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C.M. 1991/ F:45
Mariculture
Committee

Report

of the Working Group on Genetics, Helsinki

June 03-06, 1991

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(7) Citations:	
(7.1) KAPUSCINSKI, Anne R. and Eric M. HALLERMANN, 1990: Fisheries, Bull. Am. Fish. Soc., 15 (1): 2-11.	
(7.2) HALLERMANN, Eric M. and KAPUSCINSKI, Anne R., 1990: ibid.: 12-20.	
Both being xerocopied as addendum to WG-Report of 1990 (C.M. 1990 / F 15): Not added again	
(7.3) Report of the Study Group on genetic Risks to Atlantic Salmon Stocks, presented by Alan YOUNGSON, UK / Scotland (see:C.M. 1991 / M: 3) ***(not copied)***	

(1) Introductory Remarks

According to the adopted resolutions of the 77th Statutory Meeting The Hague, Netherlands, 05.-13. October 1989, the ICES-WG on Genetics was meeting this year in Finland, at the "Finnish Game & Fisheries Research Institute, Aquaculture Division", Helsinki, June 03.-06.1991 - instead of "Tvärminne Station" which were overcrowded by osther guests and guest groups. Severe thanks have to be spent to Dra. Marja-Liisa Koljonen with respect to all her efforts concerning the local organization.

Again as in 1989, the number of participating members of the WG on Genetics was low (6; see below: "list of participants") compared with the total number of 23 representatives of member countries. However, there were 6 more written contributions so that about 50% of all member countries returned informations upon their activities in concern (see foot-notes on "list of members", pages 2 and 3).

Within the days of the WG meeting a joint session was held as suggested together with the members of the ICES WG on Introductions and Transfers of Marine Organisms. During the course of this joint meeting 2 members of the ICES WG on Genetics introduced the auditory into problems of "Genetically modified organisms / GMOs: Definitions, Conceps, and Issues" (speaker: Dr. VILLWOCK / Fed.Rep.of Germany), and of "The Release of GMOs in the Marine Environment" (Speaker: Dr. SAUNDERS / Canada). The discussion of these contributions led to the reviewed "Code of Practice" (see enclosures 6.1 and 6.2, respectively, pages 43, 44). The cited background (see 'citations', 7.1 and 7.2) have already been referred and added to the 1990er report of the ICES WG on Genetics, and therefore became not again copied and enclosed to this report.

Finally, I would like to refer to the written "Report of the Study Group on Genetic Risks to the Atlantic Salmon Stocks", which was part of the WG's discussion in Helsinki (see 'Table of Contents',pos. 7.3). The results are included in the "Recommendations" (see pos. 4, page 6). Because this 'risk report' (chairman and member of the WG on Genetics: Alan YOUNGSON / U.K., Scotland) was already adopted and published during the special ICES-Meeting in Copenhagen, March 13.-15. 1991 (C.M. 1991 / M:3) it is not newly copied and enclosed to this report. The text was made available to all members of the WG.

List of Participants in the Helsinki WG-Meeting, June 03.-06.1991:

Representatives of ICES Member Countries:

1. Dr. Krzystof GORYCZKO / Poland
2. Dr. Knut JØRSTAD / Norway
3. Dr. Marja-Liisa KOLJONEN / Finland
4. Prof. Dr. Richard SAUNDERS / Canada
5. Dr. Jean-Marie SÉVIGNY / Canada
6. Prof. Dr. Wolfgang VILLWOCK / Germany (chairman)

Guests:

1. Ulrike SIENKNECHT / Germany (WG Prof. Villwock)
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* personnal, ** written contribution

(2) Reports on Genetics from the Member Countries (mainly on forms, presented in alphabetic order of the member countries) (see: annex 5.1-5.10, p. #ff).

- ***Canada*** (compiled by Richard L. SAUNDERS, Dept. of Fishieries and Oceans, Aquaculture and Invertebrate Fisheries Division. Biological Station St. Andrews: ***annex 1a***, and 2nd by Dr. Jean-Marie SÉVIGNY, Maurice-Lamontagne Institute, Mont-Joli, Quebec: ***annex 1b***).
- ***Federal Republic of Germany*** (given by Prof. Dr. W. VILLWOCK, Zoologisches Institut und Zoologisches Museum, Universität Hamburg: ***annex 2***).
- ***Finland*** (compiled by Dra. Marja-Liisa KOLJONEN, Finnish Game & Fisheries Research Institute, Aquaculture Division, Helsinki: ***annex 3***).
- ***France*** (by Prof. Dr. René GUYOMARD, Laboratoire de génétique des poissons, INRA-CRJ, Jouy-en-Josas: ***annex 4).***
- ***Ireland*** (by Prof. Dr. Noel P. WILKINS, Dept. of Zoology, National University of Ireland, Galway: ***annex 5***).
- ***Norway*** (verbal contribution by Dr. K. JØRSTAD, Institute of Marine Research, Nordnes / Bergen, and written contribution delivered by Prof. Dr. Gunnar NÆVDAL, University of Bergen, The Fishery College of Norway, Dept. of Fisheries Biology, Bergen: ***annex 6***).
- ***Poland*** (given by Dr. Krzystof GORYCZKO, Inland Fisheries Institute, Salmonid Research Laboratory Rutki, Zukowo: ***annex 7***).
- ***Portugal*** (written by Dra. Ana Maria Teia DOS SANTOS, Centro Regional de Investigação Pesqueira, Instituto Nacional de Investigação das Pescas, Aveiro: a***nnex 8***).
- ***Sweden*** (written contribution by Dr. Håkon JANSSON, Salmon Research Institute, Älvkarleby: ***annex 9***).
- ***United Kingdom, Scotland*** (presented by Dr. Alan F. YOUNGSON, The Scottish Office, Agriculture and Fisheries Department, Marine Laboratory: ***annex 10***).

(3) Brief Summary of above listed Reports (see also annexes 5.1-5.10)

The main subjects of different genetic investigations are the atlantic salmon and other representatives of salmonids, as rainbow and brown trout (e.g. Canada, Finland, France, Ireland, Norway, Sweden, UK / Scotland). Other main fin-fishes under investigation are, *Mallotus villosus* and *Reinhardtius hippoglossoides* (Canada), *Sebastes* sp. (Norway), *Merluccius merluccius* (Portugal), and such as different silurids, clupeids (France: without species nomination), tilapias, and - common carp (*T. nilotica, T. aurea, T. galilea, Cyprinus carpio:* Fed.Rep.of Germany, France: without species nomination).

Non-finfishes under investigation are especially shrimps, e.g. *Pandalus borealis* (Canada), *Nephrops norwegicus* (Portugal) and different clams, and sea urchins (Canada).

Beside classical methods, such as crossbreeding and selection, modern techniques became more and more frequent in the course of the last one-two years, e.g. studies on enzyme polymorphism, mt-DNA, "genetic fingerprin-

ting", (Canada, Fed.Rep.of Germany: partim, Finland, France, Norway, Poland, Portugal, Sweden: partim, UK/Scotland), added by investigations on immuneresponse and erythrocyte biochemical structures (e.g. Fed.Rep.of Germany).

Different groups of scientists in the ICES member countries started with the first steps of producing "transgenic fish" by application of 'growth hormon genes, partly linked to antifreeze compounds' (e.g. Canada: by winter flounder [*Pseudopleuronectes americanus*] and wolffish [*Anarrhichas lupus*] to atlantic salmon, Finland: characterization and isolation of genes coding for different enzymes, and of genes, coding for insuline and other ones being involved in disease resistance). In connection with the latter investigations scientists in some member countries started to evaluate probable risks which my arise from escaped GMOs to their wild relatives (mainly Finland, Ireland: verbal information by Dr. Dan Minchin / WG on Introductions, see also: Joint meeting and reviewed "Code of Practice", annex 6.2). Parallel to these evaluations, some member countries started to document possible consequences of interactions between wild species and related escapees from aquacultutral stocks (Canada, see SAUNDERS: annex 1a, Finland, Norway, and UK / Scotland).

Summing-up with respect to C.Res. 1989 / 2:38, pos. a:
Studies on allozyme polymorphismus should be continued and applied for stock discrimintaion (see: Atlantic salmon and other species, e.g. different gadids).

pos. b.1:
DNA fingerprinting should be continued and after having reached practical scope tried to apply to error-free specimen discrimination.

pos. b,2:
Further studies should be encouraged, mainly with concern of cautiously controlled experiments on genetic as well as on environmental interactions between GMOs, their donor species and their life conditions.

pos c:
- The members of the WG on Genetics agree with the "recommendations" formulated in the end of the "Report of the Study Group on Genetic Risks to Atlantic Salmon Stocks". From that
- the members of the WG on Genetics recommend that the "Study Group on Genetic Risks to Atlantic Salmon Stocks" should be re-considered at the 1993 Statutory Meeting of ICES. According to recommendation no. 10 of the cited "Study Group Report", 'in the interim, any specific questions (may) be directed to the North Atlantic Salmon WG, The WG on Genetics or the WG on Introductions'.
- The members of the WG on Genetics ask to look for an international funding of the "Experimental design for determining genetic, ecological and behavioural performance in native and introduced salmon" as specified in appendix I of the "Study Group Report". The WG on Genetics feels that this design and its results might act as an model for any other example similar to the atlantic salmon one.

General Statement:
The members of the WG on Genetics state, that the development of modern tools in genetic research do not replace completely the classic methods of detecting the genetic background of a given species: (Cross-) breeding and selection do still have their advantage or are at least useful.

(4) Recommendations

The Mariculture Committee recommends that the Working Group on Genetics (Chairman: Prof. Dr. W. Villwock) will work by correspondence in 1992 and will meet in Stockholm, Sweden (invited by Prof. Dr. Lennart NYMAN) for three days in 1993 (June 02.-04.). The Mariculture Committee recommends furtheron that the ICES WG on Genetics and the WG on Introductions should have another joint meeting in 1993. The Mariculture Committee accepts that the chairmen of the two WGs should stay in contact to each other, exchanging ideas and activities in the field of conservation genetic resources of wild species as well as of protecting natural environments.

The Mariculture Committee asks the WG on Genetics to continue in

a) reviewing and reporting on progress in research on biochemical markers and related techniques for species discrimination (including distinguishing between wild species and their aquacultured relatives),

b) in further evaluating and following the trends of advanced "gene technology", especially

- genetic fingerprinting, and
- production of GMOs, with concern to basic research progress as well as to applied aspects such as possible risks to donor species and environment.

c) following the development of concepts for species and environment protection in the course of aquaculture consequences.

Hamburg, September 1991

gez. W. Villwock
(Prof. Dr. W.Villwock)
Chairman
ICES-Working Group
on Genetics

International Council for the
Exploration of the Sea

Working Paper:
ICES Working Group
on Genetics

CANADIAN STUDIES ON GENE TECHNOLOGY, BIOCHEMICAL MARKERS AND MEANS OF REDUCING GENETIC INTERACTION BETWEEN CULTURED AND WILD SALMON

Compiled by

Richard L. Saunders

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Abstract

This document incorporates information solicited from individuals or groups in Canada conducting studies in genetics with particular attention to biochemical markers, gene technology including DNA fingerprinting and production of transgenetic specimens and measures to reduce risks of genetic interaction between cultured, geneticaly altered salmonids and wild stocks.

Tillman J. Benfey, Department of Biology, University of New Brunswick, Fredericton, N. B.

Production of all-female triploid Atlantic Salmon

This work is being done in collaboration with the Atlantic Salmon Federation (St. Andrews, New Brunswick). Initial attempts in 1989 to produce all-female triploid Atlantic salmon by heat shock were only marginally successful, with triploid rates ranging from 0-70%. This work was done with a synthetic strain of salmon having a high grilsing rate. Groups with the highest triploid yields have been kept for an evaluation of their growth performance in sea cages. Emphasis has now switched to the Saint John River strain of salmon favoured by the Bay of Fundy aquaculture industry. Hydrostatic pressure shock was used to induce triploidy in eggs of this strain in 1990, and hormone treatments for the production of masculinized genotypic females were initiated in 1991. Results of these latter experiments are not yet available.

Production of triploid brook trout and brown trout

Hydrostatic pressure shock was evaluated for the production of triploids of these two species in 1990. High yields of triploid brown trout were obtained from a range of treatments; results from the brook trout experiments have not yet been evaluated. Both species of trout are stocked in New Brunswick to support sport fisheries; brook trout are native to the Province whereas brown trout are an exotic species.

Stress response in all-female triploid rainbow trout

A series of experiments on cortisol and hematocrit changes during acute stress in all-female diploid and triploid rainbow trout have been completed, and data analysis is in progress. Initial results suggest little difference between triploids and diploids in stress response.

Peter E. Ihssen, Coordinator, Research Services, Ontario Minister of Natural Resources, Fisheries Research Section, Box 5000, Maple, Ontario.

Biochemical Markers

Peter Ihssen and his colleagues have identified loci which will be useful for differentiating among the Atlantic salmon stocks important to Ontario for the rehabilitation of salmon stocks in Lake Ontario. They have also identified a system of loci that are very effective for differentiating Ontario hatchery brook trout from wild brook trout. Using this system, they have examined a number of brook trout populations across Ontario in areas

that have been planted with hatchery fish. In some lakes and rivers native stocks have been completely replaced by hatchery fish. Other waters, notably in Algonquin Park, still contain uncontaminated wild stocks.

DNA Research

The University of Guelph (Roy Danzmann), in collaboration with Ihassen, is examining mtDNA variation in brook trout. The findings to date support the results from the allozyme studies. The University of Guelph (Moira Ferguson) has also initiated a project on mtDNA variation of Atlantic salmon stocks reintroduced into Ontario, in collaboration with the MNR. They are initiating a study on the mtDNA variation of walleye stocks employed in a rehabilitation project in Nipigon Bay, Lake Superior. They hope to find sufficient differentiation among stocks to identify the parental origin and breeding structure of populations resulting from adult transfers from several donor populations. University of Guelph (Moira Ferguson, Roy Danzmann, several graduate students, and post-docs) is also initiating a gene-mapping project on rainbow trout in collaboration with MNR and several other labs. Our contribution to this project will be to provide selected inbred lines (some of them gynogenetic diploids) for quantitative characters such as growth rate, seasonal maturity and temperature tolerance. MNR are also continuing collaboration with Ruth Phillips (University of Wisconsin) on nuclear DNA variation of lake trout stocks. Presently they are studying the inheritance of several nuclear genetic markers (ribosomal DNA) that, it is hoped, will prove useful for the identification of lake trout stocks used for the rehabilitation of the Great Lakes.

Genetic Risks of Introductions

Research on brook trout has shown that hatchery plantings can replace wild stocks. They have found that these newly established, hatchery-origin stocks are maintaining the quantitative genetic characters for which hatchery stocks have been selected, such as early maturity and fast growth. The Fisheries Branch of the MNR, with contributions from many of their research scientists, biologists, and managers has recently completed a major task called "Lake Trout Synthesis" (reports available from OMNR). One of the questions addressed was the impact of planted lake trout on native stocks. David Evans headed up a group looking at this question and they found that continuous planting of hatchery fish into waters inhabited by wild stocks will rapidly replace the wild stock with hatchery stocks. The interactions between the stocks are primarily predation and competition.

Work on triploid tiger trout (brook x brown) in collaboration with L. McKay and I. MacMillan of the University of Guelph has been completed. They have prepared papers on early survival and growth. They have never found a triploid tiger trout (or for that

matter, rainbow or brook trout) to produce viable sex products. However, they have found a few rare tiger trout diploids that produced viable sex products.

Moira M. Ferguson and R. G. Danzmann, Dept. of Zoology, Unviersity of Guelph

The work at the University of Guelph in association with graduate students and workers at other institutions is as follows:

1) Allozyme comparison of wild stocks of brown and rainbow trout with hatchery stocks derived from these sources (M. M. Ferguson, P. E. Ihssen, and J. D. Hynes) has show that the breeding program of the Ontario Ministry of Natural Resources has been successful in producing broodstocks that are genetically representative of natural populations.

2) Search for mtDNA markers in strains of Atlantic salmon that are being used in the Lake Ontario rehabilitation effort (M. M. Ferguson, J. Volpe, R. G. Danzmann, P. E. Ihssen, and M. Jones). The objective of this work (scheduled to begin this fall) is to find MtDNA markers so that the relative success of different strains can be evaluated.

3) Genetic characterization of wild and aquaculture strains of rainbow trout using allozymes, mtDNA, and RAPD markers (randomly amplified polymorphic DNA) (R. G. Danzmann, M. M. Ferguson, J. G. Brown, P. E. Ihssen, L. H. McKay, and I. McMillan). The group is searching for markers of loci controlling economically important traits in commercial and wild strains of rainbow trout. These markers (allozymes, mtDNA, and DNA polymorphisms using arbitrary primers to amplify anonymous regions of genomic DNA through the polymerase chain reaction) are being used to assess the relatedness of commercial stocks so that inbreeding can be counteracted and will be integrated into traditional breeding programs targeted to the private sector.

4) mtDNA variability in brook charr (R. G. Danzmann, P. D. N. Hebert, and P. E. Ihssen). Analysis of restriction fragment length polymorphisms in brook charr mtDNA has revealed distinct clonal differences between the two major hatchery strains and some wild populations in Ontario.

5) Lake sturgeon population structure (M. M. Ferguson, B. Konkle, M. Malott). RFLP analysis and PCR directed sequencing of the control region in lake sturgeon mtDNA are being used to determine genetic population structure. These data will be used to develop a genetic conservation plan, predict the impact of hydroelectric development, and proposed development of aquaculture stocks.

Michel Legault, Ministry of Leisure, Fish and Game, Québec.

Québec biologists have conducted an analysis of allele frequencies at enzymatic protein loci in the Atlantic salmon from the Port Daniel and St. Jean Rivers, Gaspé, Québec. The study involved a genetic analysis of juvenile Atlantic salmon above and below the falls on the Port Daniel to see if there were differences in known variable enzymatic protein loci. Similar comparisons were made between salmon from the Port Daniel and St. Jean Rivers. Diagnostic variation was not detected. Slight differences between samples from the two rivers suggest genetically distinct and different populations.

An attempt was made to restore a population of salmon in the Port Daniel R. using aquaculture-reared salmon and to modify the timing of returning adults. Smolt planting is planned together with possible genetic changes in the wild stock in the river. A counting fence was used to monitor ascending adult salmon.

Roger Doyle, Director, Marine Gene Probe Lab., Dalhousie University, Halifax, Nova Scotia.

The Gene Probe Lab. is involved with development and application of DNA fingerprinting techniques to provide new tools for genetic studies dealing with distribution and migration of salmon and other marine animals. Emphasis is on development of new technology for genetic improvement of broodstock for aquaculture. They are developing a suite of molecular genetic procedures (related to DNA fingerprinting) that could form the basis of a DNA-based pedigree system for salmonids, scallops, *Tilopia* and other species. Farmers will be encouraged to use a cost-effective genetic counciling service aimed at helping them improve brookstock during routine breeding operations.

DNA "pedigree probes" will be developed for highly polymorphic regions of genomic DNA. Multi-locus fingerprint probes will be developed first, then single-locus, micro- and mini-satellite VNTR probes, then allele-specific probes for transcribed genes including transferrins and MHC. A final objective is to obtain allele-specific probes and procedures (PCR, OLA, etc.) for rapid determination of first-order pedigrees. Technical progress during the project will be measured by decreasing pedigree error rates and increasing sample processing rates.

Choy L. Hew, University of Toronto, Toronto, Ontario, Garth L. Fletcher, Memorial University of Newfoundland, St. John's, Nfld., and Peter L. Davies, Queens Unviersity, Kingston, Ontario.

Enhanced salmon growth and resistance to freezing by gene transfer

This group has developed techniques for transferring genes responsible for production of antifreeze compounds by winter flounder (*Pseudopleuronectes americanus*), ocean pout (*Macrozoarces americanus*) and wolffish (*Anarhichas lupus*) to Atlantic salmon. The genes are expressed in salmon but so far the concentration of antifreeze compounds is too low to be effective in increasing resistance to freezing.

Chinook salmon growth hormone genes linked to an ocean pout antifreeze gene have been injected into newly fertilized Atlantic salmon eggs and prolactin genes have been linked to an ocean pout promoter. The fish benefitted from growth hormone gene transfer with remarkable growth in relation to controls. These studies will continue with further assessment of growth and possible smolting and sexual maturity.

The resulting transgenic fish from these studies will be reared in the laboratory. There no plans to use these fish in situations where they might escape to nature. They would be useful in aquaculture applications only if they were sterile.

Edward M. Donaldson and Robert H. Devlin, West Vancouver Laboratory, Vancouver, B. C.

Biochemical genetics for aquaculture - Studies in 1990

Isolation of specific genes involved in commercially important characteristics for use in broodstock evaluation programs, for the production of recombinant bioactive compounds, and for the production of transgenic fish containing suitably modified forms of these genes. The research program involves a variety of recombinant-DNA and molecular biological methodologies, with the aim of the research directed towards the identification of strains of fish that have desirable characteristics for the aquaculture industry and for the production of genetically manipulated broodstock containing inserted genes capable of altering the phenotype of the organism. Our efforts are initially focused on the manipulation of growth characteristics and reproduction.

a. Sockeye growth hormone genes

- two types of genes have been identified, and their sequence structure is being determined. These genes are for use in the transgenic program and for the production of recombinant growth hormone for inclusion in feed.

b. Insulin-like growth factors genes

- as for growth hormone
- collaborative investigation with Monsanto Company

c. Aromatase gene

- enzyme involved in sex steroid metabolism, for use in transgenic program to allow sex reversal of genotypic males.

d. Heat-shock proteins

- these genes are involved in stress tolerance. Two genes have been isolated and their characterization is under way. These genes may provide a valuable tool for monitoring stress responses in fish, and the promoter regions of these genes are being used in the transgenic program to allow inducible expression of introduced genes.

A research program is also under way to allow genetic determination of sex in chinook salmon. The isolation of male-specific DNA probes would facilitate the monosex female program now dominating BC's salmon aquaculture program by allowing verification of current monosex broodstock and by allowing the production of new monosex strains to be developed in one generation.

Dr. Ruth Withler, Pacific Bilogical Station, Dept. Fisheries and Oceans, Nanaimo, B. C.

DNA Fingerprinting, Chinook Salmon

A DNA probe isolated from chinook salmon that has "fingerprinting" capabilities has been used to screen parents and fullsib and halfsib offspring of chinook salmon families. The degree of band-sharing between individuals increases with increasing levels of relatedness. The DNA probe also shows stock identification potential, and will be used to screen wild and cultured stocks of chinook salmon to determine if it can be used to identify fish farm escapees on spawning grounds.

Richard L. Saunders, Biological Station, Dept. of Fisheries and Oceans, St. Andrews, N. B.

Atlantic salmon with spots on their dorsal and caudal fins - a possible genetic marker.

Salmon in one of the Miramichi River tributaries have dark spots on their dorsal and caudal fins. perhaps this provides an opportunity similar to that used by Skaala and Jørstad (Can. J. Fish. Aquat. Sci. 44:1775) as a genetic marker in brown trout. In that case, there were both visible (spots) and biochemical pecularities (tissue enzymes) that constituted the genetic marker. No such marker has been found for *Salmo salar.*

The distribution and extent of spot fin salmon in the tributory is being investigated. Reciprocal crosses between spot fin and clear fin salmon (from another stock) are being conducted to see if spots develop in cultured salmon and what spotting occurs in interstock hybrids.

Brian R. Riddell, Pacific Biological Station, Nanaimo, B. C.

Brian is chairman of the organizing committee for an "International Symposium on Biological Interactions of Enhansed and Wild Salmonids" at Nanaimo, B. C., June 17-20, 1991.

"Programs to increase fish production through artificial manipulations should be evaluated for the change in total production of both enhanced and wild stocks. The production from enhancement programs can generally be evaluated. However, their impact on production from wild stocks is seldom known, although the existence of interaction between enhanced and wild stocks is now broadly postulated."

The conference will be organized into four sessions, Production Trends, Genetic Concerns, Factors Affecting Freshwater and Marine Production and Fisheries Management.

Proceedings of the Atlantic Canada Workshop on Methods for the Production of Non-maturing Salmonids: February 19-21, 1991. Can. Tech. Rep. Fish. Aquat. Sci. 1789: 152 p. Edited by Vern A. Pepper

Abstract

There is concern among salmonid resource managers that accidental release of aquaculture fish may cause changes in genetic structure of wild populations. In consideration of the present uncertainity on this issue, the Newfoundland Region of the

Department of Fisheries and Oceans convened an Atlantic Canada Workshop to determine the "state of the art" for suppressing maturation of salmonids.

Participants were in favour of salmonid reproductive control and concluded there are potential advantages to the salmonid farming industry and resource managers. The option that was preferred for Atlantic Canada was all-female trploid stocks, Workshop endorsement was contingent on research to provide adequate resolutions of "coincident" phenomena as well as attempting to deal with the needs of the industry. Regional programs are required to demonstrate this technology to the industry.

Workshop Papers:

1) Pepper, V. A. Production of non-maturing salmonids: motives, actions and goals, using Newfoundland Region as a model

2) Johnstone, R., H. A. McLay and M. V. Walsingham. Production and performance of triploid Atlantic salmon in Scotland.

3) Donaldson, E. M., F. Piferrer, I. I. Solar and R. H. Devlin. Studies on hormonal sterilization and monosex female technologies for salmonids at the West Vancouver Laboratory.

4) Jungalwalla, P. J. production of non-naturing Atlantic salmon in Tasmania.

5) Benfey, T. J. The physiology of triploid salmonids in relation to aquaculture.

6) Friars, G. W. and T. J. Benfey. Triploidy and sex-reversal in relation to selection in the Salmon Genetics Research Program.

7) Sutterlin, A. M. and C. Collier. Some observations on the commercial use of triploid rainbow trout and Atlantic salmon in Newfoundland, Canada.

8) Henderson, E. B. Sex-reversal and induction of triploidy in Atlantic salmon: an industry perspective.

9) Stevenson, J. Maturity suppression in rainbow trout from the producer's perspective.

10) Boulanger, Y. Performance comparison of all-female, diploid and triploid brook trout.

JEAN. M. SEUIGNY

CANADA: annex 1b, pp. 16-22.

This paper not to be cited without prior reference to the author

International council for the
Exploration of the sea

This summary incorporates material that was solicited from scientists of three institutions: Maurice Lamontagne Institute, the Oceanographic Center in Rimouski and Laval University. The genetic research projects listed involve invertebrates as well as vertebrates. Although several of these projects have implications for aquaculture, many are oriented toward the description of the genetic structures of natural populations.

Ce résumé inclu du matériel obtenu de scientifiques travaillant dans trois instituts: l'université laval, le centre océanographique de Rimouski et l'institut Maurice-Lamontagne. Ces travaux de recherche en génétique portent sur les invertébrés ainsi que les vertébrés. Même si plusieurs de ces projets ont des implications pour l'aquaculture plusieurs ont, pour objectifs, la description de la structure génétique des populations naturelles.

Resumé of genetics program conducted in the laboratory of Julian J. Dodson, Département de biologie, Université Laval, Québec.

1. Introduction: My general research program concerns the ultimate and proximate mechanisms responsible for life-history pattern and population structuring in fishes. The research program is nested in the theoretical framework of Sinclair and Iles who proposed that fish populations (specifically marine populations) can be sustained where the hydrodynamical regime, or some other physical feature, is such that larvae from a particular spawning group may behave so as to maintain an aggregated distribution. The hypothesis stresses the role of behavior at different stages of the life cycle in relation to physical constraints. Adults must home to locations associated with stable physical characteristics which can be exploited by larvae to maintain aggregated distributions, thus insuring life-cycle closure. In cases where the distributional area of larvae and juveniles is different from the spawning area (or areas), population structuring will reflect the number of retention zones rather than the number of spawning grounds. Our research efforts are directed to verifying and developing different facets of this all-encompassing theory of population structure. Specifically refering to our genetic's research program, we have focused on the interaction of historical events (particularily Pleistocene glaciation events) and contemporary biological fish populations, as illustrated by mitochondrial DNA restriction fragment length polymorphisms. Two projects are presently under way.

2. Retention and population structure of smelt (Osmerus mordax) and tomcod (Microgadus tomcod) in the turbid middle estuary of the St. Lawrence R., Quebec. Larvae of anodromous rainbow smelt and tomcod originating in various spawning tributaries are retained in the St. Lawrence estuary. We proposed that smelt represent one population genetically differentiated from adjacent populations characterized by geographically distinct larval retention areas. We also analysed four landlocked populations to evaluate the phylogenetic basis of dwarf and normal-size phenotypes and their relation to anadromous smelt. A phylogenetic distinction was revealed between andromous and landlocked smelt, with only one of the two mtDNA phylogenetic groups of anadromous fish observed among landlocked smelt. Significant geographical heterogeneity in the distribution on mtDNA genotypes was observed among landlocked smelt, but no phylogenetic basis to dwarfism was evident. St. Lawrence smelt were genetically identical but distinct from adjacent populations, supporting the proposition that population genetic structure reflects the number of larval retention zones rather than spawning sites.

We are presently repeating this study with tomcod obtained from two St. Lawrence estuary spawning sites and hydrodynamically distinct sampling site along the north shore of the lower St. Lawrence estuary, the Miramichi R. and the Hudson R.. In addition, we are analyzing mtDNA genotype frequency and nucleon

diversity among age classes of two St. Lawrence tomcod stocks to evaluate the temporal stability of these parameters.

We have also undertaken a study of the phylogenetic relationships among European smelt (Osmerus eperlanus) and rainbow smelt from the east and west coast of Canada. This work is conducted in collaboration with Dr. Eric Taylor, Dalhousie University Gene Probe Laboratory.

3. Relationship between spawning mode, spawning site and phylogeographic structure in mitochondrila DNA of North Atlantic capelin. Capelin (Mallotus villosus) spawn on beaches in Alaska and British Columbia, but spawn offshore in Icelandic waters and the Barents Sea. Both modes of reproduction co-occur in the norhtwest Atlantic. The Southeast Shoal population spawns on the Grand Banks 350 km to the SE of Newfoundland at the same time as other stocks, all of which are beach spawners. These observations gave rise to 2 alternative hypotheses concerning the zoogeography and evolution of life cycle in capelin. First, the Southeast Shoal population was originally a beach-spawning population during the late Wisconsinian glaciation and is ancestral to all other northwest Atlantic capelin stocks. In such a case, present-day stocks from this area would represent a monophyletic group derived from a common ancestor nomore than 10-12,000 years ago. An alternative hypothesis is that the two modes of reproduction originally evolved in isolation. Beach spawners are hypothesized to have originated in the north Pacific and recolonized Canadian Arctic waters and the northwest Atlantic following glaciation. Bottom spawners originated in the North Atlantic and continued to reproduce where environmental conditions permitted. In such a case, genetic divergence among bottom-spawners and among beach-spawners from accross the North Atlantic would be less than that between beach- and bottom-spawners. We tested these hypotheses by comparing mtDNA restriction fragment length polymorphisms among six stocks of beach-spawning capelin (St. Lawrence estuary; Gulf of St. Lawrence; Placentia Bay, Conception Bay, Notre Dame Bay, Newfoundland; Nain, Labrador) and three stocks of bottom-spawning capelin (Southeast Shoal; Iceland; Barents Sea). We observed two major mtDNA genotype groups separated by a mean sequence divergence of 3.42%, clearly reflecting the genetic separation of the Iceland and Barents Sea stocks from the northwest Atlantic stocks. No grographical heterogeneity in the frequency of mtDNA genotypes was observed among the northwest Atlantic sampling sites. However, differences in nucleon diversities among sites did not support the view that capelin form one large panmictic population in the northwest Atlantic. Although our results do not permit the identification of the Southeast shoal stock as ancestral to Northwest Atlantic capelin, these observations refute the hypothesis that the beach- and bottom-spawning stocks evolved in isolation long before the end of the Wisconsinian glaciation.

We are presently working to validate a method for estimating the gene flow among populations of capelin in the NW Atlantic based on the phylogeny of mitochondrial DNA. Differences in the

diversity of mtDNA genotypes among NW Atlantic stocks revealed population structuring. However, mtDNA genotypes were widely distributed among stocks suggesting extensive gene flow in the NW Atlantic.

The phylogeny of mtDNA genotypes and their geographic locations indicates the minimum number of migration events, s, nevessary for their contemporary distribution to be consistent with their phylogeny. But in cases where populations are recently derived from an ancestral population, common ancestry of genotypes may not reflect ongoing gene flow but their historical associations. This situation is typical of north Atlantic species as populations were founded no more thant 12-18,000 BP. No computational method exists to distinguish between these two possibilities. However, the relationship between the NW Atlantic mtDNA clade and its ancestral group may provide an empirical evaluation of the relative importance of the 2 phenomena and the validity of gene flow estimates among NW Atlantic stocks.

In the case that N Pacific capelin repopulated the Arctic and Northwest Atlantic 4,500-6,000 BP, Pacific mtDNA genotypes would cluster with the NW Atlantic mtDNA clade an many genotypes would be common to both areas. If correct, we postulate that contemporary gene flow within either Ocean should be grater than that between any pair of Pacific/Atlantic populations. If phylogeny reflects ongoing gene flow, estimates of s should be correlated with geographic distance separating populations, with fewer migration events occuring between the 2 oceans. However, if phylogenies result from recent historical associations, estimates of s among and between sites in the two Oceans will be the same. Specific objectives of on going research are, (a) to test the hypothesis that NW Atlantic capelin populations are recently derived from N Pacific populations as revealed by a comparison of restriction fragment length polymorphisms of mtDNA and, (2) to test the hypothesis that common ancestry of genotypes in different populations is due to ongoing gene flow rather than historical association.

Résumé of the research program in aquaculture, developmental and molecular biology of the developmental biology research group.
François Dubé, Louise Dufresne and Richard Desrosiers.
Département d'Oéanographie, Université du Québec, Rimouski.

Our research interests concern the developmental biology of marine invertebrates of commercial importance, such as sea urchins, mussels, clams and scallops. We study the molecular mechanisms governing cell division cycles. Our main goal is to develop novel biotechnological tools in order to improve the growth potential of the larvae and juveniles of these marine invertebrates. One way to increase the growth potential of these organisms is to modify the molecular mechanisms controlling meiosis resumption, more specifically protein synthesis and phosphorylation, as well as the cytoskeletal organization. These studies involve the use of modern biochemical technics, such as isotope labeling of proteins, monoclonal antibodies, immunocytochemistry and molecular biology.

In order to clone genes coding for protein of particular interests, shown to be the main controlling factors of the cell cyle, cDNA libraries have been produced from mRNA of unfertilized eggs. An important team effort will concentrate on the elucidation of the promotors regulating the expression of these genes, thus controlling the cell cycle and further embryonic development. This will be achieved by site-directed mutagenesis of these genes, in order to alter their expression and increase the growth potential of the transgenic individuals produced after introduction of these new genes into the unfertilized egg.

List of research projects cunducted by our team:

1) Protein synthesis and cell cycle regulation in marine invertebrate eggs.
2) Function of the cytoskeleton during development of marine invertebrate embryos.
3) Embryonic development, differentiation and aging: a study of the molecular mechanisms.
4) Mechanisms controlling cell division during early development.
5) Production of triploid larvae from giant scallop oocytes.
6) Ultrastrutural analysis of early development of giant scallop.
7) Production of juvenile clams (Spisula solidissima) for commercial exploitation.
8) Gametogenetic cycle of clams from Magdalen Islands.
9) Analysis of commercial potential of clams raised in nurseries.
10) Developmental biology of the whelk, Buccinum undatum.

B) Study of the genetic aspects of the summer mortality of cultured mussels in the Magdalen Islands.

This year, research projects were initiated to study the genetic aspects of the summer mortality that affected mussels grown in the Magdalen Islands (Gulf of St Lawrence). Mussel growers face almost every year a problem of massive mortality of the two-year-old mussels grown in the lagunes. Recent observations indicate that the importance of this phenomenom has increased over the years and is now also affecting the one-year-old animals. There also seems to be a stock effect on the mortality. A research program was initiated to determine the possible causes of this summer mortality. Genetic markers are used to test the following hypotheses a) the presence of two mussel species in the Madgdalen Islands lagune b) the possibility that aquaculture practices influence the genetic make up of the population by selecting for animals having a shorter life cycle and earlier maturity. This study is realized using both protein and mt-DNA markers and is done in cooperation with scientists from the Government of Québec (Bruno Myrand), the Oceanographic Center in Rimouski (P. Mayzaud, L. St-Amand) and Laval University (J.J. Dodson, E. Gouderlay).

Résumé of the genetics program at the Maurice Lamontage Institute

J.-M. Sévigny, B. Sainte-Marie, M. Fréchette and L. Savard. Pêches et Océans, Mont-Joli (Qc), Canada.

The genetics research programm conducted at the Maurice Lamontagne Institute are oriented toward the description of population structure of three marine species and are concerned with the possible impacts of physical structures (topography, circulation patterns) on the gene flow in these species. Recently, a research project was also undertaken to study the genetic aspects of summer mortality that affected cultured mussels.

A) Genetic variation in the Greenland halibut , the northern shrimp and the snow crab from the Saint Lawrence system and the Northwest Atlantic.

These population genetics research projects were initiated to describe the genetic structure of different species of fishes and invertebrates including, the Greenland halibut (Reinhardtius hippoglossoides), the northern shrimp (Pandalus borealis) and the snow crab (Chionoecetes opilio). These studies will provide information on the impact of physical oceanographic characteristics of the St Lawrence on gene flow in these marine species. Because different size classes were sampled, they will assess the stability of the observed structures. Samples were collected at several sites off the Newfoundland-Labrador coast, and in the Estuary, the Gulf of Saint Lawrence and the Saguenay fjord. Allozymic and, for the snow crab, mt-DNA variations are used as genetic markers.

The population genetics studies of these species are also realized in collaboration with scientists from other fields of research. Parasitologists are studying the Greenland halibut and will use parasites as biological tags (R. Arthur, Maurice Lamontagne Institute). Morphometric characters are also taken for every specimen of snow crab studied by electrophoresis in an attempt to determine the genetics and morphometric characteristics of the populations sampled. Selective breeding is also performed with this species under controlled conditions that will permit us to determine the heritability of different characters such as size and allozymic patterns and to help understand the breeding structure of this species.

Scientists from several institutions are collaborating on these projects: D. Taylor and D. Parsons, DFO-Newfoundland; M. Moryiasu, DFO-New Brunswick; D. Pike, DFO-Iqaluit;) Samples of Pandalus borealis were also sent to Dr Yuri Kartavtsev from the Far East Science Center (USSR) and may be used to compare level of polymorphisms in different populations. These projects will continue during the next year.

Prof. Dr. W. Villwock

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Report on behalf of the German Activities in Fish Genetics.

Within the period of concern (1989-1991) immunological work on selected Tilapias was continued by the working group of the signed reporter. Very recently a master thesis ("Diplom-Arbeit") was finished by Mr. Axel JANKE, dealing with "Isolation and Analysis of mtDNA of Tilapias (Cichlidae) for Population Discrimination Purposes: 74 pp. (1991) (in German). Additionally, carp erythrocytes were investigated: The results were published in 1990 (see cited lit.: page 23). All these activities of the own working group will be continued for the next 2-3 years, partly being funded by German "Gesellschaft für Technische Zusammenarbeit mbH./ GTZ" in a co-project together with the "Institute of Aquatic Biology / IAB", Accra / Ghana and ICLARM / Manila.

Another cooperating group of scientists, including the German ichthyologist, Prof.Dr. Manfred SHARTL / Biozentrum University of Würzburg, started with "Effect of Growth Hormone on the Growth Rate of the Gilthead Seabream (*Sparus aurata*), cloning of its GH cDNA, and the Use of different Constructs for the Production of a transgenic Fish", and "Development of an inducible Fish Species Expression Vector for Gene Transfer *in vitro* and *in vivo*". Both contributions were presented at the "4th International Symposium on Genetics in Aquaculture", Wuhan / P.R. China, April 29-May 03, 1991. The investigations in concern will be continued on an international scale.

A 3rd group under the leadership of Prof. Dr. Sven PÄÄBO / Zoological Institute, University of Munich, started a few weeks ago with mtDNA-investigations, mainly on vertebrates, and among them on fish. Dr. Pääbo has been the main co-investigator of late Prof. WILSON / University of California, Berkeley.

This means, there will be in future a number of strongly working scientists and working groups dealing with genetic problems of fin-fish, hopefully interacting towards the common aim of improving our knowledge and understanding of fish genetics and the appliacability of their results.

(Prof. Dr. W. Villwock)

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CURRENT GENETIC STUDIES ON FISHES IN FINLAND

Report to ICES Working Group on Genetics
Compiled by Marja-Liisa Koljonen

1) Finnish Game and Fisheries Research Institute, Aquaculture Division, P.O. Box 202, SF-00151 Helsinki, Finland.
(Irma Kallio-Nyberg, Marja-Liisa Koljonen, Jarmo Koskiniemi)

- Population genetic studies on several brown trout and salmon populations using enzyme electrophoresis.

- Experimenental research on gynogenesis in rainbow trout is continuing. The second gynogenetic generation was established this spring.

- A research programme has begun for the evaluation of the contributions of different salmon stocks in catch samples.

- A fish stock registry has been created for grayling, vendace, whitefish, salmon, trout and char stocks.

- Genetic engineering of rainbow trout. In addition to micro-injection, several other methods are tested, high-velocity mechanical injections, receptor-mediated transformation of oocytes, treatment of sperm with genes. Jorma Piironen (Aquaculture Division), Vladimir P[illegible]mintzev, Alexei Beniumov (Department of Embryology M.V. Lomonosov, State University), Alexei Krasnov (Sevryb NII Projekt, Ptrozavodskt) and Viktor Kolesnikov (V.A. Engel'gardt Institute of Molecular Biology, Academy of Sciences of USSR)

2) Acricultural Research Center, Department of Animal Breeding, SF-31600 Jokioinen, Finland (Liisa Siitonen).

- Research connected with a rainbow trout breeding programme.

3) University of Joensuu, Department of Biology, P.o. Box 111, SF-80101 Joensuu, Finland (Jukka Vuorinen, Kari Elo).

- Electrophoretic studies, mainly on Coregonids.

4) University of Kuopio, Department of Physiology, P.O. Box 6, SF-70211 Kuopio, Finland (K. Partti-Pellinen).

- Mitochondrial DNA research on Coregonids.

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Statement to the W[illegible]roup on Genetics about the topics to be discussed at the meeting on [illegible]4.-6.6.1991 from Marja-Liisa Koljonen

a) Research on biochemical (enzyme) markers and other related techniques in Finland:

In Finland electrophoretic variation in Salmonids and Coregonids has been studied over ten years, but little attention has been paid to the use of enzyme genes as electrophoretic markers. In some cases introduction of foreign stocks has been observed with the aid of enzyme gene research. In those cases the original populations differed markedly in allele frequencies.

The basic pattern of enzyme gene variation in the most valuable species (salmon, brown trout and whitefish) is already known. The basic screening work will probably not yield much more new information than the amount of geographical variation in that particular case. However even the basic data on the population structures can be used more effectively in conservation and management recommendations.

Data on of enzyme genes could be put to practicular use in calculating effective population sizes and determining the contributions of different stocks to a mixed stock fishery. There is a need of useful discrimination programmmes for genetic data. The value of enzyme alleles as stock markers depends on the actual allele frequencies in the stocks. The allele frequencies can be altered, but large changes can affect the total genetic structure of the stock, which is not desirable. With moderate changes in allele frequencies, larger sample sizes and effective discrimination programmes, relatively reliable results could probably be achieved.

In Finland electrophoretic markers are not yet used as stock markers, but a research programme is under way on the contributions of salmon stocks in the Bothnian Bay. Some basic research on mDNA in fish has been done at Kuopio University, but there are no plans to use the mitochondrial types as tags.

b) Present trends in advanced gene technology in fisheries science and/ or aquaculture.

1) DNA fingerprinting is not performed on fishes in Finland. Some work has been done on chicken fingerprinting, but it has not yet proved useful in practice at individual level. However, it can be used to study the relationships of near relatives.

2) Research on the production of transgenic fish is being done at Kuopio University and also in the Aquaculture Division in cooperation with Soviet researchers. Production of transgenic fish is basic research at present, but it might have some practical applications in future, especially in combination with conventional selective breeding.

c) Risks involved with uncontrolled releases of genetically changed organisms. Assess methods to reduce the risks.

The risks involved with uncontrolled releases of genetically changed organisms depend on the species, host species and novel gene concerned. In the field of fisheries, direct risks to human health are not very likely, so the most probable risks are those to the environment and the ecosystem. These depend on the ability of the transgenic specimens to survive and to reproduce in nature and the very unlikely ability of the novel gene to move to other species.

Proposals to assess GMO:s outside a closed system.

To move GMOs out of the laboratory or a closed system should be allowed only with a special licence. The application for the licence should include risk analyses. Permission for release in nature should always be preceded by a controlled field test. A condition of the permission could be that the modified organisms are completely sterile.

FINLAND AND LEGISLATION ON GENETICALLY MODIFIED ORGANISMS

Compiled by Marja-Liisa Koljonen according to the memorandum on 31.1.1990 of the Finnish Ministry of the Environment

There is no actual legislation in Finland designed to regulate the risks to the environment and to human health ensuing from the use of genetically modified organisms in research and product development. The legislation on health care, environmental protection and agriculture does not define live genetically modified organisms, and this legislation, with the exception of the Decree on Infectious Diseases, cannot as such be applied to research or product development within biotechnology or genetic engineering.

The Act for the Prevention of Cruelty to Animals as a rule forbids such breeding as repeatedly leads to the birth of individuals whose delivery or the maintenance of whose vital functions calls for specific measures within veterinary medicine or other fields. The import to Finland of such animals is likewise forbidden. The Decree on Test Animals extensively regulates tests with vertebrate animals raised or acquired for test purposes. There is no legislation on the use of genetic engineerin in fish breeding. No binding regulations can be in voked in respect of field tests or to forestall their possible harmful consequences.

According to the memorandum of the Finnish Ministry of the Environment, matters related to biotechnology will be delegated within the administration in such a manner that each task rests with the central authority in the respective field. Fish breeding, like all animal breeding in Finland falls within the jurisdiction of the Ministry of Agriculture. A joint delegation on genetic engineering, intended to function as an expert board and to develop this field, has been appointed to coordinate the activitie of the different authorities in this matter.

Finnish legislation on biotechnology will be developed by supplementing the relevant specific Acts with the required regulations. Legislation on environmental protection, health care, agriculture and industrial production will be developed to supervise the use of genetically modified organisms (GMOs). The Council of the State will appoint a Comittee for the required legistlation work.

Information Form on activities of the members of the ICES-WG on Genetics.

Name and full address of the member signed below:

René GUYOMARD, laboratoire de génétique des poissons, INRA-CRJ, 78352 Jouy-en-Josas, FRANCE

(1) Actual research in fishgenetics (brief description of used techniques, aims and species of concern)

a) techniques used:
- protein electrophoresis
- mtDNA PCR and sequencing
- microsatellite single-loci electrophoresis

b) aims:
- species identification and phylogeny
- genetic diversity within species
- study of natural hybrid zones and interaction between natural and domesticated stocks

c) species: salmonids, tilipias, clupeids, silurids

(2) Planned research in fishgenetics for the next 12 months (continued and/or new):

a) gene mapping in rainbow trout and Atlantic salmon

b)

c)

In case of more than 3 different projects within pos. (1) and/or pos. (2), or, if brief explanations are wanted, please, use backside.

Deadline for return Juy 31, 1991 !

Date 09/07/91 Name GUYOMARD R.

Information Form
on activities of the members of the ICES-WG on Genetics.

Name and full address of the member signed below:

PROF. N.P. WILKINS
DEPT. of ZOOLOGY
NATIONAL UNIVERSITY of IRELAND,
UNIVERSITY COLLEGE, GALWAY, IRELAND

(1) Actual research in fishgenetics (brief description of used techniques, aims and species of concern)

a) GENETIC STUDIES IN IRELAND CONTINUE AT TWO CENTRES — CORK (T. CROSS) AND GALWAY (N.P. WILKINS)

b) T. CROSS'S GROUP is SURVEYING NATURAL POPULATIONS of SALMON USING ISOZYME AND DNA POLYMORPHISMS
N.P. WILKINS'S GROUP is STUDYING TRIPLOIDY AND SEX-REVERSAL IN ATLANTIC SALMON AND SEA-TROUT. ALSO INVESTIGATING SALMON X TR

c) HYBRIDS USING ISOZYMES, MERISTIC AND MORPHOMETRIC VARIATIONS; KARYOTYPING & CHROMOSOMAL REARRANGEMENTS IN SALMON & TROUT.

(2) Planned research in fishgenetics for the next 12 months (continued and/or new):

a)

1) CONTINUATION of ABOVE STUDIES

b)

2) CROSS & WILKINS ARE COOPERATING WITH OTHER INSTITUTES IN A REHABILITATION STUDY ON THE

c) R. SHANNON SALMON STOCKS [INVOLVING STOCK IDENTIFICATION (CROSS) AND BREEDING PROGRAMME (WILKINS)] WHICH IS ONGOING AND WILL CONTINUE FOR FORESEEABLE FUTURE

In case of more than 3 different projects within pos. (1) and/or pos. (2), or, if brief explanations are wanted, please, use backside.

Deadline for return Juy 31, 1991 !

Date 29/7/91.

Name Niall P. Wilkins

Not to be cited without prior references to the autors

ICES Working
Group on Genetics

Working Paper
Helsinki, June 1991

RECENT GENETIC STUDIES RELATED TO AQUACULTURE AND FISHERIES RESEARCH IN NORWAY

An overview compiled by

Gunnar Nævdal

Department of Fisheries and Marine Biology
University of Bergen
Bergen High Technology Center
N-5020 BERGEN

and

Knut E. Jørstad and Geir Dahle

Institute of Marine Research
P.O.Box 1870, Nordnes
N-5024 BERGEN

INTRODUCTION

This report is an updated version of genetic studies related to aquaculture and fisheries research compiled for the ICES Working Group in Genetics in 1989.

Genetics methods have traditions back to around 1960 in Norwegian fisheries research, when work on blood groups for population identification of cod was initiated by the Institute of Marine Research (Dag Møller). Later, enzyme electrophoresis and studies of other proteins came in use. Recently, these methods have been supplemented by mitochondrial DNA studies, and studies by DNA fingerprinting now are planned.

Salmomid farming started in the late 60-ties, and around 1970 work was initiated on quantitative genetics for improvement of ecomomically important traits. This work has been continued since then, and also some work on chromosome engineering has been carried out. In recent year, work on gene technology has been initiated in order to carry out genetic improvement of farmed fish.

In the following overviews these topics are dealt with:

Identification of population units and sibling species

Genetic tags applied in sea ranching for studies of gene introgression

Genetic improvement of salmonids - classical quantitative genetics

Chromosome engineering

Gene technology

IDENTIFICATION OF POPULATION UNITS AND SIBLING SPECIES

At Department of Fisheries Biology, University of Bergen in cooperation with the Institute of Marine Research, studies on species identification, species validity and intraspecies variation of redfishes (Genus *Sebastes*) in Norwegian water is completed. Similar studies on redfishes from Iceland and Greenland water are now undertaken.

Genetic studies on cod and herring stocks have been continued at the Institute of Marine Research, including analyses of new yearclasses. The last mentioned work is mainly focused on yearclass variation and identification of subpopulations by using protein electrophoresis and restriction fragment analysis of mit-DNA. Studies on resident and anadromous brown trout populations by enzyme polymorphism are continued at the same institute.

The same two institutions in Bergen are cooperating on studies of genetic composition of natural and stocked cod populations in several areas along the Norwegian coast. A central part of these investigations is use of genetically tagged cod.

At Trondheim Biological Station, University of Trondheim, the following projects are dealt with:

- Population structure and evolution of various gadoid fishes, at the moment especially blue whiting, studied by electrophoretic methods.

- Studies on homing in marine fishes (cod, plaice) by tagging/transplantation experiments.

- Biochemical genetic identification of fish eggs.

- Mathematical modelling and computer simulation of evolutionary processes (genetic drift, selection, immigration) for use in genetic resource management.

At Norwegian Institute of Nature Research, Trondheim, studies on population structure of salmon in Norwegian rivers have been continued with the aim of establishing a genetic model for wild salmon stocks in Norway. This could be used as basis for evaluating the genetic impact of reared salmon on natural salmon gene pools.

Investigations on enzyme polymorphism for use in population studies on minke whale, harp seal and hooded seal are carried out at the Department of Fisheries and Marine Biology, University of Bergen, in cooperation with the Institute of Marine Research. Similar studies on minke whale and harp seal applying mit-DNA technique are carried out at Department of Medical Biology, University of Tromsø.

GENETIC TAGGGING APPLIED IN SEA RANCHING FOR STUDIES ON GENE INTROGRESSION

A morphological genetic marker (fine spotted) in trout has been identified at the Institute of Marine Research, Bergen, and are utilized for studies on interaction between natural and reared populations.

Likewise a biochemical genetic marker has been identified in cod, and a homozygotous brood fish population has been developed. The offspring are being used in sea ranch experiments for studies of survival and interaction between natural and released cod.

Genetic analyses have been incorporated in a salmon ranching programme started at the western coast of Norway (Institute of Marine Research, Bergen). These include analyses of wild spawners used as broodstock (allozymes, DNA-fingerprinting) for evaluation of straying/genetic impact on river stocks,

and families/stock analyses with respect to survival and return rates. A pilot study of disease resistance have been initated.

GENETIC IMPROVEMENT OF SALMONIDS - CLASSICAL QUANTITATIVE GENETICS

A large scale programme for genetic improvement of salmonids have been inityiated by the fish farmers assosiations (Fiskeoppdretternes Salslag, Trondheim) and Institute of Aquaculture Research (AKVAFORSK), Ås. The breeding programme is carried out at the breeding station at Kyrksæterøra and at Sunndalsøra. The improved fish material is transferred to the fish farming industry via multiplying stations in each county.

Institute of Aquaculture Research carries out quantitative genetics on salmonids at the research stations at Sunndalsøra and Averøya, both located in the county of Møre and Romsdal, and at the Agricultural University of Norway, Ås. The following projects give an overview of the activity:

Selection for genetic improvement in cooperation with the breeding station at Kyrksæterøra, is carried out continuously on growth rate, age at maturity and survival. Genetic parameter of "new" productive traits are also estimated.

Additive genetic variations are found to be the main contribute to the variation of traits connected to fish quality (fat in flesh, intestine fat, flesh colour, belly thickness etc.) in rainbow trout. Such studies have been initiated also for Atlantic salmon. Datatomography was found to be of considerably help in the registrations of body composition in fish. Non-additive genetic variation explain a minor part of total genetic variation for this traits.

Immunological factors which may be connected to genetically determined disease resistance are identified and tested for genetic variation and covariation with productive traits and actual resistance. Also the connection between "stress" and immuneresponse is studied. These studies are carried out in cooperation with Department of Animal Breeding, Agricultural University of Norway, and Department of Microbiology and Immunology. Veterinarian University of Norway. Challenge test have shown great differences in mortality between full and half sibs families when exposured to furunkolosis, vibriosis and cold water vibriosis. A project for studying the ironbinding proteins (transferrins) and their effect on disease resistance has been started. In vitro-tests on fish patogenes will be carried out, and also cell lines and model fish will be used for studies on gene regulations.

At Aquaculture Center, Institute of Marine Research, Bergen, research on quantitative genetics on Atlantic salmon by studying the performance of a high number of sibgroups at

different commercial fish farms under different environmental are terminated. These studies were closely connected to studies on environmental impact by fish farming, field studies on fish health and studies for determination of optimal densities in net pens. Genetic/environmental interactions, mostly as different expression of genetic variations, were indicated in these studies.

At Institute of Marine Research, Aquaculture Station Matre, studies of trypsin isozymes in salmonids have been continued. These involve studies on the inheritance control as well as growth performance of selected families and specific genotypes.

CHROMOSOME ENGINEERING

Studies on the combination of triploidy and gynogenesis are carried out at Institute of Aquaculture Research, N-6600 Sunndalsøra, with the aim of producing steril all-female rainbow trout and Atlantic salmon.

GENE TECHNOLOGY

Characterization and isolation of genes coding for growth hormones, prolactine, trypsine isozymes, insuline and genes involved in disease resistance have been undertaken by several laboratories with the double aim of basic studies of such mechanisms and of transferring "valuable" genes between and within species. Both Atlantic salmon and a model fish (zebrafish) are used for such investigations.

Another aspect of these investigations have been constructions of "genome libraries" and studies on homeobox genes of salmon.

The laboratories engaged in gene technology studies on fish in Norway are listed in the Appendix.

APPENDIX

Laboratory for Biotechnology, University of Bergen
Bergen High Technology Center, N-5020 Bergen

Zoological Laboratory, University of Bergen
Allegt. 41, N-5007 BERGEN

Department of Biochemistry, University i Bergen
Årstadveien 19, N-5009 BERGEN

Department of Biotechnology,
Norwegian Technical University
N-7034 TRONDHEIM

Department of Genetics and Biotechnical Disease Control
Norwegian Veterinarian University
P.O.Box 8146 Dep., N-0033 OSLO 1

Department of Physiology
(same address)

Institute for Aquaculture Research,
Agricultural University
P.O.Box 32, N-1432 ÅS-NLH

Norwegian Fisheries High School, University of Tromsø
P.O.Box 3083, Guleng, N-9000 TROMSØ

Department of Microbiology and Plant Physiology
University of Bergen, Allegt. 70, N-5007 BERGEN

Department of Medical Biochemistry
P.O.Box 1112, Blindern, N-0317 OSLO 3

Laboratory for Microbial Gene Technology
Norwegian Agricultural University
P.O.Box 37, N-1432 ÅS-NLH

Information Form
on activities of the members of the ICES-WG on Genetics.

Name and full address of the member signed below:

Krzysztof Goryczko
Inland Fisheries Institute
Salmonid Research Laboratory
Rutki 83-330 Zukowo POLAND

(1) **Actual research in fishgenetics (brief description of used techniques, aims and species of concern)**

a) Rainbow trout family selection based on 5 strains (third year of realization). Control group of tagged fish reared in commercial farm were analized (length, weight, gutted weight). Maternal, paternal heterosis effect were calculated.

b) The interspecific hybridization among brook, sea, rainbow trout and salmon with and without polyploidization (diploids, triploids and tetraploids) is being realized. The survival, growth rate, karyology and enzyme markers of hybrids were analized.

c) The programme of "building up" the outbred Vistula sea trout broodstock in SRL Rutki for conservation purposes started in November 1990. Fertilized ova obtained from 131 wild spawners were incubated in Laboratory. Electrophoretic analyses of protein polymorphisms of brood fish was done.

(2) **Planned research in fishgenetics for the next 12 months (continued and/or new):**

a) The results mentioned in 1.a. will enable the selection of 120 brood fish in SRL Rutki to start 60 families in 1991 spring.

b) To be continued.

c) To be continued.

Date. 26.03.91. Name... K. Goryczko

Information Form on activities of the members of the ICES-WG on Genetics.

Name and full address of the member signed below:

Ana Maria Teia dos Santos

Centro Regional de Investigação Pesqueira

Instituto Nacional de Investigação das Pescas

Canal das Pirâmides

3800 AVEIRO

(1) Actual research in fishgenetics (brief description of used techniques, aims and species of concern)

a) Enzimatic study, by electrophoretic technique, of populations of Nephrops norwegicus (L.) from Portuguese Coast.

b) Enzimatic study, by electrophoretic technique, of populations of Merluccius merluccius (L.), from Portuguese Coast.

c)

(2) Planned research in fishgenetics for the next 12 months (continued and/or new):

a) DNA - mithocondrial study of populations of Merluccius merluccius (L.) from Portuguese Coast.

b)

c)

In case of more than 3 different projects within pos. (1) and/or pos. (2), or, if brief explanations are wanted, please, use backside.

Deadline for return July 31, 1991 !

Date 91|07|30 Name Ana Maria Teia dos Santos

Information Form
on activities of the members of the ICES-WG on Genetics.

Name and full address of the member signed below:

Håkan Jansson
Salmon Research Institute
S-810 70 Älvkarleby
Sweden

(1) Actual research in fishgenetics (brief description of used techniques, aims and species of concern)

a)
Studies on genetic variation in natural populations and hatchery stocks of Atlantic salmon (*Salmo salar*) and brown trout (*S.trutta*) by enzyme electrophoresis.

b)
Studies on natural hybridization between Atlantic salmon and brown trout by enzyme electrophoresis.

c)

(2) Planned research in fishgenetics for the next 12 months (continued and/or new):

a)
(continued)

b)
(continued)

c)

In case of more than 3 different projects within pos. (1) and/or pos. (2), or, if brief explanations are wanted, please, use backside.

Deadline for return Juy 31, 1991 !

Date 1991-07-18 Name Håkan Jansson

UK (SOAFD, Scotland)

PROGRESS REPORT FOR ICES WORKING GROUP ON GENETICS, 1991

1. Genetic protein variation

We have completed study of the spatial distribution of allele frequency variation in Atlantic salmon populations in Scottish rivers, examining 0-group juveniles in each of three years at single sites within river catchments. Significant differences were demonstrated among rivers. Differences within rivers among the 3 years of study were not significant. Temporal variation within a core group of sites will continue to be monitored for at least a further 2 years.

A study of allele frequency variation in farmed Atlantic salmon has been completed. Allele frequencies in 0-group juveniles were examined in each of 2 years in 16 "named" strains of salmon in culture in Scotland. Strains differed genetically between years. Overall, farmed fish in Scotland, of both Scottish and Norwegian river origin, differed genetically from a sample number of wild Scottish salmon populations. Farmed strains differed genetically from their wild source river populations. A significant directional shift between source populations and strains in MEP-2 allele frequencies was evident in one year. The shift was in favour of the 125 allele. However this could not be confirmed in the other year of study and remains in doubt.

Allele frequency variation among salmon populations in the Kyles of Sutherland river catchment has been determined. The catchment comprises 3 main rivers which share a common estuary. MEP-2 allele frequency variation is associated with water temperature - the 125 allele is more frequent in colder locations. The extent of genetic differentiation among the Kyles river is consistent with an historical average exchange of 15 fish per year. Considering only the MEP-2 locus, allele frequency differences are consistent with the average exchange of only 6 individuals.

2. Mitochondrial DNA

The frequencies of restriction fragment length polymorphisms have been determined in 15 geographical locations in Scotland and elsewhere. These data are being analysed.

3. The adaptive significance of variation at the MEP-2 locus

We have attempted to discount a direct role for variation of the MEP-2 in determining the association described previously between MEP-2 genotype in salmon and juvenile and adult performance parameters. However, intact mitochondrial preparations from homozygous MEP-2 genotypes were shown to oxidise malate at different rates at saturating substrate concentration: oxidation rate in preparations from 125 type homozygotes exceeded that in preparations from 100 type homozygotes. The homotetrameric forms of the MEP-2 allozymes are being purified chromatographically for comparative kinetic studies.

The physiological basis of performance differences among MEP-2 genotypes is being examined experimentally in the field and in captive fishes.

4. Farmed salmon escaping from culture

A two year study of the spawning biology of escaped farmed salmon has been completed. In March 1989, 184,000 growing salmon were released into a sea-loch in northern Scotland as the result of a single accident. Escaped fish returned to the nearby River Polla in 1989 (estimated to be numbered in hundreds) and in 1990 (numbered in tens). Their spawning biology was examined and compared with that in wild fish using radiotracking and field observation techniques. The accuracy of these techniques was assessed and the study was pursued further by using the presence of canthaxanthin (which had been an additive to the farmed fishes' feed) to identify farmed fish and their eggs and alevins. Farmed escapes spawned and crossed with wild fish. In 1989, farmed fish (especially females) spawned later than wild ones and tended to do so in the lower spawning areas. The results of spawning in 1990 are being analysed.

The study will be followed up by considering the extent of any genetic change to the Polla salmon population, examining protein variation initially.

A single bag-net fishery for salmon operated near Gairloch on the western coast of Scotland has been monitored for escaped farmed fish. In 1990, escapes formed 20% of the fishing station's catch.

A major survey of Scottish rivers to determine the incidence and distribution of eggs and alevins containing canthaxanthin (and therefore the progeny of female, farmed salmon) is being carried out - precipitated by the recent withdrawal of canthaxanthin as a feed additive in Scotland.

5. Salmon population structure within rivers

A long term programme has been started on the River Dee in Aberdeenshire to delineate the spatial boundaries of reproductive units, to determine effective population size at spawning and to measure exchange between units.

Wednesday,
June 05.

09.00 a.m.: Joint Meeting of the WG on Genetics and the WG on Introductions and Transfers.

Purpose and Goals of this Joint Meeting:

- Remarks by Dr. W. Villwock, Chair, Genetics WG
- Remarks by Dr. J. Carlton, Chair, Introductions WG
- Appointment of Joint Meeting Rapporteur(s)
- Genetically modified Organisms / GMOs: Definitions, Conceps, and Issues. Speaker: Dr. Villwock, Genetic WG.

10.30 a.m. Coffee Brake

11.00 a.m.
- Genetically modified Organisms: The Release of GMOs in the Marine Environment. Speaker: Dr. Sau ders, Genetic WG.

11.30 a.m. Discussion of Needs to Modify the "Code of Practice".

12.15 p.m. Lunch

01.30 p.m. Reconvene: Continue Discussion of Needs to Modify "Code of Practice".

03.30 p.m. Coffee Brake

04.00 p.m. Review of Suggested Additions to "Code of Practice".

05.00 p.m. Final Remarks (result see draft of newly formulated "Code of Practice")

Bold face = Changes and New Sections

REVISED 1991 CODE OF PRACTICE TO REDUCE THE RISKS OF ADVERSE EFFECTS ARISING FROM THE INTRODUCTIONS AND TRANSFERS OF MARINE ORGANISMS, **INCLUDING THE RELEASE OF GENETICALLY MODIFIED ORGANISMS**

I. Recommended procedure for all species prior to reaching a decision regarding new introductions. (A recommended procedure for introduced or transferred species which are part of current commercial practice is given in Section IV; a recommended procedure for the consideration of the release of genetically modified organisms is given in Section V).

V. Recommended procedure for the consideration of the release of genetically modified organisms (GMOs).

(a) Recognizing that little information exists on the genetic, ecological, and other effects of the release of genetically modified organisms into the natural environment (where such releases may result in the mixing of altered and wild populations of the same species, and in changes to the environment), the Council urges member countries to establish strong legal measures (*) to regulate such releases, including the mandatory licensing of physical or juridical persons engaged in genetically modifying, or in importing, using, or releasing any genetically modified organism.

(b) Member countries contemplating any release of genetically modified organisms into open marine and fresh water environments are requested at an early stage to notify the Council before such releases are made. This notification should include a risk assessment of the effects of this release on the environment and on natural populations.

(c) It is recommended that whenever feasible that initial releases of GMOs be reproductively sterile in order to minimize impacts on the genetic structure of natural populations.

(d) Research should be undertaken to evaluate the ecological effects of the release of GMOs.

(*) Such as the European Economic Communities "Council Directive of 23 April 1990 on the Deliberate Release into the Environment of Genetically Modified Organisms (90/220/EEC)", Official Journal of the European Communities, No. L, 117: 15 - 27 (1990).

VI. (= old Section V)