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REPORT OF THE WORKING GROUP ON GENETICS, 1983

Lowestoft, England, 27.-29. April, 1983

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REPORT OF THE WORKING GROUP ON GENETICS

1. PARTICIPANTS

The ICES Working Group on Genetics met in Lowestoft, England, April 27-29, 1983. The following members appointed by the national delegates, were present.

H. Grizel, France
R. Gydemo, Sweden (substitute for L. Nyman)
M. L. Koljonen, Finland
A. Longwell, USA
G. Nævdal, Norway (chairman)
C. Purdom, U.K.
R.L. Saunders, Canada
W. Villwoch, Federal Republic of Germany
N.P. Wilkins, Ireland

For the workshop on chromosome engineering (see below) the following specialists from non-member countries invited through the General Secretary, were present;

Kazuo Fujino, Kitasato University, School of Fisheries Sciences, Iwate, Japan
G.W. Wohlfarth, Fish and Aqua-culture Research Station, Dor, D.N. Hof-Hacarmel, Israel

For the workshop and/or the discussion on genetics of natural populations the following specialists from ICES member countries were present;

P.O. Brandal, Norway
P. Browley, U.K.
V. Bye, U.K.
A.R. Child, U.K.
B. Glebe, Canada
B. Howell, U.K.

A. Jamieson, U.K.

R. Lincoln, U.K.

S. Scott, U.K.

G. Thorgaard, USA

2. TERMS OF REFERENCE AND ITEMS FOR THE MEETING

At the 1982 Statutory meeting it was decided (C.Res. 1982/2:14) that:

> the Working Group on Genetics should meet for three days late in April 1983, in Lowestoft, England, with Dr. G. Nævdal as Chairman to: update their 1981 account of aquaculture genetics in ICES member countries; prepare the first account of genetic studies on natural populations of fish and shellfish in ICES member countries; conduct a one-day workshop on applications of chromosome engineering in aquaculture, inviting through the General Secretary specialists from non-member countries at national expence; consider how aqauculture practices might affect the genetic structure of natural populations; discuss and evaluate new approaches in aquaculture genetics.

Based upon the resolution, the following Agenda was put up:

April 27: 900 a.m. Welcome and introduction of participants.
Introductory remarks by chairman.
Updating of the 1981 account of aquaculture genetics in the ICES member countries.

230 p.m. Workshop on chromosome engineering.
Introductions by invited specialists with questions and preliminary discussion.

April 28: 900 a.m. Workshop continues.
Discussion of applications of sex reversal, gynogenesis (and androgenesis), polyploidy and new methods.
Statements and conclusions

230 f.m. Preparation of an account of genetics of natural populations within ICES member countries.

Consideration of effect of aqauculture practice on natural populations.

New approaches in aquaculture genetics.

April 29: 900 a.m. Discussion on the future of the Working Group: items for next meeting, place and time for next meeting, terms of reference. Formation of subgroups for drafting of report.

200 p.m. Discussion of draft reports. Discussion of conclusions, statements and recommendations

3. GENETICS RELATED TO MARICULTURE IN THE ICES MEMBER COUNTRIES

As a first approximation the Working Group in 1981 compiled an account of the activities related to mariculture in the ICES member countries. These activities include common quantitative genetics on aquaculture species applying techniques as selection and hybridization and also more basic research and technique of potential importance to mariculture genetics. The account was updated this year.

Sea ranching and other types of semiculture are included in the concept aquaculture. Freshwater fish are included in so far as they provide models for marine species.

In the case of those countries represented at the Working Group meeting verbal or prepared reports were presented summarizing national activities in aquaculture genetics and on genetics of wild resource populations. For countries not represented the chairman asked the delegates about such activities in their respective countries. At the time this report was prepared, replies had been made by all countries except two (Poland and the Democratic Republic of Germany).

The activities reported are listed in Appendix I.

4. GENETIC STUDIES ON NATURAL POPULATIONS OF FISH AND SHELLFISH IN ICES MEMBER COUNTRIES

At the 1982 meeting in Galway, Ireland, the Working Group found that there were sufficient reasons to include genetics of natural stocks into its general terms of reference. The following main reasons were listed:

a. Genetics of cultured and natural stocks are closely related because natural genetic variation is the basis for genetic improvement of stocks for aquaculture purpose. Further natural stocks can be influenced by aquaculture practice through development of culture based fisheries, enhancement of natural stocks and involuntary escapement of cultured organisms.
b. Genetic variation within and between natural populations is of much significance for management of fisheries. Interpretation of the results of studies on biochemical genetics are often difficult and general evaluation of such methods are needed.
c. Growing interest in the significance and conservation of genetic resources.

At the meeting in Lowestoft a first account concerning activities concerned with genetics of natural populations was compiled. Similar to the account on aquaculture genetics, the participants gave verbal communications or working papers on the activities in their countries. For the countries not represented, information was collected by the chairman through the national delegates.

The close relation between genetics of natural populations and aquaculture genetics was clearly illustrated by the fact that in several cases it was very difficult to distinguish between the two in the national reports as they were submitted.

The Working Group discussed standarization of methods and designation of phenotypes. It was generally agreed that the best way of standarizing and distribution of phenotypes, is direct exchange of samples for comparisons of known phenotypes by the different (although similar) methods used by different laboratories. A recommendation on that point was formulated (recommendation no. 8).

The group also discussed to what degree it should be concerned with genetics of natural populations. Some participants stayed with the view that the Working Group on Genetics should be concerned with genetics of natural populations only in so far as this is of actual or potential significance for aquaculture. Others were of the opinion that the Working Group should be concerned with all aspects of genetic resources in fish and shellfish. No decision was made, and thus the Council will have to decide to what degree the Working Group should be concerned with genetics of natural populations.

The activities reported are listed in Appendix II.

5. CHROMOSOME ENGINEERING WORKSHOP

5.1 INTRODUCTION

Presentations, all concerned with finfish, were made by:

Purdom: A brief review of the field
Bye: Sex-ratio control in the UK
Glebe: Sex-ratio control in salmon
Lincoln: Induced triploidy
Thorgaard: Induced triploidy, tetraploidy and diploid gynogenesis and androgenesis
Scott: The use of u-v irradiation for sperm inactivation
Fujino: Review of chromosome engineering in Japan
Brandal: Sex-ratio control and the grilse problem

A general discussion ensued on the practical applications of chromosome engineering and other genetic approaches such as hybrid DNA production and induced mutagenesis. The latter techniques should be reviewed from time to time but their near immediate application in fish was not deemed likely.

Several potentially valuable techniques have arisen from the studies of chromosome engineering. Some are already being tested in commercial practice, but other remain at the laboratory level of development. Problem areas were identified in all aspects of the subject area and are summarized below.

5.2. PRESENT STATUS

Three broad areas of activity can be identified and their present level of applicability is in the order:

a) Sex ratio control
b) Polyploid production
c) Gynogenetic reproduction
d) Androgenetic reproduction

5.3. SEX-RATIO

a) This entails the genetic control of sex differentation and is derived from the use of hormones for primary production of sex-reversed fish.

b) Significant use is now made of this approach in the UK and is developing in several other countries. Its principal advantage is the avoidance of male secondary sexual characteristics but it also is relevant in the avoidance of grilse production in salmon farming.

c) The main problem area lies in the production of sex-reversed males for the continuation of broodstock. Present techniques produce ductless gonads and there is a need to develop methods to avoid this so as to simplify the management of female-only techniques in commercial practice.
A further problem relating to males was the need to ensure that sufficient numbers were employed during spawning to avoid inbreeding.

d) Further development should include the search for situations in which males develop naturally at low frequency so as to avoid altogether the need to produce males by artificial sex-reversal.

5.4. POLYPLOID PRODUCTIONS

This is the creation of zygotes with more than the diploid numbers of chromosomes and embraces the use of hybrid situations (allopolyploidy) as well as straight crosses (autopolyploidy).

i) Triploids

a) The advantage of triploids resides in the sterility and almost complete suppression of gonad formation in females. Males do develope large gonads and secondary sexual characters, and so have less potential than females in aquaculture. In some cases, triploidy may be an advantage by conferring greater viability on otherwise poor hybrids.

b) Safe use is being made of triploids commercially in the Uk and in North America (allotriploids).

c) The principal problems in triploid productions lie in the lower survival of eggs and possibly in the slower growth rates of juvenile fish. Further research is needed into these problems areas and into other aspects of the suitability of triploids so as to create the possibility of assessing jointly the benefits and disadvantages of the procedures.

d) Future developments should include a search for oogenic transformation which might confer fertility in triploids.

ii) Tetraploidy

a) The main potential of tetraploids in breeding lies in their use as a mean for simple triploid production by crossing with diploids.

b) No commercial use of tetraploids at present.

c) The main problems seems to be the very poor survival of eggs after treatment at first mitosis and the low viability of hatched tetraploid larvae. Continued search should be made for improved egg treatment techniques, and for better hatchability.

d) Further development of the use of tetraploids must await the improvements in techniques referred to in ii)c).

iii) Aneuploidy

a) The presence of additional chromosomes, less than the complete set is usually deleterious in diploid organisms but may offer some chance of introducting hybrid material into fish genomes.

b) No commercial use is presently being made of aneuploidy in fish.

c) The problems is lack of basic research, but the use of triploid males of hybrid origin may provide a route for an experimental approach.

5.5 GYNOGENESIS AND ANDROGENESIS

There is as yet no commercial production of fish using either gynogenetic or androgenetic techniques.

i) Haploids. These are almost always inviable, and have little commercial potential, but have considerable importance for basic research.

ii) Diploids. Techniques are now available to produce these in large quantities by suppression of the completion of meiosis after gynogenetic stimulation. The gynogenetic offspring produced this way are partially inbred and, where conventional sex determination systems apply, can be used to initiate all-female production cycles. Diploid production following suppression of first mitosis either in gynogenetic or androgenetic development can be answer to the problems of producing inbred lines.
The problems encountered in tetraploid production also apply here, however, and continued research to improve viability of egg treatments and larval survival is urgently needed. The additional problem of homozygote inviability should be pursued by patient examination of strain or individual fish performance.

iii) Aneuploids. Gynogenesis and androgenesis are additional ways to produce aneuploids but differ from the use of triploid males by permitting inclusion of fragments of chromosomes into other sets. The problems remain those of lack of basic research.

5.6. CONCLUSIONS

Some aspects of chromosome engineering are rapidly developing in commercial use. The main problem areas are:

1) The production of intact sperm ducts in sex-reversed male fish.
2) The quantification of the disadvantages and advantages of the use of triploids.
3) The improvement of viability in eggs and larvae from physical shocks applied at the stage of first mitosis.
4) The lack of basic research into other areas of chromosome engineering.

All of these problems are being studied at a number of research centers throughout the world.

Although designed initially for use in fish farming, some aspects of the techniques as described may also prove commercially useful in other aquaculture groups, in particular shellfish. Because shellfish eggs are spawned at an earlier stage of meiosis than those of vertebrate fish, even a wider range of chromosome manipulations are theoretically possible in this groups. Effort to stimulate successful gynogenetic development in oysters and abalone are known to be under way.

6. CONSIDERATIONS OF EFFECT OF AQUACULTURE PRACTICES ON NATURAL POPULATIONS

An important concern in aquaculture practices is to maintain genetic diversity of natural populations, because the latter are the resources of further natural evolution as well as further progress in aquaculture investigations. Overexploitation by commercial and sportfishermen, indiscriminant transfer of animal populations (together with their parasites and pathogens), and destruction of habitats through industrial development have already reduced or even eliminated many aquatic populations and have threatened others. The concern of the ICES Working Group on Genetics is that natural genetic resources, from which aquaculture will continue to draw material have to be protected from further damage by man's impact. This intention includes efforts to minimize the possible consequences through aquaculture practices themselves, i.e.:

a) Effects of intensive fish farming.

- Introgression of genes due to non-voluntary escape of non-indegenous species or populations.
- Erosion of genes or loss of fitness due to escape of fish improved for fishfarming or with reduced genetic variation due to genetic drift.
- Replacement of natural populations by farmed exotic species due to direct competition or changes in the ecological balance.

- Transfer of pathogens from farmed to wild populations: The farmed fish may be resistant or the effect of the pathogens may be minimized by antibiotic treatment or vaccination, while the effect on the wild populations, however, may be lethal.

b) Semiculture.

- Breakdown of natural population structure as a consequence of interaction between naturally reproducing fish and hatchery reared fish.
- Erosion of genes or genotypes due to increased competition.
- Loss of fitness due to reduced genetic variability caused by genetic drift (introgression of inbred lines).
- Transfer of diseases from resistant (cultured) to non-resistant (natural) species or populations.

c) Shellfish Culture.

- Increased competition between native and introduced species or populations, with potential erosion, reduction or even replacement of the natural ones.
- Transfer of parasites and pathogens to non-resistant natural species or populations.

The potential danger that may derive from the above briefly mentioned implications of aquaculture leads to recommendations 4-7, page 16.

The following publications were of significant importance for working out this chapter:

FAO/UNEP, 1981: Conservation of the genetic resources of fish: Problems and recommendations. Report of the Expert Consultation on the gentic resources of fish, Rome, 9.-13. June 1980. FAO Fish. Tech. Pap., 217: 43 pp.

RYMAN, N. (ed), 1981: Fish Gene Pools. Ecol. Bull. (Stockholm) 34: 107-108.

7. BIBLIOGRAPHY

A reference list of papers dealing with the genetics of wild resource populations published from 1978 on was compiled (mainly by the USA representative with Genetics Abstracts as

the main source). Theoretical and methodologic papers are included, and also those dealing with non-aquatic groups when these are concerned with methods or principles of presumptive value for the genetics of species important to aquaculture or the fisheries. These references are broken down into those that use 1) isozyme techniques, 2) those that employ chromosome techniques, and 3) recent ones employing new DNA techniques.

Using the same source from 1978 on, another reference list was prepared on gynogenesis and polyploidy. This also includes works of theoretical and methodologic value.

Attention is further called to a bibliography on genetics and hybridization of salmon using more traditional breeding approaches.

Bibliographies are included as Appendix III; they serve, as well as a reference source, to indicate research trends in fishery and aquaculture research, and outside of it in areas viewed as either of present or future importance to fish breeding or to fishery and resource management.

RECOMMENDATIONS

The ICES Working Group on Genetics recommends, without any priorities, that:

1. Use of sex reversed males (producing spermatoza containing only x-chromosomes) as broodstock is recommended for producing all female populations for commercial salmonid farming.

2. Further research into the application of the technique of gynogenesis and polyploidy in fish farming should be encouraged.

3. Basic research on new techniques for chromosome and genetic engineering of aquaculture species should be encouraged.

4. Influences of aquaculture activities on natural gene pools should be carefully considered and, if possible, monitored.

5. Efforts should be directed to conserve the genetic variability providing the material of natural resources and the future use of the latter for successful aquacultural management.

6. Care should be taken when aquacultured fish (or other animals) become released into natural environments to avoid reduction of genetic variation within and between populations due to adverse interactions within and between different species, or loss of ecological balance. Use of crossbreeds between wild and domestic stocks should be considered in re-stocking programs.

7. Because of the complex and widespread problems involved, basic and applied research groups should be established on them, cooperating with aquaculturists to avoid potential negative effects as a consequence of too little informations concerning the different aspects of interactions pointed out above.

8. Exchange of samples between laboratories and institutions for comparative analysis of biochemical polymorphisms should be encouraged to facilate interpretations of results obtained by different laboratories and for precise recognation of the variability and its distributions.

9. The Working Group on Genetics should meet in St. Andrews, Canada, in September 1984 to:

a. Conduct a workshop on influence of mariculture practices on natural resource populations (i.e. sea ranching of salmon, cod, shellfish).
b. Prepare a list of institutes or laboratories within the ICES member countries which keep fish or shellfish broodstock with details of the species or strains which are maintained.
c. Consider further new approaches and results in aquaculture genetics including genetic engineering.

APPENDIX I

CURRENT AND PLANNED GENETIC STUDIES RELATED TO MARICULTURE IN THE ICES MEMBER COUNTRIES

This document incorporates material solicited from individuals or groups in ICES member countries conducting studies in genetics with particular reference to aquaculture. It updates information solicited for the first meeting of the ICES Genetics Working Group, May 12-14, 1981, in Copenhagen. It is planned that this report be updated occasionally, incorporating any appropriate activities not mentioned this year and new studies planned or started between such updates.

1. BELGIUM, No activities reported

2. CANADA

Canadian activities in the field of aquaculture genetics will be presented in a separate report (F:18) for the Mariculture Committee. In this report, compiled by R.L. Saunders, are summarized research projects on salmonids, oysters and blue mussels applying both traditional genetic methods and chromosome engineering methods.

3. DENMARK

No activities reported.

4. FINLAND

Finnish Game and Fisheries Research Institute
Fisheries Division
P.O. Box 193, SF-00131 Helsinki 13, Finland

1) Rainbow trout breeding research programme
(Olli Sumari, Liisa-Siitonen, Dan Linder, Ulf Lindström and Marja-Liisa Koljonen)

The study is being performed in co-operation with the University of Helsinki, Department of Animal Breeding.

In order to develop rainbow trout production in Finland, centralised selective breeding will be started in a few years time. For this purpose, three preliminary experiments have been arranged in which genetic and phenotypic variation in production traits and disease resistance in rainbow trout stocks and their crosses have been studied. The results of the first experiment will be published in Aquaculture (Linder et al.), the results of the second one have just been analyzed, while the third is still running. The study also includes electrophoretic analysis of the stocks.

2) Sex-reversal research on rainbow trout
(Keijo Nyholm)

At Laukaa Fish Culture Research Station studies have been made on the production of all female rainbow trout groups for commercial fish farming. In experiments trout fry have been fed with feed containing sex hormones. Thus, males have been obtained which have spermatozoa containing only x-chromosomes in their milt. With this milt eggs from normal females have been fertilized to produce all-female groups. The method and its economic value are currently being studied on a commercial scale.

5. FRANCE

A compehensive report was given in the 1981 report of the working group. This year a short verbal report concerning shellfish was given by H. Grizel. No written contribution was given this year.

6. GERMANY, Democratic Republic of ...

No activities reported

7. GERMANY, Federal Republic of ...

A short verbal statement was made by the German representative at the meeting in Lowestoft.

8. ICELAND No activities

9. IRELAND (report by N.P. Wilkins)

QUANTITATIVE GENETICS

Salmo salar

A selection programme was commenced in 1981 with 10 separate Salmo salar families reared at the Parteen Hatchery. In late 1982, after the fish had been transferred to sea-cages, this programme was terminated due to unforeseen circumstances. Individual families were mixed without distinguishing marks in a single, large sea-cage on termination. Parents may be available as broodstock from this group in 1983, in which case they will represent a founding population for the production of further generations or fully domesticated salmon for sea-cage culture. (G. Mahon, unpublished).

In Autumn 1981 separate grilse and spring fish broodstock populations were set up at Cong Hatchery Co. Mayo. The "grilse" and "Spring fish" status of the adults was confirmed by scale analysis. Grilse and spring fish offspring have been reared separately at the hatchery until large enough to be microtagged. Samples have been removed at monthly intervals for morphometric analysis. A large percentage of both groups will smoltify this year. Some will be ongrown in sea-cages to maturity, whereas the majority will be released to sea. The proportions maturing as grilse and spring fish will be monitored in sea cages and in returning/recaptured ranched fish; suitable individuals will be used as brood stock for further generations of cage culture and ranched salmon at this hatchery. (M. Murphy, unpublished).

Samples of the grilse and spring fish families are being reared at a second hatchery. Performance of the various families under the two hatchery regimes (in terms of growth rate in F-W, subsequent growth rate in sea cages and proportions maturing as grilse) is being compared at monthly intervals and will be continued. (M. Murphy).

PHYSIOLOGICAL GENETICS

Studies are continuing on haemoglobin ontogeny in Atlantic salmon. The development of the haemoglobin pattern, which was previously shown to be more rapid in grilse than in non grilse wild fish, has been studied in a group of siblings reared together in fresh water, and then ongrown in sea cages. In these fish the haemoglobin pattern developed more rapidly in those individuals which became sexually mature soonest, confirming the phenotypic association between early sexual maturation and accelerated haemoglobin development. (Wilkins, IN PRESS).

Work has commenced on cloning the salmon globin cDNA with a view to identifying the globin genes and establishing their homology to the β and non-β globins of other vertebrates. Once suitable clones are available these will be used as probes to investigate the switch controlling the expression of the various globins in salmon. (F. Gannon et al. unpublished).

The occurrence of embryonic haemoglobin in yolk sac embryos of Salmo salar has been observed. (M. Murphy & N.P. Wilkins unpublished).

SELECTION STUDIES

A survey was completed on breeding goals deemed important by shellfish farmers. (Mahon 1983). It was not possible to implement a proposed breeding programme in recent years.

PROJECTIONS

Studies are continuing on the expression of the haemoglobin genotype in Atlantic salmon reared in sea-cages. Artificial alteration in the rate of expression using dietary additives (e.g. Thyroid tissue) or parenterally administered substances (e.g. Azacytidine) will be investigated. The genetic engineering of salmon globin DNA will continue for a three year period with funds provided by the National Board for Science & Technology. (Gannon; Murphy; Wilkins).

The references are listed in Appendix III.

10. NETHERLAND

No studies in genetis related to mariculture is carried out at the moment, but genetic studies on carp and other freshwater fishes are conducted by the organization Improvement Inland Fisheries, Nieuwegein.

11. NORWAY

Experiments or quantitative genetics and selective breeding of salmonids are carried out at two institutions in Norway; The Department of Animal Genetics and Breeding, Agricultural University of Norway, Åa, and Institute of Marine Research, Directorate of Fisheries, Bergen. The practical experiments are carried out at research stations and at commercial fish farms. Mainly Atlantic salmon and rainbow trout are concerned.

Breding experiments were started about 1970 to evaluate the potential for selective breeding in salmonids. First the genetic and phenotypic parameters for the traits of greatest economic importance were studied. The results have demonstrated that there excist a large genetic variation in growth rate and age at sexual maturity; a moderate amount of genetic variation in survival, resistance against disease, meat quality characters and digestibility of food; and possibly a low genetic variation in condition factor. Genetic variation in flesh pigmentation is indicated in rainbow trout.

Inbreeding and crossbreeding experiments have shown that some non-additive genetic variation seems to excist. However, it is still not clear what emphasize should be put on crossbreeding in a future selection programme.

An extensive selection programme is carried out on Atlantic salmon and rainbow trout by the Agricultural University at the research stations at Sunndalsøra and Averøya and in cooperation with several private fish farms. The base populations of Atlantic salmon was sampled from 40 different Norwegian strains. Each year about 200 full-sib families are tested from hatching to maturation. Selection is based on individual performance and records from full- and half-sib families. The following characters are taken into consideration;

Growth rate prior to maturation, survival, meat quality and age at maturation. For rainbow trout, the selection programme is similar, and about 150 families are tested each year. The progress made during the first years of selection is very promising.

Experiments on induced polyploidy to obtain steril salmon and rainbow trout are also carried out at Sunndalsøra.

At the Institute of Marine Research, Bergen, similar experiments have been carried out since 1971, although in a smaller scale.

Due to IPN-virus in the material, the experiments had to be discontinued and started again with new material in 1978. In addition to programme for intensive fish farming purpose, a programme for genetic variation in survival and return rate of sea ranched salmon has been planned for several years, but until now only a small material have been tested for technical reasons.

New experiments on measuring genetic variation in food utilization (compared to food consumption) and variation in age at maturation caused by non-linear regression between genetical and environmental factors, are planned. Experiments with utilization of sex reversal of salmon in commercial scale are carried out by a private firm (A/S Mowi).

12. POLAND

No information received

13. PORTUGAL

No activities

14. SPAIN

No activities

15. SWEDEN (report by L. Nyman)

A stated already in the 1981 Account the studies which are relevant to aquaculture practices on fish in this country are more or less completely oriented towards salmonid fish. Two projects are directly involved in aquaculture genetics, and as such listed below, all others have aspects of both breeding pratices/"fish farm selection" and studies on natural populations. The latter type is listed in the first account of genetic studies on natural populations.

Also in the 1981 Account it was mentioned that a Steering Committee on Aquaculture had launched a large-scale program to evaluate current constraints to aquaculture development in Sweden and also give advice how future development within the total scope of aquaculture should be formalized. In 1982 8 reports on the various aspects of aquaculture were published and also a final report summarizing the views of the Steering Committee. The report summarizes the background, analyses and proposals of the different groups which briefly can be divided into four major areas: economy/marketing, administration/organization, research and education. One of the reports deals entirely with aspect of aquaculture genetics, and how population genetics theory may best be integrated with breeding practices and the economy of fish farms. It would carry too far to review all proposals in this account, suffice it to say that the report of aquaculture genetics and fish management procedures will be published in English in 1983. I would like to suggest that this report should be scrutinized by ICES Working Group on Genetics.

A new Fish Research Station for salmonids will start to operate in 1983. Research will involve a strong aquaculture genetics component in relation to the management of natural stocks and responsibility for project coordination lies with the Institute of Freshwater Research, Drottningsholm. Project 2 below will largely take place there, and so will some of the projects listed in the 1983 Account of research on natural populations. In conjunction with the formal opening of the research station the Nordic Council's Group on Gene Bank Cooperation on Fish will hold its annual meeting.

Current Projects in Aquaculture Gentics

Project 1) Calculation of genetic parameters for economically important qualities in fish stocks derived from the Swedish Salmon Research Institute

Project Head: Bengt Larsson, Swedish University og Agricultural Sciences, Department of Animal Breeding and Genetics, S-750 07 Uppsala, Sweden

To be terminated in 1983

Project 2) Studies on the genetic variation in growth rate at different temperatures in rainbow trout

Project 3) Project Head: Susanne Sylven and Bengt Larsson, address same as above

The first phase of the project runs from 1982 to 1984, and it is hoped that financial support for the continuation of the project will be granted even beyond 1984.

The experimental parts of the projects will be located at the Fish Research Station in Kälarne in a cooperation with the National Board of Fisheries, wereas data processing will take place at the Agricultural University.

16. UNITED KINGDOM

A report was compiled by C. Purdom in the 1981 Working Group report. This year a verbal account was given by C. Purdom, and more specifie report on various aspects of chromosome engineering was given at the workshop, se p. 8 in the report.

17. USA

An account of studies on aquaculture genetics and genetics of natural and stocked populations, prepared by A. Longwell, will be presented as a separate report for the Mariculture Committee (F:11).

18. USSR

(Cited from the Administrative Report from Mariculture Committee)

To rise the productivity of fish cultivated in sea cages long-term selection in a number of generations and intraspecific breeding are under way to create new breeds of sturgeons and salmons.

In 1982 special attention was paid to studies of three generations of bester of the second crossing. Selection works were aimed at the reestablishment of genetic balance broken as the result of interaction of parent genoms contrasting by many sighs. Significant variations in the viability and occurrences of abnormalities of embryonic mitosises in the progeny of spawners of the second crossing, the existence of normal individuals from the genetic point of view makes selection studies promising.

APPENDIX II

CURRENT AND PLANNED GENETIC STUDIES RELATED TO MANAGEMENT OF NATURAL POPULATIONS IN ICES MEMBER COUNTRIES

This is a first account on activities concerned with genetics of natural populations in the ICES member countries. The material is collected from individuals or groups within each country and compiled by the national Working Group members, or by the national delegates from countries not represented in the Working group.

1. BELGIUM

No activities reported

2. CANADA

Canadian activities in the field of aquaculture genetics are presented in a separate report (F:18) for the Mariculture Committee. This report, compiled by R.L. Saunders, also contain some information on genetic studies on natural and naturalized fish populations.

3. DENMARK

No activities are reported. (At the 70th Statutory Meeting in Copenhagen 1982 a comprehensive report was presented for the Demersal Fish Committee on genetic variation of cod in Danish waters. (Moth - Poulsen, T. "Genetic variation of cod from the Danish Sound. Inter-relations of stocks from adjacent waters")).

4. FINLAND

Finnish Game and Fisheries Research Institute, Fisheries Division, P.O. Box 193, SF-00131 Helsinki 13, Finland

1) The genetic differentiation and the amount of genetic variation was investigated in Finnish natural and cultivated Atlantic salmon (Salmo salar) populations using electrophoresis of enzymes. Eight enzymes were studies over all populations (representing 20 loci) GOT, IDH, LDH, ME, MDH, PGI, PGM and SDH. The study included six populations, two natural populations, two hatchery stocks and two populations reared from natural roe. The following loci were found to be polymorphic: Got-3, Mdh-3, Me-2, Pgm-1, Idh-2 and Sdh-1. The most homozygotus population was the Saimaa landlocked salmon. The project is being performed in co-operation with Department of Genetics, University of Helsinki.

2) The electrophoretic study of the genetic structure of Arctic char populations in Finland (Marja-Liisa Koljonen)

For the time being two populations have been studies using fifteen enzyme systems. The study is being performed in co-operation with ISACF (an international society for studies on Arctic char species).

University of Joensuu, Department of Biology,
P.O. Box 111, SF-80101 Joensuu 10, Finland

A research group in Joensuu is currently investigating the following:

1) Biochemical characterisation of some rainbow trout strains farmed in Finland.

2) Loss of genetic variation during artificial propagation. A study concerning one brown trout stock has been made.

3) A biochemcial method was developed to identify salmon, trout and their hybrids with live fish.

4) Stock identification of Coregonids. This large project includes the measurement of total genetic variability of some freshwater Coregonids with studies concerning their phylogeny and taxonomic status, genotype environment interactions and inheritance of protein systems. (Further information from Jukka Vuorinen)

Åbo Akademi, Department of Biology,
Porthansgatan 3, SF-20500 Turku 50, Finland

Genetic differentiation in Coregonus lavaretus s. lat. population in Finland (Mikael Himberg)

Tissue, serum and plasma proteins have been studies in different Finnish whitefish forms. Three methods have been tested: agarose and starchgel-electrophoresis and isoelectrical focusing.

21 enzymes and 3 proteins of non-enzyme character have been test-screened by starch gel -electrophoresis.

Five proteins (Est, LDH, ADH, Alb, Tf) have been studies in 510 whitefish specimens, including 8 sea-spawning whitefish populations from the Gulf of Bothnia (Ii, Hailuoto, Kalajoki, Öja, Valsörna, Molpehäller, Lokalahti and Boxö, Saltvik), and 3 river-spawning whitefish populations (Kemi-, Ii and Oulu rivers). Total proteins and esterases (liver) have been studied in 18 fresh water populations comprising different whitefish forms.

Work in progress: Genetic differentation in sea-spawning and lake and river populations of Coregonus lavaretus is being studied by considering further enzyme loci.

University of Kuopio, Department of Zoology
P.O. Box 138, SF-70101 Kuopio 10, Finland

Cytogenetic studies on the chromosomes of salmonid fish (Tuula Palva)

Mainly cultured leucocytes are used to produce high quality metaphases for karyotype studies. To check intra-individual and embryonic variation in karyotype morphology, fibroblast cultures form different tissues are also established. The chromosomes are then subsequently treated using different staining techniques (C-, G-, R-, Q-stainings). This allows the identification of individual chromosome pairs and homologus segments. Meiotic studies have also been started. The studies are carried out on both natural and hatchery populations of Salmo salar, S. gairdneri, S. trutta and Salvelinus fontinalis. Hybrids betwwen salmonid fish are also included in the study.

University of Helsinki, Department of Genetics,
P. Rautatiekatu 13, SF-00100 Helsinki 10, Finland

1) Genetic structure of Macoma baltica (Bivalvia) in the Baltic (Sirkka-Liisa Varvio-Aho and Risto Väinölä

Gene and genotype frequency differences among populations on different geographical scales and among age-classes within populations are examined at three enzyme loci.

2) Geographical pattern of allozyme variation in Baltic Sea populations of Mytilus edulis (Risto Väinölä)

3) Genetic differentation in populations of Mysis relicta and other glacial relict crustaceans (Risto Väinölä)

4) Morphological and electrophoretical variation of Hydrobia ulvae, H. ventrosa and Potamopyrgus jenkisi in the northern Baltic Sea (Irma Saloniemi)

5. FRANCE

No written contribution was given this year, but the 1981 report give some information on genetic studies on natural fish and shellfish populations.

6. GERMANY, Democratic Republic of ...

No information

7. GERMANY, Federal Republic of ...

A short verbal statement was given by Prof. Villwoch at the meeting in Lowestoft.

8. ICELAND (information by Jón Jónsson)

A program on polymorphism in blood proteins of fin and sei whales were started in 1971 and continued in 1981, then including also some tissue enzymes. Collection of samples will continue also in 1983.

The aim of this study is twofold:

a) To search for polymorphism for population and species comparison.
b) To compare tissues regarding isoenzyme patterns.

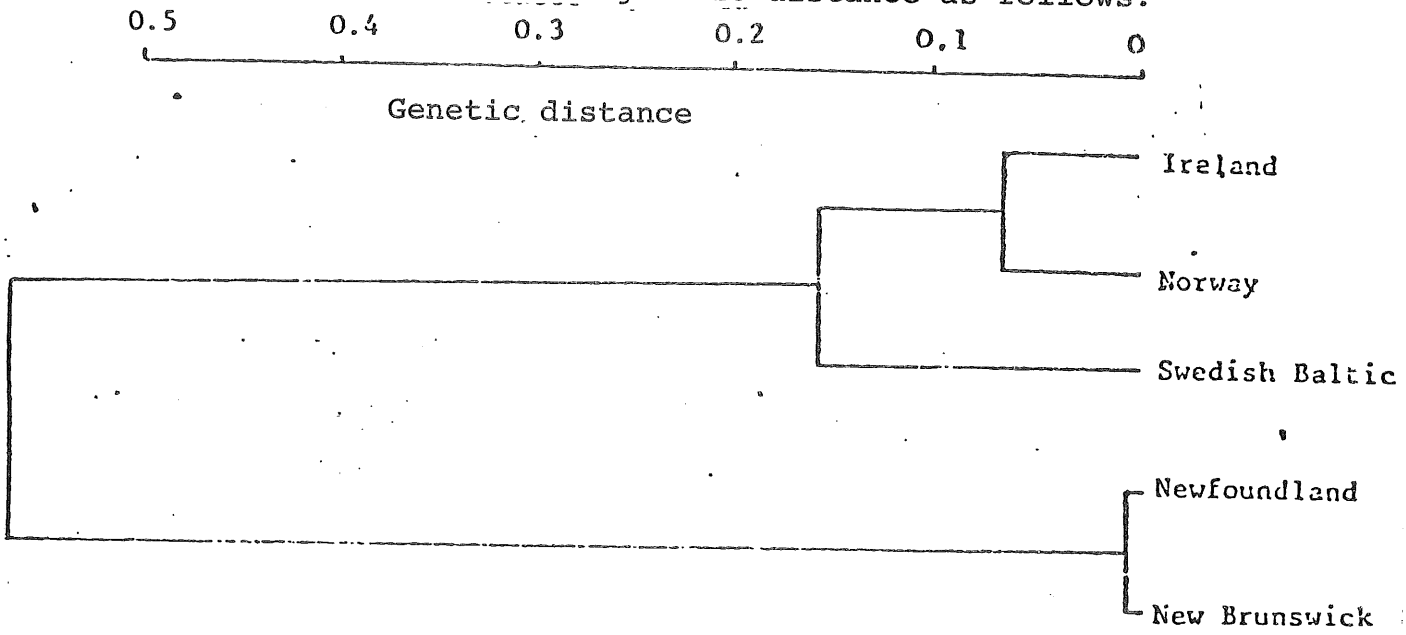
The following polymorphic systems were found: pre-alb 2, CA like esterases and GPT. When population data were provided with ten years intervals (1971 and 1981) no significant differences in gene frequencies were found.

9. IRELAND (report by N.P. Wilkins)

BIOCHEMICAL GENETICS

Salmo salar

Samples of more than 100 salmon parr from rivers in Ireland, Norway and Canada have been analysed electrophoretically at five polymorphic loci (Idh-3, Aat_s-2, Me_m-2, Mdh_s-3 and Sdh-1). The rivers were the Muster Blackwater, Bandon, Carrowniskey, Burrishoole and Moy in Ireland; the Alta in Norway and the N.W. Mirimichi and Salmonier in Canada. The results can be expressed in terms of Nei's genetic distance as follows:-



It can be seen that salmon from rivers in Newfoundland and New Brunswick are very closely related. Riverine populations from southern Ireland and northern Norway are also fairly closely related, whereas a population from the Baltic is only distantly related to either of the eastern Atlantic populations. Finally, it is clear that a major dichotomy excists between salmon from eiter side of the Atlantic. (T. Cross in Salmon Research Trust for Ireland, 1982 and IN PRESS; Cross & Healy 1983).

The genetic effects of hatchery rearing on Atlantic salmon were also investigated at enzyme gene loci. Six polymorphic enzyme loci were examined electrophoretically in a sample of wild Atlantic salmon smolts from the Burrishoole river in western Ireland and in samples of artifically-reared fry hatched in 1981 and parr hatched in 1979. These hatchery reared fish were the progeny of five generations of artifically reared sea ranched salmon which had originally come from the Burrishoole river. Selection for growth and disease resistance was practised and between ten and 30 females and similar numbers of males were used as parents in each generations. Gene frequencies differed significantly at a number of loci between the wild and the artifically reared samples. Erosion of

genetic variability, as measured by mean heterozygosity and mean number of alleles over the six loci, was evident in both hatchery reared samples. It is argued that the observed genetic changes are caused by founder effects and genetic drift rather than selection by some aspects of the artificial rearing regime. The importance of using adequate numbers of parents in hatchery rearing is stressed, since it is shown that differences between wild and reared populations are as great as between natural populations from Irish rivers. (Cross & King 1983).

Brown and Sea trout Salmo trutta

The aim of this study was to determine whether differences excist in allele frequencies at polymorphic loci between the non-migrating brown trout and the anadromous sea trout of a single river system. Samples of both kinds of trout were obtained from the L. Feagh/Burrishoole river system and were analysed at 10 polymorphic and seventeen monomorphic loci. No significant differences in allele frequencies were observed at any of the polymorphic loci, suggesting that the two forms do not represent distinct gene pools within this river system (T. Cross in Salmon Research Trust for Ireland 1982).

Eels

A comparison of allele frequencies and genotype proportions in glass eels, freshwater eels and permanent shore resident eels is in course of completion. Since all European eels are thought to originate from a single panmictic population, any local genetic differences must reflect differential survival of genotypes. Preliminary results indicate little genetic difference between the local populations investigated. Allele frequencies agree well with those reported for other European populations. The results suggest that genotypes at the loci investigated do not contribute significantly to selective survival in differing environments. (A. Nolan unpublished).

Molluscs

European oyster, Ostrea edulis

Literature research into the history of oyster transplantations among Irish oyster beds has revealed extensive artificial mixing of native stocks. Throughout the last 100 years, native oyster beds, both private and public have seen restocked with large numbers of oysters transplanted from Irish and foreign populations, so that a true native Irish oyster can no longer be said to excist. Because local oyster stocks in Ireland are distinguishable from one another on phenotypic characteristics, starch gel electrophoresis was used to test for genotypic differences between them. Allozyme patterns at 4 polymorphic and 5 monomorphic loci indicated that some genetic differences do excist, but these were small compared with differences

previously reported between Irish and Norwegian stock. They were certainly not large enough to warrant the claim that the populations represent real genetic strains or races. The results were regarded as having relevance to policies concerned with restocking and the conservation of locally adapted stocks. (Magennis et al 1983).

Japanese oyster, Crassostrea gigas

The most resently completed genetic studies on Crassostrea gigas report very little reduction in genetic variability after a number of generations of hatchery production of this species.

Genetic variability was analysed at 19 enzyme loci in a hatchery produced population available in Ireland. These oysters were the descendants of approximately 150 adult C. gigas imported from British Columbia into the Shellfish Culture Unit, Conwy (N. Wales) between 1965 and 1972; they form the basis of the breeding populations used by commercial hatcheries in Britain to-day. Levels of genetic variability calculated as polymorphism, mean heterozygosity and mean effective number of alleles per locus were 0.526, 0.176 and 1.41 respectively and compare very favourably with values observed by other workers for wild populations of this species from Japan and N. America.

In general, loci that were observed as being monomorphic in natural populations of C.gigas were also found to be monomorphic in the hatchery oysters. Loci which were weakly polymorphic in natural populations were observed to be monomorphic in hatchery oysters and loci at which three or more alleles were segregating in wild populations were as genetically variable in the hatchery stock. However, the Pgi locus - observed to be highly variable in wild populations of C.gigas - was completely monomorphic in the hatchery stock. Selective advantage of the single Pgi allele under hatchery conditions was thought to be a possible reason for this. Significant deficiencies of heterozygotes were observed at three loci (Pgm-1, Pgm-2 and Acph-3). It was considered that such deficits are probably due to selective forces acting, not on these loci themselves, but at associated loci on the same chromosome. (Gosling 1982).

Blue mussels, Mytilus edulis

Biochemical genetic studies are continuing on raft cultured Mytilus edulis at Killary Harbour, West of Ireland.

In common with many other species, mussels exhibit small deficits of heterozygotes at a number of enzyme gene loci. The cause and significance of these dificits is of interest, especially in view of reports that heterozygosity may be positively correlated with economic traits in some shellfish. The Wahlund Effect, arising from the mixing of individuals of genetically differing demes is one possible explanation for the observed deficiencies. The presence of two or more

distinct populations has been ruled out in Killary Harbour. By sampling single year classes it has further been established that the heterozygote deficits do not reflect the mixing of differing year classes in samples analysed. Studies still underway indicate that small deficits are evident even within a cohort settling within discrete periods (1-2 weeks). Genetic differences among differing settling cohorts are currently being investigated; preliminary results indicate that cohorts settling early and late within a single settling season exhibit differing allele frequencies at the loci investigated. (Gosling & N.P. Wilkins unpublished).

Scallops Pecten maximus and Chlamys varia

Genetic variability at enzyme loci in Pecten maximus is currently under investigation with a view to its use in the study of differential viability of genotypes (see below under "projections"). (E. Gosling & N.P. Wilkins unpublished).

The occurrence of heat-sensitive electromorphs at the Pgi and Pgm loci in P. maximus and C. varia is currently being documented for use in studying geographic variation in heat sensitivity (see below under "projections"). (E. Moynihan & N.P. Wilkins unpublished).

CHROMOSOMAL GENETICS

Blue mussel Mytilus edulis

Chromosomal preparations were made with cell suspensions from gill tissue of spat. A diploid number of 28 confirmed previous reports. Karyotypes were prepared from 4 cells from each of three individuals. Each cell contained 6 metacentric chromosome pairs and a variable number of submetacentric and subtelocentric pairs. The presence of subtelocentric pairs was related to the overall degree of contraction of chromosomes in the cell. (Moynihan & Mahon 1983).

Other species

The diploid number of 20 was confirmed in 33 cell counts from 7 individuals of the European oyster, Ostrea edulis. The karyotype comprised 5 metacentric and 5 submetacentric pairs. (E. Moynihan, unpublished).

In the Pacific oyster, Cressostrea gigas, the diploid number of 20 was confirmed in 26 cell counts from 4 individuals. The karyotype consisted entirely of metacentric chromosomes. (E. Moynihan, published).

The diploid number of 38 was confirmed in 21 cell counts from 7 scallop, P. maximus, individuals. The chromosomes were mainly telocentric and subtelocentric. (E. Moynihan, unpublished).

PROJECTIONS

Salmonids

Investigations will commence in Summer 1983 on the expression of the haemoglobin genotype in the European eel A. anguilla and its modification by artificial means. (Wilkins).

Biochemical genetic investigations of Atlantic salmon populations from Ireland, Iceland, Scotland, and from the high seas fishery at Faroe Islands will be continued. (Cross). Analysis of allele frequencies in sea trout and brown trout are continuing (Cross).

Shellfish

Studies on the allele frequencies of early and late settling cohorts of Mytilus edulis will continue. Where possible, differing cohorts will be ongrown in various areas to determine whether post-larval survival is the same for all genotypes in the wild. (Gosling & Wilkins).

Studies on commercially important species will continue under the aegis of the proposed C.O.S.T. - 41 action (Selected research in Aquaculture) as follows:-

(i) Coordination of research on biochemical genetic variation in natural populations

Biochemical genetic variation in commercially important crustacean and molluscan species in currently being investigated independently at six centres, at least, in five European countries, e.g. Galway, Ireland, and Brest, France. In many instances the same species are under investigations at the different centres.

Standardisation of methodologies and gene nomenclature will greatly facilitate the comparison of gene frequencies in geographically separate populations of widespread species like oysters and mussels or in cohorts of hatchery produced stocks raised in different localities. Such standardisation and comparison testing is routine in all biochemical genetic studies on domestic livestock and cultivated plants. Coordinated action in this area may result in a better understanding of the distribution of biochemical genetic variants in different populations, their role in ecological adaptation and their possible value for artificial rearing.

(ii) Concerted research on the gentics of temperature sensitive enzymes in molluscs and crustaceans

In all species which have been adequately examined heat-sensitive and heat-resistant forms of various enzymes have been shown to be genetically inherited and in some cases the frequency of heat sensitive forms varies geographically. In this action is proposed that participating laboratories throughout Europe monitor the occurrence of heat sensitive forms of specific enzymes in named species to determine whether their frequency varies between populations in colder northern waters and warmer, southern waters. Many genera of molluscs have

characteristic northern and southern species in Europe (e.g. Mytilus edulis and M. galloprovincialis, Patella vulgata and P. aspera) or species with temperature-related physiological races (e.g. the oyster Ostrea edulis). It is of interest to determine whether the frequencies of heat-sensitive enzymes differ in these natural populations distributed from the far north to the Mediterranean sea. Since the conditioning process leading to sexual maturation in hatcheries involves elevated temperature regimes, and since the cultivation of aquatic organisms in heated effluents is frequently proposed, the results of this concerted action may have significance for mariculture practice, as well as leading to a better understanding of the distribution of genetic variation in natural populations. The comparison of Mediterranean and Adriatic populations with those from Northern Europe will be particularly interesting.

(iii) Concerted research on gene-environment interaction in commercially important species

The detection of genetic variability in natural and hatchery populations of cultivable species is the first step in genetic management. The significance of the observed genetic variation in the survival, growth, reproduction and general performance of individuals or populations should be experimentally assessed before strategies are devised to improve stocks. Studies on the changes that occur in gene and genotype proportions in cohorts of natural or hatchery produced spat laid out and on-grown in different habitats or localities may indicate the importance to be attached to differing genotypes. For example, spat of the scallop Pecten maximus, derived from a single populations in Ireland, are now being on-grown at a number of sites within and outside Ireland. Since the initial spat for all these sites were derived form a single year-class of a single populations, any genetic differences observed between the introduced growing stocks at different localities will reflect differing genotype-environment interactions. Studies such as these on Pecten maximus can be extended to other commercial species such as Ostrea edulis, Crossostrea gigas, C. angulata and Mytilus edulis, and the results correlated with differing rearing and on-growing procedures.

The references are listed in Appendix III.

10. NETHERLAND No activities reported

11. NORWAY

At Institute of Marine Research, Bergen, population genetics studies on fish were started in the early sixties. Blood groups in cod, Gadus morhua, and blood protein polymorphism in cod, herring, Clupea harengus, and sprat, Sprattus sprattus, were identified and utilized for studies on population structure of these species. Enzyme polymorphism was used in a few cases. This work terminated in 1971 for capasity reasons.

In 1978 similar work was started again, this time with the main emphasize on starch gel electrophoresis of tissue enzymes. Mainly herring and cod samples have been analyzed with the aim of closer investigation of the population structure of these important commercial species in middle and northern Norway. Part of the analyses are based on fertilized eggs, newly hatched larvae and postlarvae. This work probably will continue although the financial support is somewhat uncertain. This year mainly cod samples are analyzed.

At the University of Trondheim electrophoretic studies on tissue enzymes and hemoglobins have been carried out for several years, with the aim of studying the population structure of cod in a single fjord system and the mechanisms of balancing the polymorphic systems. This year a study is started on population structure and phylogenetic relationship between different species of gadoids and platessoides. Also a program on electrophoretic (electrophocusing) identification of fish eggs (mainly gadoids) are running.

At the University of Tromsø preliminar genetic analyses of capelin Mallotus villosus, and halibut, Hippoglossus hippoglossus, have been undertaken.

At the University of Oslo population genetics of invertebrates, mainly krill, have been carried out for some years.

12. POLAND

No information received

13. PORTUGAL

No activities

14. SPAIN

No activities

15. SWEDEN (report by L. Nyman)

Genetic studies on fish populations in Sweden are primairly aimed at salmonid species. Major emphasis is on using electrophoretically detectable protein polymorphisms to study the amount of genetic variation in natural populations. Identification of populations and sibling species is imperative for conservation and management considerations of endangered stocks used in large-scale stocking operations. It is also of great importance to assess the genetic effects of fish farming practices. This problem has a dual aim. First we should consider the short- and longterm effects of using a limited number of parents for stock propagation, secondly the impact of gene frequency perturbations caused by the unnatural selection of a fish farm environment should be assessed. Most of the projects listed are unfortunately of short duration, because of the current policies of research councils. All scientists listed below who are carrying out research on salmonid fishes are members of the Wild Salmonid Watch. Also, project 2 below is an integrated part of ISACF (an international society for studies on Arctic char species).

Project 1) Evaluation of current breeding practices on population and sibling species characteristics of Arctic char

Project Head: Rolf Gydemo and Lennart Nyman, Institute og Freshwater Research, S-170 11 Drottningholm, Sweden

Funding: Institute of Freshwater Research
Duration: commenced in 1982

Project 2) Evaluation of the genetic and ecological characteristics of the Arctic char species complex in the northern hemispere

Project Head: Rolf Gydemo, Johan Hammar and Lennart Nyman, Institute of Freshwater Research, Drottningsholm

Funding: Institute of Freshwater Research
Duration: commenced in 1964

Project 3) Ecological and genetic characteristics of Arctic char populations in the country of Jämtland - with special emphasis on populations in regulated water systems

Project Head: Johan Hammar and Lennart Nyman, Institute of Freshwater Research, Drottningsholm

Funding: Institute of Freshwater Research
Duration: commenced in 1976 (?)

Project 4) Evaluation of techniques suitable for population research on Arctic char, grayling and brown trout

Project Head: Håkan Jansson, Department of Genetics, University of Uppsala, S-750 07 Uppsala, Sweden, and Rolf Gydemo, Institute of Freshwater Research

Funding: no formal support
Duration: commenced in 1983

Project 5) Genetic variation in natural populations (This project contains several sub-projects, some of which are related to fish others to moose and red deer)

Project Head: Nils Ryman and Olof Leimar, University of Stockholm, Institute of Genetics, Division for population genetics, P.O. Box 6801, S-113 Stockholm, Sweden

Funding: National Science Research Council
Duration: grant terminated June 30, 1983

Project 6) Identification and conservation of genetic resources in salmonids

Project Head: Nils Ryman and Gunnar Ståhl, address same as above

Funding: Research Council of the Environment Protection Board
Duration: grant terminated June 30, 1983

Project 7) Population structure of European perch and fourhorn sculpin and population dynamics in a heavy metal pollution gradient

Project Head: Nils Ryman and Ulf Gyllensten, address same as above

Funding: Research Council of the Environment Protection Board
Duration: grant terminated June 30, 1983

16. UNITED KINGDOM

No written contribution is given. Verbal information on studies on biochemical genetics of marine species (mackerel, cod etc.) was given by A. Jamiesen at the meeting in Lowestoft. These studies are carried out at the Fisheries Laboratory Lowestoft.

17. USA

An account of studies on genetics of natural and stocked populations together with aquaculture genetics, will be presented as a separate report to the Mariculture Committee (F:11, by Arlene Longwell).

18. USSR

No information

APPENDIX III BIBLIOGRAPHY

PAPERS RELEVANT TO UNDERSTANDING GENETIC STOCK STRUCTURE AND GENETIC MANAGEMENT IN THE FISHERIES

Largely collected from Genetics Abstracts from 1978 on. Papers on non-aquatic species cited when these have a methodologic or theoretical bearing on aquatic or resource problems. Institutional affiliation and country of authors identified for most citations.

The assistance of J. Choromanski, National Marine Fisheries Service, Milford (Connecticut) Laboratory, in compiling this list is acknowledged.

Isozyme Variation in Populations

Theoretical and procedures

Marine fish

Shellfish

Squid

Eel

Salmon

Trout

Carp

Freshwater fish

Gastropods

Gammarids

Tunicates

Chromosome Variation in Populations

Chromosomes in evolution and populations, and methodology for fish

Banding

Nucleolar organizer regions of chromosomes - methods for displaying, and their variability

Marine mammals

Oysters

Mussels

Salmon

Marine fish

Carps, Tilapia and other freshwater fish

Eels

Gastropods

Artemia

DNA Restriction Endonucleases and Hybridization Analyses as a Means of Discerning Phylogenetic and Population Differences

Theoretical background

Methodology

Applications to fish

Application to human and primate studies

Application to other mammalian studies

Application to birds

Application to plants

ISOZYME VARIATION

Theoretical and Procedures

Genetic variability and rate of gene substitution in a finite population under mutation and fluctuating selection. Takahata. National Institute of Genetics, Japan. Genetics 98, 427-440, June 1981.

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Theoretical and Non-aquatic Groups as Examples

Methods

Plaice

Flounder

Salmon

Trout

Various freshwater fish

Crustacea

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