to Genetic Interactions of Native and non-native Salmonids 19

APPENDIX 7: "Review Knowledge of the Amount of Gene Flow Between Specific Natural Populations as well as Between Cultured and Specific Natural Populations with a View to Proposing Future Studies . . . 29

1 INTRODUCTION

This Study Group is the successor to the Study Group on Genetic Risks to Atlantic Salmon Stocks that met once only at ICES Headquarters in Copenhagen, 13-15 March, 1991. The previous Study Group produced a report (C.M.1991/M:3) that has been used as a starting point for some of the current Study Group's deliberations. On completion of its work, the previous Study Group recommended that it be suspended until 1994 in order to allow time for research in hand or planned to reach completion. Although the present Study Group has recognised that much of the information necessary to respond fully to the first item of its Terms of Reference (see below) is not available, it has found that substantially more information is available now than was the case in 1991. Many other relevant studies are underway.

1.1 Terms of Reference (C.Res.1993/2:62)

A Study Group on Interactions of Wild, Ranched (Enhanced), and Reared Salmon will be established under the chairmanship of Mr A. Youngson (UK), with representation from the Anadromous and Catadromous Fish and Mariculture Committees, Working Group on Environmental Interactions of Mariculture, Working Group on Pathology and Diseases of Marine Organisms, Working Group on Introductions and Transfers of Marine Organisms, Working Group on Genetics, and Working Group on North Atlantic Salmon, and will meet in Reykjavik, Iceland from 5-6 April 1994 at national expense to:

- a) respond to a question by NASCO to evaluate the impact of salmon aquaculture on wild stocks with specific reference to genetic, disease, and parasite, ecological, and environmental impacts and to any impacts from current hatchery practices;
- b) develop preliminary plans for an ANACAT and Mariculture Committee Joint Session for the 1995 Statutory Meeting on "Interactions of Wild, Reared, Enhanced, and Ranched Salmon" for consideration by the parent Committees at the 1994 Statutory Meeting;
- c) report to the meeting of the Working Group on North Atlantic Salmon to be held in Reykjavik, Iceland from 6-15 April 1994 and ACFM.

1.2 Participants of the Study Group

E. Baum	USA
F. Caron	Canada
I. Fleming	Norway
K. Friedland	USA
G. Friars	Canada
S. Gudjonsson	Iceland
S. Helgason	Iceland
J. Jensen	Denmark
J. Jonasson	Iceland
L. Karlsson	Sweden
N. O'Maoileidigh	Ireland
R. Porter	Canada
T. Potter	UK (England)
O. Skaala	Norway
A. Youngson	UK (Scotland)

2 DEFINING BIOLOGICAL AND MANAGEMENT TERMS

In responding to the Group's Terms of Reference it has been found necessary to define the various categories of management (ranching, enhancement and rearing) that might lead to interactions between these groups and groups of wild salmon. It has been necessary also to try to define those groups of wild salmon that are considered to be of intrinsic value and therefore at risk from interaction with manipulated groups.

2.1 Wild Salmon

Wild salmon are defined as the progeny of an indigenous population that spawns naturally. The term indigenous is intended to imply a measure of temporal constancy: the term population to imply a degree of genetic separateness and a measure of genetic viability and independence. Unfortunately, it is not possible to define either term absolutely, not least because indigenous populations cannot be regarded as fixed entities. The term natural spawning is intended to indicate that volitional assortment at mating takes place and that natural selection is active among progeny.

2.2 Enhancement

Enhancement is defined as the release of salmon from a fish culture facility for the purpose of enlarging the wild population. It is not particularly intended that the fish should be recaptured at any later stage. Enhancement takes a range of forms, that may vary from the seeding of unfed fry into the same locations from which their parents were obtained to ranching itself (see below). Planting unfed fry is the simplest of enhancement techniques: the level of intervention is relatively low. However mating patterns are imposed on adults and greatly simplified compared with natural spawning. Recent

research work on the Girnock Burn, using mini-satellite genetic probes to assign progeny to their parents has revealed something of the complexity of pair formation in natural spawning and something of variation among maternal families between egg-laying and hatch. The findings are preliminary but progeny survival to hatch appears to be extremely variable, adult pair formation is not stable, adult male performance is highly variable and precocious parr contribute substantially (on average 40%) to paternity. In enhancement, no precocious parr contribute to the production of progeny and, for a time, natural selective pressure on the progeny is relaxed. Ryman (1991) has identified the potential effects of simple manipulations common in enhancement on effective population size. Favouring the progeny of particular families reduces the effective size of the managed population.

In Norway it is estimated that 8-9 million salmon fry are liberated annually.

2.3 Salmon Ranching

Ranching is defined as rearing salmon in a fish culture facility before releasing them as smolts to range freely in the ocean. Later, ranched salmon are targeted for harvesting as adults near the site of their release. In commercial ranching it is intended that the fishes' liberty is only temporary: the target for harvesting is 100%. However, harvesting is not likely to be total. Ranched salmon may well contribute to natural spawning, near the site of their release or in other locations to which ranched fish may stray.

Ranching is more interventionist than enhancement. Ranching involves all the manipulations used in enhancement and, in addition, it commonly involves the development of special genetic strains (often based on local broodstock), breeding from adults that have themselves been ranched successfully. In Iceland, particular ranching strains do not perform uniformly among ranching locations and genetic selection has been shown to improve return rates. Ranching techniques are not currently used to enhance sports fisheries, in Iceland or elsewhere, but future pressure to develop this approach may develop where salmon fisheries based on natural production are considered to be marginal or inadequate.

In Iceland, about five million smolts are released annually from three sites, currently. This compares with an estimated annual production of one million wild smolts from Iceland's rivers. In Norway, between about 250,000 and one million smolts have been released annually, in recent years.

2.4 Salmon Farming

Salmon rearing or salmon farming is defined as the production of fish retained in captivity for the duration of their lives, as a market commodity. Salmon farming involves all the manipulations involved in all the preceding forms of management. Natural selection is relaxed, mating patterns are imposed, passive selection occurs and selective breeding is practised in attempting to improve commercially valued performance traits. It is never intended that farmed salmon should spend any part of their lives at liberty. However, technical failure and human error have meant that substantial numbers of escaped farmed salmon are present in the oceans, on the coasts and in rivers throughout the North Atlantic area.

Reared salmon are present in the Faroese (27% in 1992) and Greenland (<2% in 1992) feeding areas and in many coastal and river fisheries in some (but not all) homewaters. In Norway, Scotland and Ireland (see NASWG Report for 1994) the frequencies of reared fish vary with season, with location, among years and throughout the season but in many cases frequencies are substantial. Escaped farmed salmon are common in the Magaguadavic River in New Brunswick (see also Appendices 1 and 2).

The Study Group notes that farming techniques may have a role in aiding the conservation of wild stocks. It may be possible to use farming as a means of amplifying numbers in severely depleted stocks (e.g., towards the southern limit of the species range in North America).

2.5 Local Stock

In general, genetic distance between salmon populations is correlated with geographical distance. Patterns of natural gene flow among rivers appear to comply with a "stepping-stone" model. In the context of aquaculture, local (or wild) stocks are defined as the salmon populations present in the rivers or streams near to the site(s) where farming is carried out.

3 REPORTS FROM RELEVANT WORKING GROUPS

3.1 Report of the Working Group on Pathology and Diseases of Marine Organisms (WGPDMO) (see Appendix 3)

It was the view of the WGPDMO that most of the disease organisms present in farmed salmon were also present among in wild fish. The Study Group noted however that the aquaculture industry has been responsible for the introduction of diseases/parasites into some areas which has had serious detrimental effect on wild

salmon stocks. Examples include the introduction of *Gyrodactylus salaris* and furunculosis into Norway.

The WGPDMO considered that wild fish were more likely to act as a reservoir of diseases for farmed fish than vice versa. However the Study Group concluded that while this may be true, the high frequency of diseases on farms and the concentration of pathogens have the potential to cause outbreaks of diseases in wild fish populations.

The WGPDMO considers that the proposed Joint Session meeting (see 1.1 above) would be of value from their perspective, if only as a means of taking stock.

3.2 Report of the Working Group on Application of Genetics in Fisheries and Mariculture (WGAGFM) (see Appendices 4-7)

3.2.1 Review of genetic background to Study Group's remit

The WGAGFM has expressed the view that insufficient is known of the four parameters — gene flow among wild populations, genetic structuring among wild populations, the genetic character of farmed salmon and gene flow between wild and farmed salmon — involved to be able to predict the genetic effects of interactions between wild and farmed salmon with accuracy. To define the general principles adequately by describing the range of possible outcomes, the WGAGFM has identified the need for a critical review of existing information as a possible preliminary to further well-designed experimental studies. In assessing local effects, the WGAGFM has identified the need for case specific information.

The WGAGFM has recognised the need to deploy both qualitative and quantitative genetic techniques in studies of interactions and notes parallels between the concepts of local adaptation and genotype-environment interactions.

The WGAGFM has identified a need to define the genetic characteristics of cultured salmon more closely and to monitor their development with time.

The WGAGFM stresses the importance of experimental design in studies of gene flow using genetic markers.

It is the view of the WGAGFM that a substantial research effort of genetic interactions between wild and farmed salmon is underway in a number of countries. With regard to the possibility of devoting a theme session to interactions between wild and farmed salmon, the WGAGFM considers that from the genetics point-of-view, this would be worthwhile.

3.2.2 Update on C.M.1991/M:3

In general, the content of C.M.1991/M:3 remains accurate. New genetic techniques or variants or developments of existing techniques continue to increase the power to resolve genetic differences or to use genetic markers as tools in experimental studies. Additional stock transfers between countries have been noted - surprisingly, some involving transfers among continents are very recent.

3.2.3 Research in hand

The WGAGFM has identified a large number of current studies examining a broad range of interactions from a number of view-points. These studies are all genetically based but they vary greatly in their design and in their objectives. Methodological development, monitoring of escaped or released cultured salmon, local case-histories on genetic exchange, experimental studies of performance variation, selective breeding, genetic manipulation and modelling are all covered. Many of the individual projects are major studies in themselves. Considered together it seems likely that completed studies and studies in hand will resolve many current uncertainties within the several years required to bring current studies to a conclusion.

4 STUDY GROUP'S CONSIDERATION OF QUESTION POSED BY NASCO

4.1 Genetic Interactions

The Study Group recognised that, in relation to genetic effects, clear distinctions among enhancement, ranching and farming cannot be made. All these techniques form part of a continuum and a wide range of variations are practised. Locally relevant information is essential in assessing the likely effects of these techniques where they are carried out. On the other hand, all the techniques share elements in common and it is possible to identify general principles that relate to all the techniques — as below. This can be done already — and the conclusions drawn in C.M.1991/M:3 remain accurate. In the genetic context, the findings of the various studies currently in hand (see Appendix 2) will lend balance and clarity to the various arguments.

4.2 Diseases and Parasites

In the context of disease and parasites the situation is less advanced and all the necessary work does not appear to be being pursued. The Study Group considers that new relevant studies on disease and parasite interactions between wild, enhanced, ranching and farmed salmon should be encouraged with the objective of pro-

viding a firmer basis for assessing and categorising levels of risk.

4.3 Ecological Interactions

This section formed a major part of those of the Study Group's deliberations that were independent of the material contributed by the Working Groups. The purpose of these discussions was to address the current state of knowledge about ecological and genetic interactions between cultured and wild Atlantic salmon.

It is evident that the culture of salmonids leads to divergence from their wild form, affecting performance in nature. Studies of ranched Pacific salmonids (*Oncorhynchus* spp.) suggest performance differences may occur at various life history stages (Reisenbichler and McIntyre, 1977; Leider *et al.*, 1990; Swain and Riddell, 1990; Fleming and Gross, 1993). Recent evidence suggests similar patterns exist for cultured Atlantic salmon, particularly farmed salmon.

Artificial culture of Atlantic salmon appears to lead to environmentally- and eventually, evolutionarily-induced changes to their phenotype. Fleming *et al.* (in press) contrasted Atlantic salmon of a common genetic background but different rearing histories (i.e., wild, sea-ranched, and farmed). These first-generation fish were also contrasted with a fourth-generation farmed population (Sunndalsøra, Norwegian commercial strain), and with wild and multi-generation sea-ranched populations of coho salmon.

Comparisons of hatchery-reared and wild juveniles revealed distinct differences, however, when the juveniles were reared to adulthood in the natural marine environment (i.e., sea-ranched) many of the environmentally-induced differences disappeared. Greater adult divergence from the wild state was apparent in multi-generation sea-ranched coho salmon suggesting that evolutionary changes may accumulate over generations. Continued farming of salmon juveniles through to adulthood increased environmentally-induced differences considerably (see also Lund *et al.*, 1989). Fourth-generation Norwegian farmed salmon showed the greatest phenotypic differences. These findings suggest that the proportion of a salmon's life history, and number of generations spent in culture are likely important determinants of divergence from the wild form. Such divergence is bound to be intimately linked to inferior performance and reduced fitness among cultured fish that are released or that escape.

On the other hand, successful spawnings by farmed salmon in wild populations have been documented (Lura and Sægrov, 1991; Webb *et al.*, 1991, 1993) and evidence of genetic intermixing found (Crozier, 1993). For instance, Webb *et al.* (1991) could attribute about 50%

of the redds in the River Polla during 1989 to escaped farmed females using carotenoid pigment analysis. Furthermore, observation and radio-tracking showed males to be more active than females over a greater part of the river and over a greater part of the spawning season.

Evidence is emerging, however, that cultured Atlantic salmon are reproductively inferior and that this may constrain ecological and genetic interactions. A radio-tracking study in the River Alta suggests that farmed salmon remain for a significantly shorter period on spawning grounds (five days) than wild salmon (eight days) (Okland *et al.*, in press). Elsewhere in Norway, a series of experimental studies, using arenas designed to simulate natural breeding conditions, have been conducted to compare the reproductive behaviour and success of cultured and wild Atlantic salmon (Fleming *et al.*, in prep.). In a comparison of farmed and wild salmon, farmed females were observed to display less breeding behaviour, construct fewer nests and retain more eggs unspawned, appearing to have been denied access to breeding resources and opportunities. Furthermore, farmed females were less efficient at nest covering, incurred more nest destruction and suffered greater egg mortality than wild females. The competitive and reproductive inferiority of farmed salmon was accentuated in the males, which were less aggressive, courted less frequently and partook in fewer spawnings. Even in the absence of wild salmon, farmed males exhibited inappropriate mating behaviour that led to poor fertilisation success. In these experiments, farmed fish achieved between 11 and 19% of the breeding success of wild salmon when in competition.

Furthermore, Youngson *et al.* (1993) have identified what is likely a behavioural deficiency in escaped farmed fish that has led to increased levels of hybridisation with brown trout. Such hybridisation was found to be 10 times more frequent among escaped farmed than wild Atlantic salmon females. Concern was raised regarding the negative effects this may have not only upon Atlantic salmon populations, but also upon brown trout populations.

It was mentioned (Fleming, Lamberg, and Jonsson, unpublished) that this reproductive inferiority of cultured salmon is tempered considerably when they are sea-ranched (i.e., the hatchery-reared smolts are released to grow naturally in the sea to maturity) rather than farmed. While still reproductively inferior to wild salmon (Jonsson *et al.*, 1990), sea-ranched salmon have approximately 80% the breeding success of wild salmon. Thus, the life history stage at which fish are released, either intentionally or unintentionally, is likely to be an important determinant of their performance under natural conditions.

Body size differences between cultured and wild salmon (Fleming and Gross, 1993), intensity of competition on the spawning grounds (eg, density of spawning population; Fleming and Gross, 1993) and spatial and temporal breeding patterns (Webb *et al.*, 1991) were all identified as important factors that are likely to determine the potential for ecological and genetic interactions. For instance, nests of wild females may be destroyed by nest superimposition by later spawning farmed females (Lura and Sægrov, 1991; Webb *et al.*, 1991). Interactions are thus likely to be case specific and dependent on a series of factors.

4.4 Environmental Interactions

Scope for environmental interactions (salmon on salmon) between wild and enhanced, ranched or farmed salmon form a more speculative category. The Study Group identified the following possibilities:

- a) predator attraction and increased predation rates where reared or ranched salmon are present among wild salmon;
- b) inadvertent harvesting of wild salmon among ranched salmon when groups shoal together near the harvesting site;
- c) local degradation of natural fresh water habitat caused by effluent from fresh water rearing units nearby.

5 STUDY GROUP'S CONCLUSIONS ON QUESTION POSED BY NASCO

5.1 Genetic Interactions

The Study Group considers that enhanced, ranched and farmed salmon have potential to interact genetically with wild salmon, altering the natural balance of genetic population structuring through

- a) relaxation of competition/selection;
- b) inadvertent or passive selection;
- c) selective breeding;
- d) drift;
- e) transfer of non-local stocks.

All the various categories of effect are continuously variable parameters and all have an additional component related to the numbers of fish being released or escaping to breed and especially their relative fitness (measured as gene flow). Some of the factors a-e may increase fitness but in general, they would be expected to lower it. Lower fitness may mitigate the ultimate consequences of interaction but adverse effects on wild populations might

be expected to result from single interaction events in the shorter-term. The relevant time-scales cannot be defined. Repeated interactions in succeeding generations will further complicate assessment. Work in hand may clarify some of these matters but, again, locally relevant information will be of continue to be over-riding importance.

The Study Group wishes to flag the possibility that genetically modified salmon (GMOs) may become available for use in aquaculture in the future - although they are not available or in use at present. If this innovation is made, the possibility for interactions will exist and it should be considered in relation to the specific nature of the genetic modification.

The Study Group wishes to emphasise again, that the widespread use of triploid stock in salmon rearing would reduce any possibility for genetic interaction with wild fish.

5.2 Disease Interactions

The Study Group considers that scope for disease/parasite interactions does exist but that insufficient information (with the special exceptions of furunculosis and *Gyrodactylus* transfers) is available to identify and assess the risks clearly.

The Study Group notes that cleaner fish species (wrasses) may act as vectors, independently of salmon stock movements.

5.3 Ecological Interactions

The Study Group considers that wide scope for ecological/behavioural interaction exists. This has not been widely explored and some of the effects extend outside salmon to include brown trout. This behavioural effect may be to the detriment of genetic population structure in both species.

5.4 Environmental Interactions

The Study Group considers that the possibility of salmon on salmon environmental interactions has not been explored sufficiently to reach any other than the speculations listed.

6 REPORTING TO NASWG

The Study Group communicated its findings to the North Atlantic Salmon Working Group at a joint session on 8 April. The Study Group's draft report was subsequently modified as a result of these discussions.

7 PROPOSED JOINT SESSION MEETING

The Study Group has considered Item 2b of the Study Group's Terms of Reference in consultation with the NASWG and the Chairman of the ANACAT Committee. The Study Group considers that a Joint Session Meeting in 1995 would not be the most effective means of drawing together all the strands of information that will be required to be drawn together in the future.

As an alternative proposal, the Study Group considers that the possibility of setting up a ICES Symposium meeting in 1996 should be considered, for the following reasons

- a) the Symposium rather than the Joint Session is considered to be a more appropriate forum for exchanging the information at the level required;
- b) the Symposium format would enable the requisite amount of time to be devoted to scientific exchange;
- c) the Study Group's assessment of the pattern of work known to be in progress, suggests that substantially more relevant information will be available in 1996 than in 1995.

8 FUTURE OF THE STUDY GROUP

As regards its own future, the Study Group considers that it may continue to have a role as an intermediary among the various contributing Working Groups, that it can add to their separate deliberations and that it can relieve the pressure on their own work.

Given the pace at which the relevant research is proceeding, reconvening the Study Group before 1996 would probably not be considered worthwhile. However, it may be considered that the future of the Study Group will be linked with the course of action decided at 6, above, by the appropriate authority.

9 RECOMMENDATIONS

1. That work on genetic aspects of interaction between wild and enhanced/ranched/farmed salmon should continue to be encouraged.
2. That new, relevant studies on disease/parasite interactions should be initiated.
3. That additional weighting should be given to performing behavioural ecological studies. Studies like these will identify the limits of genetic and disease interactions in specific localities. They will identify the geographical scope of effects resulting from single escapes or releases.
4. That modelling studies should be initiated to describe the general principles of interactions using existing information and the new information that is expected to become available in the near future. Studies like these will pin-point weaknesses in the range of data available, as a basis for setting future research priorities. These studies should be started now, given the inevitable lag-time in their development.
5. That a symposium session in 1996 should be considered as an appropriate forum for the Study Group's next exchanges.

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APPENDIX 1

Occurrence of, and Spawning Interactions between, Wild and Aquaculture Salmon in the Magaguadavic River, NB

Report to ICES Study Group on Genetic Risks to Atlantic Salmon Stocks

Atlantic Salmon Federation
CANADA

This study is being conducted by the Atlantic Salmon Federation (Jonathan Carr and John Anderson), with the cooperation of the Department of Fisheries and Oceans (Gilles Lacroix and Vlado Zitko), and Dalhousie University (Roger Doyle). The Magaguadavic River was chosen because it is close to the Bay of Fundy salmon aquaculture industry, and all returning adults must pass through a fishway, at the river's mouth. Fish of aquaculture origin are identified by external morphological features and scale readings (fish that smoltified in one year are presumed to be of aquaculture origin).

Results for 1992, the year the study began, and 1993 shows that about 35% of the total run of salmon of about 500 are aquaculture escapees. In 1993, swimming behaviour of wild and aquaculture adults was compared by tracking fish fitted internally with acoustic tags. Aquaculture fish tended to stay in the lower reaches of the river. Analyses of eggs sampled from redds for a carotenoid pigment found only in commercial aquaculture feed, confirm that interbreeding between wild and aquaculture salmon very likely occurs. Whether or not resulting introgression is leading to outbreeding depression is, of course, the all-important question. It is hoped to begin addressing this question in 1994 by the use of "microsatellite" DNA technology which allows progeny of parents, the latter sampled as they went through the fishway, to be identified as to their parenthood.

APPENDIX 2

UK National Report

ICES Study Group on Interactions of Wild, Ranched (Enhanced) and Reared Salmon

1. Frequency of Salmon of Farmed Origin in Coastal Fisheries

Salmon catches have been monitored in the commercial, coastal fishery at Redpoint, near Gairloch in western Scotland since 1990. A variety of techniques have been used at different stages in our investigations, including morphology, carotenoid pigment analysis and scale-reading. Salmon of farmed origin have been present in substantial numbers in every year. A range of different types is present, escaping or being released at different stages of life. Fish that have been released from fresh water and fish that have escaped recently from cages are present. All the intermediate types are present, too. The overall, frequencies are as follows. With the exception of 1991, all the figures are based on large samples taken throughout the fishing season. The figure for 1991 was based on the use of morphology only on two day-catches.

1990	- 22 %
1991	- 20-25 %
1992	- 18 %
1993	- 37 %

In 1993, sampling was extended to include additional commercial fisheries to test whether data obtained at the Redpoint fishery were likely to be representative. The results were as follows, from south to north along the Scottish coast.

Garlieston (near Dumfries in south-west Scotland)	-3 %
Kilmorie (in Mull in western Scotland)	-23 %
Redpoint	-37 %
Culkein (in western Scotland)	-23 %
Strathy (in northern Scotland)	-20 %
Bonar Bridge (north eastern Scotland)	-3 %

In general, the distribution of farmed salmon in coastal catches matches the distribution of salmon farming around the Scottish coast.

2. Genetic Comparisons of Wild and Farmed Salmon

Strains of farmed salmon in Scottish aquaculture that could be identified to their original source river were compared with wild salmon from the same source (Youngson *et al.*, 1991). Sixteen farmed strains were examined. Comparisons were performed in two consecutive year-classes (the 1988 and 1989 hatch years) of 12 of the strains. The comparisons were performed on the basis of allele frequency variation in six polymorphic loci. All the strains differed significantly from wild fish in the rivers on which they had been founded. In general, the differences appeared to be stochastic in origin. However, among the 1989 hatch year-class, frequencies of the 125 allele for *MEP-2** were elevated in every strain examined. The finding could not be confirmed among the 1990 hatch year-class. However, subsequent work has demonstrated unusually high frequencies for the *MEP-2** 125 allele among farmed salmon in Ireland (Cross, Crozier). In addition, evidence exists for the selective maintenance of *MEP-2** allele frequencies among wild salmon (Jordan *et al.*, 1990; Jordan and Youngson, 1991).

PCA shows Scottish wild populations, Scottish strains based on Scottish rivers and Scottish strains based on Norwegian rivers to group separately.

3. Spawning Behaviour of Escaped Farmed Salmon in the Wild

A study of the spawning of escaped farmed salmon in the Polla has been completed. The river was studied in autumn 1989 (Webb *et al.*, 1991) and in autumn 1990 (Webb *et al.*, 1993), following the escape of almost 200,000 growers from sea-cages nearby in February, 1989.

In the first year (1989), escaped salmon returned in substantial numbers (hundreds) to spawn. Carotenoid pigment analysis, demonstrated that about 50% of the redds made in the river in autumn, were attributable to escaped female growers. Growers spawned lower in the river and later in the season than wild fish. In both respects, the performance of the sexes differed. Observation and radio-tracking showed males to be more active than females over a greater part of the river and over a greater part of the spawning season.

In the second year, 14 of 73 spawners were identified as escaped farmed fish but only six of these showed scale patterns consistent with their being part of the documented escape. All six still contained the pigment canthaxanthin. The others were presumed to be opportunistic entrants from escapes from other sites and this is consistent with the numbers of escaped salmon entering other rivers opportunistically around the Scottish coast (see below). At spawning, only five of 54 redds examined in the Polla contained canthaxanthin. There was no evidence of substantial straying to adjacent rivers. Most of the original escape (>99%) were not accounted for in the two spawning seasons following their release. It seems likely therefore that mortality rates were high between the time when the fish escaped and the times at which they might have spawned.

4. Escaped Farmed Salmon in Coastal Fisheries

In 1990, 403 salmon from seven day-catches made by a coastal fishery in western Scotland were examined using morphology, scale-reading and pigment analysis (Webb and Youngson, 1992). Fifty-eight (22%) of the catch were classed as fish that had escaped or been released from culture. Among these fish, 65% contained canthaxanthin and had therefore escaped from sea-cages. Among the others, an additional 17 (31%) were classed as having escaped from sea-cages according to scale patterns. The remaining 4% were classed as having escaped or having been released from fresh water, according to scale patterns.

In subsequent years, the same fishery has been monitored. Although the total proportion of cultured fish in the catch has remained substantial (see above) the break-down of types (stage of life at release) has varied markedly among years.

5. Distribution of Spawning by Escaped Farmed Salmon

At emergence time in 1991, salmonid fry were sampled from 16 Scottish rivers between the Cree in the southwest and the Carron on the northeastern coast. Rivers were selected for sampling that did not support smolt-rearing units. Salmon fry containing canthaxanthin (the progeny of escaped female growers) were detected in 14 of the 16 rivers. Overall, 5% of the fry contained canthaxanthin — the greatest frequency observed was 18%. These values underestimate the contribution to spawning made by escaped fish. In the year of study only 65% of escaped growers contained canthaxanthin, fish that escape prior to the grower stage did not contain canthaxanthin and escaped male fish make no contribution to the pigment load of their progeny.

6. Behavioural Deficiency in Escaped Farmed Salmon

In the survey above, trout and salmon X trout hybrids were detected among samples. Trout were not considered. Among the 23 hybrids, eight contained canthaxanthin (35%). Among 2,350 salmon fry, 101 (4%) contained canthaxanthin. The difference was significant. Escaped farmed female salmon hybridised with brown trout 10 times more frequently than wild females: about 10% of the progeny of escaped farmed females were hybrids.

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APPENDIX 3

Extract from Report of ICES Working Group on Pathology and Diseases of Marine Organisms (WGPDMO)

Please note that this is an extract from the draft WGPDMO 1994 Report and will not necessarily be identical to the final document.

The WGPDMO considered the questions raised and concluded the following:

1. The literature on interactions of disease between wild and reared salmon is scarce. What is available contains mostly circumstantial evidence for interaction of disease between farmed and wild fish. (For example the Reports of the Furunculosis Committee, UK, 1930, 1933).
2. A number of research projects are being conducted in ICES countries:
 - in Scotland, a project on the association between sea lice, IPN, PD and furunculosis in sea trout is in progress;
 - also in Scotland, a project on diseases in wrasse (*Labridae* spp.) (cleaner fish) and cultivated salmon to identify whether either species acts as a reservoir of infection for the other;
 - in Norway, projects on the interaction of sea lice in cultivated salmon and wild salmon and sea trout;
 - in Norway, a project on the interaction of typical furunculosis in wild and cultivated salmon;
 - especially in Norway, and in some other countries, there are a number of studies into the spread of *Gyrodactylus salaris* in wild salmonid stocks.
 - in Ireland, investigations on mortalities associated with disease, particularly lice in sea trout (see Report of Sea Trout Working Group, 1993, The Department of the Marine, Dublin, Ireland);
 - in the USA studies on significant losses of Pacific salmon associated with the Erythrocyte Inclusion Body Syndrome (EIBS) virus;
 - EU countries with wild and farmed salmonid stocks routinely conducted monitoring programmes for diseases listed in EC Directive 91/67.
3. These projects are ongoing or starting as funding becomes available.
4. A joint meeting could be useful between ANACAT/Mariculture Committees, if only to spell out what the current situation is on this problem.

Conclusion

The WGPDMO concluded that most of these projects were under-resourced and with additional funding could be fruitfully expanded. Furthermore, the WG would like to draw attention to its 1992 report, C.M.1992/F:2, ref session, item 5: "Analysis of cases of disease interactions between farmed and wild populations of fish". The conclusions in the 1992 report are still valid at this time as the situation has not significantly changed since then. The WG's opinion is that most of the disease organisms present in farmed fish were also present in wild fish stocks. Furthermore, it was considered that wild fish were more likely to act as reservoirs of disease for farmed fish than vice versa.

APPENDIX 4

Extract from Report of Working Group on Application of Genetics in Fisheries and Mariculture (WGAGFM)

3. Terms of Reference (C.Res.1993/2:27)

3.1 "Prepare information for use by the Study Group on Interactions of Wild, Ranched (Enhanced), and Cultured Salmon"

On request from the WGAGFM on what type of information was needed, Alan Youngson (Chairman of the "Interaction Study Group") asked the WGAGFM to respond to the following three questions:

1. Is more work on interactions required?
2. Is enough work in progress?
3. Will a theme session at the 1995 Statutory Meeting (CR 2:62) be worthwhile from the genetics point of view?

In addition to responding specifically to these three questions (Sections 3.1.1, 3.1.2, and 3.1.3) the WGAGFM produced information for the "Interaction Group" by reviewing the most recent Study Group report on interactions (Appendix 5), and by preparing a list of current national activities in this area (Appendix 6). Also, preliminary versions of Sections 3.3 (on gene flow) and 3.4 (on combined studies) were enclosed (Appendix 7).

3.1.1 Response to Question 1

In order to assess or predict genetic effects from interaction between cultured and farmed salmon it is necessary to have information on the four parameters of interaction:

- a) The effective gene flow in the natural structure of populations;
- b) Genetic characteristics (including magnitude of local adaptation) of wild populations;
- c) Genetic characteristics of enhanced/cultured salmon;
- d) The effective gene flow between enhanced/cultured and wild populations.

Assessment of interaction in specific cases will usually require case specific information, while a more general consideration must be based on knowledge on the range of values that these four parameters (a, b, c and d) can take. Although much information undoubtedly exists, it is scattered in the literature and may be difficult to access. To this end there has not been any systematic survey or review that simultaneously covers all these interaction related parameters in salmon.

- a) The amount of gene flow between natural populations sets, so to speak, the evolutionary scene. Without reductions in gene flow between some intraspecific groupings, there would be hardly any substantial or lasting differentiation (or multiple populations at all, for that sake). Estimates of this important parameter should therefore have high priority and be carried out using the best available methodology. Unfortunately, this has not always been the case.

In salmon, published estimates of the natural gene flow between wild populations are diverse. This may not be surprising since in reality, there are probably substantial differences in gene flow between different pairs, or sets, of natural populations. It is important to be aware that the value of gene flow estimated via F_{st} or G_{st} (ie, the most common method) usually is averaged over many loci and many pairs of populations. Such mean values may not give a realistic description of the population structure (refer to Section 3.3.1). In fact they may instead hide the real variability if they are not accompanied by adequate measures of their range and variance.

However, part of the variation in estimates of salmon gene flow may also be due to differences in study design, and to how well the assumptions for such estimates are met in various studies (refer to Section 3.3). Many gene flow estimates were obtained as by-products in studies that were not properly designed to measure it. This may seriously reduce their usefulness in critical applications such as in the calibration of mathematical models in risk analyses.

The Working Group feels that there is a need for a critical review of the existing literature, and may be for new, carefully designed and properly scaled studies, with a view to obtain the best possible estimates of the actual levels of gene flow within and between hierarchies in the genetic structure of salmon.

- b) Wild populations of salmon may be characterised genetically in different ways; the qualitative genetics approach in which the statistics are based on measures at individual loci, and the quantitative approach in which the measurements are based on "statistical genes", ie the traits under study are affected by many loci and the effect of each locus is unknown.

The qualitative approach can be a rapid one, in which the type of information obtained is related to the number of different genes and their frequencies. The quantitative approach is usually more time consuming. In return, however, it can provide crucial information of a kind that as a rule cannot be obtained by qualitative methods, eg on adaptationally relevant effects of differences in gene pools (manifested as genotype-environment interactions, which is the same as local adaptations, refer to Section 2h).

The Working Group feels that whenever possible in interaction studies, the genetic characterisation of local populations should use both qualitative and quantitative methods. That would give the best result relative to gaining fundamental understanding of the natural structuring and dynamics of Atlantic salmon populations and thus the implications of ecological and genetical interaction with non-native fishes. Since there is a general lack of such studies in Atlantic salmon the Working Group recommends that they be initiated (refer also to Section 3.4).

- c) The genetic characterisation of the cultured salmon appears to be a rather neglected aspect in many studies so far. This is unfortunate, since the actual genetic differences between cultured and wild fish is one of the most important parameters of an interaction, and because the genetic composition of the cultured fish is changing due to:

- Artificial selection for production traits
- Relaxation of natural selection in the culture situation
- Random genetic drift in small broodstocks.

At present, some of the significant cultured stocks have been five generations in culture and may have changed substantially from their wild relatives. Both directional artificial selection and genetic drift is expected to result in loss of genetic variability. Hence, for assessing potential effects of genetic interactions between their offspring and wild stocks, a minimum level of information would be to have records of their effective population sizes. Loss of genetic variability is a problem that may apply to broodstocks in enhancement programs as well. Ideally, genetic monitoring programs for important captive breeding stocks should become a routine cooperation between the industry and the resource management authorities in each country.

The Working Group feels that there is a strong need for an updated genetic characterisation of broodstocks used in mariculture, and for a monitoring of genetic changes in those stocks.

- d) The effective gene flow between a specific cultured and a specific wild salmon population is addressed in several current experiments. This type of interaction is different from the natural gene flow between wild populations. Therefore the methodology for estimating gene flow is also different and usually involves some form of genetic marker (refer to Section 3.4). It may be anticipated that the gene flow estimates from such experiments will vary substantially since they may be very dependent of the specific regime for the experiment. Sources of variation would include the origin of the cultured immigrants, family differences within immigrants, the specific wild stock used, different degree of neutrality of the genetic marker itself etc.

In planned, controlled experiments, the importance of an effective and correct experimental design for getting adequate and reliable results from gene flow studies must be stressed. To improve on this, the inclusion of statistical expertise when planning specific experiments should become routine. The Working Group feels, however, that the possibility for opportunistic studies of gene flow between cultured and wild salmon has not been utilised satisfactory in the past. For example, cultured fish escapes from rearing-pen wrecks and their subsequent entering of salmon rivers may give excellent possibilities for doing studies that could not be achieved or would not be allowed in controlled experiments. To be able to utilise such situations it is important that there exist genetic base line data on wild populations as well as on the cultured stocks. In general, such base line data should be regarded as a necessary prerequisite for a rational management.

3.1.2 Response to Question 2

The partial list of the many projects currently in progress (Appendix 2) reflects a substantial research effort in many countries on the question of interaction between cultured and wild salmon. If successful, those studies should provide substantial insight into this complex question. The research area as well as the improvements on experimental designs suggested by the Working Group (above) would come in addition to the listed projects.

There are also some additional areas where specific work will be required. In particular the effects of genetic interactions on genetic fitness in the marine phase and the effects of outbreeding depression (second and later generation mixing) need to be evaluated. Exactly how this might best be done is likely to be easier to identify on the basis of the results of work currently in progress. In general the Working Group feels that agreement of research objectives, good experimental design, coordination of future work and cooperation among different disciplines will be essential for the success.

3.1.3 Response to Question 3

At the 81st Statutory Meeting in Dublin, the Mariculture Committee proposed two theme sessions for the 1995 Statutory Meeting. One of them was a theme session on interactions of wild and farmed salmon; a topic considered relevant to the activity of WGAGFM.

In general it is felt that, even though the results of much experimental work in the area which is currently underway will not be complete in 1995, sufficient results should be available to make a theme session worthwhile. It is also felt that the question of interactions, in particular, is a field where the benefits from mathematical modelling could be substantial, and that a theme session on interactions should encourage that kind of studies. The Working Group recommends that such a theme session be arranged at the 1995 Statutory Meeting, and is prepared to contribute to its implementation. It seems most natural that the session is convened by the Chairman of the Study Group on Interaction of Wild, Ranched (Enhanced) and Cultured Salmon.

APPENDIX 5

Review of the "Report of the Study Group on Genetic Risks to Atlantic Salmon Stocks" (C.M.1991/M:3)

Prepared by: The Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM) during its meeting in Copenhagen, 9-11 March 1994

For: The Study Group on Interactions on Wild, Ranched (Enhanced), and Cultured Salmon (refer to C.Res.1993/ 2:27 of the 1993 Statutory Meeting)

WGAGFM reviewed the "Report of the Study Group on Genetic Risks to Atlantic Salmon Stocks" (C.M.1991/M:3). Our viewpoints are expressed in the following section. The document was reviewed on a section by section basis (actual section headings are in bold below). Where sections are not listed, no changes or additions were put forward.

2.2 Evidence for Genetic Differentiation

2.2.1 Individual Genetic Loci

Visual Polymorphisms: The presence of a spot on the tail is a variable character which may be genetically determined and is currently being investigated (Saunders, unpublished). A further potential polymorphism, albinism, is currently being investigated and may be a useful marker for population work (Friars, unpublished). Genetic bases of these variable traits remains to be verified.

Protein Polymorphisms: Considerable new information is available but only some of it is published. However, the new information does not change the generalisations made except to some extent with respect to b) "... within most moderate and large river systems." could be changed to say "... within even some small river systems."

Non-protein Coding DNA: A large amount of new works has been done but not by WG people. Work is mostly in progress but from what is known of the results these tend to lead to the same types of generalisations as the allozyme data except that the levels of differentiation generally appear to be higher. The inability to detect differences at some loci does not mean that differences between different spatial or temporal samples do not exist. This needs to be stressed! Estimates can vary depending on which loci are used to assess gene flow. Thus large numbers of loci need to be used to derive accurate estimates. The validity of the approach to estimating gene flow based on levels of observed differentiation depends on the assumption that an equilibrium has been reached between the forces affecting genetic differentiation. If estimates are made prior to equilibrium, the gene flow will be overestimated. No known update on rRNA gene work.

Mitochondrial Genome: Considerable population data is now available with respect to mtDNA. It is, however, still largely unpublished and not fully analysed. A subjective assessment of the data again points to the same basic pattern of geographical subdivision indicated by the allozyme data.

2.2.2 Chromosome Structure

New work has been done on Q-banding and C-banding and a polymorphism for C-banding has been revealed. Population data is unpublished but shows a level of population differentiation less than for allozymes.

2.2.3 Phenotypic Variation with a Genetic Base

The use of multivariate approaches to stock differentiation as is being carried out by researchers in the Pacific area, needs to be explored with respect to the genus *Salmo*.

2.3 Estimates of Genetic Exchange among Populations

(refer to section on gene flow in the 1994 WGAGFM Report)

2.4 Evidence for Adaptive Genetic Differentiation

(refer to section on combined studies of adaptation in the 1994 WGAGFM Report)

The polygenic basis of traits is an area that needs further research. Studies of epistatic interactions could be productive.

3. Transfer of Stocks

Iceland: Two stock transfers from Norway in 1981 and 1985, involving several hundred thousand eggs have occurred and are used in land-based, contained farms.

Denmark: Since 1989, approximately 100,000 eggs from each of four European rivers (Corrib and Burrishole, Ireland, the Connon, Scotland and the Atran, Sweden) have been imported annually. These eggs are being used for stocking in a river restoration programme.

3.2 Scotland

Importation of large numbers of Canadian origin eggs from Tasmania has been carried out in 1993.

3.3 Canada

Importation of land catch eggs to Maine, involving three year classes, has been carried out. Requests for movement of the eggs into Canada are now being considered. Also increasing numbers of Atlantic salmon are now being reared on the Pacific coast.

4. Genetic Differences Between Wild and Cultured Salmon

Within stock variation should also be mentioned, maybe as a separate section after 4.1.1. This is important because stocks are not monotypic entities. In general both quantitative and qualitative studies show that most species variation is found within stocks and this may in itself be adaptive.

4.1.2 Selective Breeding

Genetic variance for disease resistance is being utilised in breeding programmes. Genetic interdependent — Genetic correlations between traits are being considered in many multiobjective selective programmes. The term "selective breeding" may not be the best one to use here. Breeding encompasses mating and selection systems. Both must be considered simultaneously. The use of "selective breeding" ignores the mating aspect.

5. Biochemical Genetic Techniques

5.1.2 Genetic Markers

This section is confusing and inconsistent. Some statements are inaccurate and inappropriate. Two particular samples of the latter are "However, the majority of polymorphisms ... are considered neutral or subject to purifying selection ..." and "... it is usual to find the majority of all possible alleles in a given population ...".

5.2 Methods

5.2.1 Allozymes

A number of new polymorphisms have been detected but the assessment remains essentially the same.

5.2.2 Chromosomes

The limited amount of new work carried out confirms this assessment.

5.2.3 Mitochondrial DNA - Restriction Enzymes

This procedure is being superseded by the methods described in 5.2.4. Most variation is revealed using 4-base restriction endonucleases. However, these produce complex fragment patterns which are difficult to screen electrophoretically in a consistent, repeatable manner. Also, it requires high quality intact mtDNA molecules in a highly purified form; a difficult and time consuming procedure.

5.2.4 Mitochondrial DNA - PCR Analysis

The use of this method has expanded through the development of new primer pairs for various regions of the mtDNA genome. This is particularly true with respect to restriction digestion of PCR amplified sections of mtDNA using 4-base restriction endonucleases which reveal the most variation. However, unpublished results for Atlantic salmon suggest that the numbers of haplotypes is still expanding, increasing the scope for finding population differences and using the variation to mark experimental stock groups. Further variation has been found in the 16sRNA/ND1 region, in addition to those previously identified for cytochrome B, as have major differences between southern and northern European stocks. Differences in haplotype frequencies have also been found regional stocks and between farm and wild stocks in some situations. Numbers of samples which can be typed per day range between 40 and 60.

5.2.5 Mini-satellite Probes - Fingerprinting

No new substantive developments with respect to salmon. Most effort is now directed at the use of single locus mini- or micro-satellite probes.

5.2.6 Mini-satellite Probes - Single Locus Probes

Substantive developments have occurred with respect to the number of loci for which probes have now been developed. The new probes developed reveal a range of levels of heterozygosity and population differentiation. Individual family identification in many experimental contexts is now feasible through the procedures are time intensive. Realistic numbers of fish which can be analysed per laboratory worker is 40 per week (based on need to use 6-7 probes) for fingerprinting.

5.2.7 Additional Techniques

Micro-satellite Probes: These are like mini-satellite probes but relate to shorter repeat sequences. They are also found to be highly variable in most cases. DNA probes have been developed but by sequencing these shorter pieces of DNA, primers have also been developed for a number of micro-satellite DNA regions. These allow PCR amplification to be used in the detection of FLP's. A large number of micro-satellite probes have been developed in Leicester (T Burke, Department of Zoology, University), Belfast (A Ferguson, Queen's University) and Halifax (R Doyle, Marine Gene Probe Laboratory, Dalhousie University). PCR based detection of variation has just started. Little of the micro-satellite work in Atlantic salmon has yet been published.

FLP's of Single Copy nDNA: Primers are now available to amplify regions of the DNA coding for structural genes. This allows detection of FLP's, as well as RFLP's, and variation by direct sequencing of amplified fragment. Studies of other species look promising. Application of this work to Atlantic salmon is only just starting.

5.3 Conclusions

The new developments mean that there is now no methodological impediment to the search for molecular markers. However, the exact number of regions which could now be screened and the extent to which these nuclear DNA regions offer useful variation for studies remains to be fully assessed.

7. Design of Experimental Studies

No comments since the actual designs used are often dictated by the available stocks and molecular markers as well as other biological and logistical constraints.

9. Conclusions

The commercial use of triploid salmon has not progressed at all in the past two years. This is largely due to the public perception of the use of genetically altered animals as a source of food and, from the point of view of farmers, the absence of the pre-maturation growth spurt. Studies of the potential ecological interactions of triploid escapes with wild salmon are needed.

APPENDIX 6

National Status Report — Summary of Research Relevant to Genetic Interactions of Native and Non-native Salmonids

Prepared by: The Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM) during its meeting in Copenhagen, 9-11 March 1994.

For: The Study Group on Interactions of Wild, Ranched (Enhanced) and Cultured Salmon

Canada - (Reported by Gerry Friars)

Laboratory/Researcher: Atlantic Salmon Federation and University of New Brunswick; J Anderson and T Dilworth

Species: Atlantic salmon

Project funding: Atlantic Fisheries Adjustment Program

Objective: To evaluate the incidence of escaped aquacultural and wild fish ascending the Magaguadavic River

Design: Monitoring of ascending fish

Methodology: Sonic tags used to track individual fish on spawning grounds. Discrimination of cultured and wild fish using scales, fins and DNA analysis (in conjunction with Dalhousie University)

Status: Ongoing

Denmark - (Reported by Michael Hansen)

Study 1

Laboratory/Research: University of Århus, Inland Fisheries Laboratory, Silkeborg and Queens University Belfast; Michael M Hansen, Volker Loeschcke, Rosaleen Hynes

Species: Brown trout

Project funding: The Danish Natural Science Research Council and the Inland Fisheries Laboratory

Objective: Determine the origin of the trout in the re-populated Karup river system

Design: Compare genetic characteristics of samples from the main river, four tributaries, another river and a hatchery strain

Methodology: mtDNA RFLP analysis, involving Southern Blotting and hybridisation to a brown trout mtDNA probe. All fish were screened with seven restriction endonucleases (two six, 2 five and three four-base cutters)

Status: Project initiated January 1992. One paper on results submitted. The project is continuing to address further questions. Methodology is changed to PCR. RFLP analysis (two restriction enzymes) of PCR amplified ND-1 segment distinguish the four most informative haplotypes.

Comments: Hatchery strain haplotype frequencies significantly different from all other samples. The genetic contribution of the stocked hatchery trout very small or absent. Present population probably descendants of an indigenous population

Iceland - (Reported by Anna Danielsdottir)

Proposed Study

Laboratory/Researcher: Marine Research Institute, Reykjavik; A K Danielsdottir

Species: Atlantic salmon

Project funding: Currently being sought

Objective: To assess the genetic interactions between wild and farmed Atlantic salmon in the river

Design: Assess the genetic structure in the River Ellidaa in different years and evaluate in relation to levels of farm Atlantic salmon in the river as determined from scale studies

Methodology: Isozymes, scale analysis

Status: Proposed work

Ireland - (Reported by Tom Cross)

Study 1

Laboratory/Researcher: University College, Cork (UCC) with two UK and one Spanish group; T Cross

Species: Atlantic salmon

Project funding: EC FAR MA-2-480

Objective: To detect molecular markers to distinguish wild and reared Atlantic salmon

Design: Four index samples from Spanish, Scottish and Irish wild populations and a Norwegian-origin farmed stock typed for variation at the four systems listed below and results compared individually and in combination

Methodology: Allozymes (UCC), plus mini-satellite nuclear DNA, mitochondrial DNA and chromosomes

Status: Two year project completed January 1993

Comments: The results of this work have been employed in a number of opportunistic and experimental studies designed here under the EC AIR1-CT92-0719 programme.

Study 2

Laboratory/Researcher: Salmon Research Agency of Ireland; T Cross (UCC) and P McGinnety, plus two UK and two Spanish partners

Species: Atlantic salmon

Project funding: EC AIR1-CT92-0719

Objective: To study the interaction of wild and reared Atlantic salmon in the field using molecular markers

Design: Field experiment where farmed and wild Atlantic salmon and their hybrids, typeable as to family using genetic profiling, are introduced as eyed eggs into enclosed stretch of river and relative fitness assessed in terms of performance. Also retrospective study of Atlantic salmon populations effected by escapes from a hatchery and sea cages, using an array of molecular methods

Methodology: Mini-satellite DNA SLPs, transcribed genes, mtDNA and allozymes

Status: In progress, three years from OCD of February 1993

Comments: Experimental and opportunistic studies

Study 3

Laboratory/Researcher: T Cross (UCC) and A Ferguson (QUB, Belfast, UK)

Species: Atlantic salmon

Project funding: Electricity Supply Board (ESB)

Objective: To assess the effect of stocking practises on the population genetics of River Shannon Atlantic salmon

Design: Survey of distribution of genetic variation

Methodology: Allozymes and mini-satellite DNA SLPs

Status: Three year study completed in December 1993.

Comments: Part of larger Atlantic salmon biology study on Shannon where extensive mitigation stocking has been used for over 30 years because of interruption of runs by hydroelectric development

Study 4

Laboratory/Researcher: N Wilkins, University College, Galway (UCG)

Species: Atlantic salmon

Project funding: ESB

Objective: Breeding of improved strains for enhancement in the River Shannon

Design: Selection of broodstock of grilse or MSW type to use in stocking different areas

Methodology: Directed breeding

Status: Three year study completed in December 1993.

Study 5

Laboratory/Researcher: N Wilkins (UCG)

Species: Atlantic salmon

Project funding: Commercial
Objective: To produce triploids to prevent farm/wild breeding interactions
Methodology: Triploid induction by heat/pressure shock, study of sea migration of triploids
Status: Ongoing

Study 6

Laboratory/Researcher: Mr P McGinnety (SRA)
Species: Atlantic salmon
Project funding: Institutional
Objective: Assess the effects of selection *MEP-2** in culture
Methodology: Allozymes, growth and maturation assessment
Status: On-going

Norway - (Reported by Geir Dahle, Knut E Jørstad and Jarle Mork)

Study 1

Laboratory/Researcher: The Norwegian Institute of Nature Research (NINA); K Hindar
Species: Atlantic salmon
Project funding: Norwegian Research Council program: "Environmental Effects of Biotechnology"
Objective: Using data on gene flow from cultured fish into natural populations to calibrate mathematical models for predicting the effect of various immigration regimes on the genetic variation within and between natural populations
Design: A genetically characterised local Atlantic salmon population is challenged by increased immigration of genetically tagged farmed Atlantic salmon. Baseline, post-challenge and F1 allele frequencies are compared
Methodology: Physical and genetic tagging, electrophoresis, mathematical modelling
Status: Started in 1994

Study 2

Laboratory/Researcher: NINA (Ims Salmon Research Station)
Species: Atlantic salmon
Project funding: NINA
Objective: Assessment of the reproductive behaviour and success of farmed, ranched and wild Atlantic salmon in the presence and absence of competition with other types

Design: Artificial spawning areas at Ims
Methodology: Ethological and biological studies
Status: On-going, results will be reported from 1994 and on

Study 3

Laboratory/Researcher: NINA
Species: Atlantic salmon
Project funding: NINA
Objective: Interaction of wild, ranched and cultured Atlantic salmon
Design: Genetically marked fish are allowed to spawn above the fish trap on the River Imsa
Methodology: Carlin tags, biopsy, electrophoresis
Status: Started in 1993, results available in 1995

Study 4

Laboratory/Research: NINA
Species: Atlantic salmon
Project funding: NINA
Objective: To study the behaviour of escaped farmed Atlantic salmon
Design: Tracking of farmed fish during upstream spawning migration in Rivers Alta, Namsen and Imsa
Methodology: Radio-tagging and tracking
Status: On-going, results to be reported when available

Study 5

Laboratory/Researcher: Inst of Marine Research, Bergen; O Skaala
Species: Atlantic salmon
Project funding: Norwegian Research Council
Objective: Measurement of gene flow from cultivated to wild populations by conducting field experiments using genetic markers
Design: Identify genetic markers in Atlantic salmon family groups and release genetically tagged Atlantic salmon smolts into a salmon river. Estimates of reproductive success of returning spawners.

Methodology: Allozyme markers; the genetic composition of the native populations of Atlantic salmon will be determined, spawning population estimates and abundance will be estimated and the genetic composition of offspring populations determined.

Status: Initiated in 1992, first estimates of returning, genetically marked fish in 1994 and possibly information on reproductive success in 1995.

Comments: DNA analyses (micro-satellites) will be incorporated; population dynamics parameters are included in study. Study will be used to model potential impacts from genetically modified organisms (GMO).

Study 6

Laboratory/Researcher: Inst of Marine Research, Bergen; O Skaala

Species: Brown trout

Project funding: Norwegian Research Council

Objective: To use trout as a case study of genetic interactions between stocks and estimate gene flow into a native population

Design: Genetically tagged farmed spawners will be released into the experimental river and the genetic make-up of subsequent juveniles monitored at different ages

Methodology: Tagging is based on a morphologic/visual marker (fine spotted body) and allozymes. Population dynamics and abundance estimates of year classes will be determined

Status: Initiated in 1989 and introduced genes present in the 1990 year class. Monitoring in year class is on-going

Comments: Estimates of reproductive success of farmed trout is about 25% less than local wild spawners. Higher mortality rates detected for offspring (all forms) carrying the introduced marker gene

Study 7

Laboratory/Researcher: University of Trondheim, Biological Station; J Mork

Species: Indifferent

Project funding: University of Trondheim

Objective: A general, interactive PC simulation programme for, eg prediction and analysis of genetic effects of interaction between cultured and wild populations

Design: Simultaneous handling of additive and interaction genetic effects from random genetic drift, gene flow (immigration) and selection on a genetically pre-characterised set of populations

Methodology: Computers, mathematical modelling, Monte Carlo simulations

Status: Functional version is being tested

Comments: Calibration of model parameters has revealed a scarcity of high-quality estimates of eg, effective population sizes, effective gene flow, fitness coefficients etc in the literature

Scotland - (Reported by Eric Verspoor)

Study 1

Laboratory/Researcher: SOAFD Marine and Fresh water Fisheries Laboratories; E Verspoor and collaborators

Species: Atlantic salmon

Project funding: SOAFD and EC AIR1-CT92-0719

Objective: To assess whether the documented interbreeding of farm Atlantic salmon, which ascended the River polla in 1989 and 1990, with the wild stock has resulted in genetic changes to the juvenile populations in the river

Design: Samples of juvenile Atlantic salmon from two year classes were collected from the lower, middle and upper reaches of the river pre-spawning of farm fish in 1989. The genetic composition of these fish will be compared with post spawning juvenile samples from the same locations taken in 1991 and differences related to the genetic make-up of adult farm Atlantic salmon ascending the river

Methodology: Allozymes, RFL analysis of PCR amplified mtDNA, mini- and micro-satellite analysis of nuclear DNA, PCR amplification of structural gene nDNA

Status: Currently underway and due to completion in 1995

Comments: This represents an opportunistic study

Study 2

Laboratory/Researcher: SOAFD Marine and Fresh water Fisheries Laboratories; E Verspoor and collaborators

Species: Atlantic salmon

Project funding: SOAFD and EC AIR1-CT92-0719

Objective: To assess whether genetic differences among stocks are relevant to their biological performance during the juvenile fresh water phase in the wild in ways that relevant to their fitness.

Design: Simultaneously spawning eggs of different regional stocks and their hybrids have been planted out within 48 hours of fertilisation in artificial redds using a random stratified planting strategy in a small experimental river where natural spawning is precluded. Performance parameters such as egg mortality, hatching and emergence timing, developmental state, maturation, growth and smoltification will be compared among groups.

Methodology: Stock groups will be genetically marked using RFLP's PCR amplified mtDNA and single locus mini-satellite fingerprinting

Status: Currently underway and due for completion in 1996

Comments: This represents an experimental study

Study 3

Laboratory/Researcher: SOAFD Marine and Fresh water Laboratories; A Youngson and J Webb

Species: Atlantic salmon

Project funding: SOAFD and Atlantic Salmon Trust

Objective: To determine the geographical distribution and relative extent of spawning of escaped farm Atlantic salmon in Scottish rivers

Design: Sampling of eggs in salmon redds in lower reaches of representative rivers across Scotland

Methodology: Identification of farm eggs on basis of canthaxanthine; allozyme analysis to detect hybrids and trout eggs

Status: Completed in 1992. Two papers have been produced (below)

Comments: This represents an opportunistic study

Webb, J.H. *et al.* 1993. Spawning of escaped farmed Atlantic salmon, *Salmo salar* L., in western and northern Scottish rivers: egg deposition by females. *Aquaculture and Fisheries Management*, 24: 663-670.

Youngson, A.F. *et al.* 1993. Spawning of escaped farmed Atlantic salmon (*Salmo salar*): hybridisation of females with brown trout (*Salmo trutta*). *Can. J. Fish. Aquat. Sci.*, 50: 1986-1990.

Study 4

Laboratory/Researcher: SOAFD Marine and Fresh water Laboratories; A Youngson and J Webb

Species: Atlantic salmon

Project funding: SOAFD and Atlantic Salmon Trust

Objective: To determine the frequency of farm escapes among Atlantic salmon in the coastal Atlantic salmon fisheries in Scotland

Design: Regular sampling of Atlantic salmon taken by four representative coastal net fisheries off Scotland

Methodology: Identification of farm fish on basis of body morphology and scale analysis

Status: Started in 1992 and ongoing

Comments: This represents an opportunistic study

Study 5

Laboratory/Researcher: SOAFD Marine and Fresh water Laboratories; E Verspoor

Species: Atlantic salmon

Project funding: SOAFD

Objective: To determine the nature and extent of population structuring of Atlantic salmon in Scotland within and among rivers so as to assess the impact of farm escapes on natural structure

Design: Geographic sampling of within and among river genetic variation and statistical analysis of differentiation

Methodology: Allozymes, mtDNA, mathematical modelling

Status: Started in 1989 and ongoing

Spain - (Reported by Eric Verspoor)

Study 1

Laboratory/Researcher: Xunta de Galicia, Lurizan; C Garcia de Leaniz and collaborators

Species: Atlantic salmon

Project funding: Xuntia de Galicia and EC AIR1-CT92-0719

Objective: To assess if genetic differences between native Spanish and non-native northern European Atlantic salmon affect the fitness of the two stock types in the wild in Spanish rivers

Design: Pure stock groups of Spanish and Scottish Atlantic salmon ova will be planted out at the eyed egg stage in the River Ulla in Spain and various performance parameters compared eg egg and juvenile mortality, hatching and emergence timing and condition, smolt production and growth performance

Methodology: Non-native stock groups will be artificially selected to fix a RFLP variant detected in a PCR amplified mtDNA fragment which is absent in Spanish stocks

Status: Currently underway and due for completion in 1995

Comments: This represents an experimental study

Study 2

Laboratory/Researcher: Xunta de Galicia, Lurizan; C Garcia de Leaniz

Species: Atlantic salmon

Project funding: Xuntia de Galicia and EC AIR1-CT92-0719

Objective: To assess differences in fresh water performance and return rate among native Spanish and non-native northern European Atlantic salmon stocks planted out a pre-smolt juveniles in the River Eco.

Design: Pure and hybrid stock groups of Spanish and northern European Atlantic salmon reared in the hatchery to the pre-smolt stage have been released into the river. Mortality and growth are being compared and return rates to the angling fishery will be monitored.

Methodology: Stock groups will be microtagged and adipose fin clipped. Fish will be monitored in fresh water by electrofishing stocked areas. Returning adults will be monitored with respect to tagged fish caught in subsequent years by anglers

Status: Currently underway and due for completion in 1995

Comments: This represents an experimental study

Study 3

Laboratory/Researcher: University of Oviedo; E Garcia Vasquez

Species: Atlantic salmon

Project funding: EC AIR1-CT92-0719

Objective: To determine if native Spanish stocks have been genetically changed as a result of historical stocking with imported Atlantic salmon ova from northern Europe

Design: Data on population numbers, stocking levels and allozyme variation in Atlantic salmon from stocked and unstocked Atlantic salmon rivers in northern Spain will be assessed in relation to allozyme variation in northern European rivers to determine whether genetic changes have taken place due to stocking

Methodology: Analysis of existing data bases

Status: Currently underway and due for completion in 1995

Comments: This represents an opportunistic study

APPENDIX 7

"Review Knowledge of the Amount of Gene Flow Between Specific Natural Populations as well as Between Cultured and Specific Natural Populations with a View to Proposing Future Studies

Introductory to the discussion in the Working Group, the central role of gene flow in most questions concerning finfish and shellfish population genetics was highlighted. It was pointed out that whether the problem under discussion concerns

- Stock identification
- Interaction between cultured and wild stocks
- Local adaptations
- Phylogenetic or speciation processes
- Gene spreading from genetically modified organisms
- Genetic resources and the precautionary principle
- Development of mathematical models for prediction purposes

Knowledge of the historic and/or present amount of gene flow between taxa at various stages of genetic differentiation is usually of paramount importance.

The discussion on this topic considered first the question of selective neutrality for genetic markers which are intended for use in estimating gene flow. It was the view of the group that, maybe with an exception for third codon substitutions, it is not possible to guarantee selective neutrality for any genetic marker. Not even non-coding DNA would be "safe" in this context because of the possibility for hitch-hiking with genes which are selected.

The discussion on methods of estimating gene flow revealed at least two principal types of need for gene flow estimates, and that the actual choice of methodology for obtaining estimates would be dependent of the need. The two principal type are discussed below (Sections 3.3.1 and 3.3.2).

3.3.1 Estimating Gene Flow Within an Established Population Structure

For this type of studies both direct and indirect methods are applicable. Direct methods would imply the use of some form of genetic marker, for which changes in frequency away from a baseline value would enable direct estimates of gene flow. In such studies more than one marker should be used in order to enable the detection of potential bias due to selection.

However, indirect methods are most often used in this situation. The most common method is based on the use of F_{st} (or G_{st}) estimates from multilocus studies, and by re-arranging the formulae of those parameters to focus on m , the gene flow (or actually $N_e m$, the absolute number of immigrants per generation). Usually, m is derived from the following basic expression from Sewall Wright's "Island model" of genetic differentiation.

$$F_{st} = 1/(1 + 4 N_e m),$$

where N_e is the effective population size and m is the proportion of immigrants in each population in each generation. It should be mentioned, however, that this formula is only an approximation of the complete expression derived by Sewall Wright, which is

$$F_{st} = (1 - m)^2 / (2 N_e - ((2 N_e - 1) (1 - m)^2))$$

and which preferably should be employed whenever critical studies are undertaken. The estimate of m by using one or another of those two expressions may differ by several percent depending on the true values of m and N_e .

Another concern in this context is that the values obtained in this way often show substantial variation depending on which locus is used. Hence the need to have estimates from many loci should be observed. A second source of

variation in this kind of estimates is the number of individuals in samples used for estimation of allele frequencies. The relative contribution by these two sources have been estimated and is described in population genetics theory. There is, however, a third variance component, and that one is usually not taken into consideration. That is the random genetic drift of allele frequencies in the populations themselves. In small populations this can cause substantial variability between generations. The expected effect of this, when sampling such populations, would be to overestimate the genetic differences between populations and hence to underestimate the gene flow. Hence, the establishment of baseline data and a monitoring of allele frequencies in the populations is considered necessary in order to assess the stability of the allele frequencies which are used, eg in estimating levels of gene flow. The F_{st} statistic is a "mean" value under the Island model of genetic differentiation, which assumptions may not be very realistic for salmon populations (they are, eg, very variable in size and their geographic interdistances are widely different). In practice, the value of F_{st} (and thereby the corresponding value of $N_e * m$) usually depends heavily on the actual set of populations included in the study. Thus, it is probably not realistic to look for such things as a fixed level of gene flow between salmon populations; the level may vary substantially between different sets of populations. Referring to the connection between gene flow and local adaptation (Section 3.1.1.a), this implies that the extent of local adaptations may also show large variability. In some instances it may be more realistic to consider groups of populations rather than the individual ones to be the real units of adaptation. In particular this may apply to situations where several small populations with substantial gene flow between them inhabit a restricted geographic area. It should also be observed that the formulae for estimating m via eg F_{st} , assumes that an equilibrium situation between genetic drift and immigration has been reached. If equilibrium has not been reached, the gene flow would tend to be overestimated.

Finally, knowledge of the actual population structure is important for relevant sampling. Samples containing mixtures from various populations would generally yield overestimates of the actual gene flow within the system.

3.3.2 Estimating Gene Flow into an Established Population Structure

In some situations, for example when assessing the risk that a genetically modified organisms (GMO) would be able to enter an existing population and proliferate there, the indirect methods discussed above would not be feasible. Since the organism in question would not be "part of the system", it's expected performance cannot be assessed based on observations or estimates of the natural gene flow in the system. Rather, such situations would call for specific experiments with model organisms that mimic the GMO as good as possible. Again it is the effective gene flow which is important, hence a genetic marker (rather than counting immigrants) is necessary. Ideally, the marker gene itself should be as selectively neutral as possible in order to yield unbiased results.

3.4 "Propose Studies of Local Adaptations of Specific Natural Populations using Combined qualitative (eg Gene Markers) and Quantitative (eg Family Studies) Genetic Approaches"

There is an increasing international awareness of the need for a sustainable use of natural resources. For fish stocks, a sustainable use would include eg conservation of existing genetic resource. The term "substantial" should in this context reflect eg how many generations it took to establish it, and how large changes to the original gene pool it represents. The identification and assessment of such adaptations are important for practical management.

A local adaptation develops by differential mortality of genotypes for fitness-related traits causing a directional change in population allele frequencies. In mathematical models of genetic differentiation (evolution, adaptation), the main forces which determine the speed and thus the magnitude of an adaptation over time would be:

- Population size (through the effect of random genetic drift)
- Gene flow (the proportion of non-selected immigrants and their gene frequencies)
- Selection coefficients (the intensity of selection)

While the action of genetic drift increases genetic differences between populations, gene flow has a homogenising effect. For environmental forces which are uniform for populations, natural selection may be a homogenising factor while it may increase genetic differences when environmental factors vary between populations. These evolutionary forces may interact in very complex ways and eventually a situation will be reached where the differentiating and the homogenising effects neutralise each other in an equilibrium. In this situation the mean fitness of the local population is at its maximum, ie this is the highest achievable local adaptation under the given circumstances. The number of generations needed to reach this stage is determined by the intensity (ie, the selection mortality each generation) of the natural selection.

3.4.1 Methodology and Application Areas

Adequate methods for assessing population size and its variation are available. Also, qualitative genetic methods exist for direct and indirect assessment of gene flow. With respect to natural selection one may conclude, from the enormous mortality commonly observed from egg to adult stage in most fish species, that there is a large potential for natural selection and thus adaptation. However, the actual selection intensity for multilocus, fitness-related traits cannot be assessed without knowledge of the heritability of those traits in the actual environment (ie how strongly the genotype is reflected in the phenotype on which the environmental factors act).

A list of quantitative genetic traits which probably are related to fitness would include, e.g.:

- Relative fecundity
- Relative egg size
- Time of emergence
- Yolk sac absorption
- Survival to first feeding
- Survival to end of first year
- Survival to smoltification
- Extent of precocious maturity
- Disease resistance in fresh water
- Resistance to low or high pH
- Sea absence pattern
- Growth rate at sea
- Survival at sea
- Disease resistance at sea
- Migration route
- Time of upstream migration
- Susceptibility to angling
- Rate of sexual development
- Time of spawning
- Kelt survival (multiple spawnings)
- Disease resistance as adult in fresh water

Estimating heritability is a field of quantitative genetics. A better understanding of the nature and magnitude of local adaptations, and of the potential risks to them posed by certain human activities (like populations bottlenecks and increased gene flow) therefore call for studies which combine methodology from qualitative and quantitative genetics. Until results from such studies are available, predictions of effects of specific changes in, eg immigration regimes are bound to be hypothetical.

3.4.2 Sample Outline of a Combined Study of Adaptation

In the following, some important aspects to be observed in the design of such combined qualitative and quantitative genetics studies will be outlined. In order to yield parameter estimates that are valid for wild stocks, it is essential that the actual performance tests are carried out under natural conditions.

3.4.2.1 Assessment of Genetically Effective Population Size

Usually N_e , the genetically effective population size is not simply the number of spawners counted one year. Most natural populations have overlapping generations, and N_e must be adjusted according to formula I:

$$I: N_e = N_0 t l$$

where N_0 is the number of individuals born in a specific generation, t is the mean age at reproduction and l is an individual's probability of survival to that age.

Furthermore, N_e is heavily affected by the proportion of male and female spawners (formula II):

$$II: N_e = 4(N_m N_f) / (N_m + N_f)$$

where subscripts m and f represent males and females, respectively. Formula II tells that with unequal sex proportions, the effective size is always smaller than the actual size. Likewise, there are formulae which allow adjustment to be made for cyclic changes of population size when records of such as available (formula III):

$$\text{III: } N_e = n / \Sigma (N_i^{-1})$$

where n is the number of generations in the cycle, and N_i is the population size in the i-th generation of the cycle. The formula shows that the effective size is closer to the smaller than to the larger size in the cycle. Finally, N_e is affected by differences in sizes of the offspring groups between families (formula IV):

$$\text{IV: } N_e = 2N / (1 + (V_k / k))$$

where k and V_k are the mean and variance of progeny number per individual. The ratio N_e/N is about 0.75 in many organisms.

3.4.2.2 Assessment of Genetically Effective Immigration

Immigrants may be less reproductively fit than native individuals (scale 0-1). Due to this, m, the genetically effective immigration may be different from the actually observed proportion that the immigrants constitute of the total spawning group. In assessing the m parameter one should observe the pitfalls and assumptions discussed in 3.3.1 and 3.3.2 (above).

3.4.2.3 Outline of an Assessment of Genetic Parameters for Fitness-Related Traits

Objective: To estimate genetic and environmental variance as well as genotype-environment interaction for fitness-related traits. The genotype-environment interaction on the quantitative context is a manifestation of genetic adaptation. The present design will require the use of genetic markers (eg PCR micro-satellites) to establish the full pedigree of the population under study, and hence represents a merging of quantitative and qualitative genetics techniques. It is realised that this approach is costly but it is important that correct procedures be involved from the start. A study of only fresh water stage related traits would be less expensive and could be performed in a much shorter time.

The specific goals of the present study would be to:

- Estimate the contribution of each stock and the families (individuals) within stocks in each river.
- Estimate the stock-by-river interaction and the family within stock interaction.

The following experimental design assumes two rivers, each with a native salmon population.

1. Take a random sample of 50 females from each of two salmon stocks. Mate the females artificially using a nested mating design with one male to two females.
2. Mix all groups at the eyed egg stage and distribute the eggs from the mixed pool into the river-gravel of each river.
3. Sample 10-15 individuals from each full-sib group at the parr and/or smolt stage and identify pedigrees. At this stage one can estimate the parameter for eg growth rate and survival in the fresh water stage.
4. Recapture adults, take samples from each fish to identify pedigrees and record traits of interest.
5. Assessment of the reproductive success of the returning adults by repeating point 3).
6. The design proposed here will involve unequal numbers for the stock, family and river effects. Sufficient numbers to test family and family-by-river effects are more feasible in the use of fresh water traits than in the data on returned fish. Hence, the estimation of narrow-sense heritability and genetic correlations using sire-components of variance and covariance may be more meaningful in fresh water traits than in traits measured on returned fish.