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Mariculture  
Committee

**Report**  
of the Working Group on Genetics  
1993

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**(1) Introductory remarks.**

According to the adopted resolutions (C.Res. 1992/2:49) of the 80th Statutory Meeting, Rostock-Warnemünde, Germany, Sept. 24 - Oct. 1 1992, the ICES-Working Group on Genetics was meeting this year in Sweden, at the "Swedish Salmon Research Institute", Älvkarleby, June 08-10, 1993. The participants have to express their severe thanks to Dr. Håkan JANS-SON and his cooperants, who were organizing the meeting at its best.

Again, the number of participants (see below) was small- much too low for an extended discussion of the recommended items. Even the written contributions were lesser in number than ever before so that the participants finally felt themselves misplaced. However, they were discussing the items of concern, but they did not come through to specific resolutions because of their limited number and the fact that most of the Älvkarleby attendants had primary interests related to genetic problems of natural fish stocks. This 'combination' - few attendants and a more or less concentrated interests to just one main sector of items within fish genetics - led only to some general conclusions which are repeated in position, "(5) Conclusions (with statements of wg.-members, pp. 6-10)".

**(2) List of working-group members (attendants at Älvkarleby-meeting\*; written contributions by\*\*, excused, but without statements\*\*\*).**

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**(3) Reports on genetics (verbal or written), presented in alphabetic order of the member countries (see also "Appendix 7a-7 f/g", pp. 11-28).**

- **7a Canada** (compiled by Jean-Marie SÉVIGNY, Dept. of Fisheries and Oceans, Institute Maurice-Lamontagne, Quebec, and Richard L. SAUNDERS, Dept. Fisheries and Oceans, Biological Station St. Andrews, N.B.).
- **7b Finland** (given by Marja-Liisa KOLJONEN, Finnish Game and Fisheries Research Institute, Aquaculture Division, Helsinki).
- **7c Germany** (given by Wolfgang VILLWOCK, University of Hamburg, Zoological Institute und Zoological Museum, Hamburg).
- **7d Norway** (compiled by Geir DAHLE, Knut JØRSTAD, Institute of Marine Research, Nordnes [Bergen] and Gunnar NÆVDAL, Dept. of Fisheries and Marine Biology, University of Bergen, Bergen).
- **7e Sweden** (given by Håkan JANSSON, Salmon Research Institute, Älvkarleby)
- **7f Scotland / UK** (given by Alan F. YOUNGSON and Eric VERSPOOR, SOAFD, Marine Laboratory, Aberdeen).

**(4) Brief summaries of above listed reports.**

As in the foregoing years, most of the papers deal with the different salmonids, especially with the Atlantic salmon, *Salmo salar* L. A very few contribute to genetic research on fresh-water fishes, as tilapias and others.

Only the Canadian report includes crustaceous species.

The main techniques are applied to stock discrimination and stock assessment of salmonids and other marine finfish species.

Electrophoretic techniques seem to have become introduced to nearly every reporting research group, mainly used for species or population discrimination purposes, respectively. Gene technology (in a broad sense) is nearly exclusively applied for the detection of genes coding "growth hormon" and being responsible for other special loci, and being involved in disease resistance.

"Genetic fingerprinting" is on further progress, however not consequently used but more "randomly" applied and not synchronized between the different groups of interest.

**(5) Conclusions.**

The make-up of the attending group (as well as the study of the written contributions) demonstrate the inhomogeneity on all levels of the real cooperating part of the present working-group of genetics a fact, that results in a lack of possibilities for the formulation of concrete recommendations to the items of concern. Therefore, the attending members of the Älvkarle-

by-meeting tried to come out with some general recommendations which might be helpful for reorganizing the Working-Group itself, finally resulting in a much more successful way of international cooperation than it has been true in the past and, by this to come to a real support of the Mariculture Committee.

The above mentioned considerations are the following (unanimously agreed by the attendants of the Älvkarleby-meeting, Friday, June 10, 1993):

(1) The present status of the Working Group on Genetics was discussed as a priority item in light of the poor attendance of Group members at the current and previous meetings. It was noted that those attending had primary interests related to the genetics of natural fish stocks as opposed to the genetics of those under culture. This was considered to be at odds with the direction of the group by, and the reporting of the group to, the Mariculture Committee.

The fundamental division of genetic interests in the marine sector into those relating to natural fisheries and to aquaculture was agreed by the attending members, even though the two areas clearly share a common methodology and have some overlapping interests. The division arises because the two have fundamentally different concerns:

**Fisheries genetics** is concerned with an understanding of the distribution and dynamics of genetic variation in natural fish stocks and its relevance to their management. This relates both to defining stocks for stock assessment as well as identifying and conserving biodiversity.

In contrast, **aquacultural genetics** is concerned with the manipulation (e.g. selective breeding, gene insertion) and management of the genetic composition (e.g. maintenance of heterozygosity, avoidance of inbreeding) of cultured populations toward the end of improving the quality and quantity of production.

Addressing both these concerns within a single group was felt to be problematic by allowing neither area of concern to be properly addressed. This may in part account for the poor attendance of the current Working Group, particularly by aquacultural geneticists. Those attending therefore put forward the suggestion that the current working group on genetics be split into two

- **Working Group on Aquacultural Genetics:** this would, as the current group does, report to the Mariculture Committee.
- **Working Group on Fisheries Genetics:** this would be constituted to report to Committees concerned with fish stock assessment and the conservation of biodiversity e.g. ANACAT or Fish Stock Assessment.

In this manner the appropriate committees for the respective areas of concern will be best served. However, it was recognized that there would potentially be a value in having overlapping, joint meetings on some occasions to exchange information on methodology and deal with issues of joint concern e.g. effects of escapes from culture on natural populations and sea ranching.

It was felt that the opinion of other non-attending members with regard to this proposal should be solicited by correspondence. A deadline for replies of 15 July is in order to ensure report to the Secretary General, or the Chairman of the Mariculture Committee, respectively, is sent on time (personal remark: the M.C. Chairman became informed preliminarily).

(2) The poor attendance of members meant that expertise needed to properly address the resolution adopted at the ICES Statutory Meeting, Warnemünde, Germany 1992 (C.Res.1992/2:49) with regard to the Working Group on Genetics was lacking. This accounts in part for the limited response to the specific requests for information. This is not to dismiss in any way the importance of the questions raised. Most certainly these questions will continue to be of great importance to fisheries and aquaculture management likewise.

2.1: The request to review and report on progress in research on biochemical markers and related techniques for species and stock discrimination is too general to allow for a specific response in the available time. The value of biochemical markers and the extent to which they are available is highly dependent on the species and the scientific aims involved. (this also leads to the proposal to divide the WG into two groups, each of which focuses on a different related set of scientific aims.) Furthermore, even within a species, the discriminatory capacity of a particular type of genetic probe (e.g. "fingerprinting" probes) can be highly variable. A general consensus appears to be emerging that in most situations it is unlikely to find natural fixed biochemical differences among stocks

which allow them to be unequivocally distinguished. However, enough genetic variation exists among and within most stocks to allow genetically marked stock groups to be generated for experimental studies or in the aquaculture context.

The Committee should also refer to the information contained in the appendices and to the already published reports of different recent symposia and workshops (e.g. see Biotechnology of Aquatic Animals. IUBS symposium Nov. 25-27, 1991, Toba City, Japan: This report pos. 8) where this topic has been covered in more detail than our group is able to do in the limited time available with the limited expertise present.

**(N.B. The reference should be to distinguishing between wild and cultured stocks not "species").**

2.21: "Genetic fingerprinting" is a method which depends on the the level of variability revealed by a given probe and its distribution among stocks. Furthermore, it is unclear as to whether the question asked relates to basic research development or applied methods. At present it remains primarily a basic research tool and has not yet been developed to the point of being used routinely in fisheries or aquacultural work.

The use of the phrase "gene technology" is uncertain and potentially ambiguous. It may lead to the conclusion that molecular methods are all directed at the genetic engineering of the genome of various species. The use of the more general term "methods in molecular genetics" avoids this pitfall. This distinction is important where the methods are used simply to reveal natural genetic variation for the analysis of the genetic structuring of wild populations of marine species.

2.2.2: The request for a working definition for a GMO seems to duplicate work already carried out by the joint meeting in Helsinki between the WGs on Genetics, and on Introductions and Transfers of Marine Organisms as well as by committees in other organizations e.g. EC and FAO where official definitions have now been formalized.

According to the feeling of the present members, the production of GMO's is just at the level of basic research (see papers in appendices) and it makes no sense to repeat all the statements which have already been formulated and published. No new aspects of the issue of GMO's which might require new deliberations were identified.

(3) Difficulties arise in trying to define general concepts in relation to the protection of the environment from aquacultural species. In this regard, however, we refer the Committee to the paper of the Special Study Group on the Genetic Risks to Atlantic Salmon Stocks whose recommendations have not yet been fully followed as far as we know. For example, the use of sterile strains of cultivated salmon has not been encouraged or steps taken to make it mandatory in the future.

The few limited genetic studies undertaken to date are insufficient to draw general conclusions on the genetic impact on natural populations or environments, respectively. Therefore there has been little new development of genetic concepts (i.e. principals) to guide aquacultural practices beyond those indicated by a consideration of the general body of population genetics knowledge and theory. The evaluation of impacts has to be continued, making use of new molecular methods as they become available, and management principals revised according to new findings. To do this is crucial to deal with the very valid concern of potential negative genetic impacts on natural systems that might arise from aquacultural activities (statements see encl. pp. 6-10).

#### **(6) *Personal closing remarks.***

According to my information of 1992 I hereby announce my resignation from the chair of the WG on Genetics in its present shape. It is impossible to chair a group of scientists who are in their majority not ready to cooperate in a constructive sense (no response on the yearly questionnaires, no show at the meetings without excusing themselves, etc.pp.).

However, there is another basic lack in the understanding of the member countries with respect to their delegates: Those who excused themselves for not joining the meetings more and more often pointed out that they will not get any financial travel support (as myself, too). The members of the ICES Statutory Meeting should address their member countries to chan-

ging this non-support or otherwise the meetings might be degraded to a rich peoples' talking circle, that does not seem to be the right way for continuing hard and necessary work.

In case, the Mariculture Committee might follow the conclusions of the Älvkarleby attendants I would be interested to cooperate further on in the "Aquaculture Working Group". Otherwise I am ready to stay in the WG on Genetics, however no longer as chairperson. By the way: Some suggestions for a successor failed, as it is documented e.g. by Dr. David THOMPSON, Lofestoft, UK.

Thanks to everybody who were supporting my efforts and contributed the one or the other way to the existence of the Working Group on Genetics up to the present moment.

Hamburg, September 1993

(Prof. Dr. W. Villwock)  
Chairman in Charge  
ICES Working Group  
on Genetics



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LAXFORSKNINGSINSTITUTET

Tidpunkt - Date

93-06-21

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Dear Wolfgang

My answers to the questions in your letter of 15.06.1993 are as follows: 1) I agree that the WG on Genetics will be split in two. 2) I prefer to belong to the WG on Fisheries Genetics. 3) I suggest Eric Verspoor as chairman for the WG on Fisheries Genetics.

Yours Sincerely

Håkan Jansson



Helsinki 21.6 1993  
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2. -7 / 8 -

Prof. Dr. Villwock  
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GERMANY

Dr. Villwock

I wanted to make still some remarks to the paper we wrote in Älvkarleby.

1) I feel that it is not our task to define how often the new WG:s should meet and how often they want to have joint meetings. It will depend on their own decisions and by making now binding involvements we unnecessarily make it more difficult for them to organise their meetings. I think that we can only note that we would see it usefull if they would organise joint meetings according to the needs.

2) I think that one argument more for the separation of the groups is that the back ground schooling of the people of these groups often differs clearly so that aquaculture people have learnt quantitative genetics and fisheries intrested people population genetics. One of the main differences in the attitudes of these two groups is that mariculture people tend to change the genetic structure of the cultured fish populations and the population geneticists usually try to conserve the natural populations and their biodiversity, in spite of fishing and aquaculture. Thus the sea ranching work should belong to the both WG:s according to its aims. If the aim is enhancement of wild population it comes to Fisheries WG, if selective breeding for sea ranched stocks as they do in Island, it belongs to WG on Aquaculture Genetics. It should also be conserved if the name of the Fisheries WG, should be something more clearly related to conservation and sustainable use. It might also clarify the question which should be discussed if the Convention on Biological Diversity had mentioned.

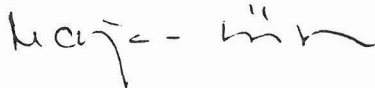
3) I am also dissatisfied with the aims for the Aquaculture WG, I dont understand the sentence about GMO:s. The tasks for this WG are also to negative. They are mostly people who are intrested to advance mariculture and thus they object should also be to develop methods for making aquaculture more safely and succesfully. This should also include work done for ordinary selective breeding not only GMO:s and other modern techniks one of the tasks might for example be how to combine these GMO:s and selective breeding with a most succesfull way.

4) There exists no Fish Stock Assessment Committee, to which to report. There is Advisory Committee on Fishery Management, which is the the roof Committee for the other standing comittees, which can have WG:s. From these I believe three could be relevant as reporting for Fisheries WG on Genetics: Demersal Fish Committee, Pelagic Fish Committee and Anadromous and Catadromous Fish Committee (, which I believe might be most intrested to have us as their WG, at least now most of the genetic management problemms are related to salmonids.). I think that a letter to the chairmans of these Committees is needed to introduce us and our intrest on working for fishery management and genetically sustainable consumption.

In addition I should have wanted emphasise that when new members are to be schosen they are people, who are actively making research in this field. Probably it not any more unclear but I would be intrested in working in the Fisheries WG on Genetics (or what ever tha name will be). For the Aquaculture Group I can recommend Liisa Siitonen from Finland, who is working with selective breeding of rainbow trout at our Istitute.

I hope this helps you in writing the paper ready.

With best wishes,

A handwritten signature in dark ink, appearing to read 'Marja-Liisa' followed by a stylized flourish.

Marja-Liisa Koljonen





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7 July 1993

Dear Professor Villwock

#### ICES WORKING GROUP ON GENETICS

The draft report of the recent meeting of the WG which you circulated last month outlines quite accurately the problems of the current WG and makes sensible suggestions for the future. The division of the existing WG into two new WG's each considering different aspects of genetics and reporting to different committees should make the objectives of each new WG more precise. However, after some discussion, my stock assessment colleagues and I believe that the proposed terms of reference for the new WG on Fisheries Genetics may still be too broad. Whilst undoubtedly the type of population genetics that most of us are familiar with can make a useful contribution to monitoring biodiversity within and between populations, which may be linked to stock identification for management purposes, any attempts to relate biodiversity to stock assessment and population dynamics will be much more complex and will require the involvement of experts in the latter field. Our suggestion would be that this is a specialised subject which could benefit from the attention of a specific ad hoc study group meeting. In fact we have some reservation on the need for a permanent WG on Fisheries Genetics at all and suggest that the possibility of 'study groups' being formed to solve specific problems or when significant advances in genetic research have been made could be considered. This reservation may be particularly relevant at the moment as the ICES Study Group on Stock Identification will be meeting this August in Copenhagen and will, no doubt, make recommendations on the application of various methodologies, including genetics, in fisheries management. Perhaps we should wait until this group has reported before making any firm recommendations to the Statutory Meeting.

With respect to the suggestion of proposing me as a candidate to chair the Fisheries Genetics WG I have to decline the invitation. I am afraid that to develop the WG in the directions that are currently suggested would be moving into areas outside my research experience. Unfortunately my existing commitments at Lowestoft make it impossible for me to devote the time required to carry out the task satisfactorily. Thank you, however, for considering me.

Best wishes.

Yours sincerely

David Thompson

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Prof Dr W Villwock  
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Dear Wolfgang

As per the meeting in Alvkarleby, this is to confirm that I am in full agreement with the proposals put forward. I have nothing to add to the responses to the questions put to the Working Group by the Mariculture Committee.

I look forward to the reaction of ICES to the proposal!

Until then....

Yours sincerely



Eric Verspoor

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

International Council for the  
Exploration of the Sea

Working Paper:  
ICES Working Group  
on Genetics

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

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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 transgenic specimens and measures to reduce risks of genetic interaction between cultured, genetically altered salmonids and other aquatic organisms and wild stocks.

**Robert Devlin, Department of Fisheries and Oceans, West Vancouver Lab,  
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#### Genetic fingerprinting

DNA probes from chinook salmon have been developed and are capable of distinguishing different Pacific salmon stocks, and can identify related individuals from mixed groups of families as would be found in nature. These probes have been used to successfully identify escaped farmed fish among wild fish recovered from spawning habitat and can therefore be used to assist with impact assessments of such accidents.

#### Sex identification

Molecular characterization of the Y-chromosomal DNA probe isolated from chinook salmon is continuing. Although this probe does not appear to be sex-limited in other salmonid species, a new probe has been identified which can distinguish genetic males and females in four salmonid species.

#### Transgenic fish

Work is continuing on the production of transgenic Pacific salmon with improved growth performance and with altered reproductive capabilities (sterilization). Transgenic coho salmon have now matured and transmitted the new genetic information and altered phenotypic characteristics to the next generation. Considerable effort is under way to improve large-scale methods of triploidy induction for use in the biological containment of GMO's.

**James D. Reist, Department of Fisheries and Oceans, Central and Arctic Region,  
Freshwater Institute, Winnipeg, Manitoba R3T 2N6**

#### Identification of arctic fishes at inter- and intra-specific levels

This research program concentrates upon the use of allozymes and morphological data to identify taxa of arctic fishes of both the inter- and intra-specific (i.e. genetic stock) levels. The work is in direct support of fisheries management in the Canadian arctic. In order to clarify taxonomic ambiguities between North American and Eurasian arctic fishes, Dr. Reist, with a co-worker (Dr. D. Bodaly) at this institute, also conduct similar genetic research on Holarctic taxa. This research has to date focused upon arctic coregonids and charrs. Future research will likely include marine fish species such as Greenland halibut as well as freshwater species of importance in fisheries. Future techniques to be applied will also likely include mtDNA and related DNA work in an effort to establish the

genetic uniqueness of stocks and/or taxa for conservation purposes. All work is of a survey nature conducted on natural populations.

**Peter Ihssen, Ontario Ministry of Natural Resources, Fishery Research Lab,  
Maple, Ontario**

Mapping of production traits in rainbow trout using genetic polymorphisms at the mtDNA and nDNA level

In collaboration with the University of Guelph (Moir Ferguson, Roy Danzmann, Ian MacMillan), a project, in part funded by an INSERC grant to Moira Ferguson and Roy Danzmann, was initiated to study the relationship between desirable production traits and DNA markers. Rainbow trout stocks genetically differentiated for growth rate, seasonal spawning time, and age of maturity are being interbred and backcrossed to produce strains in which the genes effecting these production traits segregate. Simultaneously, a set of genetic markers is being developed (University of Guelph) that will permit classical linkage studies between quantitative genetic traits and DNA markers. Restriction Fragment Length Polymorphisms (RFLP's), in conjunction with nuclear DNA polymorphisms (determined by the PCR technique using arbitrary primers to amplify anonymous regions of genomic DNA) will be used to develop an extensive marking system for the rainbow trout genome. These markers, once identified, and linked to production traits, will then be used in breeding programs to produce strains with desirable features.

Genetic differentiation of brook trout (*Salvelinus fontinalis*)

An extensive survey of brook trout populations in Ontario (with an emphasis on Algonquin Park populations) is in progress in collaboration with the University of Guelph. Allozyme variation and quantitative genetic characters (growth, age of maturity) in conjunction with mtDNA variation (University of Guelph) are being used to assess the glacial history of stocks and the genetic impact of recent hatchery supplemental stockings. Many of Ontario's rivers and lakes still contain pure native brook trout stocks; however, for some lakes and rivers, the native gene pool has been significantly augmented by hatchery stock genes. These findings will be used to develop management plans for brook trout that have as one of their major objectives the preservation of the genetic diversity of Ontario's brook trout stocks.

Application of mtDNA, nDNA and allozyme polymorphisms to identify progeny derived from adult transfers originating from several walleye (*Stizostedion vitreum*) populations

Three walleye stocks (Ombabika Bay, Georgia Lake, Lac des Mille Lacs) are being used as donor stocks to re-establish a walleye population in Nipigon Bay Remedial Action

Plan (RAP). In order to determine the relative genetic contribution of the donor stocks and the breeding structure of the newly established population, genetic markers (mtDNA, nDNA and allozyme) that differentiate the three stocks were developed. To date sufficient markers at all three molecular levels have been found to accurately discriminate among the three stocks. In particular, a nDNA marker developed via the randomly amplified polymorphism DNA (RAPD) method was found that distinguished the Ombabika Bay from the Georgia Lake stock. These two stocks could not be very precisely differentiated using the other two systems (allozyme, mtDNA) alone.

Genetic impacts of supplemental stocking on the effective population size of lake trout (*Salvelinus namaycush*) stocks: application of nuclear DNA techniques

In collaboration with McMaster University (Brad White) and a graduate student (Wendylee Stott), a research project was initiated to develop a DNA-based genetic marking system that will permit an accurate assessment of the genetic contribution of supplementally planted fish to native populations, and to estimate their effective population size. Fingerprinting, sequencing and PCR-based techniques will be used to find nuclear genetic markers that are needed for this application. The genetic marking system will be applied to a series of lake trout populations that are postulated to have been genetically impacted by supplemental stocking to varying degrees.

**John M. Anderson, Atlantic Salmon Federation, P. O. Box 429, St. Andrews,  
New Brunswick E0G 2X0**

Identification of escaped aquacultured salmon

Studies in 1992 on the Magaguadavic River in New Brunswick, which is in close proximity to the salmon aquaculture industry in the Bay of Fundy, shows that 34% of the salmon returning to spawn are escapees from the sea cages. External appearances, notably fin conditions, could detect only about 50% of the escapees. The remainder were identified by scale analysis. The results from DNA fingerprinting to investigate the possibility of discriminating between salmon of wild and aquaculture origin are not yet available.

The investigation continues in 1993.

**Gerald W. Friars, Salmon Genetics Research Program, Atlantic Salmon Federation,  
P. O. Box 429, St. Andrews, New Brunswick E0G 2X0**

Management of genetic variability in reared Atlantic salmon stocks

A major concern in relation to farmed escaped salmon is that these fish may be inbred and thus by interbreeding with wild salmon, may produce progeny of reduced genetic variability. While some reared strains have been shown to be inbred, well planned breeding programs are designed to minimize inbreeding, since this effect is detrimental to performance. The measures used to avoid inbreeding in the Atlantic Salmon Federation (ASF) in eastern Canada, are described. An allozyme study which investigated seven enzyme loci in seven strains of salmon reared by the ASF, two of which had artificial selection applied, is then detailed. It is shown that in all four year-classes of Saint John River-origin strains (the stock most used by the eastern Canadian cage industry), there is little evidence of inbreeding when levels of genetic variability are compared with those in a wild Saint John River sample. Also, selection applied to one year-class of this strain has not reduced variability compared with controls. In contrast, substantially lower variability was evident in a strain of mixed population origin which had been sea ranched for a number of generations. In this strain, select and control fish had also diverged in genetic composition. This strain is not used extensively by the cage industry.

**Jean-Marie Sévigny (and associates as indicated)**

Use of the genetic variability at the liver MDH locus to determine the specific composition of redfish (*Sebastes* sp.) in the Gulf of St. Lawrence (J. M. Sévigny<sup>1</sup>, Y. deLafontaine<sup>2</sup>, J. J. Dodson<sup>3</sup>, B. Morin<sup>1</sup> and P. Gagné<sup>3</sup>)

Juveniles and adult of *S. fasciatus* and *S. mentella* are difficult to separate on a morphological basis and there are no readily available criteria to identify their larvae. This identification problem has imposed a limit to our understanding of the biology of the *Sebastes* species in the Gulf of St. Lawrence. For the last few years, genetic variability at the liver MDH locus has been studied systematically in larvae, juveniles and adults of the Gulf of St. Lawrence in an attempt to describe the life cycle characteristics (spawning time, fecundity, cohort analysis, etc...) of those sibling species.

<sup>1</sup>Ministère des Pêches et Océans, Institut Maurice-Lamontagne, C.P. 1000, Mont-Joli, Québec G5H 3Z4

<sup>2</sup>Environment Canada, Conservation et Protection, Centre Saint-Laurent, 105, McGill, Bureau 400, Montréal, Québec H2Y 2E7

<sup>3</sup>Département de biologie, Université Laval, Sainte Foy, Québec G1K 7P4



Description of new genetic markers for the redfish species (*Sebastes*) of the Gulf of St. Lawrence using PCR (B. Desrosiers<sup>4</sup> and J. M. Sévigny)

Redfish (*Sebastes*) from the Gulf of St. Lawrence are difficult to identify. The variability at the liver MDH locus indicates the presence of two genetically different groups (*S. fasciatus* and *S. mentella*). However, the two groups share an allele at this locus and it has been hypothesized that the two groups hybridized in the Gulf of St. Lawrence. The goal of the present project is to describe other genetic markers using polymerase chain reaction (PCR).

Genetic variation in the Greenland halibut, the northern shrimp and the snow crab from the St. Lawrence system and the northwest Atlantic (J. M. Sévigny<sup>1</sup>, B. Sainte-Marie<sup>1</sup> and L. Savard<sup>1</sup>)

These population genetics research projects were initiated to describe the genetic structure of the Greenland halibut (*Reinhardtius hippoglossoides*), the northern shrimp (*Pandalus borealis*) and the snow crab (*Chionoecetes opilio*) in eastern Canada. 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 St. Lawrence and the Saguenay fjord. Allozymic and, for the snow crab, mtDNA variations are used as genetic markers.

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.

Development of locus-specific VNTP-probes for the delineation of breeding population of the snow crab (*Chionoecetes opilio*) (U. Kuhnlein<sup>5</sup>, J. M. Sévigny<sup>1</sup> and B. Sainte-Marie<sup>1</sup>)

This project will be undertaken in 1993. VNTP-probes will be developed. They will be used to delineate breeding populations of snow crab.

<sup>4</sup>Département d'Océanographie, Université du Québec, Rimouski, Québec G5L 3A1

<sup>5</sup>Département d'Agriculture, MacDonald College, 21,111 Chemin Lakeshore, Sainte-Anne-de-Bellevue, Québec H9X 1C0



Study of the genetic aspects of the summer mortality of cultured mussels in the Magdalene Islands (J. M. Sévigny<sup>1</sup>, J. J. Dodson<sup>3</sup>, M. Fréchette<sup>1</sup> and M. Alunno-Bruscia<sup>3</sup>)

A multidisciplinary research project was initiated to study the genetic aspects of the summer mortality that affected mussels grown in the Magdalene Islands (Gulf of St. Lawrence). Genetic markers are used to test the following hypotheses: a) the presence of two mussel species in the Magdalene Islands lagoons, b) the possibility that aquaculture practices influence the genetic makeup of the population by selecting for animals having a shorter life cycle and earlier maturity. Both protein and mtDNA markers are used to detect genetic variability of mussel.

**François Dubé, Dépt. d'Océanographie, Université du Québec à Rimouski, 310 allée de Ursulines, Rimouski, Québec G5L 3A1**

Our research deals with the embryonic development processes of various marine invertebrate species, particularly bivalves. The blue mussel (*Mytilus edulis*), the giant scallop (*Placopecten magellanicus*) and the hard clam (*Spisula solidissima*) have been used in the various studies on intracellular mechanisms responsible for the initiation of the cellular cycle following the fecundation. Intracellular calcium ions and pH variations have been analyzed in the hard clam ovocyte. These variations seem to play a key role in the initiation of embryonic development. Protein synthesis and phosphorylation have also been studied on hard clam and blue mussel ovocytes. Protein synthesis and phosphorylation patterns have been quantitatively and qualitatively characterized by unidimensional gel electrophoresis and autoradiograms. Precise identification of protein substrates involved in the cell division control represent a major component of our research. The giant scallop ovocyte has been used for the development of a new triploid embryo production method. The efficiency of 6-dimethylaminopurine (6-DMAP) to produce triploid larvae has been demonstrated. These studies will continue over the next years. A more complete molecular characterization of some proteins involved in the regulation of the cellular cycle of these invertebrate species will be initiated.

**Choy L. Hew, Department of Clinical Biochemistry, Banting Institute,  
100 College St., University of Toronto, Toronto, Ontario M5G 1L5**

**Garth L. Fletcher and David R. Idler, Marine Sciences Centre, Memorial Univ. of  
Newfoundland, St. John's, Newfoundland**

**Peter L. Davies, Biochemistry Department, Queens Univ., Kingston, Ontario**

**Richard L. Saunders, Dept. of Fisheries and Oceans, Biological Station,  
St. Andrews, New Brunswick**

This team of researchers has been working for some time on the production of Atlantic salmon with genes from other fishes which produce antifreeze proteins. Salmon mariculture in Atlantic Canada is constrained by the relatively small area in which temperature does not reach the lower lethal (-0.7 to 0.9°C) for salmonids. It is hoped that

salmon can be bred having increased resistance to freezing which some marine fishes have. The gene is expressed in salmon developed from eggs into which the gene was injected. The work continues with production to subsequent generations from transgenic individuals.

More recently, the team has been producing transgenics incorporating chinook salmon growth hormone. The "all fish" gene construct has been designed to be useful in aquaculture. Transgenic individuals show growth rates several times greater than non-transgenic sibs. Some male transgenics matured and have been used as sires for control females. Emphasis is on production of sufficient numbers of first,  $f_1$ , and subsequent generation transgenics to conduct studies on growth dynamics, smolt development, sexual maturation and heredity.

### **Policy and guidelines for research and release of genetically modified aquatic organisms in Canada**

The Dept. of Fisheries and Oceans has developed a draft Policy and Guidelines on the use of transgenic aquatic organisms. The draft document is at the initial public comment stage. It will form the basis for regulations under the Fisheries Act on research, utilization, and safeguards within which the relevant industry groups can make plans.

The draft guidelines consider the information required for conduct of research with transgenic organisms, information relating to the transgenic organisms, information on conditions of use in research laboratories and aquaculture and the receiving environment, possible interactions between the transgenic organisms and the environment, monitoring, control and emergency response plans.

## CURRENT GENETIC STUDIES ON FISHES IN FINLAND

Report to ICES Working Group on Genetics

Marja-Liisa Koljonen

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

- a) Population genetic studies by enzyme electrophoresis on Baltic salmon and brown trout stocks in connection of conservation and enhancement projects.
- b) Selective breeding programme for rainbow trout.
- c) Genetic stock identification of Baltic salmon stocks.

Agricultural Research Center, Department of Animal Breeding, SF-31600 Jokioinen, Finland (Kari Elo).

- a) Species identification, phylogenetic analysis and genomic variation in Coregonids using random amplified polymorphic DNA (RAPD).

University of Joensuu, Department of Biology, P.O. Box 111, SF-80101 Joensuu, Finland (Jukka Vuorinen).

- a) Electrophoretic studies on Coregonids evolution.

University of Kuopio, Department of Physiology, P.O. Box 6, SF-70211 Kuopio, Finland (Otso Järvisalo).

- a) Growth hormone gene transfer project on rainbow trout.

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

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Name and full address of the member signed below:

Prof. Dr. W. Hesse  
University of Hamburg  
Zoologisches Institut  
und Fischereimuseum  
Martin-Luther-King-Platz 3  
D-20046 Hamburg  
Fed. Rep. of Germany

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

a)

b)

SEE ENCLOSURE:  
reported research was  
continued

c)

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

a)

b)

SEE ENCLOSURE!  
referred research will be  
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: April 30. 1993!**

Date .....

Name .....

Prof. Dr. W. Villwock

Fed.Rep.of Germany: annex 2, p. - 23 -

Zool. Inst. u. Zool. Museum  
Universität Hamburg  
Martin-Luther-King-Pl. 3  
D-2000 Hamburg 13

June 04, 1991

Fed.Rep.of Germany

***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 applicability of their results.

(Prof. Dr. W. Villwock)

Bibliography (partim) concerning aspects of 'fishgenetics, aquaculture and immune response' (with special reference to applied aspects).

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- GROTH, V., RENWRANTZ, L., VILLWOCK, W., 1984a: Bloodgroup determination in fish by means of lectin agglutination tests. Oral presentation on the 2. status seminary of the German - Israelian cooperation on aquaculture in Hamburg, FRG, 5.-6.3.1984.
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- GROTH, V., VILLWOCK, W., RENWRANTZ, L. 1987: Demonstration of carbohydrate containing molecules in isolated erythrocyte plasma membranes from the teleost *Cyprinus carpio* L. Oral presentation on the 3. status seminary of the German-Israelian cooperation on aquaculture in Tiberias, Israel, 27.4.-1.5.1987. Published in: European Mariculture Society, Bredene, Belgium, EMS Special Publication (in press).
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- OBERST, S., VILLWOCK, W., RENWRANTZ, L. 1988b: Immunbiologische Methoden zur Differenzierung zwischen verschiedenen *Tilapia*-Arten. Verhandlungen der Deutschen Zoologischen Gesellschaft, 81. Jahrestagung in Bielefeld, 23.-28.5.1988. G. Fischer Verlag Stuttgart, New York, 1988.
- OBERST, S., VILLWOCK, W., RENWRANTZ, L., 1989: Antisera from *Tilapia* species to differentiate among erythrocytes from *T. aurea*, *T. galilaea* and *T. nilotica* by agglutination assays, and a comparative analysis of hemoglobins. J. Appl. Ichth. 1, 18-27.
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Not to be cited without prior references to the autor

ICES Working Group  
on Genetics

Working Paper  
Älvkarleby, Sweden,  
June 1993

GENETIC STUDIES  
RELATED TO  
AQUACULTURE AND FISHERIES  
RESEARCH IN NORWAY  
IN 1993

An overview compiled by

Geir Dahle, Knut Jørstad  
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P.O.Box 1870, Nordnes,  
N-5024 BERGEN - Norway

and

Gunnar Nævdal  
Department of Fisheries and Marine Biology  
University of Bergen  
Bergen High-Technology Center  
N-5002 BERGEN - Norway

## INTRODUCTION

This report represent an updated version of genetic studies related to aquaculture and fisheries research compiled for the ICES Working Group of Genetics in 1993.

Studies on genetic variation and population structure of commercial important marine species for better resource management, and studies on quantitative genetics of productive traits with the aim of genetic improvement for aquaculture are still beeing carried out. Interrelations between natural and farmed populations have become of increasing importance, both in relation to aquaculture and sea ranching.

In the following oveview 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 and Marine 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* from Iceland and Greenland waters are in progress. The Institute of Marine Research has included DNA.fingerprint (multilocus) analysis in the redfish studies.

Genetic studies on cod and herring stocks have been continued at the Institute of Marine Research, including analysis of new yearclasses. The last mentioned work is mainly focused on yearclass variation and identification of subpopulations by using protein electrophoresis, restriction fragment analysis of mtDNA and DNA fingerprinting (multilocus). Studies on resident and anadromous brown trout populations by enzyme electrophoresis 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 investigation is use of genetically tagged cod.

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



- 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.

Investigation on enzyme polymorphism for use in population studies on harp seal and hooded seal are nearly completed at the Department of Fisheries and Marine Biology and Institute of Marine Research. Studies on the same species by use of multilocus DNA probes will be terminated this year. Similar studies on minke whale and harp seal applying enzym electrophoresis and sequencing of specific mtDNA regions are carried out at Department of Medical Biology, University of Tromsø.

#### GENETIC TAGS APPLIED IN SEA RANCHING FOR STUDIES OF GENE INTROGRESSION

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

Likewise a biochemical genetic marker has been identified in cod, and a homozygous 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. Genetically tagged cod have been released for three years, and data on recoveries are now being collected.

Genetic analysis 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 initiated.

## GENETIC IMPROVEMENT OF SALMONIDS - CLASSICAL QUANTITATIVE GENETICS

The large scale programme for genetic improvement of salmonids initiated by the Norwegian Fish Farmers Association and Institute of Aquaculture Research (AKVAFORSK), Ås, are continued. The breeding programme is carried out at the breeding station at Kyrksæterøra and at Sunndalsøra.

Institute of Aquaculture Research continue to carry out quantitative genetics on salmonids at the research stations at Sunndalsøra and Averøy, 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 parameters of "new" productive traits are also estimated.

Additive genetic variation 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 and Atlantic salmon. Datatomography was found to be of considerable help in the registration of body composition in fish. Non-additive genetic variation explain a minor part of total genetic variation for these 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 immunresponse is studied. These studies are carried out in cooperation with Department of Animal Breeding, Agricultural University of Norway, and Department of Microbiology and Immunology, Vetereinarian University of Norway. Challenge tests have shown great differences in mortality between full and half sibs families when exposed to furunculosis, vibrosis and cold water vibrosis. A project for studying the ironbinding proteins (transferrin) and their effect on disease resistance has been started. In -vitro tests on fish patogens are carried out, and also cell lines and model fish are are used for studies on gene regulations.

At Institute of Marine Research, Aquaculture Station, Matre, studies of trypsin isozymes in salmonids have continued. These involve studies on the inheritance control as well as growth performance of selected families and specific genotypes. These trypsin studies show a correlation between genetic variation and growth rate in the Atlantic salmon. Identification of salmon with the "right" trypsin variant based on DNA markers will be carried out.

## CHROMOSOME ENGINEERING

Pilot studies on production of triploidy are carried out at Institute of Aquaculture Research, Sunndalsøra, with the aim of producing sterile halibut using heat shock.

## GENE TECHNOLOGY

Characterization and isolation of genes coding for growth hormones, prolactin, trypsin, isozymes, insulin and genes involved in disease resistance have been undertaken by several laboratories with the double aim of basic studies of these 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 construction of "genome libraries" and studies on homeobox genes of salmon.

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

Laboratory for Biotechnology, Univesity of Bergen,  
Bergen High-Technology Center, N-5020 BERGEN.

Department of Biotechnology,  
Norwegian Technical University,  
N-7034 Trondheim.

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

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

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

# **STUDIES ON BIOCHEMICAL MARKERS FOR STOCK DISCRIMINATION AND MONITORING OF GENETIC CHANGES IN ANADROMOUS SALMONIDS.**

by Håkan Jansson  
Salmon Research Institute  
S-810 70 Älvkarleby  
SWEDEN

## **Population genetic structure of Atlantic salmon (*Salmo salar* L.) from two northern Swedish rivers.**

Atlantic salmon parr from R.Kalix älv were analysed by isozyme electrophoresis. Significant genetic heterogeneity was found at three of five polymorphic loci. Three distinct clusters were revealed. Each cluster comprised salmon from a restricted part of the river. The result is evidence of at least three distinct salmon populations within R.Kalix älv. The main difference was found between salmon upstream and downstream a partially impassable waterfall (Jokkfall).

Isozyme analyses of smolts from R.Torne älv did not reveal any genetic heterogeneity. Three samples were taken on different occasions during the migrating season. The result can be interpreted in two ways: 1) The sampling strategy did not reveal genetic differences due to overlap of migration times of local populations. 2) There is genetic homogeneity within the river.

## **Introgression from hatchery stocks of brown trout (*Salmo trutta* L.) into a natural population.**

The influence of stocking with two non-native brown trout stocks into R.Dalälven was studied by isozyme electrophoresis. The introgression from the hatchery stocks into the native natural population was estimated to be less than one percent.

## **Loss of genetic variation in a hatchery stock of brown trout (*Salmo trutta* L.).**

The degree of genetic variation in the Weichsel (Vistula) stock of brown trout at the Kälärne hatchery was measured by isozyme electrophoresis, and compared to earlier studies of the same stock. The loss of genetic variation based on number of polymorphic loci was estimated to 45%. This dramatic reduction of genetic variation can be attributed to the use of a restricted number of breeders.

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

---

Name and full address of the member signed below:

A F Youngson and E Verspoor  
SOAFD Marine Laboratory  
PO Box 101  
Victoria Road, Torry  
Aberdeen AB9 8DB  
Scotland

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

- a) Studies of genetic population structure in Atlantic salmon, by examining variation in protein-coding loci and in mitochondrial and nuclear minisatellite DNA.
- b) Studies of adaptive genetic variation among Atlantic salmon populations, assessing performance variation in the field and testing hypotheses by means of laboratory experiments.
- c) Studies of social factors in Atlantic salmon, at spawning and during emergence and dispersal of juveniles using genetic techniques to identify parents and families.

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

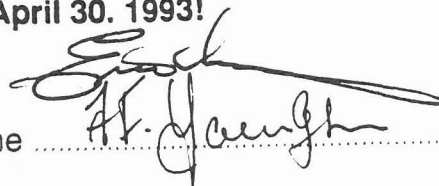
- a) Identification of novel mtDNA markers for stock delineation using PCR methodology to allow non-destructive genetic typing.
- b) A study of the performance of genetically different stocks drawn from widely separated catchments in Scotland. Differences in performance will be assessed among stocks and their crosses, competing in a single, managed stream, using planted green ova.
- c) A study of the effects of redd distribution and competition among families during emergence, on variation in survival among families and the utilisation of available fry habitat in rivers.

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: April 30. 1993!**

Date 8<sup>th</sup> April, 1993

Name

  
A. F. Youngson

2d. Development of a genetic model for the typing of phenotypic variation observed for IDDH in Atlantic salmon, using starch-gel electrophoresis.

2e. Retrospective analysis of the genetic consequences of the spawning of escaped farmed salmon in the River Polla in northern Scotland.

2f. Computer modelling of Atlantic salmon populations within rivers to describe population sub-structuring.