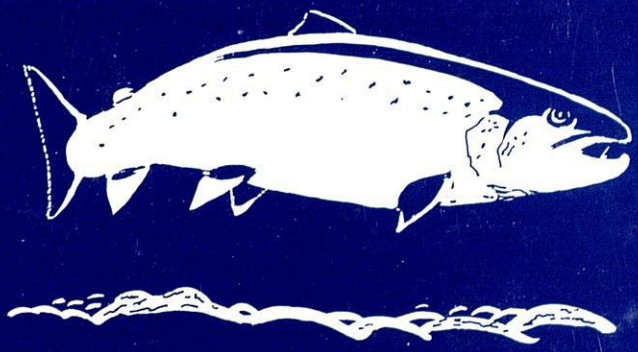
ATLANTIC SALMON TRUST

ATLANTIC SALMON FEDERATION

GENETICS AND THE MANAGEMENT OF THE ATLANTIC SALMON

Besinger Liddell Memorial Atlantic Salmon Fellowship 1987/1988

T. F. CROSS



The Atlantic Salmon Trust
Moulin, Pitlochry
Perthshire PH16 5JQ
Telephone: Pitlochry (0796) 3439

Price £2.50

HONORARY SCIENTIFIC ADVISORY PANEL

Gordon Bielby, B.Sc. (South West Water Authority) (Chairman)
W. J. Ayton, B.Sc., M.Sc. (Regional Rivers Division, Welsh Water)
G. D. F. Hadoke, M.A., M.Sc.(Econ.) F.I.F.M.
M. M. Halliday, Ph.D. (Joseph Johnston & Sons Ltd.)
G. Harris, Ph.D. (Welsh Water Authority)
G. J. A. Kennedy, B.Sc., D.Phil. (Department of Agriculture for Northern Ireland)
E. D. Le Cren, M.A., M.S., F.I.Biol., F.I.F.M.
D. H. Mills, M.Sc., Ph.D., F.I.F.M. (Department of Forestry and Natural Resources, Edinburgh University)
I. Mitchell, B.Sc., (Tay Salmon Fisheries Co. Ltd.)
D. J. Piggins, B.Sc., Ph.D. (Salmon Research Trust of Ireland, Inc.)
J. Solbe, B.Sc., C.Biol., F.I.F.M., M.I.Biol. (Unilever Research)
D. Solomon, B.Sc., Ph.D., M.I.Biol., M.I.F.M.
E. Twomey, M.Sc. (Department of the Marine, Dublin)
Professor Noel P. Wilkins (Department of Zoology, National University of Ireland)
Sir Ernest Woodroofe, B.Sc., Ph.D., F.Inst.P., F.I.Chem.E.

Observers: K. O'Grady b.Sc., Ph.D., M.I.F.M., F.L.S. (National Rivers Authority)
W. Shearer, B.Sc., M.Sc., C.Biol., M.I.Biol. (Department of Agriculture and Fisheries for Scotland)

Director: Rear Admiral D. J. Mackenzie
Deputy Director: Captain J. B. D. Read, RN

GENETICS AND THE MANAGEMENT OF THE ATLANTIC SALMON

T. F. Cross, Ph.D.,
Dept. of Zoology,
University College,
Cork, Ireland

May 1989

Report arising from the 1987/1988 Bensinger Liddell Memorial Atlantic Salmon Fellowship, sponsored by the Atlantic Salmon Trust and the Atlantic Salmon Federation

ISBN 1 870875 09 5

Printed in Scotland by Woods of Perth (Printers) Ltd., 3-5 Mill Street, Perth PH1 5JB — Telephone 0738 22244/5

FOREWORD

We are increasingly warned by geneticists of possible dangers of stocking rivers with surplus juveniles from salmon farms and of escapees from those farms; even from well-intentioned, but unwise, programmes of enhancement with hatchery juveniles reared specifically for the purpose. Furthermore, there are potential genetic problems where populations have been reduced below a critical level, by pollution or other damage; and where stocks have been eliminated, genetic considerations must play an important part in planning rehabilitation. Geneticists are not dogmatic about these matters and readily admit that their advocacy of policies of prudence relies on partial evidence and on their scientific judgement. But they are strikingly of one mind.

The Atlantic Salmon Trust was quick to recognise the importance of the contribution that the geneticists could make to the conservation of salmon when it published the excellent monograph by Professor Noel Wilkins, "Salmon Stocks - A Genetic Perspective", in 1985. In 1988, the Trust, jointly with DAFS, sponsored an international Workshop, which was attended by the principal research workers in this field. A non-specialist account of this Workshop, written by Professor Wilkins, is included as an addendum in this present Blue Book.

We were delighted that, in awarding the Bensinger-Liddell Fellowship to Dr. Tom Cross on the basis of his excellence as a fishery scientist, we were able to further our promotion of an understanding of the application of genetics to the conservation of salmon. He has drawn together brilliantly the results of his discussions about the latest developments with those prominent in

the field of fish genetics in Norway, Sweden and North America and has carried our earlier monograph one stage further. His veiws on the application of genetics to the management, conservation and enhancement of salmon stocks make fascinating reading and there are many lessons to be learned. As a non-specialist, I have been the better able to appreciate these practical aspects of his message as a result of diligent reading of his lucid explanation of the scientific aspects in his early chapters.

The Trust is most grateful fo him for his work in connection with the Fellowship and for writing this excellent monograph.

Sir Ernest Woodroofe,Ph.D.,F.Inst.P.,F.I.Chem.E.,
Chairman, Hon.Scientific Panel,
Atlantic Salmon Trust.

CONTENTS

1. INTRODUCTION

Of late it has been realised in both Europe and North America, that to properly manage salmonid fish species, consideration must be given to genetic principles. Thus I was awarded the 1987/1988 Bensinger Liddell Memorial Salmon Fellowship to study advances in salmon and trout genetics and to consider their implications for management. Using the fellowship funding, a brief visit to Sweden and Norway was made in August 1987, while a more extensive tour of North American laboratories was undertaken in April and May 1988. Much of the trip in North America involved visits to salmonid geneticists on the West Coast. While the species of salmon (*Onchorhynchus kisutch*, coho; *O. tshawytscha*, chinook; *O. nerka*, sockeye; *O. gorbuscha*, pink and *O. keta*, chum) and trout (*Salmo gairdneri*, rainbow/steelhead and *S. clarkii*, cutthroat) differ from the salmon (Atlantic salmon, *Salmo salar*) and trout (brown/sea trout, *S. trutta*) of Britain and Ireland, the much greater numbers of each Pacific species leads to a far bigger commercial and recreational catch and thus to more scientific investigations. In the case of genetic work, this is particularly true of Washington, where many of the advances in salmon and trout genetics were first made. The major laboratories visited, their areas of expertise, and the name of a contact person are given in Appendix 1. Since many of the investigators visited specialised in more than one area of salmonid genetics or formed part of a larger co-operative programme, a description in chronological order is not attempted. Instead the various areas of genetics which have been studied in salmon and trout are described, incorporating information acquired during the visits with previously published material. A list of further reading is provided for those who

wish to obtain background information and technical details or wish to delve further into the subject.

Three areas of salmonid genetics are described. These are:-

(i) Biochemical systematics and population genetics,
(ii) Quantitative genetics or selective breeding,
(iii) Genetic engineering and other manipulations.

Many studies in the first area relate directly to the management of wild salmon and to stock enhancement and so the broadest coverage is given to this subject (Section 2). The latter two areas relate primarily to techniques used in salmon farming. However, since they have implications for the management of wild and enhanced stocks because of introductions or inadvertant release of such modified fish, they are also described (Sections 3 and 4). In each area the methodology is briefly described and major results are presented and discussed. The possible applications of these genetic techniques in the management and enhancement of Atlantic salmon are then summarised (Section 5).

2. BIOCHEMICAL SYSTEMATICS AND POPULATION GENETICS

Most of the recent research in this area has utilised one or other of two techniques - protein electrophoresis and mitochondrial (mt) DNA analysis. The former was developed as a genetic technique in the late 1960's, with the latter being initiated more recently in the late 1970's. In order to fully understand what follows here and in later sections, a brief description is given of some principles of molecular genetics.

Genetic Principles

Genes and chromosomes: Deoxyribonucleic acid (DNA) is the genetic material of animal cells and occurs chiefly in the cell nucleus. Genes consist basically of specific DNA molecules. Genes are arranged in specific linear array in chromosomes, of which a number, characteristic of a particular animal, occur in each cell of the body. For example, 60 chromosomes occur in most cells of Atlantic salmon. Thirty of these chromosomes, all carrying a different set of genes, are inherited from the mother. Another 30, mirror images of the maternal chromosomes, are inherited from the father. In most cells the chromosomes occur in what are termed homologous pairs, i.e. maternal and paternal chromosomes carrying the same genes occur in pairs. The complete set of chromosomes (60 in this case) is termed the diploid (2N) number of the animal. In the formation of ova, or sperm, reduction division to half (30 for Atlantic salmon), referred to as the haploid (N) number of chromosomes, occurs. This is not a complete maternal or paternal set of chromosomes since independent assortment (shuffling) occurs at reduction division, thus increasing genetic

variability. When sperm and ovum fuse, the diploid (2N) number is re-established.

The genetic code: DNA consists, amongst other components, of two complementary chains of four kinds of base (adenine, guanine, cytosine and thymine). A specific sequence of three bases, termed a triplet, codes for one specific amino acid (a building block of a protein) or gives some other specific instruction. Proteins are constructed in the cell cytoplasm with the assistance of ribonucleic acid (RNA) molecules which carry the exact instructions from the DNA in the nucleus to the site of protein manufacture. Thus the sequence of nucleic acids in the nuclear DNA is termed the genetic code.

The gene locus: The place on a homologous pair of chromosomes where a pair of genes of similar function occur is termed a gene locus. One of these genes comes from the mother and the other from the father. We refer to a particular locus as, for example, the locus determining protein A, i.e. the place on the chromosomes where the "blueprint" for protein A occurs.

Protein structure: A protein molecule consists of one or more chains with the amino acids arranged in specific linear sequence. Each amino acid has a certain electrical charge. Therefore, a protein has a composite charge based on amino acid composition. Nearly all enzymes are proteins. Enzymes are organic catalysts which speed the conversion of a specific chemical substrate into a product. This specificity should be stressed.

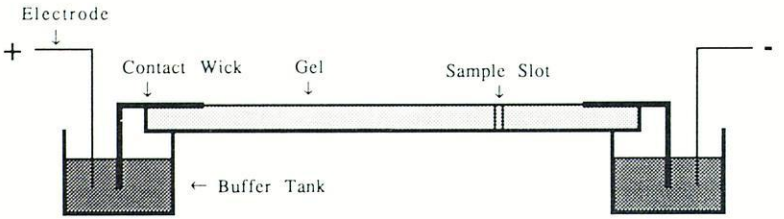
Forces which act on genes: Three forces act either directly or indirectly on genes. These are (a) mutation, (b) selection and (c) drift.

Mutation acts directly on genes and in its commonest form results in a change in a single nucleic acid in a DNA molecule. When such an altered gene codes for a protein, the mutation will usually result in a different amino acid being produced in the protein. This may alter the composite electrical charge on the protein. Mutations happen at random and are essentially rare events, but they are the source of most genetic variability and "raw material" of evolution. The latter two forces act indirectly on genes through the whole organism, in that they alter the likelihood of an individual producing offspring. In genetic terms, they alter the fitness of an individual. Selection is a process which acts on individuals in a certain deterministic way, e.g. in a fast flowing stream only fish of a certain genetic composition survive to reproduce. Drift on the other hand, is a random process, whereby animals survive to leave offspring by chance alone. Drift is a potent force in small populations, while selection is independent of population size.

Protein Electrophoresis

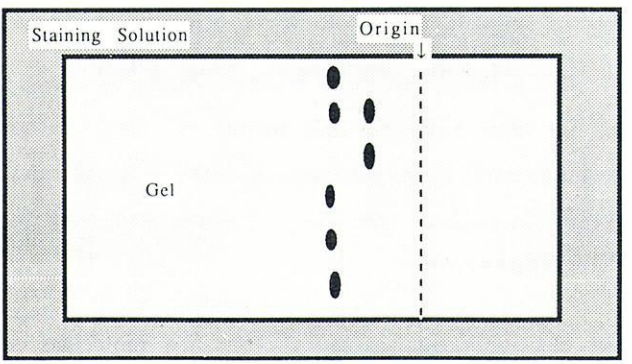
Protein electrophoresis is a technique for molecular separation, based on the concept of passing an electrical charge through a mixture of proteins. Different proteins, as I said earlier, will have different composite charges due to variation in amino acid composition. Thus, different proteins move at different speeds in response to electrical current, i.e. have different mobilities. The proteins are usually separated in a gelified medium, often consisting of hydrolysed starch. A typical electrophoretic set-up is shown in Figure 1. Protein solutions from small pieces of tissue (muscle, liver, heart, eye, serum etc.) from individual fishes are applied to the gel at right angles to the direction of current flow. Protein samples are usually applied on

a)



Side View

b)



Top View

Figure 1. Some details of the equipment and procedures involved in enzyme electrophoresis. In (a), tissue homogenates from individual fish are placed in a gel at right angles to the direction of an electric field, which is applied for several hours. After separation of the enzymes and other proteins (b), the gel is immersed in a specific stain for a particular enzyme. Bands are produced where the enzyme in the gel interacts with its substrate.

filter paper squares. An electric current is passed through the gel for several hours, causing the proteins to migrate. When the current is switched off, the gel is immersed in a stain. If this is a general protein

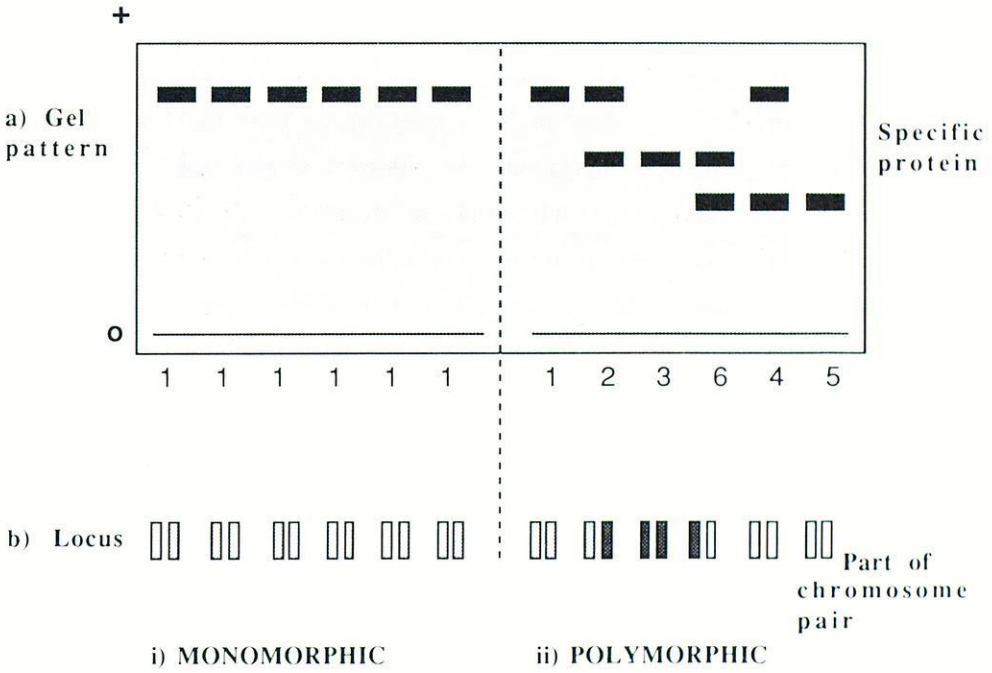


Figure 2 (a) The patterns observed on gels stained for a (i) monomorphic and (ii) polymorphic enzyme, with (b) the genetic composition resulting in the observed patterns. Each channel on the gel represents an individual salmon. 1, 3 and 5 are homozygotes; 2, 4 and 6 are heterozygotes.

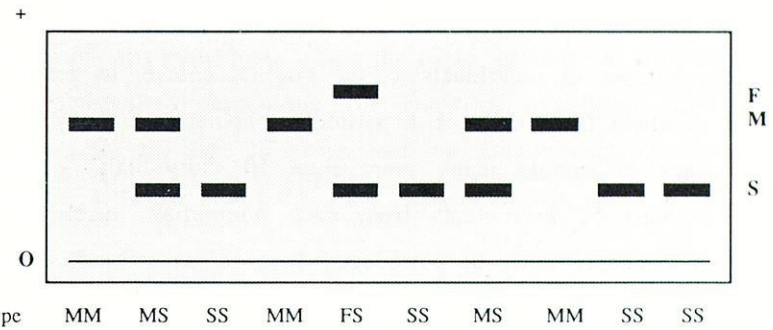
stain, many bands will appear on the gel, emmanating from each sample. Each band represents an individual protein. However, because of the multiplicity of bands a genetic interpretation is impossible, unless a single type of protein can be isolated. This is made possible by using a specific stain which isolates either a non-enzymatic protein or an enzyme. An

example of a specific stain of the first kind which has been utilised in Atlantic salmon is for transferrin. Transferrin is the iron-binding protein of the serum. By saturating the samples with radioactively-labelled iron and placing the finished gel on an X-ray film only the transferrin bands appear on the film.

Enzyme staining works in a different way. Here the finished gel is immersed in a solution, containing the substrate of the target enzyme and an appropriate stain. The only bands to appear are those of the specific enzyme. Many enzymes, particularly in salmonid fishes, are determined by more than one gene locus, so several sets of bands can appear on a specifically-stained gel. Proteins are only two steps removed from the DNA, so they give an accurate picture of the genetic composition of the fish. This picture is also uninfluenced by the environment, which can influence external body traits such as fin ray number. Currently, workers on Pacific salmon can assay more than 100 protein (mostly enzyme) loci. A specific protein may appear as a single band in all individuals examined. Alternatively, different individuals may have single bands of different mobilities, while other individuals may have combinations of two band types, often with extra bands of intermediate mobility (Fig. 2a). The genetic explanation of these observations are as follows. In the former case, the same maternal and paternal gene occurs in all individuals sampled, and the gene locus is referred to as *monomorphic* (Fig. 2b). In the latter case, two or more genes (in this case three) occur in the sample of individuals and the locus is referred to as *polymorphic*. Of course only two genes can occur in any single individual. Each animal is referred to as a homozygote for that locus if the two genes are the same and a heterozygote if the genes are different (Fig. 2a and b).

Protein polymorphism is just a specific case of genetic polymorphism. An everyday example is polymorphism for coat colour in sheep. Here,

black sheep are one type of homozygote, while white sheep can be either heterozygote or the other homozygote. We say that white coat colour is dominant and black is recessive. So, in this case, you can not tell the exact



Number of individuals tested (n)= 10. Number of genes (2n)= 20.

Frequency (q) of an allele, e.g. $F = \frac{2 \text{ Homozygotes(F)} + \text{Heterozygotes with F}}{2n}$

e.g. qF= 1/20= 0.05, qM= 8/20= 0.40, qS= 11/20= 0.55.

Heterozygosity (H)= Number of heterozygotes/ Number of salmon

e.g. 3/10= 0.3.

Figure 3. A typical electrophoresis gel stained specifically for a polymorphic enzyme with three alleles, showing how allele frequencies (genetic composition) and heterozygosity (extent of genetic variability) are calculated. Each channel on the gel represents an individual salmon.

genetic make-up of a white sheep. Luckily, this is not the case with proteins. They are said to be co-dominantly inherited. The different genes

occurring at a polymorphic locus are referred to as alleles. The allele or gene frequency at a polymorphic locus can be calculated merely by counting the number of each banding pattern on a gel; scoring homozygotes as two doses of the same allele and heterozygotes as one each of two kinds and then dividing the numbers for each allele by the total number of genes (twice the number of individuals - Fig. 3). Of course, to get an accurate estimate of allele frequencies in a particular population or species, it would be necessary to sample many more than 10 individuals. (One should sample at least 50 individuals from each population, taken at random). Two other concepts must be introduced here to understand what follows. These are heterozygosity and genetic distance.

Heterozygosity is a measure of genetic variability. It is calculated for a polymorphic locus by dividing the number of heterozygous individuals observed by the total number of fish sampled. For the example given in Fig. 3, heterozygosity (H) is 0.3. For monomorphic loci, heterozygosity is zero. Mean heterozygosity ($\bar{H}$) can be calculated over all the loci sampled, both monomorphic (H=O) and polymorphic (H greater than 0). The heterozygosities for the individual loci are simply added and divided by the number of loci assayed.

The formula for calculating mean genetic distance ($\bar{D}$) between samples, be they from different species, races or populations, is rather complicated. Suffice it to say that this measure becomes greater with larger genetic differences between samples, e.g. as gene frequencies between samples become increasingly different. Genetic distance is greatest in two samples which are monomorphic for different genes at a specific locus. As with heterozygosity, mean genetic distance ($\bar{D}$) is averaged over all loci assayed. To get an accurate measure of mean heterozygosity or genetic distance, it is necessary to assay at least 10 loci and preferably more than 20.

One shortcoming of protein electrophoresis is that it does not uncover all the variability in the DNA at the loci assayed. Nevertheless, results from electrophoresis undertaken with care by experienced workers should be comparable from different laboratories. Another question often raised concerning electrophoretic data is whether the loci assayed are representative of all gene loci. Despite these limitations, electrophoresis has been the standard technique for studying population genetics of salmonid fishes for the last 20 years, and has greatly expanded knowledge of relationships between and particularly within species.

Electrophoretic Differences between Salmonid Species

Genetic differences between species in the same genus, such as Atlantic salmon (*Salmo salar*) and trout (*S. trutta*) are large ($\bar{D}$ values are high) since many monomorphic enzyme loci are fixed for different alleles, i.e. different forms of a particular enzyme, having different mobilities, occur in the two species. This situation is found to occur at more than one third of monomorphic loci in salmon and trout. At other monomorphic loci the species share one allele. Thus, when making inter-species comparisons, monomorphic loci, are of most importance and differences in allele frequency at polymorphic loci are of minor importance. Indeed, the latter differences may be considered as "background noise". By careful choice of loci and the use of several loci to confirm identification, *single* fish may be assigned to a particular species. This can be useful for confirming the species identity of potential record specimens and has been used in this way in recent years, by at least one Water Authority. It is also possible to

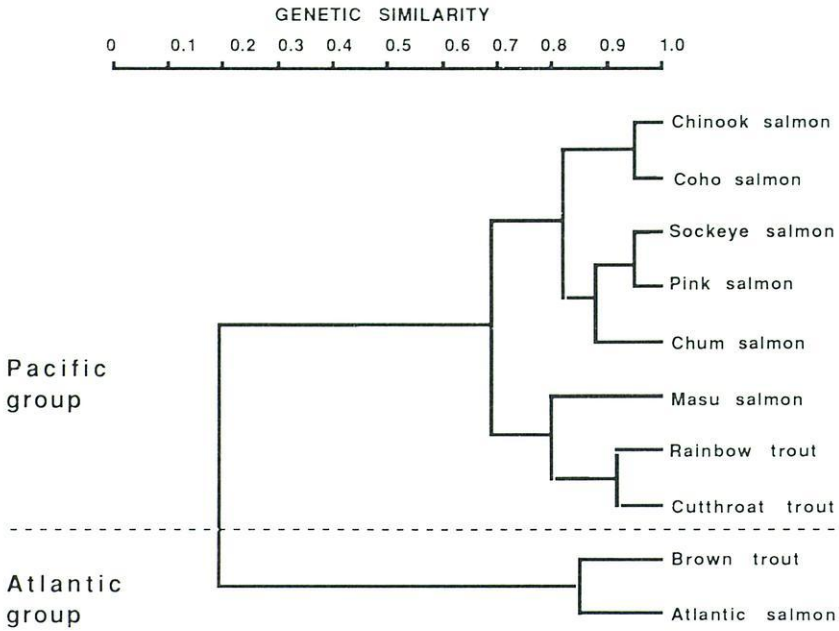


Figure 4. Branching diagram, based on electrophoretic assay of more than 18 enzyme loci, showing the relationship between the Pacific and Atlantic salmonid species. The closer to the right hand side of the diagram that species are interconnected, the more closely they are related. (Adapted from Ferguson and Fleming, 1983).

identify the species of origin of smoked fillets using a particular type of electrophoresis. Furthermore, a study on the genetic relationship between Atlantic and Pacific salmon and trout species undertaken by Andrew Ferguson and Colin Fleming of Queens University, Belfast has yielded some interesting results (Figure 4). They showed that Atlantic salmon and brown trout are quite closely related but both are distantly related to rainbow trout. This species clusters closely with the Pacific group including cutthroat trout and the *Oncorhynchus* species. In view of these results, splitting of the genus *Salmo* into Atlantic and Pacific genera, with different

names and with the latter being closely allied to *Oncorhynchus*, is perhaps indicated. One thing is very clear. We should not think of the rainbow trout as a close relative of Atlantic salmon or brown trout.

Hybrids between Species

Interspecific hybrids, especially first generation (termed "F_1") hybrids can be identified by the experienced worker using electrophoresis. The presence of F_1 Atlantic salmon x brown trout hybrids has been reported at low numbers in commercial "salmon" catches around Britain and Ireland by Ron Payne and his co-workers, and in juveniles from an English river by Tony Child and David Soloman. Relatively large numbers of hybrids seem to occur when one species is present in much smaller numbers than the other. Such a situation is reported from eastern Canada where the brown trout has been introduced. F_1 Atlantic salmon x brown trout hybrids seem to have low fertility. This fact, coupled with differences in spawning behaviour keeps the species separate. There is the slight possibility of record "sea trout" being F_1 hybrids with Atlantic salmon. Some fisheries managers may like to have the identity confirmed, and protein electrophoresis provides a simple and unambiguous way of doing this.

Electrophoretic Variation within Species

Within species of salmonid fishes, most electrophoretic differences seem to be at polymorphic loci, i.e. all of the units that make up the species share identical alleles at monomorphic loci. So these loci are useless as

discriminators. (Two sub-specific units are widely used. These are race and population. Races often occupy different landmasses or regions and are much more genetically distinct than populations. Their formation is usually thought to be related to isolation before or during the last Ice-Age. Populations are neighbouring groups which are largely reproductively isolated from each other and thus exhibit genetic differences, but of much smaller magnitude than between races. The population is often equivalent to the stock of the fisheries manager). Differences at polymorphic loci are either (a) quantitative, e.g. different races or populations each with the same alleles, but at different frequencies or (b) semi-qualitative, e.g. two groups sharing one allele but with other alleles being specific to a particular group. The consequence of these sorts of differences is that a large sample of individuals (50+) must be taken from a race or population to get a statistically valid estimate of differences. A single individual *cannot* be assigned unambiguously to a particular population or race. The racial and population structures of Atlantic salmon have been excellently reviewed by Noel Wilkins in an earlier booklet published by the Atlantic Salmon Trust, so my comments in this area will be selective.

Racial variation: Three major races of Atlantic salmon are recognised, based on allele frequency differences at several polymorphic loci (Fig. 5). It is of interest to note that Icelandic salmon are part of the European race. Earlier work by Ron Payne and other researchers, using the serum transferrin locus, had suggested the presence of western and eastern Atlantic races and also detected the presence of two population groupings in Britain and Ireland. The latter were termed Celtic and Boreal for southern and northern groupings respectively. However, when polymorphic enzymes were studied in salmon from British and Irish rivers, no such groupings were apparent. The answer to this apparent

contradiction may lie in the suggestion that transferrin evolves much faster than metabolic enzymes. Thus the effect of isolation for a short time might show only in transferrin. However, many authorities feel that races should not be proposed on the evidence from a single locus. When transferrin frequencies are added to allele frequencies at other polymorphic loci, and an average taken, the difference virtually disappears. Therefore, these two groupings do not exhibit a mean genetic distance typical of races. So what do we call them? I would propose "population groupings". In summary, I am not suggesting that the Celtic and Boreal groupings are any less real than races, just more recently evolved.

Electrophoretically-defined races also occur in other salmonid species. Andrew Ferguson and his co-workers have described two races of brown trout in Britain and Ireland, based on time of re-invasion after the last Ice Age. It should also be mentioned that they did *not* find separate races of brown trout in Britain and Ireland nor racial differences between sea trout and non-migratory *Salmo trutta* from the same locations. Fred Allendorf and Fred Utter have described races of rainbow trout and cutthroat trout from the Pacific North West, corresponding to the eastern and western slopes of the Rocky Mountains. These mountains were much higher in earlier geological times and separate rivers ran east and west, each with isolated trout stocks. Today, rivers traverse the Rockies but the separate races remain in different stretches.

The existence of distinct electrophoretically-defined races of Atlantic salmon in Europe and North America has facilitated the estimation of continental proportions in the feeding aggregation off West Greenland. Salmon are extensively fished from this aggregation with over 2000 tonnes per annum being taken in the early 1970s. Though currently controlled by quota to less than 1000 tonnes per annum, it is still a cause of concern for producing nations (mainly Canada, Scotland, Ireland and southern Norway).

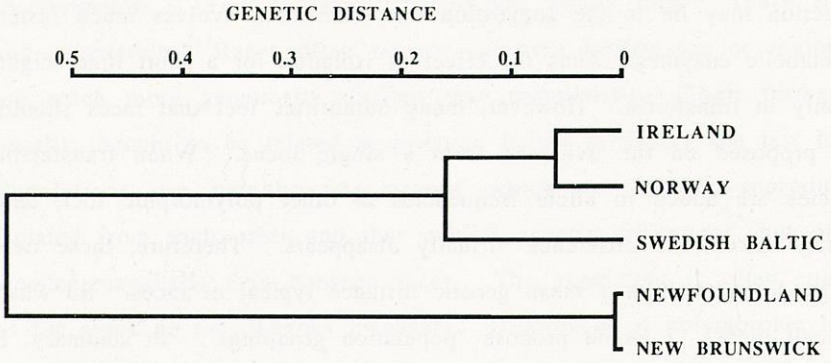


Figure 5. Branching diagram, based on electrophoretic assay at several polymorphic enzyme loci, showing the macro-geographic relationship between Atlantic salmon populations. Three races can be identified from (i) western European rivers, (ii) rivers entering the Baltic Sea and (iii) eastern North American rivers (From Cross, 1983, *Ann. Rept. Sal. Res. Trust of Irl. Inc.).*

Previous electrophoretic methods for estimating continental proportions were based on single loci. While these methods could estimate the proportions in a large sample of fish, they could not, in the majority of cases, assign individual salmon to continent of origin. Recent work by Eric Verspoor (now at the DAFS Marine Laboratory in Aberdeen) has shown that by using all currently available polymorphic loci, it is possible to be over 99% sure of the origin of a single fish. The currently favoured method of estimating continental proportion is based on scale feature analysis, since scale samples are easier and cheaper to collect. However, the increased power of electrophoresis, and the advent of mtDNA analysis (described later) should be considered in future choices of methods to investigate this fishery.

There is widespread agreement amongst fisheries geneticists and managers that there should be a total ban on inter-racial movements. There is little practical genetic evidence to support this idea. Yet, theoretically it is felt that since races have been separate for many thousands of years and experience different environments, such transfers would be unsuccessful. The history of *Gyrodactylus* in Norway confirms the danger of inter-race movements. This ectoparasitic fluke occurs on Baltic *Salmo salar* and apparently the fish exhibit considerable resistance, so there is little ill-effect. However, when Baltic salmon parr infested with *Gyrodactylus* were transferred to a Norwegian hatchery, and released in rivers, problems occurred. *Gyrodactylus* moved to native salmon which were extremely susceptible and currently fish populations have been decimated in 30 rivers. So it seems that genetic differences in disease susceptibility can occur in different races. It is also known that differences in virulence occur between salmonid disease organisms from different regions. This provides another reason for prohibiting inter-race transfers. While there is opposition amongst fisheries managers to such transfers, they may be carried out by some individuals in the salmon farming industry, in ignorance of the possible consequences, unless legislation prohibits such movement.

Population differences: Research over the last 20 years has demonstrated small but statistically significant allele frequency differences between most populations of all salmonid fish species investigated. Such genetically distinct populations or stocks breed in different water bodies or different parts of the same water body, but usually intermingle during part or all of the rest of their lives. In the case of Atlantic salmon, there can be different populations in the various tributaries of a big river. (The most recent and comprehensive publication on Atlantic salmon population

structure is by Gunner Stahl, and by Fred Utter and his co-workers for Pacific salmon). These genetic differences are maintained by accurate homing to natal spawning areas. Complete reproductive isolation is not necessary to maintain genetic discreteness but these results suggest that very few wanderers spawn successfully in another area (not more than one or two individuals per generation).

The meaning of these differences is much more difficult to explain. Because forces other than natural selection act on populations, such differences cannot be taken to imply adaptation to local conditions. Put another way, one cannot say that natural selection has moulded each population to the conditions of its natal river. This is because the proportional importance of natural selection and genetic drift is not known at present. As stated previously, (page 5) genetic drift is a chance process and is most potent when population numbers are low. Furthermore, some researchers have argued that drift is the major force acting on molecular traits. So all that we can say at present, is that individual populations may be adapted to their environment, but that the extent of this adaptation is unknown. Hopefully future experiments will shed more light on this area since it is vital to know the extent of adaptation when contemplating inter-population transfers. A study by Brian Riddell and co-workers on Atlantic salmon from the Miramichi river in New Brunswick showed that parr from fast and slow-flowing tributaries had different (presumably adaptive) body shapes. These characteristics were still evident in their offspring reared together in the same hatchery. Thus we know that there is some adaptation. Whether the variable proteins detected by electrophoresis are themselves adaptive or linked to adaptive loci is currently unknown. What we do know is that allele frequencies at the polymorphic loci provide a specific "mark" for a population. This has been of great use in the studies described below. Before continuing, I should stress that a comprehensive

investigation of population structure in each salmonid species is an essential prerequisite for other work of more direct use to managers.

Table 1. Mean heterozygosity (a measure of genetic variability) observed in several species of salmonid fishes, derived from electrophoretic studies of more than 20 enzyme loci[1].

SPECIES	MEAN HETEROZYGOSITY ($\bar{H}$)
Pink salmon	0.039
Chum salmon	0.045
Coho salmon	0.015
Sockeye salmon	0.018
Chinook salmon	0.035[2]
Cutthroat trout (Coastal)	0.063
(Interior)	0.023
Rainbow trout	0.060
Atlantic salmon	0.024

Probably the best measure of genetic variability as detected by electrophoresis is mean heterozygosity ($\bar{H}$) (see page 10). Table 1 shows estimates of mean heterozygosity in various salmonid species. (Bear in mind that this is presumed to be a good estimate of genetic variability in the entire genome i.e. all the genes of the animal). Different species vary from less than 2% to more than 6%. It is not clear why this is so, though it

1 From Allendorf and Utter, 1978 - see Further Reading

2 Recent studies have increased this figure considerably.

has been discussed well in a 1979 publication by Fred Allendorf and Fred Utter. What is clear from Table 1 is that mean heterozygosity is low in Atlantic salmon. Amongst Pacific salmon the species of high variability like chinook are currently being investigated, whereas low variability species like coho are not being considered at present. Using these criteria we might decide for example, not to attempt genetic stock identification on Atlantic salmon. However, workers on Pacific salmon are currently investigating almost twice as many enzyme loci as have been assayed in Atlantic salmon. Thus a study is urgently needed to investigate all of these "new" loci in Atlantic salmon in order to detect other polymorphic systems.

Within a species, it is important to maintain the highest possible heterozygosity in each population. Heterozygosity is reduced when very few parents found the next generation. This is a type of inbreeding, known as "bottlenecking". "Bottlenecks" can be naturally induced, as happens when a few individuals found a population in a new area or when numbers are reduced by natural disasters like drought or volcanic eruption. They can also be artifically induced by pollution or breeding regime. The latter will be discussed later. We know that reduced heterozygosity can result in lowered performance in terms of survival and growth. Fred Allendorf and his colleagues at the University of Montana are looking in particular at the implications of reduced heterozygosity in western North American trout. They have found a significant positive correlation between heterozygosity and condition factor in rainbow trout. Theorising that this phenomenon might result from increasing developmental stability with increased heterozygosity, they use a test of fluctuating assymmetry which quantifies such stability. The fluctuating assymmetry test simply measures the extent of variation in characters such as number of rays in paired fins, on the left and right side of the fish. Reduced fluctuating assymmetry, i.e. more developmental stability, correlates tightly with greater

heterozygosity. Fred Allendorf's group have also found that the rainbow trout spawning at the middle of the breeding season are the most heterozygous. This finding has important implications for the rainbow trout industry where efforts are being made to greatly extend the spawning period; the object being to have eggs available throughout the year. This is initially achieved by selecting early or late spawners. When farmers do this they may be inadvertently reducing heterozygosity drastically. Of course, individual fish vary in heterozygosity and every trout or salmon cannot possibly be heterozygous at all polymorphic loci. However, it is vitally important to maintain *average* heterozygosity and managers should ensure that their actions do not lead to erosion of genetic variability.

Mixed Fishery Analysis

Perhaps the most important management usage of electrophoretic data by western North American fisheries biologists at present is mixed fishery analysis of ocean-caught chinook salmon-the so-called Genetic Stock Identification (GSI) programme. This is a co-operative study involving laboratories from California to Alaska, many of which were visited. More than 50 scientists and technicians are involved. The method is based on a mathematical model called the EM algorithm. This is a maximum likelihood method, which establishes the most probable composition of a mixture if one knows the genetic composition of donor populations. In practice, all wild riverine and hatchery populations of chinook salmon from California to Alaska are assayed for all electrophoretically-detectable polymorphic loci. The allele frequencies then form part of a data base of all populations.

The fact that all populations differ genetically makes the method feasible. Also since allele frequencies in all but the smallest wild populations are relatively constant over time, a data base, once established can be used for many years. As we shall see in the section on artificial rearing, the allele frequencies of the populations from small hatcheries may vary between year classes. Thus each year class or cohort of reared populations must be typed. Having established the data base, large samples (300+ fish) are taken from the coastal commercial and recreational fisheries at regular intervals throughout the season. The samples are assayed within a few days of collection and the results analysed using the EM algorithm. Information on stock composition of the various fisheries is then provided to the managers who can make decisions about adjustment of catch or closure of the fishery. Closure, for example, is indicated if small stocks are heavily represented and therefore being overfished. Decisions are made within one week of sampling.

Managers in the Pacific North West are very enthusiastic about this technique which provides the first comprehensive data on composition of wild populations in coastal fisheries. (The micro-tagging of all reared smolts provides proportions of hatchery chinook caught and also provides the possibility of validation of GSI. This is currently being explored).

Chinook salmon were chosen for initial investigation because, they (a) have a very wide geographic distribution, (b) are the most valuable species of Pacific salmon and (c) exhibit high levels of genetic variability (high mean heterozygosity). Initial work is in progress on chum, pink and sockeye salmon and steelhead trout. Despite their commercial importance, coho salmon have not yet been included because of their very low heterozygosity (Table 1). The inclusion of chinook salmon in the GSI programme has led to a considerable increase in the number of loci assayed and thus to polymorphisms detected, due to the coastwide attention and

particularly the co-ordination efforts of Jim Shaklee of Washington State, Department of Fisheries.

More enzyme loci are now being electrophoretically assayed in chinook salmon than any other species except *Homo sapiens* and thus the results are of great biological significance. Therefore it is felt that coho should also be included in the GSI programme despite their low heterozygosity. Whether similar attention should be applied to one or more of the three races of Atlantic salmon (Figure 5) is a more difficult question. Considering the western European race, the catch may not be large enough to sustain such a research effort financially. Furthermore, Atlantic salmon are managed on a fixed season basis, not a flexible quota system. So the management system would need to be changed, if GSI were to be useful. Such a change could make enforcement even more difficult than it is at present. However, GSI might be useful in Atlantic salmon management under selected circumstances, such as estimating the effects of restocking or of farmed escapes. We have experimentally applied GSI to Atlantic salmon from the Faroes fishery in 1982 and 1983. We used several riverine populations from Scandinavia, Scotland and Ireland; a highly abbreviated data base. Our hope was that populations would form three distinct clusters corresponding to landmasses and that we could then use average landmass allele frequencies in the analysis. This was not realised (Figure 6). For example, populations in the data base from western Ireland and southern Norway were almost identical for these characters.

One thing demonstrated by the study was the extent of both inter and intra-year variation in the stock composition of the Faroes fishery. However, accurate estimation of riverine populations would require formation of a data base of all western European salmon populations and also the assay of as many "new" loci as possible. The latter is by far the quicker and easier option and, in addition, would provide data useful in

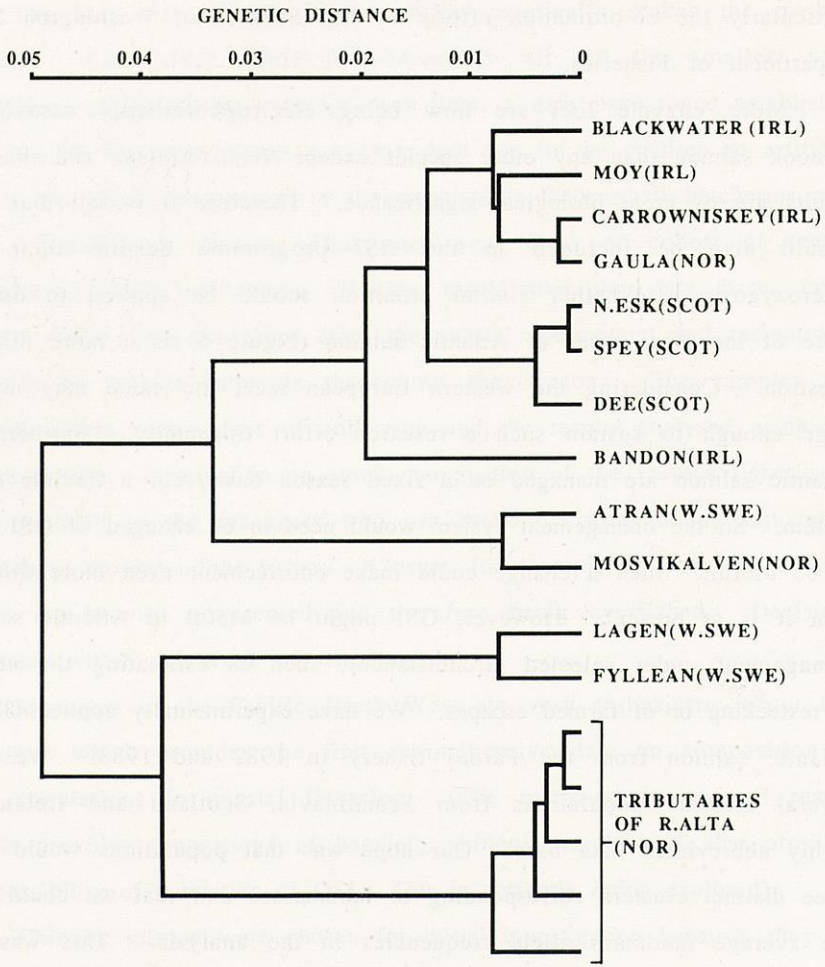


Figure 6. Branching diagram, based on electrophoretic data from the same polymorphic enzyme loci as used in Figure 5, showing the relationship between populations of Atlantic salmon within the western European race. The data were used by Cross and Healy (1983) in attempting stock discrimination in the Faroes fishery.

studying many other aspects of salmon genetics.

Hatcheries

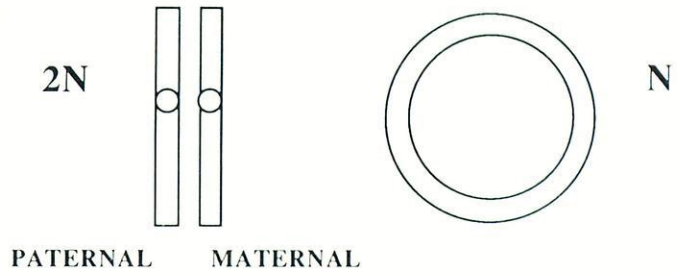
Hatchery programmes have been the sole reason for the continuing survival of Atlantic salmon in many areas, particularly on rivers dammed for hydroelectric generation. However, it is now realised that captive breeding programmes may (i) alter the genetic composition and (ii) lower the genetic variability of offspring when compared with their wild parents. These phenomena, as noted earlier, are inbreeding effects caused by using too few or a non-random sample of adults as broodstock. Such effects have been detected by electrophoretic assay of polymorphic enzymes, as changes in gene frequencies and reduction in heterozygosity, in most hatchery populations of Atlantic salmon in Ireland and Scandinavia, brown trout in Sweden and rainbow and cutthroat trout in western North America. The consequences of the first of these changes (variation in gene frequencies) is unknown, depending as it does on whether a particular genetic composition is adaptive for a specific population. Stated another way, one is asking whether the genetic composition of a particular population has been moulded largely by natural selection or by chance. As noted previously, the relative importance of these two forces is as yet unknown. In contrast there is a great deal of experimental evidence on the consequences of loss of genetic variability resulting in lowered performances in terms of survival and growth. A small amount of inbreeding occurs in all populations that are founded by less than 100 fish of each sex, equally represented. However substantial levels of inbreeding occur when less than 40 of each sex are used as parents. The consequences of a further

fish. Like all forms of marking used in fisheries biology, there are certain shortcomings to genetic tagging. To retrieve tissue samples for electrophoresis smaller fish must be sacrificed or larger specimens biopsied (enough enzyme for assay can sometimes be found in a sliver of adipose fin). Also fish chosen because they are homozygous for a particular allele may have a different survival potential from the general donor population. Such a difference must be quantified and a correction factor applied to the results, if necessary. Despite these limitations, genetic tagging is the most efficient (and sometimes the only) way of obtaining results in certain cases.

Analysis of DNA Structure

Since the 1970's, methods of DNA structural analysis have been developed and some of these are now being used in the investigation of salmonid fishes. Put simply, such analyses describe the structure of the genetic material itself and not the products of the genes, as in protein electrophoresis. Many more genetic differences are likely to be detected by DNA analysis, since protein electrophoresis usually fails to detect base changes which either do not result in amino acid changes (called synonomous mutations) or result in the substitution of an amino acid of similar charge. Most fisheries-related work on DNA analysis has concentrated on the mitochondrial genome. Here the DNA is in the form of a single circular chromosome, 18,000 base pairs long (see page 4). This chromosome is haploid (N) and maternally inherited, in contrast to the nuclear chromosomes which occur in diploid pairs (2N) - one maternal and the other paternal in origin (Fig. 8) The reason for the mitochondrial chromosome being chosen is because it is small compared with the nuclear genome and is therefore more manageable. Work with a variety of animals

also suggests that mtDNA evolves up to ten times faster than nuclear DNA. This property is of obvious interest to fisheries geneticists since it means



Nuclear DNA

Occurs in linear array on homologous pairs of chromosomes in nucleus. Diploid. Genes for enzymes detected by electrophoresis located here.

Mitochondrial DNA

Single ring chromosome in mitochondria. Haploid. Can be seperated from nuclear DNA by density gradient centrifugation.

Figure 8. Distinguishing features of nuclear and mitochondrial DNA.

that geologically more recent population separations could be detected. In recent years a great deal of work has been done on nuclear DNA using probes. These probes are templates that remove a short defined piece of the nuclear DNA, which can then be studied further. This is how the so-called "genetic fingerprinting", as used to identify human criminals works. Such techniques are now being tried in several laboratories with salmonid fishes and results should be published in the near future. Here, however, I intend to concentrate on mtDNA techniques in salmonids since some results from these methods are already available.

The traditional method of studying mitochondrial DNA (mt DNA) is to separate the nuclear and mtDNA by density gradient centrifugation. The purified mtDNA is then divided into aliquots and each aliquot incubated with a different enzyme. These enzymes are of bacterial origin and are called restriction endonucleases. Their mode of action is to cut the DNA wherever a sequence of four or six bases specific for each enzyme occurs. The resulting fragments are separated electrophoretically on the basis of size. Size markers are run on the same gel, so it is possible to determine the size of the DNA fragments from each fish. Thus it is possible to say whether a given fish has one or more specific 4- or 6-base sequences (called "restriction sites") in its mitochondrial genome. This same procedure is then followed for as many other restriction enzymes as possible. The degree of difference either between and within species can then be estimated.

The most comprehensive paper on the use of these methods in salmonid fishes is by Ulf Gyllensten and Allan Wilson (in Ryman and Utter, 1987). They compare a variety of Atlantic and Pacific salmonid species and produce a phenogram which is very similar to that derived by protein electrophoresis (see Fig.4). They also demonstrate the usefulness of mtDNA analysis in studying direction of hybridisation and presence of introgression between species and sub-species. MtDNA, because of its mode of inheritance is shown to be particularly sensitive for detecting inbreeding in hatchery populations of Atlantic salmon and brown trout. There is also some evidence that it may be possible to identify individual fish to their specific populations, which is not possible at present with enzyme electrophoresis.

Though few large-scale population surveys in salmonid fishes, using mtDNA analysis have yet been reported, much interest is being expressed in this method. For example, the National Marine Fisheries laboratory in

Seattle has an mtDNA section initiated by Fred Utter. Few results have yet been produced using mtDNA in large scale management-orientated surveys. The equipment required for mtDNA analysis, particularly the ultracentrifuge, is much more expensive than enzyme electrophoresis equipment. Also the assay of each individual fish is at least twice as expensive when using mtDNA analysis (though more information may be forthcoming). Finally mtDNA analysis requires researchers trained to a much higher level than enzyme electrophoresis. Ulf Gyellensten and Allan Wilson suggest applying both methods to a particular problem and point out the similarities and differences between what the methods measure. Such an approach would give a great deal more analytical power and also allow a thorough appraisal of mtDNA analysis as a management orientated technique.

Methods of mtDNA analysis are still evolving. During the study tour, Allan Wilson's laboratory in University of California at Berkeley was visited. There, a method of mtDNA analysis is being used which eliminates the need for an expensive ultracentrifuge and reduces the time taken for each analysis. The basis of the method is the so-called polymerase chain reaction (PCR). In this method a crude tissue extract containing both mitochondrial and nuclear DNA is taken and a selected section of mtDNA is amplified (thousands of copies are made). This is done by identifying runs of about 20 bases on each side of the sequence of interest, which are then made in the laboratory. When attached to the native mtDNA they act as endmarkers confining copying to the specific sequence. The treated extract together with an enzyme which promotes copying is then placed in a computerised heating block which heats and cools regularly over 24 hours making multiple copies. The extent of amplification is checked electrophoretically and the DNA divided into single strands before running on a long polyacrylamide gel in the presence of four types of "Sequenaze"

enzymes. As a result, the entire base sequence of the selected section of mtDNA can be determined. During my visit, this method was demonstrared with salmonid fishes including Atlantic salmon. The whole process takes 48 hours.

DNA analysis of either the mitochondrial or nuclear genome will obviously provide a great deal of management-related genetic information in the future. However, the methods are still experimental and are evolving rapidly. Thus most of the major management orientated studies (e.g.GSI) at present utilise enzyme electrophoresis. As suggested earlier, studies which utilise both methods are strongly recommended. Such studies should highlight the advantages and disadvantages of each method. They should also provide much more information, since the methods differ in scope and emphasis.

3. QUANTITATIVE GENETICS

The traits involved in most breeding programmes, such as growth rate, are influenced by the genetic composition of many gene loci and hence they are referred to as quantitative traits. These traits are described by their mean ($\bar{x}$) and variance (δ^2) and no attempt is made to distinguish the effects of individual gene loci. Such traits are also influenced by environmental conditions. We know, for example that the growth rate of fishes is influenced by water temperature. What one needs to know in designing a breeding programme, is what proportion of total variance (V_p) of a trait is due to a particular kind of genetic variance called additive genetic variance (V_a). This proportion is termed heritability (h^2) and ranges from 0 (no genetic variance) to 1, (all variance due to additive genetic variance). Another perhaps simpler way to describe heritability is through response to selection. Suppose the mean weight of a cage of salmon after 1 year in the sea is 2kg. The largest (i.e. fastest growing) 10% are selected as broodstock. Their mean weight is 3kg, so strength of selection (S) is 3kg - 2kg = 1kg. The offspring are reared in the same way and their mean weight after a year in the sea is measured. Suppose this weight is 2.3kg. Therefore response to selection (R) is 2.3kg - 2.0kg = 0.3kg. Heritability (h^2) is R/S - in this case 0.3/1.0 = 0.3.

The methods of estimating heritability are fully described in Falconer (1981). A note of caution should however be expressed about such measures. They apply only to the particular strain and environment in which the estimate is made. So, if a selective breeding programme is being designed one cannot rely on heritability estimates from elsewhere and should repeat the measurements oneself.

The longest and most comprehensive selective breeding programme for Atlantic salmon and rainbow trout so far undertaken is that of Trygve Gjedrem and his group in the Agricultural University of Norway. Their basic plan has four parts;-

(i) identification of breeding goals,

(ii) selection of the most suitable strains,

(iii) identification of the optimal type of intrastrain selection for the chosen traits,

(iv) selective breeding.

(i) Identification of breeding goals may seem a simple task but it should be borne in mind that goals must be chosen which remain relevant for many years. In other words the farmer or rancher's needs for the next 10 to 20 years must be anticipated, since for example Atlantic salmon generation time is four or more years, so even three generations would take a minimum of 12 years. The breeding goals identified by Gjedrem (1983) were growth rate to market size, late maturity, carcass quality and disease resistance.

(ii) Selection of the most suitable river strains involves rearing all available river strains together and studying their performance with respect to the chosen breeding goals. The logistical problems involved in collecting eggs from many different rivers are considerable. It should also be noted that a very large rearing facility is required to adequately test several strains whilst avoiding inbreeding. In the Norwegian programme the six best riverine populations were chosen for future work and crossed to maximise genetic variability.

(iii) The heritability of each of the chosen traits was then estimated. Some of these heritabilities are given in Table 2. The type of selection to be used was then chosen. In general, mass selection is applied with a trait

of high heritability (>30%) and family selection with traits of lower heritability (<30%). Mass selection is where all available parents of a strain are considered and a proportion that are best for a particular trait chosen. In family selection, on the other hand, the best families are chosen as broodstock. In the Norwegian case, family selection was chosen, since it was intended to select for fast growth and late maturity together. This technique, called index selection usually means reduction in individual trait heritability. Thus family selection was judged to be more powerful. The difficulty with family selection is that the progeny of each female must be held in individual tanks until marking is possible (at the end of the first summer) and many families must be held to prevent inbreeding (requiring a large facility). In the Norwegian programme, as in any properly designed programme, a control group is maintained in the same facility, so the actual response to selection can be gauged. Over the years selection for fast growth and late maturity has continued and 3% improvement in growth *per year* is reported. Disease resistance, largely to *Vibrio anguillarum*, which showed low heritability and also carcass quality are no longer included as breeding goals.

Table 2. Calculated heritability for traits of commercial importance in rainbow trout and Atlantic salmon. (From Kinghorn, 1983 - see Further Reading).

SPECIES	TRAIT	HERITABILITY
Rainbow trout	$2^1/2$yr prespawning weight	17 - 21%
Atlantic salmon	$3^1/2$yr prespawning weight	31 - 37%
	Late maturity	42%

It is obvious that such a programme is economically beneficial to the farming industry. However these programmes demand a considerable investment and are by their nature, long term. So if such a programme is initiated it must be continued for several generations. An example of the problems that can arise with such programmes is illustrated by the experience in British Columbia. There, until two or three years ago the primary farmed species was coho salmon, and various Government agencies and academic institutes are running selection programmes on that species. The industry however, has recently largely changed to chinook salmon, making the ongoing programmes somewhat redundant. Strain selection is currently underway with chinook salmon, but it will be some time before selective breeding can begin, at least in the public sector.

As stated earlier, most selection programmes are related to farming, with few programmes being concerned with ranching. The Atlantic Salmon Federation in New Brunswick has attempted to run a selection programme based on ranching but this has been hampered by poor returns, so effort is now focused in producing faster growing salmon for the rapidly emerging farming industry. Some interest is also being expressed in quantitative genetics in Iceland where a big ranching industry is envisaged. One might wonder how fish selected for a particular trait such as growth would perform in the wild. Selective breeding might lead to a change in genetic composition and possibly to a reduction in genetic variability. If this were so, then the possibility of spawning with wild fish would have to be minimised.

Perhaps because of rather different backgrounds, there has been very little discussion in the past between population geneticists (largely researchers interested in wild salmon) and quantitative geneticists (with an animal production background and concentrating on farmed stock). This situation needs to be rectified if both healthy wild stocks and salmon

aquaculture are to co-exist. At present we do not know the genetic composition (as displayed by electrophoresis or DNA analysis) of many reared strains. As stated earlier this knowledge urgently needs to be acquired.

4. GENETIC ENGINEERING AND MANIPULATION OF SEX

Three topics will be discussed in this section:-

(a) manipulation of chromosomal composition,

(b) manipulation of sex,

(c) gene transplantation.

All of these techniques are either still experimental or used to a limited extent in the farming industry. They are included here because they could influence wild stocks, either through ranching of these forms or because of escapes from farms.

Manipulation of Chromosome Number or Type.

Gynogenesis: Salmonid fishes are diploid (2N) organisms (see Fig. 9) with chromosomes occurring in homologous pairs, one of maternal and one of paternal origin. It is possible to produce a haploid embryo with maternal chromosomes only, by irradiation of the sperm. The radiation dose is adjusted to knock out the genetic material of the sperm without inhibiting mobility and therefore the ability to start the egg dividing. The resulting embryo is called a haploid gynogen (all female). Survival of haploids is very poor so the "zygote" is made diploid (2N) by applying a temperature or pressure shock at one of two particular times shortly after "fertilization". (The most common shock is immersion of the eggs in a water bath held at 28^0C for a short period). A shock applied at the earlier of these times affects meiosis, the reduction cell division that produces the haploid egg. This type of diploid gynogen is relatively easy to produce but there is still some genetic variation between progeny. A later shock affects mitosis

(ordinary cell division). Thus all of the offspring are genetically identical (cloned individuals). However, it has proved very difficult to produce these forms.

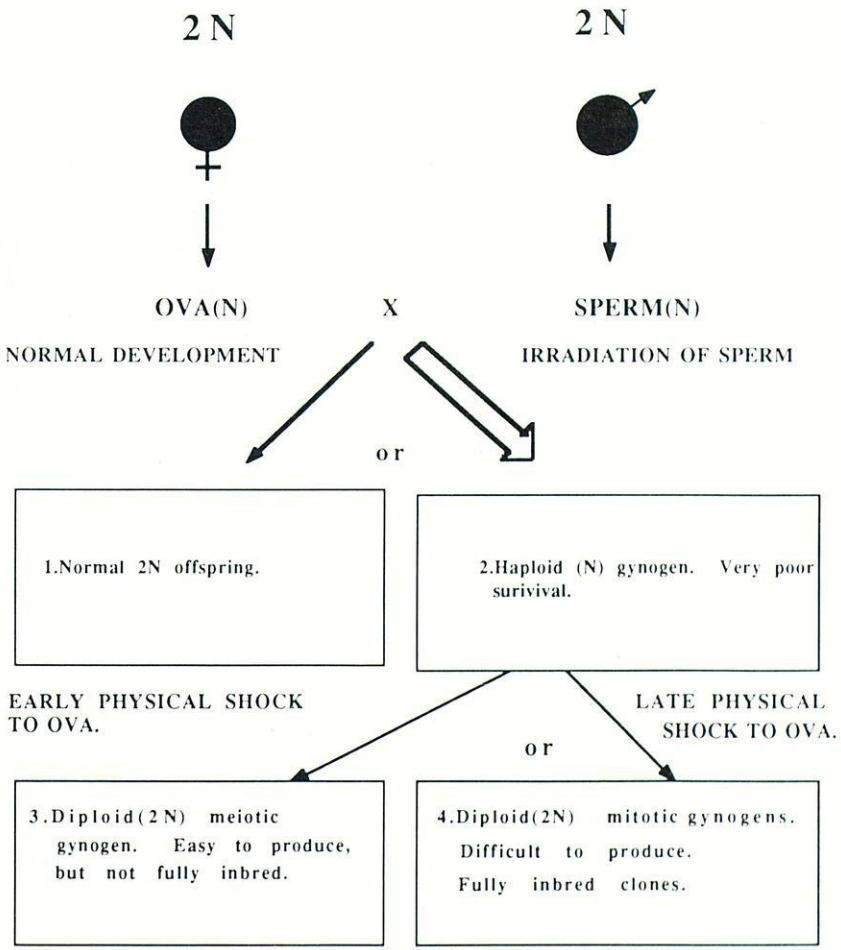


Figure 9. Manipulation of chromosome number: diagram showing the method of production of (i) normal offspring, (ii) haploid gynogens, (iii) diploid meiotic gynogens and (iv) diploid mitotic gynogens.

The applied relevance of such work lies in the production of strains of genetically identical totally - homozygous individuals. Two of these strains could then be crossed (using the sex manipulation techniques outlined below) to produce variable offspring and so the value of genetic variability could be quantified.

Triploidy: Of more applied relevance is the production of triploid (3N) salmonids. Triploidy is achieved by allowing fertilisation with normal sperm and then applying a physical shock to prevent loss of the second polar body of the female. The resulting fish have one set of paternal and two sets of maternal chromosomes. Such fish are sterile and therein lies their value to the farming industry. When a fish matures sexually, body growth ceases, secondary sexual characteristics such as the kype in males are formed and susceptibility to disease increases. Hence the farmer would prefer sterile production fish, provided performance prior to maturity is equivalent to normal (diploid) fish. Unfortunately, triploid males, though not fertile, develop secondary sexually characteristics, so only females should be triploidised. How this is done is described below (page 41).

Androgenesis: Another form of ploidy manipulation has been practised by Gary Thorgaard and his coleagues at State University of Washington at Pullman. They have managed to produce diploid androgen (all male) rainbow trout (2N). This is done by destroying the genetic material of the egg by radiation. The dose is adjusted to allow the remainder of the egg to remain viable so cell division can occur. As with gynogens, haploid (N) survival is poor, so heat shock is applied to produce diploids (2N). Viability of diploid androgens is low at present, but as it improves in the future, one could see a practical application of this

technique. At the moment eggs can not be satisfactorily cryopreserved but sperm can. In Norway for example sperm are being collected from wild Atlantic salmon populations at possible risk of extinction. If production of diploid androgens was possible in the future, using these sperm, then a stock could be reconstituted while maintaining genetic integrity.

Manipulation of Sex

Methodology: Distinct sex chromosomes occur in some salmonid fish species (Thorgaard 1977). In these species and probably in all salmonids the female has two X chromosomes (referred to as the homogametic sex), whereas the male has one X and one Y chromosome (referred to as the heterogametic sex). However, the functional sex of a salmon or trout can be changed by incorporating a large dose of male or female hormone in the food at first feeding. Thus chromosomal males can be turned into functional females and *vice versa*. In farming, particularly with rainbow trout, males sexually mature a year, on average, earlier than females. Aiming to market their fish prior to sexual maturity but at adequate size, trout farmers therefore prefer females. All female stock can be produced directly by administering female hormone at first feeding. However, this method means that hormonally treated fish will be offered for sale and this is forbidden in certain countries. Thus an indirect method of producing all female stock has been developed. In this method chromosomal females are converted into functional males in the *parental* generation using male hormone (Fig 10a). Milt from these functional males is then used to fertilise normal ova resulting in all XX and thus all-female progeny. These offspring have not been hormonally treated themselves

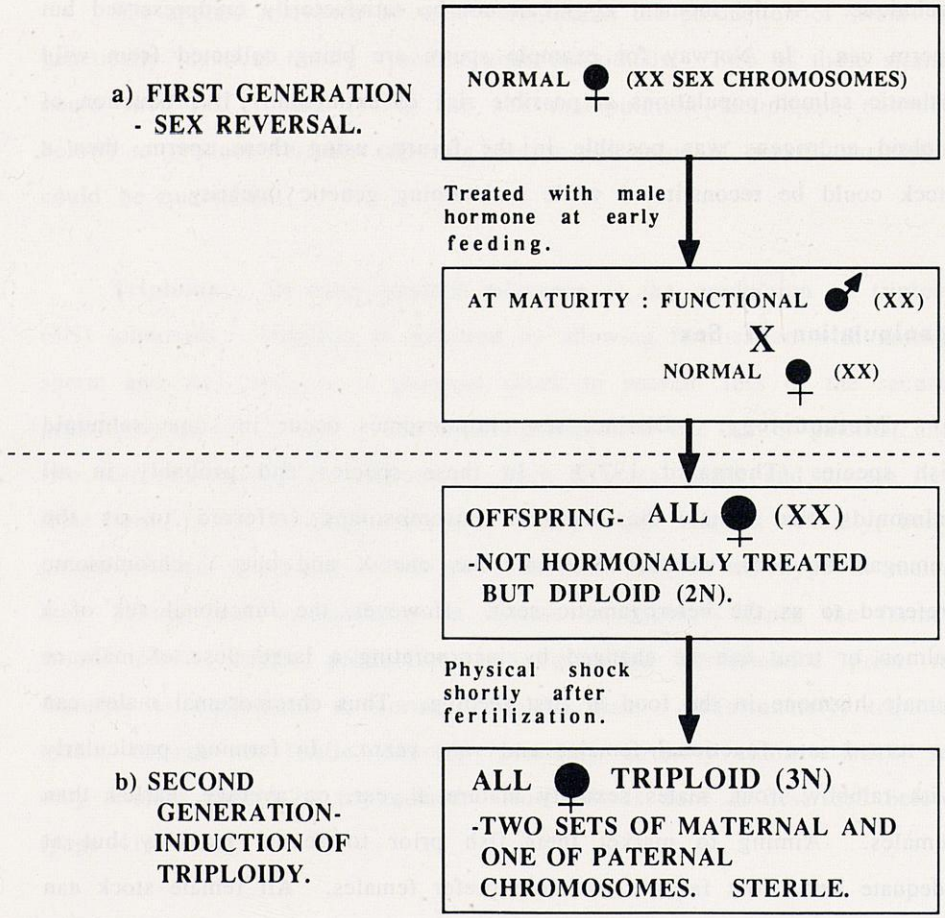


Figure 10. Method of production of sterile Atlantic salmon by sex reversal and induction of triploidy.

and therefore can be marketed without legal problems. All female rainbow trout produced in this way are now available commercially in the United Kingdom and provide the farmer with more flexibility in terms of fish size and time of sale. For those farmers seeking larger fish, the induction of triploidy (3N), as described on page 40, has been combined with all female production (Fig 10b). In this process, a physical shock is applied to the all-female ova shortly after fertilisation to induce triploidy and thus sterility. Triploid all-female rainbow trout eggs are now also available in Great Britain.

Possible applications to Atlantic salmon: The technique of producing sterile all-female fish is now being viewed with interest by British and Irish salmon farmers as a way of eliminating grilsing. Grilse maturity has an environmental as well as a genetic component and the higher sea temperatures experienced in the south of the European farming range seem to induce considerably more early maturity. Several commercial and research establishments in Britain and Ireland are at present conducting trials, the results of which should be available in the near future. As discussed later (Section 5) if sterile fish were to be used extensively in salmon farming, the potential genetic effect of farmed escapes on wild stocks would be negligible.

Considerable work on manipulation of sex and ploidy is also in progress with Pacific salmon, particularly in the West Vancouver Laboratory in British Columbia by Ed Donaldson and his colleagues. Many of their experimental groups of fish have been or are currently being tested in enhancement or farming programmes.

Gene Transplantation.

Methods: Perhaps the most widely publicised aspect of genetic engineering has been gene transplantation. The basic idea is to transfer the gene coding for a useful substance from one species to another and so improve a specific aspect of the performance of the recipient. The classic example was the transfer of the rat pituitary growth hormone gene into the mouse, thus promoting "rat-type" growth. In practise, the gene is located in the donor species and excised using appropriate enzymes. The gene is then incorporated into a bacterial chromosome. The bacteria are grown and thus multiple copies of the gene are cloned. The target gene, accompanied by one or more marker genes is removed from the bacteria and injected using a microneedle into a fertilised egg. The idea is to inject the gene into the nucleus and have it incorporated into the DNA of the recipient species. The gene should then be passed on genetically to subsequent generations. Presence of the gene can be confirmed using a marker gene or genes, whereas inheritance can be checked by breeding experiments.

Transgenic salmonid fishes: Researchers in many laboratories throughout the world are working on the production of these so-called transgenic animals and some are using salmonid fishes as recipient species. For example Norman Maclean and David Penman of Southampton University have sucessfully transferred mammalian growth hormone gene into rainbow trout. Also, in one of the laboratories visited in St. John's, Newfoundland, Garth Fletcher is experimenting with transferring the winter flounder gene for an "antifreeze" protein into Atlantic salmon. One aim of this research is to permit caged salmon to survive the Newfoundland winter.

Transgenics are still at the experimental stage, but if in the future, they are adopted for farming, then the possible genetic impact of transgenic escapes on wild stocks will need to be considered.

5. POSSIBLE APPLICATIONS OF GENETICS TO ATLANTIC SALMON MANAGEMENT

In the preceeding sections the recent advances in salmonid genetics both in Europe and North America have been described. In this section the possible applications of these methods to the management, conservation and enhancement of wild Atlantic salmon stocks are discussed. The section begins with consideration of undepleted wild populations. Restocking is then discussed and finally the possible genetic impact of escapes from farms or ocean ranching programmes is considered.

Wild populations

Electrophoretic surveys of population structure have been carried out in several Atlantic salmon producing countries. A recent workshop at Aberdeen jointly organised by the Department of Agriculture and Fisheries for Scotland and the Atlantic Salmon Trust demonstrated that many more such surveys are in progress (e.g. in eastern Canada, Iceland, Norway, Sweden, Finland, Scotland, England, Wales, Northern Ireland, Republic of Ireland and Spain). A summary of the populations involved will be published shortly. This proliferatrion of electrophoretic surveys is timely because of the huge expansion of farming. This expansion has resulted in an increase in potential escapes. Also the planned increase in ranching and restocking programmes may cause variation in the genetic composition of wild populations. Thus, every effort should be made to type as many populations as possible in their native state. Since electrophoretically-detected gene frequencies in large wild populations seem fairly constant

over a few generations, such data can be used as a baseline against which to assess later human-induced changes. Efforts should also be made to extend the number of loci that can be assayed in Atlantic salmon. As mentioned earlier, workers with Pacific salmon are currently investigating more than twice as many enzyme loci as in the Atlantic species, so these might be the first new loci to investigate in *Salmo salar*. Also, as DNA analysis assumes more importance as a management tool, the two techniques should be used on the same samples.

One cannot predict the population structure of salmon in a particular river without an electrophoretic investigation. Thus an electrophoretic survey, along with other surveys carried out by the fisheries biologist should be considered as a necessity when managing the salmon of a specific river. It is vital, for example, to know whether one freely interbreeding population occupies a river or whether there is an isolated population in each tributary.

As more polymorphic enzyme loci are discovered and as DNA analysis becomes routine, it should be possible to substantially improve mixed fishery analysis in Atlantic salmon. Thus it should be possible to routinely identify single salmon from the West Greenland fishery to continent of origin or achieve improved stock separation in the Faroes fishery. While it would be beneficial, for instance, to apply a genetic stock identification programme to the Irish coastal drift-net fishery, it is doubtful whether the resource is big enough to justify the expense involved in forming a database and in analysing fishery samples. However, ongoing or future surveys may provide the major part of a population database, thus making such a programme substantially cheaper. The other area in which mixed fishery analysis could be useful is in quantifying the smolt contribution of the different tributaries of a catchment. This of course, would assume that genetically-distinct populations occupied the different tributaries. If so,

such an approach might be more economical and accurate than the current method which involves electrofishing surveys to assess parr numbers.

Reared strains

Atlantic salmon have traditionally been reared for enhancement but are now also grown in increasing numbers for farming and ranching. In all cases reared strains are of course founded from wild populations and the usual intention is to ensure that reared strains are genetically identical to the wild founders (unless artificial selection is being applied). Unfortunately, as many electrophoretic surveys have demonstrated, this is seldom the case. Most reared strains tested show reduced genetic variability (measured as mean heterozygosity), loss of rare alleles and changes in frequency of common alleles, compared with their wild founder populations. Furthermore, different derivative strains from the same wild population often differ in common allele gene frequency (i.e. have different genetic composition). Thus where inbreeding has occurred, each reared strain must be individually assayed and one cannot refer to "strain A derived from wild population A". Rather, individual strains must be identified by year and location of hatching and the group of parents used, e.g. "strain A 1988 from rearing station X". As stated earlier all of this results from using too few and/or non-randomly selected wild founders.

Douglas Tave, in a recent book entitled "Genetics for fish hatchery managers" presents a detailed discussion of the avoidance of such inbreeding effects. He stresses that while the use of 40 parents of each sex will prevent major inbreeding in the short term, many more parents will be required to completely eliminate inbreeding. Many Atlantic salmon hatcheries do not currently use this many parents and thus a radical

change in thinking is required. This is particularly important in enhancement hatcheries which are attempting to preserve wild populations in their original form. However, it is also an important consideration in hatcheries producing smolts for farming or ranching, since progressive inbreeding will decrease performance and response to artificial selection. A dual approach is required to assess levels of inbreeding in current hatchery strains. Firstly, breeding records (where they exist), should be examined. This will give a preliminary indication of possible inbreeding. Then, the genetic composition of each reared strain should be assessed using either enzyme electrophoresis or DNA analysis, and the results compared with the wild founder populations. Where severe inbreeding is detected, then breeding should not be continued from that strain. Where slight inbreeding has occurred, then an increase in the level of inbreeding can be arrested by subsequent use of adequate parental numbers. Inbreeding may be eliminated by outcrossing with the wild founder population (if this is still possible). Such manipulations should be monitored genetically, as should the creation of all new reared strains. Only by adopting the suggestions outlined here can we be sure of the genetic composition of reared strains. This is not the case at present and may be the reason for the lack of success of some enhancement efforts. Extra work and expense would be involved for fisheries managers if such a scheme were adopted but I believe that its incorporation is essential to optimise future restocking programmes. This has now been realised by North American salmon managers but has, as yet, to achieve wide recognition in Europe.

In the discussions which follow on restocking the use of non-inbred reared strains will be assumed. While this is not the current situation, it will be realised from what I have said above, that hatchery managers should aim to achieve this situation as soon as possible.

Restocking is deemed necessary in two situations. These are (i) where salmon still remain in the system, (though their continued existence may be threatened) and (ii) where salmon are extinct. Since different considerations apply in each case, they will be discussed separately.

Considerations in restocking a system where salmon still remain: Before considering restocking itself, I feel that it is important to consider the reasons for stocking and also to assess possible alternatives. This is important because restocking is a costly practise, and post-release survival of stocked salmon is always poorer initially than that of wild fish.

The extent of population decline must also be accurately quantified. Trapping or electrofishing surveys over a *long time series,* usually provide good data, whereas anglers catch data or opinions can be wildly inaccurate. The classic reason for restocking has been the construction of dams or reservoirs which eliminate spawning areas. Restocking may be the only option with total elimination of spawning areas but partial destruction or limitation of access might be aleviated by habitat manipulation. This area is well reviewed by David Soloman in an Atlantic Salmon Trust publication. Another possibility for increasing salmon numbers is by kelt reconditioning. Here kelts are captured immediately after natural spawning, are kept in cages and are eventually weaned back onto food. Much better survival to second spawning (up to five times higher), is achievable than in the wild, so many more eggs are available from previously-spawned females. This method has been successfully used in Newfoundland (Pepper and Parsons, 1987) and also in New Brunswick.

Such options are preferable to restocking because they utilise the potential of the wild stock.

Sometimes restocking or other methods of population enhancement are suggested because of decline in rod catches due to poaching, pollution or heavy netting at sea. In the former cases it is more sensible to first control the poaching or pollution and only then to consider enhancement (if it is still necessary). With heavy coastal netting, as is the case in Ireland, enhancement improves the number of fish returning to the river *but* also provides many more salmon to the nets. Thus, the price of enhancement is prohibitive for private fishery owners.

If restocking seems the only option and is economically feasible, the next consideration, again before thinking about genetics, is to analyse the detailed aims. With mitigation restocking, as in the case of a hydroelectric scheme, this may involve "maintaining the salmon run" in the entire river. The aim of the private fishery owner will usually be more specific, eg. "maintain or improve the rod catch on a certain stretch or stretches". Such an aim will determine the location of release. Furthermore, selection of ages and numbers of fish to release will require a detailed knowledge of survival and cost of production of the various stages from egg to pre-smolt.

Genetics can now be considered in choosing the donor population. Current genetic wisdom dictates that it is best to use native salmon as parents of individuals for restocking. Thus, it is important to know whether there are one or more populations in the catchment so that suitable groups of parents can be chosen. To investigate this, an electrophoretic or DNA survey should be undertaken of salmon from several locations within the catchment. These data will also serve as a baseline against which to compare the reared strains produced. Furthermore, the points concerning parental number, as discussed earlier (page 26) must be considered.

It is ideal if a rearing station already exists on the river to be restocked. If not, then the manager must decide whether to build a hatchery or perhaps to arrange contract rearing by an existing hatchery on a nearby catchment. The first of these options is expensive and takes time, so contract rearing, if feasible could be used in the interim. However, it should be borne in mind that the waters of each river differ slightly in chemical and physical properties, so there may be different selection pressures. Thus rearing of fish for enhancement of one river on another catchment may not be as effective as rearing locally.

Fears have been expressed that reared salmon in general, either because of selection for living in crowded conditions and being constantly fed or because of relaxation of natural selection, will be less fit than wild fish when released. Such ideas are unproven and would, in any case, be very difficult to establish because of environmental effects. It has, for example, been noticed in some enhancement operations that a higher proportion of reared than wild salmon return after one winter at sea, i.e. as grilse. One could postulate a genetic theory to account for this, but a more likely explanation is that fast growth promoted by good feeding in the hatchery influences time of maturity.

It is essential that the effects of restocking be monitored by marking the introduced fish in some way to distinguish them from wild salmon. This has not often been done in the past, but provides the only method of evaluating a stocking programme. Salmon from their first autumn in freshwater onwards, can be marked by adipose fin-clipping, Panjet tattooing or microtagging. Unfortunately these methods are not usually practical on the younger stages which are often used in restocking. Furthermore, none of these marks are passed to the next generation. Genetic marks, aş described earlier (page 27) satisfy these criteria and their use might be considered. However, they are time consuming to

produce, and many specific crosses must be made to avoid inbreeding. Also, in their production, one is perhaps altering the genetic make-up which is optimal for survival in a particular area. Thus there should be detailed discussion between the manager and population geneticist prior to adopting this method of marking.

If too few fish remain in a river for rehabilitation using native salmon, then a donor population will need to be chosen from elsewhere. Selection of the best donor population is difficult. A similar problem arises in restocking a fishless river and the details will be discussed below.

Restocking a fishless system: Restocking a system where salmon are extinct or very few remain involves choosing the most suitable donor population or populations. Firstly though, one must be sure that the causes of the population extinction or reduction have been rectified. Usually these causes are pollution or lack of access due to weirs or dams.

Transfers between the major races (eastern North America, western Europe and Baltic) are not recommended, as noted on page 17. The transfer of salmon from Finland (Baltic race) to Norway (western European race) probably resulted in the *Gyrodactylus* problem. Inter-racial transfers for restocking purposes will probably not be considered in future but may be envisaged by the farming industry. The possibility of escapes (discussed below) means that such transfers should be opposed.

However, within races and particularly for the well studied western European race, electrophoretically-derived genetic distance is not positively related to geographic distance (Fig.6). In other words, two populations, 100Km apart, can be less genetically different than two 10Km apart. Notwithstanding such results it still makes more sense genetically to choose nearby rather than distant donor populations, since it can be assumed that environmental factors, which act as selective agents, are more similar in

nearby rather than in distant rivers. Nor is it sensible to import salmon from another landmass or country because of the risk of disease transfer. Biologists in the Salmon Enhancement Programme (SEP) in western Canada consider a number of factors in addition to genetic composition when choosing a donor population. Such a system would also be useful with Atlantic salmon and might consider factors such as:-

(1) Geographic similarity: fast or slow flow; large or small catchment; degree of branching.

(2) Geological similarity: type of rock underlying the catchment.

(3) Similarity in water chemistry and physics: pH, temperature, nutrient status, etc.,

(4) Similarity in run characteristics: largely a grilse or multi-sea-winter salmon river (if known from historical records).

(5) Ecological similarity: similar prey species; bird, fish and mammalian predators; similar competitors.

(6) Genetic similarity: nearby population if possible; not from another country, landmass or race.

(7) Broodstock for enhancement hatcheries should ideally be obtained from the wild in each genertation (to avoid domestication).

(8) Surplus young from commercial farms should not be used for restocking. (Surplus parr destined to be S2 smolts, sometimes of foreign parentage are often offered by fish farmers to managers. Such fish should not be accepted on two counts: - a) Pre S2 smolts are the slower growing component of the population and therefore selection for reduced growth rate is being applied and b) foreign salmon are more likely to be more genetically different from the original natives than local fish, *even if this is undetectable using current methods.* Even if the parr offered were rising S1's and S2's from a local population, they would still need to be examined using population genetic methods to check for inbreeding).

A method tried by Lars Hansen in rehabilitating the river running through Oslo, was to stock with more than one donor strain. Three suitable strains were used and returning adults were caught in upstream traps, where distinctive marks allowed strain identification. The strains were ranked in terms of biomass of returning adults, with one of the two best strains producing a much higher proportion of grilse than the other. In this experiment adults were not allowed upstream to breed naturally.

Finally, it has been suggested by some authors that several donor strains should be used for restocking and allowed to spawn and interbreed naturally. This might result in progeny with higher genetic variability than normal, ensuring good performance. Also, natural selection could act on these fish moulding a genetically-optimal population. These ideas contravene the theory that each population has a specific suite of adaptations, maintained by locally co-adapted gene complexes. If this theory is correct, then mixing of populations might be counter productive. This is an area which has not been tested practically.

The Extent of Current Use of Genetic Techniques in Farmed and Ranched Atlantic Salmon

Farming: Artificial selection, mainly for faster growth to market size and later sexual maturity, and manipulation of sex and ploidy to produce all-female sterile triploid fish, are the two genetic techniques currently in major use by the salmon farming industry. Artificial selection, as stated earlier, has been practised in Norway by both Government agencies and private companies for more than 20 years. This programme is one of the major reasons for the use of eggs of Norwegian origin in the other European salmon farming countries. The excellence of the Norwegian

efforts has discouraged other countries from starting breeding programmes of their own. This situation needs to be rectified for the good of the industries in these other countries. At present, there is a reliance on a potential competitor for the "raw material" of the farming industries in countries other than Norway. Also, there are suggestions from Ireland that fish of Norwegian origin are more susceptible to local variants of diseases. Until properly-organised strain selection programmes are undertaken in for example, Scotland and Ireland, the merits of the local strains will not be known. Furthermore, if local fish were used for farming, then the potential impact of farmed escapes on wild populations would be reduced.

Recently the salmon farming industries in Scotland and Ireland have begun to experiment with sterile (all-female triploid) stocks. This development is designed to totally eliminate "grilsing", which becomes a bigger problem as one moves south in Europe. Unfortunately, because of commercial secrecy, very little is being published about this development, but it is one which should be welcomed by salmon conservation organisations, as will be discussed below.

Ranching: Iceland is the only country around the Atlantic where ranching is economically feasible at present. This is because of the virtual absence of legal or illegal coastal netting in that country. There seems to have been very little genetic manipulation of the salmon used, so far, in ranching in Iceland, (except for the possibility of inadvertent inbreeding in their production). There has, however, been a proposal by Tygre Gjedrem for a programme of artificial selection for ranched salmon in Iceland. Furthermore, there are reports from Norway that there will be a surplus of reared smolts for farming in 1989. This may encourage an expansion of

ranching in that country, using fish which are possibly inbred and may have been subjected to several generations of artificial selection.

Sterile fish are unlikely to be used in ranching Atlantic salmon, since such fish, because of lack of circulating sex hormones, would not return to their point of freshwater release.

Possible Genetic Consequences of Escapes from Sea Farms or Ranching Programmes.

An often-cited scenario, concerning the escape of reared salmon is that escapes from sea farms or wanderers from ranching programmes run rivers at random and spawn with native salmon. The offspring which result are less fit and so the population declines. Furthermore, reared fish of the same genetic composition may enter many rivers and thus isolation between populations, which over the centuries have become adapted to their particular riverine environment, is broken down.

Does this happen? We know (i) that escapes occur at least in Norway, Scotland and Ireland and that fears are being expressed from Iceland about wandering of ranched fish ; (ii) that some of these fish run rivers as spawning time approaches and (iii) that spawned-out farmed escapes have been observed. We do not know exactly (a) how many salmon escape from farms or wander from ranching facilities, (b) how many survive to run rivers or (c) how many different rivers one group of reared fish enter. In the case of farmed escapes, a field experiment by Lars Hansen and his colleagues in Norway, has shown that post smolts and maturing fish are recaptured in coastal nets in highest numbers, with most of the intermediate stages giving much lower recaptures. Also we do not know whether the salmon which run rivers spawn with native fish or amongst

themselves, nor do we know anything about the fitness of their offspring. It seems unlikely however, that reared salmon would spawn only amongst themselves, especially if much larger numbers of wild fish were present. The genetic consequences of interbreeding with native salmon are likely to be greater when the escapes or wanderers are of foreign origin and/or have been subjected to many generations of artificial selection, since they may differ greatly in genetic composition from the native fish.

However, even salmon which originated in a nearby river can be different from their wild ancestors in terms of genetic composition, and level of variation, if too few parents were used to found the strain. The trouble is that we do not know the genetic make-up of many reared strains - particularly those of Norwegian origin - which are widely used by farmers in Scotland and Ireland.

Electrophoretic studies, which give good measures of genetic composition and extent of variation, could be carried out rapidly and should be undertaken immediately. Arising from such studies it should then be possible to investigate the effects of interbreeding between reared and wild salmon. Investigations of this type could also include the potentially more powerful techniques of DNA analysis.

The adoption of certain steps by the farming and ranching industries should lessen the potential genetic impact.

Farming:

Short Term Measures: 1. Adopt a quality standard for cages to reduce numbers of escapes.

2. Initiate a reporting system, whereby all escapes are communicated *quickly* to the relevant government departments.

3. Initiate a system of marking farmed fish.

4. Assist in the organisation and funding of the genetic work outlined above.

Long Term Measures: 1. Investigate the use of native strains for farming (taking care to avoid inbreeding in their production).

2. Investigate the use of all-female triploids. Such sterile fish would not constitute a genetic threat if they escape.

Ranching: Use local stocks for ranching; *not* foreign strains produced by the farming industry. If or when reared smolt surpluses occur in the various countries producing salmon for farming, there will be increased pressure to ranch foreign strains. This pressure should be resisted until a lot more experimental work is undertaken, especially studies which quantify the extent of wandering.

Managers can assist by not using farmed escapes as broodstock in restocking programmes. (These can be identified fairly easily by extensive fin erosion - see Further Reading). Where upstream traps or fish fences exist on rivers, access for escapes should be prohibited. Such measures will reduce the possible impact, whilst allowing genetic work to proceed.

Summary of Ways in which Genetics can help Atlantic Salmon Management.

1. The number of wild salmon populations on which electrophoretic surveys and possibly DNA analysis are undertaken should be extended, particularly in areas where intensive farming or ranching is taking place or restocking is envisaged.

2. The possibility of using electrophoretic mixed fishery analysis with Atlantic salmon should be borne in mind in certain cases, especially as further populations and enzyme loci are assayed.

3. In the future production of reared strains for enhancement or commercial use, care must be taken with parental number to avoid inbreeding.

4. Extant reared strains used for enhancement, should be compared with their wild founder populations, using enzyme electrophoresis or mtDNA analysis, to detect any loss of genetic variability or alteration of genetic composition (indications of inbreeding). Reared strains used for farming or ranching, especially those which have been subjected to selective breeding, should also be investigated in this way. Any loss of genetic variability may have implications for future performance of such strains. Knowing the genetic profile of reared strains is also a prerequisite to investigating the genetic consequence of reared fish escaping from farms, or wandering from ranching operations.

5. When enhancing a river system where salmon still occur, certain alternatives should be considered before deciding on restocking. (This assumes that a population survey has shown a substantial decline in numbers). Habitat improvement or kelt reconditioning should first be considered as biologically less disruptive and possibly cheaper methods of enhancement. It should be noted that where interceptory fisheries take a large proportion of returning salmon, restocking or any other enhancement strategy for increasing the adult run to a river, will always be inefficient. If restocking is chosen then *native fish should be used as broodstock*, even if reared elsewhere. The strain reared for restocking should be assayed genetically and *all restocked fish should be marked*, so that progress can be monitored. If the remaining native population is very small then a suitable donor strain must be chosen. This process is outlined below.

6. Restocking is essential for enhancing a river where salmon are extinct or severely depleted. In choosing donor strains, genetic identity is a useful criterion, but should be used in conjunction with geographical, geological,

chemical, behavioural and ecological considerations. It is not recommended that surplus parr from farming operations, particularly those of foreign origin, be used for restocking. As was stated above, genetic monitoring is again essential. Experimentation with more than one donor strain is possible, where efficient upstream traps exist.

7. It is recommended that selective breeding programmes using native stocks be initiated in Scotland, Ireland and other Atlantic salmon farming countries (with the exceptions of Norway and Canada where such programmes already exist).

8. Salmon escaping from farms or ranching programmes *may* be genetically damaging to wild populations. The extent of such effects must be established. Meanwhile every effort should be made to quantify the number of escaping fish and how many of these survive to spawn. The salmon farming industry might help to reduce the numbers of escapes by adopting quality standards for cages. In the longer term, the use of either sterile or native salmon for farming could eliminate or minimise the possible impact. Ranching should not be allowed with strains of foreign origin. It should be confined to native fish until a lot more is known about the genetics of wild and reared Atlantic salmon.

9. Far greater dialogue should be promoted between salmon managers and population geneticists. Until the adaptive significance (if any) of the isolated population structure of the Atlantic salmon is known, this structure must be preserved.

6. ACKNOWLEDGEMENTS

I want in particular, to thank the Atlantic Salmon Trust and the Atlantic Salmon Federation for awarding me the 1987/1988 Bensinger Liddell Memorial Fellowship. I am very grateful to the people in the laboratories and other establishments listed in the Appendix, and many others, who gave freely of their time and whose discussion and ideas greatly contributed to this interpretive report. The hospitality shown by all those visited was tremendous and I am particularly grateful to Howard Bern, Allan Wilson, Conrad Mahnken, Jim Shaklee, Fred Allendorf, John Spence and their wives and also the Atlantic Salmon Federation, for personal accommodation. The visits during the trips were the source of much stimulation and ideas which I hope will lead to useful research in years to come. The aims of this report were to explain modern genetic techniques to salmon managers and conservationists and to attempt to forge closer links between geneticists and those concerned with the welfare of the Atlantic salmon. I found the writing of such a report to be a very interesting exercise and hope that it partially achieves these aims.

I also wish to thank my employers, University College, Cork, for allowing me the time to make these trips and for providing a great deal of secretarial assistance. Finally, I want to thank my wife Mary, who looked after things in my absence, typed and proof read the manuscript and who shared all of the hard work but missed the enjoyable parts.

7. FURTHER READING

Several books should be of great interest to anyone wishing to delve further into salmonid fish genetics. These books also contain many references to original research contributions. Each is listed below with comments.

Ferguson, A. 1980. *Biochemical systematics and evolution.* Blackie, Glasgow and London, 194p. - As well as clear descriptions of methods and background theory, this book contains an excellent chapter on the use of population genetics in fisheries biology.

Gall, G.A.E. and C.A.Busack eds. 1986. *Second International Meeting on Genetics in Aquaculture.* Elsevier Science Publishers B.V., Amsterdam, Oxford and New York, 386p. - This volume, like the proceedings of the First International Meeting listed below, contains original papers on many of the aspects discussed in this report.

Kapuscinski, A.R. and L.D.Jacobson. 1987. *Genetic guidelines for fisheries managers.* Minnesota Sea Grant, Research Report No. 17, 66p.- Covers many of the aspects contained in this report but from a North American viewpoint, and requires somewhat more specialised knowledge.

Ryman, N. and F.M.Utter. 1987. *Population genetics and fisheries management.* University of Washington Press, Seattle and London, 420p.- The present standard work in this area, it contains chapters by Stahl on Atlantic salmon, Gyllensten and Wilson on salmonid mitochondrial DNA and Allendorf and Utter on genetic aspects of artificial culture, amongst many others.

Tave, D. 1988. *Genetics for fish hatchery managers.* Avi Publishing Company, Inc., Westport, Connecticut, 299p. - This book is aimed at the fish rearing sector, and gives a clear description of the numbers of broodstock necessary to maintain genetic variability, with and without artificial selection.

Wilkins, N.P. and E.Gosling, eds. 1983. *First International meeting on Genetics in Aquaculture.* Elsevier Science Publishers B.V., Amsterdam, Oxford and New York, 425p. - Contains comprehensive reviews by Ryman on salmonid population genetics, Gjedrem on quantitative genetics and Purdom on ploidy manipulation.

Some references which relate to particular sections are as follows:-

Section 2

Aebersold, P.B., Winans, G.A., Teel, D.J., Milner, G.B. and F.M.Utter. 1988 *Manual of starch gel electrophoresis: A method for detection of genetic variation.* NOAA Technical Report NMFS 61, U.S. Department of Commerce, 19p.

Allendorf, F.W. and F.M.Utter. 1979. Population genetics. In *Fish Physiol*ogy, Vol.8, eds. W.S.Hoar, D.J.Randall and J.R.Brett, Acdemic Press, New York, pp407-454.

Cross, T.F. and J.A.Healy. 1985. An investigation of the use of electrophoresis to estimate the stock composition of salmon in the oceanic fishery off the Faroes. *ICES, ANACAT Fish Comm.*, C.M./M:29, 10p.

Cross, T.F. and R.D.Ward. 1980. Protein variation and duplicate loci in the Atlantic salmon, *Salmo salar* L. *Genetical Research, 36,* 147-165.

Ferguson, A. and C.C.Fleming. 1983. Evolutionary and taxonomic significance of protein variation in the brown trout (*Salmo trutta* L.) and other salmonid fishes. In *Protein Polymorphism: Adaptive and Taxonomic Significance*, eds. G.S.Oxford, and D.Rollinson, Academic Press, London and New York, pp85-99.

Grant, W.S., Milner, G.B., Krasnowski, P. and F.M.Utter. 1980. Use of biochemical genetic variants for identification of sockeye salmon (*Oncorhynchus nerka*) stocks in Cook Inlet, Alaska. *Can. J. Fish. Aquat. Sci., 37,* 1236-1247.

Ryman, N. and G.Stahl. 1980. Genetic changes in hatchery stocks of brown trout (*Salmo trutta*). *Can. J. Fish. Aquat. Sci., 38,* 1562-1575.

Taggart, J.B. and A.Ferguson. 1986. Electrophoretic evaluation of a supplemental stocking programme for brown trout, *Salmo trutta* L.*Aquaculture and Fisheries Management, 17,* 155-162.

Utter, F.M., Campton, D., Grant, S., Milner, G.B., Seeb, J. and L.Wishard. 1980. Population structures of indigenous salmonid species of the Pacific Northwest. In *Salmonid Ecosystems of the North Pacific*, eds. W.J.McNeil and D.C.Himsworth, Oregon State University Press, Corvallis, pp285-304.

Wilkins, N.P. 1985. *Salmon Stocks: A Genetic Perspective.* Atlantic Salmon Trust. 30p.

Section 3.

Falconer, D.S. 1981. *Introduction to quantitative genetics.* 2nd. ed., Longman, London.

Kinghorn, B.P. 1983. A review of quantitative genetics in fish breeding. *Aquaculture, 31,* 900-902.

Section 4.

Thorgaard, G.H. 1977. Heteromorphic sex chromosomes in male rainbow trout. *Science, 196,* 900-902.

Section 5.

Hansen, L.P., Lund, R.A. and K.Hindar. 1987. Possible interaction between wild and reared Atlantic salmon in Norway. *ICES* C.M: 14, 18p.

Pepper, V.A. and P.Parsons. 1987. An experiment on the aquaculture potential of Atlantic salmon, *Salmo salar* L. kelts in Newfoundland, Canada. *Aquaculture and Fisheries Management, 18,* 327-344.

Soloman, D.J. 1985. *Salmonid enhancement in North America.* Atlantic Salmon Trust, 40p.

8. APPENDIX.

Laboratories visited during the course of the 1987-1988 Bensinger Liddell fellowship

Genetics Laboratories

Address	Area of expertise	Contact person/s
Dept. of Genetics, University of Stockholm, Stockholm, Sweden.	Population genetics (P. G.)	N. Ryman, G.Stahl
Norwegian Salmon Growers Breeding Station, Kyrksaeterora, Norway.	Quantitative Genetics (Q.G.)	K.Gunnes.
Dept of Animal Sciences, Univ. of California, Davies, CA95616, USA	Q.G., P.G./Genetic Stock Identification (G.S.I.)	G.A.E.Gall.
Dept. of Biochemistry, Univ. of California, Berkeley, CA94720, USA	DNA analysis	A.Wilson.
Northwest and Alaska Fisheries Centre Nat. Mar. Fish. Serv., 2725, Montlake Boulevard East, Seattle, WA98112, USA.	PG./G.S.I.	G.A.Winans, C.Mahnken.
Washington State,Dept. of Fisheries, Room 115, General Administration Building, Olympia, WA98504, USA.	P.G./G.S.I.	J.B.Shaklee.
Programme in Genetics and Cell Biology, Washington State University,Pullman, WA99164, USA	Ploidy and sex manipulation (P and S.M) Transgenics	G.Thorgaard.
Dept. of Zoology, Univ. of Montana, Missoula, MN59812-120/USA	P.G.	F.W.Allendorf.
Dept. of Fisheries and Oceans Pacific Biological Station, Nanaimo, B.C. V9R5K6, Canada.	Q.G, P.G./G.S.I.	R.Withler, B.Riddell.
Dept. of Fisheries and Oceans, West Vancouver Lab., Vancouver, B.C. Canada.	P. and S.M.	E.Donaldson.

Faculty of Medicine, Memorial Univ., St. John's, Nfld, Canada, A1C5SF	Human P.G.	R.H.Payne.
Atlantic Salmon Federation, P.O. Box 429, St. Andrews, BOG2XO	Q.G.	G.W.Friars, J.M.Anderson.

Other laboratories and facilities visited

Address	Area of expertise	Contact person/s
Directorate of Nature Management, Fish Research Division, Tungasletta 2, N-7047, Trondheim, Norway.	Salmon ranching and restocking/farmed escapes	L.P.Hansen.
Dept. of Zoology, Univ. of California, Berkeley, CA94720, USA	Endocrinology of smoltification	H.A.Bern.
B.C.Salmon Farmers Assoc., 2459A Bellevue Ave., West Vancouver, B.C., V7VIE, Canada.	Salmon farming (mainly coho and chinook salmon)	W.Pennell.
Hardy Sea Farms, Suite 720, 1140 West Pender St., Vancouver, B.C. V6E4G1, Canada.	do.	J.A.Spence.
Northwestern Atlantic Fisheries Centre, Dept. of Fisheries and Oceans, P.O.Box 5667, St. John's, Nfld., AIC5X1, Canada.	Kelt reconditioning, salmon management and enhancement.	V.Pepper, J.Pippy.
Salmon Demonstration Farm, Lime Kiln Bay, St. George, New Brunswick, Canada.	Atlantic salmon farming	E.Henderson.

9. CAREER SUMMARY.

Tom Cross was born in Cork, Ireland and graduated in Zoology from University College, Cork. He obtained a Ph.D. on the "Biochemical genetics of hybrid fishes" from the National University of Ireland. He then held research positions in the DFO laboratory in St. John's, Newfoundland and in the Dept. of Genetics, University College, Swansea. From 1980 to 1983 he was Assistant Director of the Salmon Research Trust of Ireland and then moved to his present position as Lecturer in Zoology at University College, Cork. His major research interests are in the genetics, biology and culture of the Atlantic salmon.

REPORT ON WORKSHOP ON GENETIC PROTEIN VARIATION IN ATLANTIC SALMON

by Prof. N. P. Wilkins, representing the Atlantic Salmon Trust.

The workshop was held in the Marine Laboratory, Aberdeen, December 14-16, 1988 and was attended by 18 researchers.

The programme comprised of three sections:

- reviews of previous work and compilation of bibliography;
- standardisation of nomenclature for alleles and loci;
- discussion of progress and plans for the future.

REVIEW OF PREVIOUS WORK

This session was opened by papers from Wilkins (Ireland) and Stahl (Sweden), followed by short presentations of work from 1983 to date, given by other participants. The results indicate that protein electrophoresis is useful in visualising and quantifying differences between Atlantic salmon populations, (a) in different continents; (b) in different geographic regions within a continent; (c) between river systems within some regions and (d) within different tributaries of certain catchments. Although the degree of genetic differentiation between populations of Atlantic salmon is considerably less than that between populations of brown trout, it is nevertheless sufficient to justify a population-by-population management approach rather than a mixed-fishery approach to the resource. Salmon populations do show genetic evidence that they are reproductively

separate; consideration of the genetic implications of this was left to the final section.

Work carried out since about 1980 has concentrated less on blood proteins (as the earlier work had done) and more on tissue enzymes. This reflects the easier availability of solid tissue samples than of fresh blood from commercial catches, and the greater range of enzymatic proteins which can be investigated in solid tissues.

Data were compiled on all papers and research reports, published and unpublished, which were prepared since 1970. These will be collated to provide the first complete international bibliography on protein genetic variation in Atlantic salmon.

STANDARDISATION OF NOMENCLATURE

In the long run, this will prove the most valuable and enduring product of the workshop. Since 1980, geneticists working on populations of Atlantic salmon have examined: up to 10 different tissues (e.g. heart, liver, brain, white muscle etc.); up to 30 different types of enzymes; populations from over 10 countries, comprising of up to 100 separate rivers. These facts alone suggest the absolute necessity for a common nomenclature and common standards to apply to all workers in the various countries, if results are to be directly comparable from study to study and from time to time.

For the nomenclature of the various enzymes and the manner in which they are written, the meeting adopted the broad outlines of the scheme proposed by the Fish Genetics Nomenclature Committee of the American Fisheries Society. Thus for example, phosphoglucose isomerase (PGI) will

useful. But otherwise is it valuable? Paradoxically, we still do not know the answer to this question which could help us to predict or even determine the effects of escapees.

In resume, the group was confident that genetic protein variants have a more valuable role than ever both in the basic and applied studies on Atlantic salmon. In almost all countries such studies have confirmed that natural stocks are reproductively separate and genetically different, for whatever unknown reason. Protein studies have also shown that artificial cultivation results in a loss of some variants and alteration in the frequency of others. We need to know the significance of these changes and until we do, caution should be shown in the legitimate and valid use of hatchery material. For stocking purposes, protein electrophoresis can help in ensuring that levels of genetic variability are maintained and in monitoring the effects of some transfers and introductions.

Finally, although there is less work in train than the group felt was needed, it was clear that protein studies were no longer being seen as highly expensive academic studies: they are more and more being applied to specific problems and the outcome looks bright for a fuller description of the genome of the Atlantic salmon and its population structure using this technique.

N.P.Wilkins

Dept. of Zoology,
University College,
Galway, Ireland.

ATLANTIC SALMON TRUST PUBLICATIONS

		£
Atlantic Salmon: Planning for the Future (Proceedings of the 3rd International Atlantic Salmon Symposium, Biarritz, 1986)	edited by D. Mills and D. Piggins	45.00
The Biology of the Sea Trout (Summary of a Symposium held at Plas Menai, 24-26 October, 1984	by E.D. Le Cren	1.50
Salmon Stocks: A Genetic Perspective	by N.P. Wilkins	1.50
Report of a Workshop on Salmon Stock Enhancement	by E.D. Le Cren	1.50
Salmonid Enhancement in North America	by D.J. Solomon	2.00
Salmon in Iceland	by Thor Gudjonsson and Derek Mills	1.00
A Report on a Visit to the Faroes	by Derek Mills and Noel Smart	1.00
Problems and Solutions in the Management of Open Seas Fisheries for Atlantic Salmon	by Derek Mills	1.00
Scotlands King of Fish (out of print)	by Derek Mills	1.85
Atlantic Salmon Facts	by Derek Mills and Gerald Hadoke	0.50
The Atlantic Salmon in Spain	by C.D. de Leaniz, Tony Hawkins, David Hay and J.J. Martinez	1.50
Salmon in Norway	by L. Hansen and G. Bielby	1.50
Water Quality for Salmon and Trout	by John Solbe	2.50
The Automatic Counter - A Tool for the Management of Salmon Fisheries (Report of a Workshop held at Montrose, 15-16 September, 1987)	by A. Holden	1.50
A Review of Irish Salmon and Salmon Fisheries	by K. Vickers	1.50
Water Schemes - Safeguarding of Fisheries (Report of Lancsaster Workshop)	by J. Gregory	2.50
Genetics and the Management of the Atlantic Salmon	by T. Cross.	2.50