Mariculture Committee **ICES CM 1998/F:1**

REPORT OF THE

WORKING GROUP ON THE APPLICATION OF GENETICS IN FISHERIES AND MARICULTURE

Cork, Ireland
30 March–3 April 1998

International Council for the Exploration of the Sea
Conseil International pour l'Exploration de la Mer

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1 INTRODUCTION

As decided by the ICES Council in ICES C.Res.1997/2:23 adopted at the Annual Science Conference in Baltimore, USA, the Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM; Chairman: J. Mork, Norway) met at the National University of Ireland, UCC Cork, Ireland, 30 March–2 April 1998 to address its Terms of Reference (Annex 1).

1.1 Attendance

There are currently 51 appointed members and observers in WGAGFM. Nineteen of these members, from twelve countries, attended the 1998 WG meeting in Cork, Ireland (Annex 2). Countries represented (number of persons in parenthesis) were Belgium (2), Canada (2), Denmark (1), Germany (1), Finland (1), France (1) Iceland (2), Ireland (1), Norway (2), Poland (2), Sweden (2), and UK (2).

In addition, ten observers from Ireland attended various parts of the meeting: A. Norris, M. O. Stefansson, P. Galvin, G. C. Mouzakitis, S. Martin, M. Cross, P. McGinnity, A. Langston, J. Coughlan, E. Dillane.

As in the four previous years, the representation on the quantitative genetics was lower than on the qualitative genetics side. The composition of the qualitative and quantitative sub-groups during the meeting were:

Qualitative genetics sub-group: **T. Cross,** (leader), P. Boudry, P. Bossier, G. Dahle, W. Davidson, A.K. Danielsdottir, A. Ferguson, M. M. Hansen, E. Kenchington, M.-L. Koljonen, M. Luczynski, N. Lundblad, J. Trautner, E. Verspoor, F. Volckaert, E. R. Wenne.

Quantitative genetics sub-group: **J. Jonasson** (leader), J. Nilsson.

1.2 Organization of the Meeting

Prior to the meeting, certain members agreed to prepare position papers related to specific issues in the Terms of Reference (ToR), and to chair the respective sessions. During the meeting, these position papers were first presented and discussed in plenary. Thereafter, each topic was discussed in *ad hoc* sub-groups which prepared an updated text for a final plenary discussion and editing for the WG Report.

* J. Mork chaired "Selective fisheries". (ToR point a1).
* E. Kenchington chaired "Genetically modified organisms (GMO)". (ToR point a2).
* J. Jonasson chaired "Genetic management of new species in mariculture". (ToR point b).
* T. Cross chaired "Genetic management of pelagic fishes" (position paper co-authored with Gary Carvalho, University of Hull, UK). (ToR point c).
* M. M. Hansen chaired "Sampling strategies in studies of genetic structure". (ToR point d).
* A. Danielsdottir compiled the National Activity Reports. (ToR point e).

The session chairmen were responsible for leading the respective colloquia, the subsequent plenary sessions, and for preparing the final report text from their sessions.

All members had been asked to collect national activity reports from their respective countries and bring them (on diskette) to Cork. A preliminary report on national activities could thus be compiled during the meeting.

The Working Group decided that, as in the four previous years, the preparation of the WG Report should mainly be done by the members present at the meeting. This year, the Chairman will put a preliminary version on the (external) WGAGFM homepage and notify the participants by e-mail to check the contents. The participants should direct their comments on specific sections to the chairpersons for those sections, who in turn send the updated versions to the Chairman for inclusion in the WG Report which is submitted to ICES.

2 TERMS OF REFERENCE FOR 1998

2.1 Selective Fisheries

[Based on a position note by J. Mork, Norway. Adopted by WGAGFM in Cork]

Overview of the treatment of the selective fisheries topic in WGAGFM

1994 (Copenhagen): WGAGFM suggested that the theme "Genetic effects from selective fishing gear" be put on the terms of reference for 1995, and be subject to a combined treatment from the quantitative and qualitative geneticists in WGAGF.

1995 (Copenhagen): WGAGFM restricted the treatment to a principle level, and concluded that the complexity of the problem calls for input from external expertise, such as modellers and fishery biologists. Also, estimates of genetic parameters from aquaculture could be utilised, although the controlled environment in aquaculture might cause some representativity problems. A number of potential selecting factors from current fishery practice were identified, and it was concluded that among expected effects from current practices are reduced growth and traits correlated with that. It was further concluded that, in practice, genetic changes to a population are inevitable effects of all harvesting which is not random for genotypes. Net gear does not catch randomly. However, although one must probably 'live with' genetic changes due to harvesting, one should gain knowledge about the type and amount of genetic change going on in order to avoid changes that may reduce, e.g., the productivity or the evolutionary potential of populations.

1996 (Faro): Simulation modelling which links quantitative genetics, fish biology and fish exploitation rates in a consistent way is now under way. In cooperation with Dr K. Stokes (MAFF), a newly developed simulation model was tried out on data from English cod landings between 1980 and 1990. An important outcome of the study was the importance of traits correlated with growth (e.g., age at maturity). For example, although net gear selection in general is expected to change the population mean towards slow-growing, late-maturing individuals, a minimum-length net gear selection regime for the North Sea cod actually appears to select for fast growth and early maturity. WGAGFM recommended further studies along the line represented by simulation modelling, and emphasized the need for reliable estimates of parameters going into the models. These parameters will often be different for different populations.

1997 (Gdynia): WGAGFM undertook a review of published studies on qualitative and quantitative genetic effects of harvesting, and a comprehensive literature list was produced. Among the main conclusions from this review were that:

- most empirical studies have been made on already overfished stocks and may be biased;
- the background environmental 'noise' makes phenomena in natural populations extremely difficult to interpret;
- in the short term, modelling may therefore the best option for revealing general aspects;
- realistic modelling requires quality input parameters produced specifically for each stock.

1998 (Cork): It was noted that recent computer software available for use in the management of brood stocks has some qualities and options which might make them useful for application on the selective fishery problem. It was also noted that some research milieu are actively exploring new techniques and data to bring this research field forward.

It was further noted that the potential impact from fisheries probably varies between species, as do the measures that can be taken to avoid impact. One measure that most certainly removes the selection factor is a total banning of fishery in specific areas or time periods ('Boxes'). A more detailed discussion of this point is planned for the WG meeting in 1999 (refer to the suggested Terms of Reference for 1999, Section 3.3, point d).

Summary of recommendations on selective fisheries

1) WGAGFM emphasised that stocks should be monitored for relevant traits (e.g., age at maturity, growth rates, spawning period, migration patterns, etc.) so that potential selection effects can be identified as early as possible.
2) WGAGFM reviewed recent literature which emphasizes, e.g., the effect of the age composition of the spawning stock as a significant factor for year class strength. In this connection, it was noted that some regulation regimes in current use may have effects on age composition which in fact are not considered beneficial for stock recruitment.
3) In the monitoring of biological traits of populations, time series of data which make it possible to sort out effects of environmental changes would be especially valuable, and efforts should be made to identify and/or produce such

data (e.g., age at maturity data during medium-term shifts in the temperature regime, e.g., from an upward trend to a downward trend, could make it possible to identify the variance component due to temperature and thus make it possible to reduce substantial 'noise' in the data sets).

2.2 Genetically Modified Organisms (GMOs)

[Based on a position paper by E. Kenchington, Canada. Adopted by WGAGFM in Cork]

The 1996 WGAGFM report presented an overview of GMO research and research policies, and highlighted concerns associated with the current status of GMO fish research with respect to mariculture applications (ICES CM 1997/F:4). That report made six recommendations concerning GMOs with the intention of balancing the reality of transgenic research with guidelines to minimize the potentially detrimental effects of the escape of such organisms into natural environments. These recommendations were directed toward transgenic research, which is biotechnology-oriented, and not to the basic and medically-oriented research which uses aquatic organisms as models (e.g., zebrafish), primarily in the fields of developmental biology and the regulation of gene expression. In particular, organisms that can interbreed with and introduce recombinant DNA-derived genetic material into native populations were the focus of attention. Given the complexity of aquatic ecosystems, it was not possible to fully assess the potential impact that a transgenic organism may have if it escapes into and becomes a member of a natural population. The implementation of policy guidelines regulating both research and rearing of transgenic aquatic organisms by a number of Member Countries was considered both timely and necessary.

RISK ASSESSMENT

The 1996 WGAGFM report recommended that all ICES Member Countries should consider risk assessment protocols for GMO management. Some countries have adopted risk assessment and risk management processes to reach a decision on the environmental release of aquatic GMOs; risk assessment and management being, in this context, the process of identifying hazards posed by a particular action, quantifying their probabilities, and determining their likely consequences (Hallerman and Kapuscinski, 1995). The main purpose of the risk assessment protocols is to distinguish the serious risks from the lesser ones that in turn will help in decision making. The major environmental, social and economic components that must be assessed to determine the probability models for the spread of GMO genomes into the natural gene pools have been summarized by the Report to the Aquatic Nuisance Task Force Generic Non-indigenous Aquatic Organisms Risk Analysis Review Process, Washington, D.C., February 9, 1996 by the Risk Assessment and Management Committee of the US Aquatic Nuisance Species Task Force. These include 1) elements of organism establishment, 2) risks to the environment if the organism becomes established, 3) economic impacts if the transgenic organism becomes established, and 4) social impacts if the transgenic organism becomes established. Each applicant must identify potential hazards associated with the use of GMOs and the application is rated accordingly. The United States Department of Agriculture has also published Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish (available through the US Dept. of Agriculture, Office of Agricultural Biotechnology, Rm 3868-South Bldg, 14th and Independence Ave., S.W., Washington, DC 20250-0904, Document No. 95-04, Pt. I, II). Decision-making is assisted by a series of flow charts and accompanying worksheets. In these documents, the critical recommendations are made in Section IV.B where Risk Management of GMO is considered when insufficient information is available on the impact of the GMO on the environment in the event of an escape. The protocols allow for the culture of such organisms provided that the project provides sufficient barriers to ensure no/negligible accidental escape of GMO. These barriers include 1) physical or chemical barriers (e.g., water temperature, salinity) which induce 100 % mortality in any life stage of the GMO before reaching an accessible ecosystem, 2) mechanical barriers that physically hold back any life stage of the GMO from leaving the project site (e.g., screens), 3) biological barriers that prevent any possibility of GMO reproduction or survival, and 4) scale of experiment, i.e., maintaining an experimental size small enough so that accidental escape of all organisms would not have adverse ecological effects. These protocols are exceptional in that they acknowledge the release of GMO on small scales (contrary to the WGAGFM recommendations made in 1996).

Discussion of these risk assessment protocols during the 1997 WGAGFM meeting raised several points. It was felt that the risks associated with transgenic fish are different from those associated with polyploids, and that these different organisms should be treated separately. A review of the more extensive literature on polyploid fish and shellfish, including evaluation of fitness measures, was suggested in order to contextualize the environmental risks associated with these organisms. Further, a differentiation between environmental risk and food/consumption risk should be drawn, as some decisions are made on the basis of consumer appeal and not on a scientific basis. This Working Group has only addressed the former, i.e., environmental risk, which in turn can be broken into genetic effects and potential ecological impacts on wild stocks. The later impacts depend upon the interaction of the environment with the phenotype, regardless

of the genetic makeup, and an understanding of the phenotypic variance associated with the GMO is critical before impacts can be assessed.

Little confidence was placed in the calculation of probabilities associated with the potential impact of release. Namely, effective population sizes are mostly unknown in aquaculture species, therefore modelling of the fate of a transgene would be of limited use. The recent developments of transgenic plant risk assessment could be of use for risk assessment of GMO in aquaculture. As most aquaculture species are not genetically domesticated, the use of transgenic technology in such species is likely to be difficult to control in the environment. As interactions between cultivated stocks and wild populations are still of concern (e.g., in salmonid species), the utilisation and commercial production of GMO need to be clearly stated and documented before any large-scale development is initiated. This documentation should include: strategies for effective confinement of the GMO and their gametes, the genetic nature of the released GMO (e.g., triploid, F1 hybrid, pure line, etc.), and fertile broodstock management in the case of sterile GMOs.

Another major concern was the assumption in many of the genetic risk assessment documents that triploidy provides 100 % sterility, and that this procedure will reduce significantly the risk associated with escape of transgenic organisms. These concerns were discussed in some detail in the 1996 WGAGFM report. Triploidy has been advocated as a mechanism to induce sterility in GMO organisms as a step toward their possible release into open systems, but this should be restricted to particular species and sexes. Functional sterility in salmon can only be produced by all female triploids induced by hormonal sex reversal; male triploids, although incapable of producing viable sperm, maintain secondary sexual characteristics which can potentially interfere with wild stocks should they escape. There is currently no method which can ensure 100 % functional sterility of male salmon, however the percentage of males producing viable sperm can be reduced to very low levels. In shellfish, certified triploid oysters (*Crassostrea gigas*) were obtained and confirmed by biopsy and after a season of grow out in the field, about 15 to 20 % of the supposed triploids reverted to a heteroploid mosaic state (Allen *et al.*, 1997). This observation reinforces concerns over the proposed use of triploids for population control, for introduction and testing of non-native species and for release of genetically modified organisms (Allen *et al.*, 1997).

The biological containment of transgenic animals should be considered in parallel to physical containment. An approach being developed under the EU (Biotechnology: Biosafety) is the production of reversibly sterile transgenic fish. This involves the inhibition of gonadotropin releasing hormone (GnRH) from the hypothalamus, thus blocking the hypothalamic-pituitary-gonad axis of sexual maturation. To achieve inhibition of GnRH production, constructs are introduced to fish that express antisense GnRH, which in theory should hybridise with the sense strand of GnRH mRNA, thus blocking translation of the protein. The antisense GnRH has been expressed under the control of an Atlantic salmon histone H4 promoter, but experiments still need to be performed to determine the effect of this on sterility. The level of antisense expression needs to be determined that will successfully block translation of GnRH. Brain-specific promoters will be identified in order to have the correct tissue expression of the antisense molecule. Induction of fertility for broodstocks can be obtained by injection of pituitary extract, which should allow for gonad development. Lines of fish that can be produced in this way and shown to be 100 % sterile could be used as a starting point for other transgenes to be introduced. This research offers the most promising solution to effective sterilization of marine organisms. However the accidental release of large numbers of sterile fish has its own impact on the environment, some of which were discussed in the 1996 WGAGFM report.

Recommendation

A distinction between the potential impacts of polyploid and transgenic organisms should be drawn in the development of risk assessment protocols.

UPDATE OF GMO RESEARCH SINCE THE 1997 WGAGFM REPORT

The 1996 Annual Meeting of the National Shellfisheries Association (USA) generated a number of abstracts detailing advances in the production of GMO shellfish (cf. J. Shellfish Res., 16(1)). Research advances in the Pacific oyster (*Crassostrea gigas*) include the successful mating of tetraploid and diploid oysters to produce triploids without altering meiosis (Guo *et al.*, 1996). An international patent concerning tetraploids is currently under application. In the eastern oyster, *Crassostrea virginica*, a bacteriophage P1 high molecular weight genomic library has been generated (Pierce, 1997) for genetic physical mapping studies and genome targeting leading to genetic engineering.

Powers *et al.* (1997) reported genetically engineered abalone with enhanced growth using both gene transfer and ploidy manipulation protocols. The first abalone promoter, beta-actin, was developed (Gomez-Chiarri *et al.* 1994) and coupled to reporter genes luciferase and beta-galactosidase and to the coho salmon growth hormone gene. These three constructs were successfully transferred into abalone by electroporation. The majority of the embryos became transgenic and

retained the constructs for more than a year, and the transmission of these transgenes to the next generation is being evaluated (Powers *et al.*, 1997). This research team has also successfully generated triploid abalone that grow significantly faster than their diploid counterparts, and are using triploid manipulation of transgenic abalone to create unique strains of abalone for aquaculture purposes. The sterility of triploid abalone should then be carefully examined (see above). Further success in abalone was reported in using sperm as a carrier to introduce foreign DNA (6.4 kb antifreeze promoter and CAT gene) into the oocyte of the Japanese abalone (*Haliotis divorsicolor*; Tsai *et al.*, 1997). Electroporation efficiency, while 10–100 times greater than that of microinjection, is still limiting in the transgenic production of the millions of eggs produced by some fish and shellfish species. This is partly due to the inability to control the placement of the transfer gene within the egg. Electroporation of sperm and subsequent sperm-mediated transfer has been successful in a number of fish species including carp, catfish, tilapia (Muller *et al.*, 1992) and salmon (Symonds *et al.*, 1994), but has not previously been attempted in marine molluscs. Tsai *et al.* (1997) were able to produce fertilization rates of 95–99 % with 65 % of the trocophore larvae transgenic.

The first successful gene transfer in bivalve molluscs was reported in the model clam species *Mulinia lateralis* (Lu *et al.*, 1996; Chen *et al.*, 1997). The small size of the bivalve egg and the opacity of the oocyte, rendering microinjection techniques technically difficult, have previously hampered research in this field. Electroporation was used to introduce a pantropic pseudotyped retroviral vector in the dwarf surfclam, *Mulinia lateralis*, producing ~30 % transgenic F1 offspring (Lu *et al.*, 1996; Chen *et al.*, 1997). These pantropic retroviral vectors have a very broad host cell range and infection of *Mulinia* was well tolerated and did not affect the survival rate of the embryos. The authors suggest that pantropic pseudotyped retroviral vectors provide a useful method for the stable introduction of foreign genetic information into surfclams and may facilitate the introduction of desirable genetic traits (e.g., Miahle *et al.*, 1995) into commercially important shellfish and crustaceans.

In marine algae, the gene *crtO*, which converts beta-carotene to canthaxanthin to produce the economically valuable ketocarotenoid astaxanthin, was cloned from a green alga and transferred to a cyanobacterium which does not normally produce this carotenoid (Harker and Hirschberg, 1997). Astaxanthin is responsible for imparting the pinkish colour to the flesh of many marine organisms such as salmonids and crustaceans. Animals cannot synthesize astaxanthin and must obtain it from their diets. This research paper, which also elucidates the biosynthesis pathway in detail, will facilitate the gene transfer of *crtO* into higher plants and ultimately improve the nutritional and economic value of salmonids.

Advances in finfish transgenic research over the past year have been more modest, with the major advances appearing in the recent progress with modified proviruses to introduce the DNA construct (Gaiano *et al.*, 1996). This method may improve specificity in the absence of reliable fish embryonic stem cells (Volckaert and Ollevier, 1997). Reviews have been published by Ferraris and Palumbi (1996), Iyengar *et al.* (1996) and Vockaert and Ollevier (1997), amongst others. A summary of transgenic studies on aquatic organisms from 1985 to 1997 was compiled by G. Mouzakitis (Dept. of Zoology and Animal Ecology, Lee Maltings University College, Cork, Ireland) and is presented in Table 1. This list may not be comprehensive.

NATIONAL REPORTS ON TRANSGENIC RESEARCH ON MARINE AND ANADROMOUS SPECIES

Canada

To date, research has been limited on transgenic aquatic organisms in Canada. There are at least six laboratories conducting transgenic research, all on fish. Aqua Bounty Farms market a construct consisting of an ocean pout antifreeze promoter with a salmonid growth hormone gene. This same company, a subsidiary of AF Proteins Inc., is rearing eggs of Atlantic salmon from GMO parents in a land-based hatchery on Prince Edward Island. The company is also conducting research into the energetics (oxygen consumption, food conversion, etc.) of the GMO fish, production of female-only fish through sex reversal, triploid GMO and sensory testing of the final product (quality of flesh, smoking, etc.). Laboratories in Toronto, Ontario (Hew) and St. John's, Nfld. (Fletcher) are working on the development of different fish constructs. The transgenic salmon (growth hormone) in Vancouver, B.C. (Devlin) have produced new generations.

Ireland

Atlantic salmon promoters are being characterized for all fish constructs. Transgenics are not at present performed in Ireland but by collaborators on EU projects.

France

Research on transgenic fish (rainbow trout) is conducted at INRA in Paris and Rennes. Work on anti-sense mRNA in GnRH is in progress to obtain reversible sterile transgenic fish. Research has been initiated on transgenesis of shellfish

and crustaceans at IFREMER-CNRS in Montpellier. The objective is the introduction of desirable traits such as disease resistance in these species.

REFERENCES

Allen Jr., S.K., X. Guo, G. Burreson and R. Mann, 1997. Heteroploid mosaics and reversion among triploid oysters, *Crassostrea gigas*. Fact or Artifact. Abstract. J. Shellfish Res., 16(1): 514.

Chen,T.T., J.-K. Lu, S.K. Allen, T. Matsubara and J.C. Burns, 1997. Production of transgenic dwarf surfclams, *Mulinia lateralis*, with pantropic retroviral vectors. Abstract. J. Shellfish Res., 16(1): 475.

Ferraris, J.D. and S.R. Palumbi (eds.), 1996. Molecular Zoology: Advances, Strategies and Protocols. John Wiley and Sons, N.Y. 580 pp.

Gaiano, N., M. Allende, A. Amsterdam, K. Kawakami and N. Hopkins, 1996. Highly efficient germ-line transmission of proviral insertions in zebrafish. Proc. Nat. Acad. Sci. USA, 93: 7777–7782.

Gomez-Chiarri, M., L. Hereford and D. Powers, 1994. Cloning of an actin promoter from the red abalone *Haliotis rufescens*. Abstract. Third International Marine Biotechnology Conference, Tromso, Norway, p. 125.

Guo, X., G.A. DeBrosse, and S.K. Allen, 1996. All-triploid Pacific oysters (*Crassostrea gigas* Thunberg) produced by mating tetraploids and diploids. Aquaculture, 142: 149–161.

Hallerman, E.M. and A.R. Kapuscinski, 1995. Incorporating risk assessment and risk management into public policies on genetically modified finfish and shellfish. Aquaculture, 137: 9–17.

Harker, M. and J. Hirschberg, 1997. Biosynthesis of ketocarotenoids in transgenic cyanobacteria expressing the algal gene for B-C-4-oxygenase, *crtO*. FEBS Letters, 404: 129–134.

Iyengar, A., F. Muller and N. MacLean, 1996. Regulation and expression of transgenes in fish: a review. Transgenic Res., 5: 147–166.

Lu, J.-K., T.T. Chen, S.K. Allen, T. Matsubara and J.C. Burns, 1996. Production of transgenic dwarf surfclams, *Mulinia lateralis*, with pantropic retroviral vectors. Proc. Natl. Acad. Sci. USA, 93: 3482-3486.

Miahle, E., E. Bachere, V. Boulo, J.-P. Cadoret, C. Rousseau, V. Cedeno, E. Saraiva, L. Carrera and R.R Colwell, 1995. Future of biotechnology-based control of disease in marine invertebrates. Mol. Mar. Biol. Biotechnol., 4: 275–283.

Muller, E., Z. Ivics, F. Erdelyi, T. Papp, L. Varadi, L. Horvath, N. MacLean and L. Orban, 1992. Introducing foreign genes into fish eggs with electroporated sperm as a carrier. Mol. Mar. Biol. Biotechnol., 1: 276–281.

Pierce, J.C., 1997. A bacteriophage P1 high molecular weight genomic library from the oyster, *Crassostrea virginica*. Abstract. J. Shellfish Res., 16(1): 477.

Powers, D.A., V. Kirby and M. Gomez-Ghiarri, 1997. Genetic engineering abalone: gene transfer and ploidy manipulation. Abstract. J. Shellfish Res., 16(1): 477.

Symonds, J.E., S.P. Walker, F.Y.T. Sin and I. Sin, 1994. Development of a mass gene transfer method in chinook salmon: optimization of gene transfer by electroporated sperm. Mol. Mar. Biol. Biotechnol., 3: 104–111.

Tsai, H.-J., C.-H. Lai and H.-S. Yang, 1997. Sperm as a carrier to introduce an exogenous DNA fragment into the oocyte of Japanese abalone (*Haliotis divorsicolor suportexta*). Transgenic Res., 6: 85-95.

Volckaert, F. and F. Ollevier, 1997. Transgenic fish. The future of fish with novel genes. Proc. First Int. Symp. "Genetics and Aquaculture in Africa", Abidjan, Ivory Coast.

Table 1. Summary of Transgenic Studies on Aquatic Organisms, 1985–1997.

Species	Method	Promoter/Gene	Authors
Rainbow Trout	Mi	mMT/rGH	(MacLean *et al.* 1984)
(*Oncorhynchus mykiss*	Mi	SV40/hGH	(Chourrout *et al.* 1986)
and *Salmo gaidneri*)	Mi	mMT/rGH	(MacLean *et al.* 1987a)
	Mi	mMT/hGH	(Guyomard *et al.* 1989)
	Mi	mMT/rGH	(Penman *et al.* 1988)
	Mi	mMT/rGH	(Penman *et al.* 1990)
	Mi	mMT/rGH	(Penman *et al.* 1991)
	Mi	RSV/rtGH	(Inoue *et al.* 1993)
	Mi	opAFP/csGH	•(Devlin *et al.* 1995)
Cutthroat Trout	Mi	opAFP/csGH	(Devlin *et al.* 1995)
(*Oncorhynchus clarki*)			
Atlantic Salmon	Mi	wfAFP	(Fletcher *et al.* 1988)
(*Salmo salar*)	Mi	mMT/CAT	(McEvoy *et al.* 1988)
	Mi	mMT/hGH	(Rokkones *et al.* 1988)
	Mi	wfAFP	(Hew *et al.* 1992)
	Mi	opAFP/csGH	(Du *et al.* 1992)
Chinook Salmon	Mi	opAFP/csGH	(Devlin *et al.* 1995)
(*Oncorphynchus tshawytscha*)	Sp	RSV/Gal	(Sin *et al.* 1993)
Coho Salmon	Mi	opAFP/csGH	(Devlin *et al.* 1995)
(*Oncorphynchus kisutch*)			
Tilapia	Mi	mMT/hGH	(Brem *et al.* 1988)
(*Oreochromis niloticus*)	Mi	mMT/rGH-CAT	(Rahman *et al.* 1992)
	Mi	RSV/bGH	(Phillips *et al.* 1992)
	Mi	MMT/rGH	(Rahman *et al.* 1992)
	Mi	caBA/Gal	(Alam *et al.* 1996)
	Mi	CMV/tiGH	(Martinez *et al.* 1996)
	Mi	CMV/tiGH	(Estrada *et al.* 1996)
Medaka	Mi	cCR	(Ozato *et al.* 1986)
(*Oryzias latipes*)	Mi	RSV/CAT	(Chong *et al.* 1989)
	Mi	fLuc/luc	(Tamiya *et al.* 1990)
	El	mMT/rGH	(Inoue *et al.* 1990)
	Mi	mMT,vTK,rCCK,cBA/hGH	(Lu *et al.* 1992)
	Mi	SV40,RSV,chMT,dhsp70/luc	(Sato *et al.* 1992)
	Mi	rtMT/CAT	(Kinoshita *et al.* 1994)
	Mi	medaka-actin/Gal	(Takagi *et al.* 1994)
	Mi	RSV/Gal, CMV/Gal	(Tsai *et al.* 1995b)
	Mi	rtMT/CAT	(Kinoshita *et al.* 1996)
Goldfish	Mi	mMT/hGH	(Zhu *et al.* 1985)
(*Cassarius auratus*)	Mi	mMT/hGH	(MacLean *et al.* 1987b)
	Sp	RSV/Neo	(Yoon *et al.* 1990)

Table 1. Summary of Transgenic Studies on Aquatic Organisms, 1985–1997.

Species	Method	Promoter/Gene	Authors
	Mi	opAFP	(Wang *et al.* 1995)
(*Ictalurus punctatus*)	Mi	mMT/rGH, RSV/rtGH,	
		RSV/csGH, RSV/rtV	(Hayat *et al.* 1991)
	Mi	RSV/rtGH, RSV/csGH	(Dunham *et al.* 1992)
	Mi, El	RSV/rtGH	(Powers *et al.* 1992)
Loach	Mi	mMT/hGH	(Zhu *et al.* 1986)
(*Misgurnus anguillicaudatus*)	Sp	opAFP/csGH	(Tsai *et al.* 1995a)
Common Carp	Mi	RSV/rtGH	(Chen *et al.* 1989)
(*Cyprinus carpio*)	Mi	RSV/rtGH	(Zhang *et al.* 1990)
	Mi	mMT/rGH, RSV/rtGH	
		RSV/csGH, RSV/rtV	(Hayat *et al.* 1991)
	Mi, El	RSV/rtGH	(Powers *et al.* 1992)
	Mi	RSV/rtGH	(Chen *et al.* 1993)
	Mi	caBA/csGH	(Moav *et al.* 1995)
Northern Pike	Mi	RSV/bGH, RSV/rtV	(Gross *et al.* 1992)
(*Esox lucius*)			
Pacific Oyster	Bo	dhsp70/luc, CMV/luc	(Cadoret *et al.* 1997)
(*Crassostrea gigas*)			
Dwarf Surfclam	El	PPRV(Gal)	(Chen *et al.* 1996)
(*Mulinia lateralis*)	El	PPRV(Gal)	(Kan *et al.* 1996)
Abalone	El	d-actin/Gal	(Powers *et al.* 1995)
(*Haliotis rufescens*)	El	aBA/luc,Gal	(Powers *et al.* 1996)
	Sp	opAFP/CAT	(Tsai *et al.* 1997)
Artemia	Bo	dhsp70/luc	(Gendreau *et al.* 1995)
(*Artemia franciscana*)			

Studies published prior to 1992 were compiled from Chen and Powers (1990), Brem (1993) and Beaumont (1994). Studies published from 1992 to 1997 were compiled from ASFA (Aquatic Sciences and Fisheries Abstracts), Biological Abstracts, and MedLine databases.

Methods: Mi = microinjection; El = electroporation; Sp = sperm vector; Bo = particle bombardment.

Promoters: MT = metallothionein promoter: mMT = mouse MT, chMT = chinese hamster MT, rtMT = rainbow trout MT; BA = beta-actin: cBA = chicken BA, caBA = carp BA, aBA = abalone BA; SV40 = SV40 promoter; opAFP = ocean pout antifreeze protein promoter; wfAFP = winter flounder antifreeze promoter and protein; RSV = Rous sarcoma virus long terminal repeat promoter; XeF1a = Xenopus laevis enlongation factor 1 alpha promoter; CMV = promoter of the immediate early gene of the human cytomegalovirus; cCR = chicken crystallin promoter and gene; fLuc = firefly luciferase; vTK = viral thymidine kinase; rCCK = rat cholecystokinin; dhsp70 = drosophila heat-shock protein 70; P-elem = drosophila P-element; d-actin = drosphila actin promoter; PPRV(Gal) = pantropic pseudotyped retroviral vector containing Gal; MLV(XeF1a/Gal) = Moloney murine leukemia virus containing XeF1a/Gal; NLS:CMV/Gal = plasmid containing CMV/GAL was coupled to the SV40 T antigen nuclear localization sequence prior to microinjection.

Genes: GH = growth hormone: rGH = rat GH, hGH = human GH, bGH = bovine GH, rtGH = rainbow trout GH, csGH = chinook salmon GH, coGH = coho salmon GH, tiGH = tilapia GH; CAT = bacterial chloramphenicol acetyltransferase; opAFP = ocean pout antifreeze protein; wfAFP = winter flounder antifreeze protein; rtV = rainbow trout vitellogenin; Neo = neomycin resistance; Gal = beta galactosidase; Hygro = hygromycin resistance; luc = firefly luciferase.

References (for Table 1)

Alam, M. D. S., A. Popplewell and N. MacLean, (1996). Germ line transmission and expression of a lacZ containing transgene in tilapia (*Oreochromis niloticus*). Transgenic Research, 5: 87–95.

Beaumont, A. R., (1994). The application and relevance of genetics in aquaculture. *In* Genetics and Evolution of Aquatic Organisms. A. Beaumont (ed.).

Brem, G., (1993). Transgenic Animals. *In* Genetic Engineering of Animals. A. Puhler (ed.), VCH Publishers, N.Y., pp. 83–171.

Brem, G., B. Brenig, S.-G. Hoerstgen and E. L. Winnacker, (1988). Gene transfer in tilapia (*Oreochromis niloticus*). Aquaculture, 68(3): 209–220.

Cadoret, J. P., V. Boulo, S. Gendreau and E. Mialhe, (1997). Promoters from *Drosophila* heat shock protein and cytomegalovirus drive transient expression of luciferase introduced by particle bombardment into embryos of the oyster *Crassostrea gigas*. J. Biotech., 56(3): 183–189.

Chen, T. T., K. Kight, C. M. Lin, D. A. Powers, M. Hayat, N. Chatakondi, A. C. Ramboux, P. L. Duncan and R. A. Dunham, (1993). Expression and inheritance of RSVLTR-rtGH1 complementary DNA in the transgenic common carp, *Cyprinus carpio*. Mol. Mar. Biol. Biotech., 2(2): 88–95.

Chen, T. T., J. K. Lu, S. K. Allen, T. Matsubara and J. C. Burns, (1996). Production of transgenic dwarf surfclams, *Mulinia lateralis*, with pantropic retroviral vectors. J. Shellfish Res., 15: 475–476.

Chen, T. T. and D. A. Powers, (1990). Transgenic Fish. Trends in Biotechnology,. 8: 209–215.

Chen, T. T., Z. Zhu, C. M. Lin, L. I. Gonzalez-Villasenor, R. Dunham and D. Powers, (1989). Fish genetic engineering: a novel approach in aquaculture. Proc Nat. Shellfish Assoc. Aquaculture, Los Angeles.

Chong, S.S.C. and J.R. Vielkind, (1989). Expression and fate of CAT reporter gene microinjected into fertilized medaka (*Oryzias latipes*) eggs in the form of plasmid DNA. Theor. Appl. Genet., 78: 369–380.

Chourrout, D., R. Guyomard and L. M. Houdebine, (1986). High efficiency gene transfer in rainbow trout (*Salmo gairdneri*) by microinjection into egg cytoplasm. Aquaculture, 51(2): 143–150.

Devlin, R. H., T. Y. Yesaki, E. M. Donaldson, S. J. Du and C. L. Hew, (1995). Production of germline transgenic Pacific salmonids with dramatically increased growth performance. Can. J. Fish. Aquat. Sci., 52(7): 1376–1384.

Du, S. J., Z. Gong and G. L. Fletcher, (1992). Growth enhancement in transgenic Atlantic salmon by the use of an 'all fish' chimeric growth hormone gene construct. Biotechnology, 10: 176–181.

Dunham, R. A., J. Eash, J. Askins and T. M. Townes, (1987). Transfer of the metallothein-human growth hormone fusion gene into channel catfish. Trans. Amer. Fish. Soc., 116: 87–91.

Dunham, R. A., A. C. Ramboux, P. L. Duncan, M. Hayat, T. T. Chen, C. M. Lin, K. Kight, V.-I. Gonzalez and D. A. Powers, (1992). Transfer, expression, and inheritance of salmonid growth hormone genes in channel catfish, *Ictalurus punctatus*, and effects on performance traits. Mol. Mar. Biol. Biotech., 1: 380–389.

Estrada, M. P., J. Figueroa, M. Krauskopf and L.-F. J. De, (1996). Ectopic expression of tiGH in muscle cells of transgenic F2 tilapias. Biotechnology, 13: 46.

Fletcher, G. L., M. A. Shears, K. J. King, P. L. Davies and C. L. Hew, (1988). Evidence for antifreeze protein gene tranfer in Atlantic salmon (*Salmo salar*). Can. J. Fish. Aquat. Sci., 45: 352–357.

Gendreau, S., V. Lardans, J. P. Cadoret and E. Mialhe, (1995). Transient expression of a luciferase reporter gene after ballistic introduction into *Artemia franciscana* (Crustacea) embryos. Aquaculture, 133: 199–205.

Gross, M. L., J. F. Schneider, N. Moav, B. Moav, C. Alvarez, S. H. Myster, Z. Liu, E. M. Hallerman, and P. B. Hackett, (1992). Molecular analysis and growth evaluation of northern pike (*Esox lucius*) microinjected with growth hormone genes. Aquaculture, 103: 253–273.

Guyomard, R., D. Chourrout, C. Leroux, L. M. Houbedine and F. Pourrain, (1989). Integration and germ line transmission of foreign genes microinjected into fertilized trout eggs. Biochimie, 71: 857–863.

Hayat, M., C. P. Joyce, T. M. Townes, T. T. Chen, D. A. Powers and R. A. Dunham, (1991). Survival and integration rate of channel catfish and common carp embryos microinjected with DNA at various developmental stages. Aquaculture, 99: 249-256.

Hew, C. L., P. L. Davies and G. Fletcher, (1992). Antifreeze protein gene transfer in Atlantic salmon. Mol. Mar. Biol. Tech., 1: 309-317.

Inoue, K., S. Yamada and S. Yamashita, (1993). Introduction, expression, and growth-enhancing effect of rainbow trout growth hormone cDNA fused to an avian chimeric promoter in rainbow trout fry. J. Mar. Biotech., 3: 131–134.

Inoue, K., S. Yamashita and J.-I. Hata, (1990). Electroporation as a new technique for producing trangenic fish. Cell Diff. Devel., 29: 123–128.

Kan, L. J., T. T. Chen, S. K. Allen, T. Matsubara and J. C. Burns, (1996). Production of transgenic dwarf surfclams, *Mulinia lateralis*, with pantropic retroviral vectors. Proc. Natl. Acad. Sci. USA, 93: 3482–3486.

Kinoshita, M., H. Toyohara, M. Sakaguchi, K. Inoue, S. Yamashita, M. Satake, Y. Wakamatsu and K. Ozato, (1996). A stable line of transgenic medaka (*Oryzias latipes*) carrying the CAT gene. Aquaculture, 143: 267–276.

Kinoshita, M., H. Toyohara, M. Sakaguchi, N. Kioka, T. Komano, K. Inoue, S. Yamashita, M. Satake, Y. Wakamatsu and K. Ozato, (1994). Zinc-induced activation of rainbow trout metallothionein-A promoter in transgenic medaka. Fisheries Sci. (Tokyo) 60: 307–309.

Lu, J. K., T. T. Chen, C. L. Chrisman, O. M. Andrisani and J. E. Dixon, (1992). Integration, expression, and germ-line transmission of foreign growth hormone genes in medaka (*Oryzias latipes*). Mol. Mar. Biol. Biotech. 1(4-5): 366–375.,

MacLean, N., D. Penman and S. Talwar, (1987a). Introduction of novel genes into the rainbow trout. *In* Selection, Hybridization and Genetic Engineering in Fish. Vol II. K. Tiews, (ed.), Heenemann, Berlin.

MacLean, N., D. Penman and Z. Zhu, (1987b). Introduction of novel genes into fish. Biotechnology, 5: 257–261.

MacLean, N. and S. Talwar, (1984). Injection of cloned genes into rainbow trout eggs. J Embryol. Exp. Morphol., 82: 187.

Martinez, R., M. P. Estrada, J. Berlanga, I. Guillen, O. Hernandez, E. Cabrera, R. Pimentel, R. Morales, F. Herrera, A. Morales, *et al.*, (1996). Growth enhancement in transgenic tilapia by ectopic expression of tilapia growth hormone. Mol. Mar. Biotech., 5(1): 62–70.

McEvoy, T., M. Stack, B. Keane, T. Barry, J. Sreenan and F. Gannon, (1988). The expression of a foreign gene in salmon embryos. Aquaculture, 68(1): 27–38.

Moav, B., Y. Hinits, Y. Groll and S. Rothbard, (1995). Inheritance of recombinant carp ss-actin/GH cDNA gene in transgenic carp. Aquaculture 137: 179–185.

Ozato, K., H. Kondoh, H. Inohara, *et al.*, (1986). Production of transgenic fish: introduction and expression of chicken crystallin gene in medaka embryos. Cell Diff., 19: 237–244.

Penman, D. J., A. J. Beeching, A. Iyengar and N. MacLean, (1988). Introduction of metallothein-somatotropin fusion gene into rainbow trout: analysis of adult transgenics. Bull. Aquacult. Assoc. Can., 88: 137–139.

Penman, D. J., A. J. Beeching, S. Penn and N. MacLean, (1990). Factors affecting survival and integration following microinjection of novel DNA into rainbow trout eggs. Aquaculture, 85: 35–50.

Penman, D. J., A. Iyengar, A. J. Beeching, A. Rahman, Z. Sulaiman and N. Maclean, (1991). Patterns of transgene inheritance in rainbow trout (*Oncorhynchus mykiss*). Mol. Reprod. Devel., 30: 201–206.

Phillips, P. C., C. C. Kohler and W. L. Muhlach, (1992). Procedural protocol, survival to hatching and plasmid DNA fate after microinjection into Tilapia zygotes. J. World Aquaculture Soc., 23: 98–113.

Powers, D. A., L. Hereford, T. Cole, T. T. Chen, C. M. Lin, K. Kight, K. Creech and R. Dunham, (1992). Electroporation: A method for transferring genes into the gametes of zebrafish (*Brachydanio rerio*), channel catfish (*Ictalurus punctatus*), and common carp (*Cyprinus carpio*). Mol. Mar. Biotech., 1: 301–308.

Powers, D. A., V. Kirby and G. M. Gomez, (1996). Genetic engineering abalone: Gene transfer and ploidy manipulation. J. Shellfish Res., 16(1): 477.

Powers, D. A., V. L. Kirby, T. Cole and L. Hereford, (1995). Electroporation as an effective means of introducing DNA into abalone (*Haliotis rufescens*) embryos. Mol. Mar. Biol. Biotech., 4: 369–376.

Rahman, M. A. and N. Maclean, (1992). Production of transgenic tilapia (*Oreochromis niloticus*) by one-cell-stage microinjection. Aquaculture, 105: 219–232.

Rokkones, E., P. Alestrom, H. Skjervold and K. M. Gautvik, (1988). Microinjection and expression of a mouse metallothein human growth hormone fusion gene in fertilised salmon eggs. J. Comp. Physiol. B 158: 751–758.

Sato, A., J. I. Komura, P. Masahito, S. Matsukuma, K. Aoki and T. Ishikawa, (1992). Firefly luciferase gene transmission and expression in transgenic medaka (*Oryzias latipes*). Mol. Mar. Biol. Biotech., 1: 318–325.

Sin, F. Y. T., A. L. Bartley, S. P. Walker, I. L. Sin, J. E. Symonds, L. Hawke and C. L. Hopkins, (1993). Gene transfer in chinook salmon (*Oncorhynchus tshawytscha*) by electroporating sperm in the presence of pRSV-lacZ DNA. Aquaculture, 117: 57–69.

Takagi, S., T. Sasado, G. Tamiya, K. Ozato, Y. Wakamatsu, A. Takeshita and M. Kimura, (1994). An efficient expression vector for transgenic medaka construction. Mol. Mar. Biol. Biotech., 3: 192–199.

Tamiya, E., T. Sugiyama, K. H. Masaki, A. Hirose, T. Okoshi and I. Karube, (1990). Spatial imaging of luciferase gene expression in transgenic fish. Nucleic Acid Research, 18: 1072.

Tsai, H. J., C. H. Lai and H. S. Yang, (1997). Sperm as a carrier to introduce an exogenous DNA fragment into the oocyte of Japanese abalone (*Haliotis diversicolor suportexta*). Transgenic Research, 6: 85–95.

Tsai, H. J., F. S. Tseng and I. C. Liao, (1995a). Electroporation of sperm to introduce foreign DNA into the genome of loach (*Misgurnus anguillicaudatus*). Can. J. Fish. Aquat. Sci., 52: 776–787.

Tsai, H. J., S. H. Wang, K. Inoue, S. Takagi, M. Kimura, Y. Wakamatsu and K. Ozato, (1995b). Initiation of the transgenic lacZ gene expression in medaka (*Oryzias latipes*) embryos. Mol. Mar. Biol. Biotech., 4: 1–9.

Wang, R., P. Zhang, Z. Gong and C. L. Hew, (1995). Expression of the antifreeze protein gene in transgenic goldfish (*Carassius auratus*) and its implication in cold adaptation. Mol. Mar. Biol. Biotech., 4: 20-26.

Yoon, S. J., E. M. Hallerman, M. L. Gross, *et al.*, (1990). Transfer of the gene for neomycin resistance into goldfish, *Carassius auratus*. Aquaculture, 85: 21–33.

Zhang, P., M. Hayat, C. Joyce, V.-L. I. Gonzalez, C. M. Lin, R. A. Dunham, T. T. Chen and D. A. Powers, (1990). Gene transfer, expression and inheritance of pRSV-rainbow trout-GH complementary DNA in the common carp, *Cyprinus carpio* (Linnaeus). Mol. Reprod. Devel., 25: 3–13.

Zhu, Z., G. Li, L. He and S. Chen, (1985). Novel gene transfer into the fertilised eggs of goldfish (*Carassius auratus*). Z. Angew Ichthyol., 1: 32–34.

Zhu, Z., K. Xu, G. Li, Y. Xei and L. He, (1986). Biological effects of human growth hormone gene microinjected into the fertilized egg of the loach *Misgurnis anguillicaudatus*. Kex Tong Acad. Sin., 31: 988–990.

Summary of recommendations on Genetically Modified Organisms (GMOs)

A distinction between the potential impacts of polyploid and transgenic organisms should be drawn in the development of risk assessment protocols.

2.3 Genetic Management of New Species in Mariculture

[Based on a position paper by J. Jonasson, Iceland. Adopted by WGAGFM in Cork]

INTRODUCTION

In recent years, the importance of selective breeding programmes in aquaculture has been demonstrated. The gap between the demand for fish and the supply is widening, due to a growing human population and a decline in production from capture fisheries. Selective breeding has contributed greatly to the steadily increasing productivity of terrestrial agriculture, but only about 1 % of production in aquaculture is based on improved stocks (Gjedrem, 1997). There is thus a great disparity between the need for increased aquaculture production and the genetic quality of the stocks available to meet the demand.

Substantial realised selection responses reported for a number of aquatic species (Table 2) demonstrate the possibilities in selective breeding for aquatic animals.

Table 2. Response to selection for growth rate.

Species	Mean	Gain per Generation (%)	Number of Generations	Author
Coho salmon	250 g	10.1	4	Hershberger *et al.*, 1990
Rainbow trout	4.0 kg	13.0	2	Gjerde, 1986
Atlantic salmon	4.5 kg	14.4	1	Gjerde, 1986
Channel catfish	-	12.0–18.0	1	Dunham, 1987
Channel catfish	67 gr.	20	1	Bondary, 1983
Tilapia	50–200 g	12–17	5	Eknath *et al.*, 1998
Oyster	24 g	19.4	1	Jarayabhand *et al.*, 1995
Tilapia	-	14	2	Jarimopas *et al.*, 1986
Shrimp (P. vannamei)		4.4	1	Fjalestad *et al.*, 1997

At present, selective breeding programmes for fish and shellfish are scarce. Breeding programmes based on family selection are rare in aquaculture. In Norway, breeding experiments started in 1971 for Atlantic salmon and rainbow trout. Today over 240 families of Atlantic salmon and 120 families of rainbow trout are tested each year in Norway. Similar breeding programmes for Atlantic salmon exist in Canada, Iceland and the Faroe Islands. Over 200 families of rainbow trout are being tested in Finland. Two breeding programs for Arctic char are running in Iceland and Sweden. Commercial breeding studies for common carp are being conducted in Hungary, Israel and in the former Soviet Union and a national breeding programme for tilapia in the Philippines started in 1993. Research in developing a breeding program for oysters is under way in France and for red abalone in Iceland.

The high fecundity of fish and other aquatic animals opens for very high selection intensities and consequently for large selection responses. On the other hand, this also means that a very small number of individuals can make a large contribution to the gene pool in successive generations and, hence, the rate of inbreeding can be high. Detrimental effects of inbreeding are reduced fitness, depression for important economic traits and loss of additive genetic variance. Because genetic variability is essential for selection response, reduced variability limits the scope for further and long-term genetic improvement. Use of a small number of parents also leads to highly variable selection responses. Consequently, it is of crucial importance to restrict the rate of inbreeding when selective breeding programmes are implemented for aquatic animals.

BREEDING GOAL

The breeding goal specifies which traits are to be improved. Ideally, the breeding goal should include all traits of economic importance that show genetic variation. These traits would be increased growth rate, age at maturity, disease resistance and carcass quality traits.

ESTABLISHMENT OF A BASE-POPULATION

All cultured fish and shellfish species show variation around the mean for most production traits. The amount of variation is measured and expressed as the phenotypic variance of each trait, e.g., body size, fat content, etc. The phenotypic variance is partitioned into genetic variance and environmental variance. The degree of genetic variance is the most important in animal breeding and is divided into additive and non-additive variation. Utilising the additive genetic variance will usually be the most effective selection method (Falconer, 1989). When little or no additive genetic variation exists and it is either difficult or impossible to improve a phenotype by selection, the breeding technique that can be used to improve the productivity is hybridisation (cross-breeding). Hybridisation improves productivity by exploiting the non-additive genetic variation, usually done by crossing inbred lines, strains or even species.

When establishing the baseline, the results shown in Table 2 indicate substantial additive variation for production traits for a number of fish or shellfish species. The baseline must be large enough in the beginning of the selection work to give response to selections for generations to come. Exploiting the non-additive genetic variation should not be ruled out. This base must have a wide array of genotypes (large genetic variation) in order to maximise genetic gains in both the short and long term. This can be achieved by the introduction of several stocks into the base. The ideal way, before intense selection is applied, is that one or two generations of random mating should be conducted to allow the mixing of genes from the original populations if more than one stock is used. This procedure allows a safeguard against the narrowing of the genetic variance in a population. In general this is not done, rather several stocks are tested and the best families of the stocks are mixed to form a base population.

SELECTION METHODS

Different methods of selection are available; each characterised by the type of information that the selection decision is based on. The choice of the method depends on several factors among which the heritability of the trait(s), the nature of the traits to improve (e.g., normally distributed or binary trait) and the reproductive capacity of the species are the most important. Three selection methods are important to consider in fish species: individual selection (or mass selection), family selection or a combination of the two (combined selection).

Individual selection: Selection is based on the individual's own performance or phenotype. Since records on relatives are not used, no tagging is required and individuals from different families can be reared together. However, a prerequisite for using individual selection is that the trait(s) selected for can be measured on the breeding individual itself while being alive. The method is thus difficult to practice, for carcass quality traits where the trait can not be recorded on the breeding individual it self. The method will be inefficient for binary traits, like survival and early sexual maturity, at high (>90 %) or low frequencies (<10 %)..

Family selection: When the trait of interest can not be measured on the breeding candidates themselves while alive, selection decisions have to be based on the phenotypic records obtained from relatives. Whole families are selected or rejected as units according to the mean value of the family. Mean values of phenotypic records of sibs or of BLUP (Best Linear Unbiased Predictions) breeding values of the sibs could be used. The families may consist of full- or halfsibs, whereas families of more remote relationships are of little practical significance. To obtain an acceptable rate of genetic gain and a low rate of inbreeding, the number of family groups tested when applying family selection needs to be high.

Combined selection: This method optimally combines all available sources of information about the breeding value of an animal. In fish breeding this means information recorded on the breeding candidate itself and its full- and half-sibs. Combined selection maximises the rate of genetic gain and is therefore generally considered to be the best selection method. When sib records are used to estimate the breeding values, siblings will tend to have more similar breeding values than found under individual selection. Thus, compared to individual selection schemes, the probability of selecting large numbers of sibs from a limited number of families is increased. Consequently, the need to restrict the number of selected individuals from each family is even more important in a combined selection program. In a mass selection programme without tagging, the number of selected individuals from each family must be restricted at or shortly after fertilisation. However, in populations where family identity can be attained through physical tagging or DNA profiling, the restriction may be implemented after the performance test.

MINIMISING INBREEDING

In a mass selection system, the size and structure of the population is important in order to control inbreeding. When applying selection, strict control should be kept on the number or offspring per dam and the use of milt from a wide array of sires. A subdivision of the population would allow the use of sires and dams from different subpopulations to avoid inbreeding. Gjerde *et al.* (1996) used stochastic simulation to evaluate the optimum size of a breeding population in a mass selection programme for a normally distributed trait. He concluded that optimum population size is dependent on the heritability of the trait as well as the rate of inbreeding allowed. As an example, in a population of the size of 9600 the optimum number of full sib groups for the next generation is 369 if the heritability is 0.4 and the rate of inbreeding is 0.25 % per generation. A lower number of breeders is needed for lower heritabilities. With a less stringent restriction on the inbreeding this number was reduced substantially to around 100 for an inbreeding rate of 1 % and 50 for an inbreeding rate of 2 % per generation. By applying family selection, levels of inbreeding can be reduced by avoiding mating between full- and half-sibs. Secondly through pedigrees, inbreeding over generations can be monitored. The optimum number of families is yet to be simulated. When, in a population consisting of 100 families, individuals from the 20 best families are selected each year the rate of inbreeding per generation is expected to increase 0.6 % per generation. By increasing the number of families to 200 and keeping the same selection intensity, the inbreeding level will be reduced to 0.3 % per generation.

ESTIMATION OF GENETIC PARAMETERS

During the test of genetic material in the base population and families, reliable phenotypic and genetic parameters for traits of importance should be used. Estimating heritability for traits as well as genetic correlations between the traits is important. The parameters are crucial for the prediction of expected genetic gain resulting from selection, for decisions of which selection method to apply, and for estimation of breeding values. Improvement of software for running breeding programs and revolutionary development of computer hardware has enabled geneticists to run much larger and complex breeding programmes than previously and selecting for many traits simultaneously. Multi-trait selection is most efficiently accommodated by use of a selection index, which requires information on the phenotypic and genetic (co)variances structure as well as economic weights for the traits included in the selection criterion. Economic weights should reflect the relative economic value for each trait, based on detailed knowledge about the production system and the market situation.

GENOTYPE BY ENVIRONMENTAL INTERACTION (GXE)

For several aquaculture species the farming is widespread and takes place under different climatic conditions and under a wide range of production systems. If the rank order of different genetic groups (e.g., stocks or families) varies between different types of production environments, an interaction between genotype and environment (GxE) is present (for review, see Bowman, 1972). If the level of re-ranking is important, independent selection may have to be carried out in two or more distinct sub-populations, each targeting specific types of actual production environments. Alternatively, a single breeding population may be serving a range of production environment types, if the selection is based on average performance across target environments. This strategy, however, requires that random samples of individuals from each of the genetic groups under evaluation are tested in several production environments, thus substantially increasing the costs involved. However, due to the high fecundity, this is more feasible in aquatic species.

Even for situations where no re-ranking of genotypes between production environments is seen, GxE may affect the absolute and relative magnitude of the genetic, environmental and phenotypic variances and thus lead to heterogeneity of variances between environments.

Both types of GxE are important for selection decisions. If a particular genotype is superior in one production environment but is less superior in another, selection based on performance in the first environment may not lead to

lower genetic improvement in the second. If the GxE is due to heterogeneous variances between the involved environments, a possible solution might be to base the selection within each class of environments on breeding values estimated on the basis of different sets of genetic parameters.

Maintaining genetic variation – methods

CONTROL OF SELECTION RESPONSE

Monitoring genetic changes is an important task in breeding programmes, not only for traits under selection but also to detect possible correlated responses in other traits. This should be a part of the internal quality control of all breeding programmes. It is also useful documentation for the marketing of the genetic resources towards customers/farmers. Rates of genetic gain and inbreeding obtained in selected lines often differ from those predicted due to unrealistic assumptions implied in the prediction models. Also, many genetic correlations are not known, and hence it is important to monitor possible correlated responses.

Appropriate methods for estimating genetic change should account for environmental trends. Such estimates can be obtained by keeping an unselected control and by applying divergent selection. Alternatively, BLUP methodology can be used if adequate connectedness exists between data for different years or generations (Henderson, 1984). Conducting divergent selection is often very costly and establishing connectedness between generations may be difficult due to biological constraints in several aquatic species. Hence, it is important to investigate whether the genetic ties that can be obtained via relatives representing different generations and years of hatching and rearing are sufficient to obtain unbiased estimates of genetic change with sufficiently low sampling variances. Further, the design of family groups, the degree of relationship between animals hatched in different years, and the proportion of selected individuals that contribute to genetic ties between data for different years should be studied with respect to accurate and unbiased estimation of genetic change.

PRESERVING THE GENETIC MATERIAL

After a few generations of selection, the genetic material becomes more and more valuable. Therefore, ways to preserve and split the material to reduce the risk of loss due to disease outbreak or other causes are essential. Establishment of at least two breeding nucleus populations is recommended. Cryopreservation of milt is recommended where possible to preserve the genetic material. This can also be used to monitor genetic gain over generations where milt from 'old' sires for pervious generations is used again and performance of offspring is tested with 'younger' material.

Summary of recommendations on management of new species in aquaculture

WGAGFM recommends that research be initiated on new species for developing breeding programmes. Research programmes to estimate genetic parameters for the most valuable traits are recommended. This will give scientists as well as the industry information to establish breeding programmes. It is recommended that research should start for the following species: halibut, turbot, sea bass, sea bream and oyster species.

REFERENCES AND FURTHER READING

Eknath, A.E:, Dey, M.M., Rye, M., Gjerde, B., Abella, T.A., Sevilleja, R.C., Tayamen, M.M., Reyes, R.A. and Bentsen,H.B., 1998. Selective breeding of Nile tilapia for Asia. *In* Proc. 6th World Congress on Genetics Applied to Livestock Production, Armidale, Australia, 10–11 January 1998.

Falconer, D.S., and Mackay, T.F.C. 1996. Introduction to quantitative genetics. Fourth edition. Longman Group, London and New York.

Fjalestad, K.T., Carr, W.H., Lotz, J., Sweeney, J.N. and Gjedrem, T. 1997. Genetic variation and selection response in body weight and disease resistance in Pacific White Shrimp (*Penaeus vannamei*). (Abstract). *In* Proc. 6th Int. Symp. on Genetics in Aquaculture, Stirling, Scotland, 24–28 June 1997.

Gjedrem, T. 1985. Improvement of productivety through breeding schemes. *GeoJournal*, 10(3):223–241.

Gjedrem, T., 1997. Selective breeding to improve aquaculture production. World Aquaculture, March 1997, 33–45.

Gjerde, B., Gunnes, K. and Gjedrem, T., 1983. Effect of inbreeding on survival and growth in rainbow trout. Aquaculture, 34: 327–332.

Gjerde, B.,Gjøen, H.M. and Villanueva, B. 1996. Optimum designs for fish breeding programmes with constrained inbreeding. Mass selection for a normally distributed trait. Livest. Prod. Sci., 47: 59–72.

Henderson, C.R., 1984. Applications of linear models in animal breeding. University of Guelph, Guelph, ON, Canada, pp 462.

Jarimopas, P., 1986. Realized response of Thai red tilapia to weight-specific selection for growth. (Abstract) *In* Proc. First Asian Fisheries Forum, Manilla, Philippines, 26–31 May 1986.

Jonasson, J., Gjerde, B. and Gjedrem, T. 1997. Genetic parameters for returne rate and body weight of sea-ranched Atlantic salmon. Aquaculture, 154: 219–231.

Jonasson J. and Gjedrem T. 1998. Genetic correlation for body weight of Atlantic salmon grilse between fish in sea ranching and land-based farming. Aquaculture, 157: 205–214.

2.4 Genetic Aspects of Management of Pelagic Marine Species

[This document was produced for the Working Group by Prof. G.R. Carvalho, who was unable to be present at the meeting. It is reproduced in almost complete form below. The presentation in Cork was made by Tom Cross.]

MOLECULAR GENETIC ANALYSIS OF STOCK STRUCTURE IN MARINE PELAGIC FISHES

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ABSTRACT

Foremost among the various challenges facing fisheries biologists has been the development of techniques to estimate population discreteness and associated patterns of dispersal and gene flow, so-called stock structure analysis. Although pelagic marine fishes have been studied intensively using a variety of molecular genetic markers, the levels of differentiation detected are typically low, often in contrast to estimates of phenotypic divergence. It is not entirely clear whether the lack of genetic structuring in pelagic fishes arises entirely from extensive migrations and the apparent lack of barriers to gene flow, or whether it is related to inappropriate sampling or molecular analysis. Here, some recent developments in molecular technology are reviewed, especially microsatellite analysis, and critical consideration is given to how such markers can be employed to estimate genetic differentiation in species exhibiting high mobility in open waters. Comparisons are made between microsatellites and other genetic markers (protein and DNA) in their ability to detect population structuring. Particular emphasis is placed on the need to design sampling programmes that take account of aspects of the biology of the species under study, and the scale and nature of sample collection.

INTRODUCTORY REMARKS

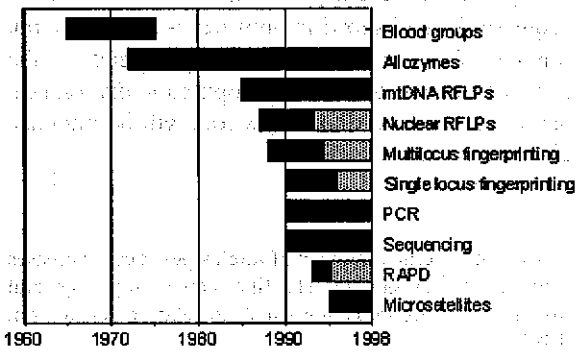
Although a vast array of markers are available to describe population structure (Park and Moran, 1994), it is the attempts to describe and monitor the levels and distribution of genetic variability in natural populations that has dominated fish and fisheries biology. Molecular genetic approaches are based on the premise that migration and mating patterns among proximate populations will determine the extent to which individuals share a common gene pool, and that a comparison of samples taken from each, can be used to estimate their integrity. Thus, where populations exchange few individuals, opportunities for genetic differentiation arising from local adaptation and random genetic change will be high, resulting in a discrete population structure. It does not necessarily follow, however, that a lack of detectable genetic structure represents a freely interbreeding population, due either to inadequate sampling, or limitations of the molecular tool employed. Further, the attempts to describe fish genetic population structure in freshwater and marine systems require fundamentally different sampling strategies, a point frequently overlooked, despite the marked contrasts in patterns displayed (Ward *et al.*, 1994). With the ever-increasing range of molecular markers to adopt (Park and Moran, 1994; O'Connell and Wright, 1997), it becomes imperative that an appropriate balance of marker sensitivity and representative sampling strategy be employed in population studies. Indeed, it appears that deficiencies in the matching of sampling design to the biology and environment of the species under study has led to a biased view of fish population structure, and that spatially-based protocols alone can be misleading. Here, some recent advances and applications in

molecular methodology are described with emphasis on microsatellite analysis, and how such developments can improve our understanding of the dynamics and structure of wild fishes, with special reference to pelagic species, is considered.

DEVELOPMENTS IN MOLECULAR TOOLS

Allozymes have been the 'workhorse' of fish molecular population genetics since the early 1970s (Utter, 1994), and continue to play a prominent role in the description of intraspecific genetic diversity in fishes, and will most likely continue to do so in the near future (Figure 1; Ward and Grewe, 1994; Carvalho and Hauser, 1994). Its general ease of application, rapid processing of large sample size, effective and usually simple determination of Mendelian-based allele frequency dynamics, taken together with the direct comparability with numerous published studies, has secured its popularity, especially as an initial investigative tool, or where resources and expertise are limited. Nevertheless, as many species or populations became recognised as endangered or threatened, or which exhibited limited allozymic variation, a requirement for non-invasive, highly polymorphic markers fuelled a determined search for markers at the DNA level (Park and Moran, 1994).

Figure 1. Modifications to the 'molecular toolbox' applied to fish and fisheries research since 1960. Solid areas show the period when the respective method was commonly used; shaded sections show restricted applications.



Two notable advances in molecular methodology facilitated the application of nucleic acid sequence variation to natural fish populations: the discovery of highly polymorphic repetitive, short nucleotide sequences (variable number of tandem repeats (VNTR)), dispersed throughout the genome of many eukaryotes (Wright, 1993), and the ability to amplify specific genes or nucleotide sequences rapidly via the polymerase chain reaction (PCR; Saiki *et al.*, 1988). Not only did the advent of PCR remove the necessity for labour-intensive cloning of DNA sequences and complex hybridization protocols, but it became possible to obtain DNA variability measures using minimal amounts of tissue stored conveniently in alcohol or other DNA-stable solutions (Whitmore *et al.*, 1992; Dessauer *et al.*, 1996). Fish genetic research was marked in the early 1990s by a surge of studies on nuclear and mitochondrial DNA variation (Park and Moran, 1994; Ward and Grewe, 1994; Ferguson *et al.*, 1995; O'Reilly and Wright, 1995), many of which revealed enhanced discrimination among conspecific populations, as well as providing new opportunities for the analysis of parentage, social structure and estimation of reproductive success under natural conditions (Fleischer, 1996; O'Connell and Wright, 1997).

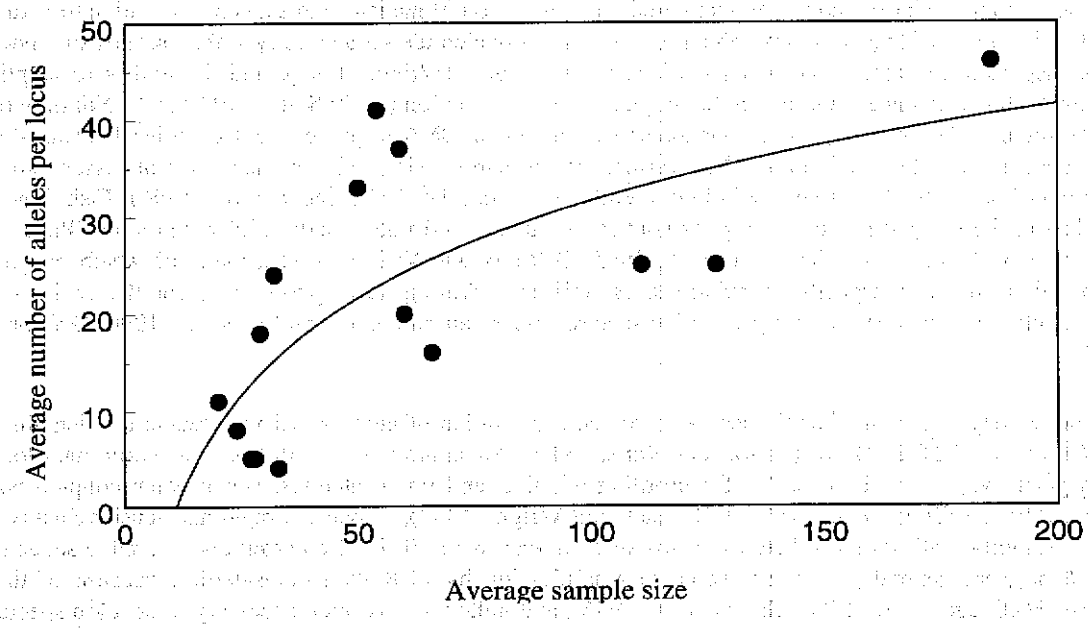
Foremost among the variety of new methodologies has been the exploitation of nuclear loci with repeat unit lengths of between 1 and 64 bp, or VNTR DNA (Tautz and Renz, 1984; Nakamura *et al.*, 1987). The early multilocus fingerprinting has given way to single-locus VNTR profiling of mini- and microsatellites, which when coupled with PCR (Galvin *et al.*, 1995; McGregor *et al.*, 1996; O'Connell and Wright, 1997), provides a rapid and sensitive assay of nuclear sequence variability, with levels of heterozygosity often in excess of 70 %. Microsatellites, or simple sequence repeats (SSRs: 1-6 bp), are generally more amenable to amplification by PCR than minisatellites because of their smaller size (but see McGregor *et al.*, 1996; Galvin *et al.*, 1996), and although it is often necessary to develop species-specific primers, there are numerous cases of sufficiently conserved sequence variation in flanking regions to enable amplification of loci across closely related species (Angers *et al.*, 1996; O'Connell and Wright, 1997). Recent advances such as semi-automated multilocus genotyping using colour fluorescent detection with automated DNA sequencers (Olsen *et al.*, 1996), and multiplexing of dinucleotide and tetranucleotide loci allowing amplification in the same reaction (O'Reilly *et al.*, 1996) have simplified and accelerated the screening of large population sample sizes (50–100 individuals), so rendering microsatellites one of the most rapidly expanding source of molecular markers in population and fisheries biology (Wright and Bentzen, 1994; O'Reilly and Wright, 1995; Bruford *et al.*, 1996; Jarne and Lagoda, 1996; O'Connell and Wright, 1997). Even where primers for PCR do not exist, it is usually possible to identify and

characterise several polymorphic loci within two to three months, and if automated facilities are available, an experienced researcher can process up to 150 fish at four loci in a week.

Despite the undoubted advantages of using microsatellites to examine genetic relationships among individuals and populations, there are several difficulties in their application which should be considered prior to their use, including: (1) the nature of the mutation model which best describes variation at microsatellite loci, (2) the associated choice of statistically appropriate distance measures, (3) the problem of 'stuttering' at dinucleotide loci which may often occlude adjacent alleles, (4) the detection of null alleles which may artificially inflate the number of homozygous genotypes, and (5) the choice of appropriate sample size in cases of high allelic diversity (O'Reilly and Wright, 1995; Bruford *et al.*, 1996; O'Connell and Wright, 1997).

The minimum sample size required to detect representative microsatellite variability depends critically on the scale of taxonomic resolution required, such as whether the investigator is interested in the identification of social groups, populations or species (Bruford *et al.* 1996), the level of relatedness amongst genotypes (Jones and Avise, 1997; Shaw, 1997), and the mean number of alleles per locus (Chakraborty, 1992). Several cold-water fishes such as Atlantic cod display > 50 alleles at some loci (Ruzzante *et al.*, 1996), and it is usual to find 30–40 alleles per locus (Figure 2). In populations of high allelic variability, it may be unrealistic to obtain sufficiently large sample sizes, leaving the employment of numerical re-sampling techniques such as bootstrapping as the only feasible option. O'Connell and Wright (1997) point out that it is impossible to provide an optimum sample size for the application of microsatellites to population analysis, though a minimum of 50 individuals would be required for loci showing between 5–10 alleles, but even larger sample sizes would be more appropriate. Similarly, the number of loci to include in a study depends on the levels of variability, and question being tackled. Although 1 or 2 loci may be adequate to detect population divergence, the need to estimate variance and confidence limits across loci means that data based on too few loci will be dubious. Goudet (1996) suggests that at least 5 loci are required for meaningful estimates of *F*-statistics.

Figure 2. Allelic diversity of microsatellites detected in samples of varying size. The average number of alleles per locus increases with sample size, though the increase is slower above approximately 50 individuals per sample. The line shows only a general relationship between allelic diversity and sample size, and is not meant to provide a rigorous statistical correlation. Data from O'Connell and Wright 1997 (Table 3), Bentzen *et al.*, 1996 and Rico *et al.*, 1997.



The quest to obtain enhanced polymorphism and heterozygosity has brought its own problems of analysis and interpretation, and it may sometimes be necessary to reduce the number of alleles analysed through the process of 'allele binning', where alleles within designated boundaries are pooled into composite alleles, and various population parameters are estimated for allele groups instead of specific alleles (Taylor *et al.*, 1994; O'Reilly and Wright, 1995). Although such practices can ease the statistical analysis and interpretation of closely adjacent alleles, it may underestimate genetic diversity and differentiation.

Although most molecular markers that have been used to describe population structure are assumed to be predominantly selective neutral, recent attention has come to bear on the use of DNA loci either known or likely to be under selection, such as variability at the MHC loci (Edwards and Potts, 1996; Sanjayan *et al.*, 1996) and some single copy nuclear DNA loci (e.g., Fevolden and Pogson, 1998). As pointed out by Ferguson (1994), there has been little attempt to distinguish between neutral in the evolutionary sense, and effectively neutral as far as their use as markers are concerned, and that the chances of obtaining population-specific markers are much greater in polymorphisms subject to selection. In circumstances where diversifying or stabilising selection is contributing to the spatial distribution of alleles, the persistence of patterns over time, or among year classes (Butler and Cross 1996), may be an informative indicator of the extent of gene flow, especially if the selection coefficients to maintain the differentiation are unrealistically high (Fevolden and Pogson, 1998). On the other hand, selection may act fast enough to cause differentiation within the lifetime of individuals (Mork and Sundnes, 1985) and may bring about genetic differences among fish growing up in different nursery areas, so causing apparent substructuring within a panmictic population (Smith *et al.*, 1990).

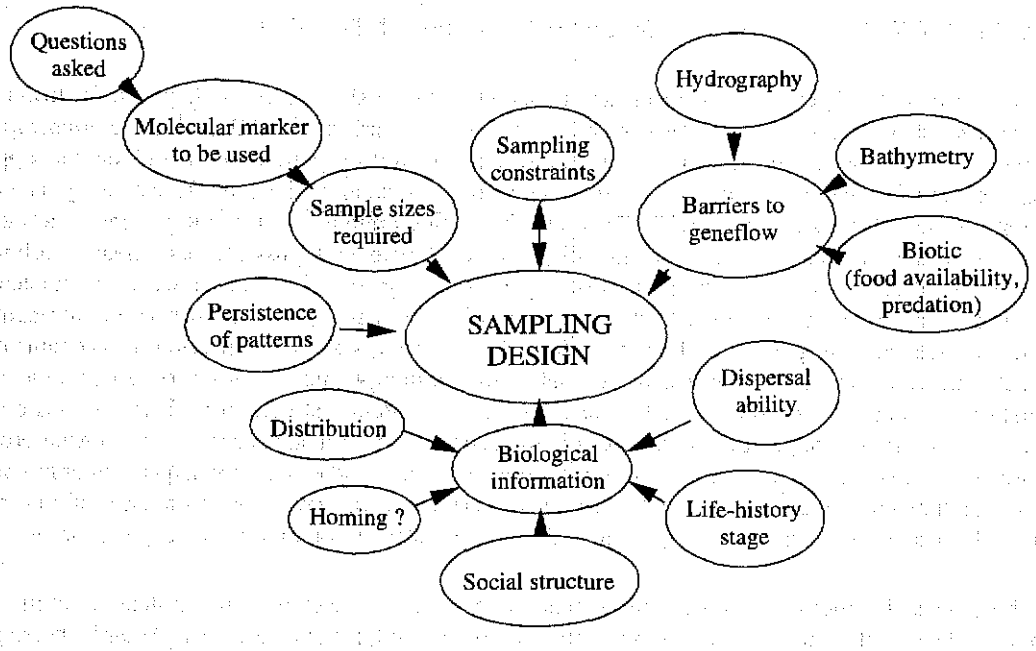
SAMPLING DESIGN AND THE DETECTION OF GENETIC DIFFERENTIATION

Clear patterns in the extent of genetic structuring are apparent across fish species, with generally lower levels of divergence in marine fishes compared to freshwater and anadromous (Ward *et al.*, 1994). Such relationships between habitat patchiness and vagility and the extent of genetic structuring follow predictions based on the opportunities afforded by physical isolation and ability to reinforce divergence through natal or site-specific spawning. The low levels of genetic divergence normally found in pelagic marine species is interpreted as being due to higher between-habitat continuity, larger effective population sizes, pelagic dispersal of larvae and possibly less clearly defined spatial separation of breeding sites. The readiness to interpret such patterns along such lines can, however, be misleading or at least an oversimplification, since it is well established that high dispersal capacity in the marine environment does not necessarily result in high rates of gene flow (Palumbi, 1994, 1996). Factors such as behavioural mechanisms limiting random dispersal, selection against immigrants or for balanced polymorphisms, complex oceanographic circulation patterns, and historic barriers to dispersal, may all serve to promote population structuring. There are thus considerable opportunities for the evolution of population biodiversity in the oceans, and it remains uncertain as to what proportion of the published studies on fish genetic structure represent biological reality, rather than inadequate sample collection or inappropriate use of molecular methodology and analysis. Nevertheless, the generalized patterns of lower levels of divergence across divergent taxa does support the notion of enhanced rates of population exchange in the sea.

Despite these broad-based patterns, the apparent genetic homogeneity detected using molecular tools sometimes contrasts with high levels of population polymorphism, as exemplified for example, by Atlantic herring (*Clupea harengus*, Parrish and Saville, 1965). *C. harengus* in the North Atlantic typically exhibits apparent genetic homogeneity across wide geographic areas, and yet displays marked population polymorphism in morphological, behavioural, life history and physiological traits. The lack of correspondence between genotypic and phenotypic differentiation is typically interpreted according to the paradigm of panmixia, whereby it is assumed that rates of gene flow among populations is too great to allow genetic structuring to develop. However, it is becoming increasingly clear (Bembo *et al.*, 1996; McQuinn, 1997) that variations in the intensity, timing and spatial scale of sampling can significantly underestimate or obscure the probability of detecting structuring, even where it may exist. For example, there is a common tendency to sample marine fishes without clear separation of feeding and spawning individuals. Furthermore, samples are often taken randomly with respect to hydrographic features and processes such as water depth and ocean fronts, or locally upwelling waters, often resulting in the comparison of either non-representative population samples, or the grouping of ecologically ambiguous assemblages. Additionally, the molecular tool adopted, and associated differences in detectable variability, mutation rates and responses to demographic processes (Carvalho and Hauser, 1994; Ward and Grewe, 1994), will further influence the extent of structuring detected. Finally, the focus on spatial relationships has tended to obscure subtle interactions arising from temporal dynamics and persistence.

Such limitations in sampling design usually arise from the lower accessibility and greater geographic expanse of many marine fish populations compared to freshwater species, making the collection of samples, often taken from commercial vessels, more a function of opportunism, rather than of design. While it is difficult to completely compensate for such constraints, the effectiveness of a sampling programme can be improved by considering several central questions relating to the biology of the species under study, and the scale and nature of sample collection (Figure 3). The absence of biological information such as size, age, sex and maturity stage makes it difficult to compare different studies meaningfully.

Figure 3. Salient points for consideration in the design of a sampling programme. Sampling will depend on the biological information available, the sample size requirements and possible barriers to gene flow. Most importantly, the nature of the question asked (e.g., relatedness, population structure, species identification) will determine the molecular marker to be employed, which in turn will dictate the sample sizes. It is also important to consider features of the environment, in particular barriers to gene flow by hydrographic or bathymetric features or indeed biotic interactions, such as predation and food availability. Biological information important to the design of sampling programmes include the distribution of a species, its social structure (e.g., schooling, lekking), its dispersal abilities and whether it is likely to home to natal or first spawning sites. In addition, it is important to consider which life-history stage is most likely to show population separation (larval/feeding/spawning), and when mixing among populations may occur. Important additional considerations are whether a time series of samples is required to demonstrate the persistence of the observed patterns. Finally, sampling will always be dependent on sampling constraints such as the availability of vessels, human resources and the fishing method.



For example, modifications to the usual practice of opportunistic sampling, and the integration of molecular and morphometric techniques, enabled the detection of allozymically distinct populations of the European anchovy, *Engraulis encrasicolus*, in Adriatic waters (Bembo *et al.*, 1996). Here, samples were collected at regular intervals throughout the Adriatic over a two-year period, and subjected to allozymic and morphometric analysis. In contrast to many allozyme studies on pelagic marine teleosts (Carvalho and Hauser, 1994; Ward and Grewe, 1994), significant genetic heterogeneity was observed on a geographic and local scale. Furthermore, the temporal analysis demonstrated a marked persistence of geographically differentiated populations from the north-western and southern-central waters, with a clear correspondence between allele frequencies at two loci, and the geographic distribution of morphologically distinct anchovies. Such patterns were subsequently related to the distribution of anchovy colour phenotypes characteristic of waters contrasting in depth, hydrographic features and circulatory patterns (Zore-Armanda, 1969; Tortonese, 1983). There was therefore a clear association between allele frequencies, morphology, geographic distribution and hydrography. The most compelling evidence for the existence of two Adriatic anchovy stocks was provided not by the spatial patterns in allele frequencies alone, but by their temporal persistence, often across short stretches of water (40–50 km). Such observations emphasize the point that it is not the description of genetic differences that necessarily matters: it is the dynamics of persistence over time that can facilitate the identification of stock-environment correlates. In the above anchovy case, such correlates were represented by the correspondence of distinct allozyme and morphological morphs with features of oceanography.

A factor relating to the evolution of populations can further complicate the estimation of gene flow estimates. Most genetic models such as the island model of Sewall Wright (1978) are based on the premise of genetic equilibrium, whereby a balance has been attained between genetic drift which causes divergence among populations, and gene flow, which tends to homogenise them. The time for such equilibrium to be reached depends on effective population size, which in marine populations, with sizes typically in excess of 10^5–10^6, may require millions of generations to attain. The effect of such genetic disequilibrium is that some patterns may arise from extant microevolutionary forces such as drift

and gene flow, whereas others may be relic patterns arising from historical oceanographic conditions (Reeb and Avise, 1990). The implications of such historical effects on gene flow suggest that it is important to employ molecular genetic techniques of high sensitivity, which allow for the detection of slight allelic divergence using one or a few nucleotide differences, as well as enabling the characterisation of more distantly related alleles. It follows therefore that more recently diverged alleles, with high nucleotide similarity, may have been less influenced by changes in the distant past, such as climate, than those which have persisted for millions of years (Baker *et al.*, 1993). The recent increase in availability of high resolution molecular markers (Park and Moran, 1994; Ferguson *et al.*, 1995; O'Reilly and Wright, 1995; O'Connell and Wright, 1997) thus has profound consequences on our ability to both detect and interpret the relative roles of historical and contemporary factors in determining population genetic structure.

PATTERNS OF GENETIC DIFFERENTIATION

The major determinants of genetic differentiation among populations include the degree of physical isolation and extent of gene flow among conspecific assemblages, the extent of habitat heterogeneity and associated nature of selection pressures, and the stability and size of populations over time. Among fishes, general global patterns of genetic differentiation are discernible: based on allozyme data, freshwater fishes show the highest levels of genetic differentiation (mean $G_{ST} = 0.22$) and marine fishes the lowest (mean $G_{ST} = 0.06$), with anadromous species showing intermediate levels (mean $G_{ST} = 0.11$) (Ward *et al.*, 1994). The lower levels of differentiation in marine species likely arise from higher gene flow among subpopulations, presumably due to the relative absence of barriers to dispersal in the sea. While such general patterns in fishes have been confirmed by the DNA-based approaches, the higher resolution afforded by such methods, and the ability to compare several markers simultaneously, have occasionally shown additional scales of population structuring, together with new information on the dynamics of variability in the mitochondrial and nuclear DNA genomes.

The early studies on mitochondrial DNA population differentiation were conducted on terrestrial vertebrates and, in accordance with predictions based on the higher mutation rates of mtDNA and its smaller effective population size relative to nuclear DNA, showed generally higher levels of population differentiation compared to allozymes (Avise, 1996). Although some studies on fishes confirmed such patterns (Reeb and Avise, 1990; Pogson *et al.*, 1995; Hansen and Loeschke, 1996), there are instances where no additional divergence was revealed, or cases where nuclear differentiation was greater (Ferguson *et al.*, 1991; Ward and Grewe, 1994; Ward *et al.*, 1994; Turan *et al.*, 1998). In consequence, there has been an increasing number of studies undertaken which compare the utility of different methods (Pogson *et al.*, 1995; Bentzen *et al.*, 1996; O'Connell *et al.*, 1997; Prodöhl *et al.*, 1997), though often not simultaneously on the same samples. Such approaches indicate differential responses of various nuclear and mitochondrial markers to changes in gene flow (Miller and Kapuscinski, 1996; Lu *et al.*, 1997), population size (Hauser *et al.*, 1995; Dueck and Danzmann, 1996) and possible effects of selection (Ferguson, 1994; Carvalho and Hauser, 1994; Fevolden and Pogson, 1998), as well as contrasting patterns depending on the region of DNA examined (Yang, 1996; Turan, 1997; Kocher and Carleton, 1997). Studies that utilise samples collected at different times and locations, although useful, are confounded by variability in the distribution of genetic variants, and data should be interpreted with caution. For example, it is well established that North Atlantic herring (*Clupea harengus*) taken from the Balsfjord herring on the Norwegian coast are allozymically distinct from local coastal populations (Jørstad and Nævdal, 1983; Jørstad *et al.*, 1994). In contrast, a recent study using allozymes and mtDNA RFLPs on samples taken from the same fjord (Turan *et al.*, 1998) failed to detect any such differentiation. A comparison of sampling methods, however, revealed that fish in the early work were collected from deep waters (ca. 100 m), whereas the recent samples were obtained from pelagic trawls taken at 15–20 m. Indeed, the existence of a genetically distinct, deep-water resident population, is supported by data showing the existence of specific spawning grounds in intertidal areas, and the associated presence of Balsfjord-characterised eggs from the innermost part of the fjord (Jørstad *et al.*, 1994; Jørstad, pers. comm.). Thus, in studies designed to compare the sensitivity of molecular markers, small-scale differences in spatial distribution require, at the very least, detailed information on sampling practices, or where possible, utilisation of the same samples. Clearly, it is not usually possible to analyse samples retrospectively, though as new methodologies are developed, such practices are feasible utilising the ever-increasing repository of alcohol-preserved samples available in many research laboratories and museums.

A recent example of the simultaneous application of different markers to the same samples is provided by recent studies on *Clupea harengus* (Turan, 1997; Turan *et al.*, 1998). Although there have been numerous molecular genetic studies on this species (Smith and Jamieson, 1986, reviewed in Turan *et al.*, 1998), our present understanding of herring stock structure is equivocal, and marked phenotypic heterogeneity is often not associated with detectable genetic divergence. *C. harengus* exhibits apparent genetic homogeneity on an oceanic scale, whereas, heterogeneity is observed locally among Norwegian fjords (Jørstad and Nævdal, 1981, 1983; Jørstad *et al.*, 1994; Turan, 1997; Turan *et al.*, 1998). The assumption has been that across oceanic distances, panmixia is the rule, facilitated by extensive migrations arising from the pelagic habit (Ward *et al.*, 1994; Graves, 1996). The localised divergence among fjord populations, both from each other (Turan, 1997), and from the coastal Atlanto-Scandian herring, appears to originate from the existence of distinct

spawning grounds within respective fjords. However, it is uncertain the extent to which such patterns are compromised by the inclusion of non-spawning fish in samples, or the dominant use of allozymes, markers known to have high sensitivity to low levels of gene flow (Carvalho and Hauser, 1994; Ward and Grewe, 1994).

A comparison of population structure using allozymes, mtDNA RFLPs, and microsatellites simultaneously on the same *C. harengus* individuals taken from the N.E. Atlantic, excluding fjord populations (Turan, 1997), revealed contrasting levels of differentiation depending on the marker system employed ($F_{ST} = 0.12^{NS}$ allozymes; 0.03^{NS} mtDNA-RFLP ND 5/6; 0.03^{***} microsatellites (Turan, 1997)). Microsatellite data exhibited the highest levels of divergence, revealing significant genetic heterogeneity among samples shown simultaneously to be homogeneous using allozymes and RFLP mtDNA analysis at the ND 5/6 genes. Indeed, this was the first demonstration of large-scale genetic divergence in *C. harengus* taken from open waters, demonstrating the effectiveness of these markers in detecting differentiation in a highly mobile, pelagic teleost. Such patterns of genetic structuring support the recent proposal (McQuinn, 1997) that herring comprise a series of metapopulations, maintained by behavioural isolation of adults, through homing to specific spawning grounds. Indeed, the small, but genetically significant, extent of straying among seasonal spawning groups and transience of some populations are proposed to be sufficient to homogenise gene pools, but maintain phenotypic differentiation. Such an assertion depends critically also on the levels of marker polymorphism, which in turn are affected by the mutation rate. It is possible, though further such studies are required on a temporal basis, that the high allelic diversity and rate of substitutions at microsatellite loci are sufficient to maintain genetic differentiation among populations experiencing occasional gene flow.

Microsatellites are proving effective for describing population structure in fishes, especially in cases where low levels of marker polymorphism have been detected previously, (O'Connell and Wright, 1997), though there are cases where alternative markers reveal higher measures of divergence, especially among salmonids (O'Connell and Wright, 1997). Although few published F_{ST} values based on microsatellites are available to examine global patterns of genetic differentiation in fishes (Table 3), data based on comparisons of microsatellite variability among freshwater, anadromous and marine fishes indicate a tendency toward higher levels in the latter. Such preliminary patterns support the notion that the higher effective population sizes of most marine fishes may result in higher overall levels of genetic diversity (Ryman *et al.*, 1994). Surprisingly perhaps, the highest estimates of genetic divergence have thus far been observed among herring (Turan, 1997), previously shown to yielded low distance measures based on protein or mtDNA markers.

Although the high mutation rate of microsatellite loci affords high levels of polymorphism for population studies, the step-mutational process, if prevalent, may produce convergent or parallel mutations. In such cases, allelic identity does not necessarily signify identity by descent, thus confounding estimates of genetic differentiation based on allelic comparisons (di Rienzo *et al.*, 1994). Such convergence may have given rise to the lower than expected differentiation of NW Atlantic and Barents Sea cod populations (Bentzen *et al.*, 1996), in contrast to the previously established east-west differences seen at protein, nuclear RFLP and mtDNA loci (Pogson *et al.*, 1995; Bentzen *et al.*, 1996). It is possible that the lack of divergence at several microsatellite loci could have originated through convergent evolution neutralising the effects of genetic drift, thus indicating that these markers may be less informative for broad-scale geographic surveys. Although moderately variable nuclear RFLP loci may offer certain statistical advantages for population discrimination over hypervariable microsatellites at some geographic scales (Pogson *et al.*, 1995; Bentzen *et al.*, 1996), the practical advantages of PCR-based technology far outweigh those of classical Southern blot procedures, especially where available tissues yield small quantities of DNA.

Thus, although the hypervariability of microsatellites yields a powerful array of genetic markers, it is necessary to consider carefully certain statistical and interpretative aspects of genetic differentiation estimates.

CONCLUDING REMARKS

The initial surge of molecular characterisation of fish populations provided by the advent of allozyme electrophoresis has in the last decade shown little evidence of abating. Indeed, the availability of PCR-based technology, combined with the enhanced resolution provided by highly repetitive DNA, has fuelled the pursuit of data which describe population structuring and migration patterns in wild fishes. Much of our ability to resolve stock structure, especially in highly mobile species, depends not only on the variability and microevolutionary responses of marker systems employed, but also critically on a more effective integration of biological and environmental data in the design of sampling programmes. While it remains difficult to sample populations repeatedly from expansive marine waters, much can be gained from the simultaneous comparison of protein and DNA markers in the same individuals, as well as the inclusion of some temporal sampling where possible.

The new DNA technology permits the improved description of population structuring in species largely inaccessible using conventional protein markers, either due to limited polymorphism, or high sensitivity of allozymes to low levels of gene flow. Although it is tempting to escalate such descriptive studies in the search for global patterns, it is the application of such approaches to tackle the underlying mechanisms that generate and maintain population structuring that may prove most insightful. Perhaps foremost among the recent applications which hold particular promise are studies aimed at monitoring the dynamics of microgeographic differentiation, and the analysis of historical populations. Elucidation of the oceanographic, spatial and temporal determinants of small-scale structuring would greatly facilitate our understanding of the variability in larval survival and recruitment dynamics, including the critical relationship between larval and adult populations (Ruzzante *et al.*, 1996). Studies on the extent to which juveniles, identified individually with microsatellites, become associated with returning adults to spawning grounds, for example, may provide significant data on the maintenance of seasonal spawning groups of pelagic fishes, and the origin of metapopulation structuring (Fontaine *et* al., 1997; McQuinn, 1997).

Table 3. Comparison of expected heterozygosities within samples and F_{ST} values derived from microsatellite studies on marine, freshwater, and anadromous species. Note the higher heterozygosity of the marine species compared to freshwater fish.

Species		% H	F_{ST}	Reference
Marine				
Sea bass	*Dicentrarchus labrax*	84 (69–93)	0.007	Garcia de Leon *et al.*,. 1997
Atlantic cod	*Gadus morhua*	88 (83–90)	0.015	Bentzen *et al.*, 1996
Whiting	*Merlangius merlangus*		0.006	Rico *et al.*, 1997
Pacific herring	*Clupea pallasi*	89	0.023	O'Connell *et al.*, 1996
Atlantic herring	*Clupea harengus*	90–92	0.035	Turan ,1997
Freshwater				
Atlantic salmon	*Salmo salar*	63		Tessier *et al.*, 1997
Brook char	*Salvelinus fontinalis*	71		Angers and Bernatchez, 1996
Rainbow trout	*Oncorhynchus mykiss*	74–95	0.016	O'Connell *et al.*, 1997
Stickleback	*Gasterosteus aculeatus*	73		Rico *et al.*, 1993
Bluegill sunfish	*Lepomis macrochirus*	27–63		Colbourne *et al.*, 1996
Common carp	*Cyprinus carpio*	60		Crooijmans *et al.*, 1997
Northern pike	*Esox lucius*	21		Miller and Kapuscinski, 1997
Anadromous				
Atlantic salmon	*Salmo salar*	80–91		O'Reilly *et al.*, 1996
		89		O'Connell and Wright, 1997
		83 (54–95)	0.075	Fontaine *et al.*, 1996
		68–78	0.054	McConnell *et al.*, 1997
Cutthroat trout	*Oncorhynchus clarki clarki*	69–71		Wenburg *et al.*, 1996
Steelhead trout	*Oncorhynchus mykiss*	41-72		Wenburg *et al.*, 1996

REFERENCES

Angers B. and Bernatchez L. 1996. Usefulness of heterologous microsatellites obtained from brook charr, *Salvelinus fontinalis* Mitchill, in other *Salvelinus* species. Mol. Ecol., 5: 317–319.

Baker, C.S., Perry, A., Abernethy, B., Alling, A., Bannister, J., Calambokidis, P., Lambertsen, R.H., Lien, J., O'Brien, S.J., Urban, J., Vasquez, O., Wienrich, M., Palumbi, S.R. (1993) Abundant mitochondrial DNA variation and world-wide population structure in humpback whales. Proc. Natl. Acad. Sci. USA, 90: 8239–8243.

Bembo D.G., Carvalho G.R., Cingolani N., Arneri E, Gianetti G. and Pitcher T.J. 1996 - Allozymic and morphometric evidence for two stocks of the European anchovy *Engraulis encrasicolus* in Adriatic waters. Mar. Biol.126: 529-538.

Bentzen P., Harris A.S. and Wright J.M. 1991 - Cloning of hypervariable minisatellites and simple sequence microsatellite repeats for DNA fingerprinting of important aquacultural species of salmonids and tilapias. *In* DNA Fingerprinting: Approaches and Applications. Birkhäuser Verlag, Basel. 400 pp.

Bentzen, P., Taggart, C.T., Ruzzante, D.E. and Cook, D. 1996 - Microsatellite polymorphism and the population structure of Atlantic cod (*Gadus morhua*) in the northwest Atlantic. Can. J. Fish. Aquat. Sci. 53: 2706-2721.

Bruford, M.W., Cheesman, D.J., Coote, T., H. Green, H.A.A., Haines, S.A., O'Ryan, C. and Williams, T.R. 1996 - Microsatellites and their application to conservation genetics. *In* Molecular Genetic Approaches in Conservation. Oxford University Press: Oxford, pp. 278-297.

Butler A. and Cross T.F. 1996 - Genetic differences between successive year classes of two strains of rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquaculture Res. 27: 643-649.

Carvalho, G.R. and Hauser, L. 1994 - Molecular genetics and the stock concept in fisheries. Rev. Fish Biol. Fish. 4: 351-373.

Carvalho G.R. and Hauser L. (1995) Genetic impacts of fish introductions: a perspective on African lakes. *In* Species Changes in African Lakes (Pitcher T.J. and Hart P.J.B., eds.). Chapman and Hall, London. pp. 457-494

Chakraborty R. 1992 - Sample size requirements for addressing the population genetics issues of forensic use of DNA typing. Hum. Biol. 64: 141–159.

Colbourne J.K., Meffe B.D., Wright J.M. and Cross M.R. 1996 - DNA fingerprinting of bluegill sunfish (*Lepomis macrochirus*) using $(GT)_n$ microsatellites and its potential for assessment of mating success. Can. J. Fish. Aquat. Sci. 53: 342-349.

Dessauer, H.C., Cole, C.J. and Hafner, M.S. 1996 - Collection and storage of tissues. *In* Molecular Systematics (2^{nd} ed). (ed) Hillis, D.M., Moritz, C. and Mable, B.K., Sinauer Associates, Massachusetts, pp. 29-50.

Di Rienzo, A., Peterson, A.A., Garza, J.C., Valdes, A.M., Slatkin, M. and Freimer, N.B. 1994 - Mutational processes of simple sequence repeat loci in human populations. Proc. Natl. Acad. Sci., USA, 91: 3166-3170.

Dueck L.A. and Danzmann R.G. 1996 - Matriarchal population structure of introduced rainbow trout (*Oncorhynchus mykiss*) in the Lake Ontario watershed. Can. J. Fish. Aquat. Sci. 53: 2100-2114.

Edwards S.V. and Potts W.K. 1996 - Polymorphism of genes in the major histocompatibility complex (MHC): implications for conservation genetics of vertebrates. *In:* Smith T.B. and Wayne R.K. (eds.) Molecular Genetic Approaches to conservation. Oxford University Press, New York. pp. 214-236.

Estoup, A., Tailliez, C., Cornuet, J.M. and Solignac, M. 1995. Size homplasy and mutational processes of interrupted microsatellites in two bee species, *Apis mellifera* and *Bombus terrestris* (Apidae). Mol. Biol. Evol. 12: 1074–1084.

Ferguson A. 1994 - Molecular genetics in fisheries: current and future perspectives. Rev. Fish Biol. Fish. 4: 379-383.

Ferguson M.M., Danzmann R.G. and Hutchings J.A. 1991 Incongruent estimates of population differentiation among brook charr, *Salvelinus fontinalis*, from Cape Race, Newfoundland, Canada, based upon allozyme and mitochondrial DNA variation. J. Fish Biol. 39 (Suppl. A): 79–85.

Ferguson A., Taggart J.B., Prodöhl P.A., McMeel O., Thompson C., Stone C., McGinnity P. and Hynes R.A. 1995 - The application of molecular markers to the study and conservation of fish populations, with special reference to *Salmo*. J. Fish Biol. 47 (Suppl. A): 103–126.

Fevolden S.E. and Pogson G.H. 1998 - Genetic divergence at the synaptophysin (*Syp1*) locus among Norwegian coastal and North-East Arctic populations of Atlantic cod, *Gadus morhua*. J. Fish Biol., in press.

Fontaine, P-M., Dodson, J.J., Bernatchez, L. and Slettan, A. 1997 - A genetic test of metapopulation structure in Atlantic salmon (*Salmo salar*) using microsatellites. Can. J. Fish. Aquat. Sci., 54: 2434-2442.

Fleischer, R.C. 1996 - Application of molecular methods to the assessment of genetic mating systems in vertebrates. *In* Molecular Zoology. Advances, Strategies and Protocols. Wiley-Liss, New York, pp. 133–162.

Galvin, P., McGregor, D. and Cross, T. 1995- A single locus PCR amplified minisatellite region as a hypervariable genetic marker in gadoid species. Aquaculture, 137: 31-40.

Galvin, P., Taggart, J., Ferguson, A., O'Farrell, M. and Cross, T. 1996- Population genetics of Atlantic salmon (*Salmo salar* L.) in the River Shannon system in Ireland: an appraisal using single locus minisatellite (VNTR) probes. Can. J. Fish Aquat. Sci. 53: 1933–1942.

Garcia de Leon, F.J., Chikhi, L. and Bonhomme, F. 1997 - Microsatellite polymorphism and population subdivision in natural populations of European sea bass *Dicentrarchus labrax*. Mol. Ecol. 6: 51-62.

Goudet, J. 1995 - F-STAT (1.2), a computer-program to calculate F-statistics. J. Hered. 86: 485-486.

Graves J.E. 1996 - Conservation genetics of fishes in the pelagic marine realm. *In* Avise J.C. and Hamrick J.L. (eds.) Conservation Genetics - Case Histories from Nature. Chapman and Hall, London. pp. 335-366.

Grooijmans, R.P.M.A., Bierboom, V.A.F., Komen, J., van der Poel, J.J. and Groenen, M.A.M.1997 - Microsatellite markers in the common carp (*Cyprinus carpio*). Anim. Genet. 28: 129–134.

Hansen, M.M. and Loeschke, V. 1996 - Genetic differentiation among Danish brown trout populations as detected by RFLP analysis of PCR amplified mitochondrial DNA segments. J. Fish Biol. 48: 422-436.

Hauser, L., Carvalho, G.R. and Pitcher, T.J. 1995- Morphological and genetic differentiation of the African clupeid *Limnothrissa miodon* 34 years after its introduction to Lake Kivu. J. Fish Biol., 47 (Suppl. A): 127–144.

Jarne, P. and Lagoda, P.J.L. 1996. Microsatellites, from molecules to populations and back. TREE, 11: 424-429.

Jones, A.G., and Avise, J.C. 1997 - Microsatellite analysis of maternity and the mating system of the Gulf pipefish *Syngnathus scovelli*, a species with male pregnancy and sex-role reversal. Molecular Ecology, 6: 203-214.

Jørstad, K.E. and Nævdal, G., 1981. Significance of population genetics on the management of herring stocks. ICES CM1981/H:64.

Jørstad, K.E. and Nævdal, G. 1983 - Genetically distinct populations of herring. Contrib. XI. Intern. Cong. Genet., New Delhi, India.

Jørstad, K.E., Dahl, G. and Paulsen, O.I. 1994 - Genetic comparison between Pacific herring (*Clupea pallasi*) and a Norwegian fjord stock of Atlantic herring (*Clupea harengus*). Can. J. Fish. Aquat. Sci. 51(Suppl. 1): 233-239.

Lu, G., Li, S., and Bernatchez, L. 1997 - Mitochondrial DNA diversity, population structure, and conservation genetics of four native carps within the Yangtze River, China. Can. J. Fish. Aquat. Sci. 54: 47-58.

McGregor, D., Galvin, P. and Cross, T. 1996- PCR amplification of a polymorphic minisatellite VNTR locus in whiting (*Merlangius merlangus* L.). Anim. Genet. 27: 49-51.

McQuinn, I.H. 1997 - Metapopulations and the Atlantic herring. Rev. Fish Biol. Fish. 7: 297-329.

Miller, L.M., and Kapuscinski, A.R. 1997. Historical analysis of genetic variation reveals low effective population size in a Northern pike (*Esox lucius*) population. Genetics, 147: 1249–1258.

Mork, J. and Sundnes, G. 1985. O-group cod (*Gadus morhua*) in captivity: differential survival of certain genotypes. Helgol. Meeresunt. 39: 63-70.

Nakamura, Y., Leppert, M., O'Connell, P., Wolff, R., Holm, T., Culver, M., Martin, C., Fujimoti, E., Hoff, M., Kumlin, E. and White, R. 1987. Variable number of tandem repeats (VNTR) markers for human gene mapping. Science, 235: 1616–1622.

O'Connell, M. and Wright, J.M. 1997. Microsatellite DNA in fishes. Rev. Fish Biol. Fish., 7: 331-364.

O'Connell, M, Dillon, M.C., and Wright, J.M. 1996. Development of microsatellite markers for genetic discrimination of Prince William Sound herring populations. Rep. Alaska Dept. Fish and Game, 31 pp.

O'Connell, M, Danzmann, R.G., Cornuet, J.M., Wright, J.M. and Ferguson, M.M. 1997. Differentiation of rainbow trout populations in Lake Ontario and the evaluation of the stepwise mutation and infinite allele mutation models using microsatellite variability. Can. J. Fish. Aquat. Sci., 54: 1391–1399.

Ohta, T., and Kimura, M. 1973. A model of mutation appropriate to estimate the number of electrophoretically detectable alleles in a finite population. Genet. Res. Camb. 22: 201-204.

Olsen, J.B., Wenburg, J.K., and Bentzen, P. 1996. Semiautomated multilocus genotyping of Pacific salmon (*Oncorhynchus* spp.) using microsatellites. Mol. Mar. Biol. Biotech., 5: 259–272.

O'Reilly, P. and Wright, J.M. 1995. The evolving technology of DNA fingerprinting and its application to fisheries and aquaculture. J. Fish Biol. 47 (Suppl. A): 29-55.

O'Reilly, P., Hamilton, L.C., McConnell, K. and Wright, J.M. 1996. Rapid analysis of genetic variation in Atlantic salmon (*Salmo salar*) by PCR multiplexing of dinucleotide and tetranucleotide microsatellites. Can. J. Fish. Aquat. Sci., 53: 2292-2298.

Palumbi, S.R. 1994. Reproductive isolation, genetic divergence, and speciation in the sea. Ann. Rev. Ecol. Syst. 25: 547-572.

Palumbi, S.R. 1996. Microspatial genetic structure and speciation in marine taxa with high dispersal abilities. *In:* Ferraris J.D. and Palumbi S.R. (eds.) Molecular Zoology: Advances, Strategies and Protocols. Wiley-Liss, New York, pp. 101–118.

Park, L.K. and Moran, P. 1994- Developments in molecular genetic techniques in fisheries. Rev. Fish Biol. Fish. 4: 272-299.

Parrish, B.B., and Saville, A. 1965- The biology of the north-east Atlantic herring populations. Oceanogr. Mar. Biol. Ann. Rev. 3: 323–373.

Pogson, G.H., Mesa, K.A., and Bouttilier R.G. 1995. Genetic population structure and gene flow in the Atlantic cod *Gadus morhua*: a comparison of allozyme and nuclear RFLP loci. Genetics, 139: 375–385.

Prodöhl, P.A., Walker, A.F., Hynes, R., Taggart, J.B., and Ferguson, A. 1997. Genetically monomorphic brown trout (*Salmo trutta* L.) populations, as revealed by mitochondrial DNA, multilocus and single-locus minisatellite (VNTR) analysis. Heredity, 79: 208–213.

Reeb, C.A. and Avise, J.C. 1990. A genetic discontinuity in a continuously distributed species: mitochondrial DNA in the American oyster, *Crassostrea virginica.* Genetics, 124: 397–406.

Rico, C., Zadworny, D., Kuhnlein, U., and Fitzgerald G.J. 1993. Characterisation of hypervariable microsatellite loci in the threespine stickleback *Gasterosteus aculeatus.* Mol. Ecol., 2: 271–272.

Rico, C., Rico, I., and Hewitt G.M. (1996) 470 million years of conservation of microsatellite loci among fish species. Proc. R. Soc., Lond. 263: 549-557.

Rico, C., Inbrahim, K.M., Rico, I., and Hewitt, G.M. 1997 - Stock composition in North Atlantic populations of whiting using microsatellite markers. J. Fish Biol. 51: 462-475.

Ruzzante, D.E., Taggart, C.T. and Cook, D. 1996-Spatial and temporal variation in the genetic composition of a larval cod (*Gadus morhua*) aggregation: cohort distribution and genetic stability. Can. J. Fish. Aquat. Sci. 53: 2695-2705.

Ryman, N., Utter, F. and Laikre, L. 1994. Protection of intraspecific biodiversity in exploited fishes. Rev. Fish Biol. Fish. 4: 417-446

Saiki, R.K., Gelfand, D.H., Stoffel, S., Scharf, S.J., Higuchi, R., Horn, G.T., Mullis, K. and Erlich, H.A. 1988- Primer-directed enzymatic amplification of DNA with thermostable DNA polymerase. Science, 239: 487–491.

Sanjayan, M.A., Crooks, K., Zegers, G., and Foran D. 1996. Genetic variation and the immune response in natural populations of pocket gophers. Cons. Biol. 10: 1519–1527.

Smith, P.J., and Jamieson, A. (1986) Stock discreteness in herrings: a conceptual revolution. Fish. Res. 4: 223-234

Shaw, P.W. and Boyle, P.R. (1997) Multiple paternity within the brood of single females of *Loligo forbesi* (Cephalopoda: Loliginidae), demonstrated by microsatellite DNA markers. Mar. Ecol. Progr. Ser. 160: 279-282.

Smith, P.J., Jamieson, A. and Birley A. and Birley, A.. 1990.Electrophoretic studies and the stock concept in marine teleosts. J. Cons. int. Explor. Mer., 47: 231-245.

Tautz, D. and Renz, M. 1984. Simple sequences are ubiquitous repetitive components of eukaryotic genomes. Nucleic Acids Res. 12: 4127-4138.

Taylor, E.B., Beacham, T.D., and Kaeriyama, M. 1994. Population structure and identification of North Pacific salmon (*Oncorhynchus keta*) revealed by analysis of minisatellite variation. Can. J. Fish. Aquat. Sci. 51:1430–1442.

Tortonese, E. (1983) Distribution and ecology of endemic elements in the Mediterranean fauna (fishes and echinoderms). NATO Conf. (Ser I: Medité. OR Ecosystems) 1: 57-83.

Turan, C. 1997 Population structure of Atlantic herring, *Clupea harengus* L., in the North-East Atlantic using phenotypic and molecular approaches. PhD thesis, University of Hull, U.K.

Turan, C., Carvalho, G.R., and Morke, J. 1998. Molecular genetic analyis of Atlanto-Scandian herring (*Clupea harengus*) populations using allozymes and mitochondrial DNA markers. J. Mar. Biol. Ass., U.K. 78: 1–15.

Utter, F.M. 1994. Perspectives of molecular genetics and fisheries into the 21st century. Rev. Fish Biol. Fish. 4, 374-378.

Ward, R.D., and Grewe, P.M. 1994. Appraisal of molecular genetic techniques in fisheries. Rev. Fish Biol. Fish. 4: 300-325.

Ward, R.D., Elliott, N.J., Grewe, P.M., and Smolenski A.J. 1994. Allozyme and mitchondrial DNA variation in yellowfin tuna (*Thunnus albacaers*) from the Pacific Ocean. Mar. Biol. 118: 531-

Whitmore, D.H., Thai, T.H. and Craft, C.M. 1992. Gene amplification permits minimally invasive analysis of fish mitochondrial DNA. Trans. Am. Fish. Soc. 121: 170–177.

Wright, J.M. 1993. DNA fingerprinting in fishes. *In* Biochemistry and Molecular Biology of Fishes, Vol 2 (ed) Hochachka, P.W. and Mommsen, T. Elsevier, Amsterdam, pp. 58-91.

Wright, J.M., and Bentzen, P. 1994. Microsatellites: genetic markers for the future. Rev. Fish Biol. Fisheries 4: 384-388.

Wright, S. 1978. Evolution and the Genetics of Populations. University of Chicago Press, Chicago.

Yang, Z. 1996 - Among site variation and its impact on phylogenetic analysis. TREE, 11: 367-372.

Zore-Armanda, M. 1969. Water exchange between the Adriatic Sea and eastern Mediterranean. Deep Sea Res. 16: 171–178.

Following consideration of the document presented above and discussion among members of the Working Group, the following recommendations were made:

Summary of recommendations on pelagic fish genetics

1) In view of their ecological and commercial importance, WGAGFM suggests that more molecular genetic work be undertaken on marine pelagic species. The choice of species should be made on the basis of their current status (particular attention being given to species with depleted stocks) and to the likely use of the results in management. Multi-country studies utilising an array of suitable molecular markers are most likely to yield most information.
2) Sampling should be undertaken on spawning individuals or their progeny. To do this, the biology of the species must be known.
3) A survey of variation throughout the range of the species should be undertaken, but such a study should incorporate a temporal element.

2.5 Sampling Strategies in Studies of Genetic Structure

[Based on a position paper by M.M. Hansen, Denmark. Adopted by WGAGFM in Cork.]

Practical sampling strategies in studies of genetic population structure of marine and anadromous species

INTRODUCTION

In studies of the genetic structure of fish populations, much emphasis is usually put on the choice of genetic markers and the statistical analyses applied. The issue of conducting adequate and representative sampling of populations often receives less attention, though even the best available statistics and genetic markers cannot compensate for inadequate sampling.

There are several factors that are important to be aware of in relation to sampling, in particular sample size, life stage of the individuals sampled, and the extent of the geographical area in which sampling has taken place.

SAMPLE SIZE

The optimal sample size depends primarily on the type of problem addressed, the statistical tests and the properties of the genetic markers that are applied. In studies of phylogeny and phylogeography, it is mainly the genetic relationships among alleles and haplotypes that are of interest, such as the phylogenetic relationships among mtDNA haplotypes, rather than differences in allele or haplotype frequencies among populations. Consequently, for such purposes sample sizes need not be that high.

In studies of genetic relationships and differentiation among populations, it becomes really important to have sufficiently large sample sizes. However, it is still unclear what constitutes a 'sufficiently large sample size'. Previously, when allozyme electrophoresis was the prevalent technique, the number of alleles per locus was usually not higher than two or three. Under these circumstances, as a rule of thumb Allendorf and Phelps (1981a) suggested that there should be less than 5 % probability of not detecting an allele present at a frequency of 5 % (i.e., $[1 - 0.05]^n < 0.05$). This corresponds to sampling approximately 60 genes, translating into sample sizes of 30 individuals if disomic loci are studied.

With the increased application of highly variable markers, such as mini- and microsatellites, the situation has become more complicated. Particularly in marine species the number of alleles per locus may be very high, often > 50. Also, the statistics applied have changed accordingly, and most test procedures are now based on numerical resampling and exact tests. The problem to be concerned about relates to the power of the tests applied. With a large number of alleles, e.g., 40, in a sample size of 50 individuals, there may not be sufficient power for detecting deviations from Hardy-Weinberg equilibrium. Hardy-Weinberg equilibrium is, however, a condition that must be fulfilled before most other tests and

analyses applied are valid, and insufficient power for detecting deviations from equilibrium therefore compromises the very basis of the study undertaken. Similarly, for tests for genetic differentiation among populations, it is important to consider power in relation to the number of alleles and sample sizes. In general, genetic differentiation among populations of marine fishes is much smaller than differentiation among populations of anadromous fishes (Ferguson *et al.*, 1995). This, combined with the large number of alleles at many of the loci studied, again may result in little power for detecting population subdivision unless sample sizes are large.

There can be little doubt that there is a positive relationship between sample size and the power of tests. However, for most of the exact tests applied, this relationship is not simple and may vary considerably among the different tests. As a consequence, it is not possible to give any firm recommendations for sample sizes. However, WGAGFM wants to stress that the issues of sample sizes and the specific test procedures to apply for population studies using highly variable molecular markers are so important and basic to the field that a specific workshop should be organised to treat these topics. This workshop should involve experts in the statistical treatment of population genetic data along with fish population geneticists.

From a more empirical point, WGAGFM would recommend that preliminary studies are undertaken before the start of the main studies. Such preliminary studies would involve a small number of samples (3–4 samples from geographically separate areas) and relatively small sample sizes (approximately 50). The degree of polymorphism observed in these samples and the amount of genetic differentiation observed among samples would serve as a basis for the further planning of sampling and sample sizes.

Also, for marine species it is often not a problem to obtain very large sample sizes (200 or so). Subsets of samples (but not necessarily all sampled individuals) could be analysed until satisfactory statistical power would be obtained. For instance, a marginally significant deviation from Hardy-Weinberg equilibrium could suggest that more individuals from the sample should be analysed in order to possibly verify this deviation.

LIFE STAGE OF SAMPLED FISH AND EXTENT OF GEOGRAPHICAL AREA SAMPLED

The biological and geographical features to consider in sampling are not necessarily the same for anadromous (salmonid) and marine species, but there are some issues that are common to both groups. A population can be defined as a group of individuals that are more likely to reproduce with one another than with individuals from other such groups. Consequently, since the aim is to identify and quantify genetic differences among reproductive units, the optimal sampling strategy would appear to involve sampling of sexually mature individuals. In addition, many fish species undertake migrations between foraging areas and geographically distinct spawning places and often exhibit rather precise homing. Good sampling procedures therefore also should involve sampling of spawning individuals directly at the spawning places (or, for some live-bearing species like redfish, at the time of fertilisation).

Sampling of marine species often is a tedious task and it may be difficult to accurately identify the spawning grounds. The requirements for sampling spawners directly at the spawning places therefore often have been relaxed, and studies have instead been based on taking samples from diverse geographical areas without particularly sampling mature individuals at the spawning grounds. While this approach may be useful for assessing large-scale variation within species, it may render it impossible to detect any small-scale population structure (if at all present). In principle, analysis of such 'mixed stock samples' should result in the Wahlund effect (homozygote excess). However, in the case of PCR-amplified single locus molecular markers (microsatellites and PCR'able minisatellites) mutations in the primer-binding regions may result in null-alleles (e.g., Pemberton *et al.*, 1995) which will also lead to homozygote excess. Consequently, apparent Wahlund effect is not necessarily a good indicator of the presence of small-scale genetic differentiation (cf. Rico *et al.*, 1997).

WGAGFM wants to stress the importance of sampling marine fishes at the spawning grounds at the time of fertilisation. This requires precise information from fisheries biologists regarding the reproductive biology, the geographical location of spawning grounds, migratory behaviour, etc., of the species studied. Also, it is very important to record biological information on the individual fish sampled in order to be able to verify that it is indeed likely to belong to the specific spawning population. The minimum information required includes age, sex, length, weight, maturity, precise location and date of sampling. In the case of pelagic spawners, physical oceanographic information is important for precise definition of spawning sites

There are some specific cases where sampling of spawning individuals directly on the spawning grounds is problematic or would be directly erroneous. The long-standing debate whether cod spawning in the coastal waters of northern Norway consist of two reproductively isolated populations (coastal and Arctic cod) is one such example (cf. Mork *et al.*, 1985; Dahle, 1991; Arnasson and Palsson, 1996). The foraging areas of the two types are geographically distinct, but

they spawn in the same geographical areas, possibly upholding some sort of reproductive isolation. Sampling of fish at the spawning grounds would in this case lead to the inclusion of both coastal and Arctic cod in the samples, resulting in a 'mixed-stock' sample. While coastal and Arctic cod in the samples could be distinguished on the basis of independent morphological and/or physiological features, the optimal sampling procedure would probably be to sample populations in their separate foraging areas. Overall, this example demonstrates the importance of being as much aware as possible of the general biology of the species and populations studied.

In salmonid fishes, it is customary to sample juveniles from the nursery areas in rivers. This is partly a remnant from studies based on allozymes, as it is almost impossible to do non-destructive sampling of eye, liver and heart tissue, and because sampling and killing larger numbers of adult individuals may be detrimental to the populations studied. Even though non-destructive sampling of, for instance, adipose fin tissue is sufficient for analyses of DNA-markers, juveniles are still easier to sample, as they are present in the rivers all year round and may be sampled in large numbers.

The genetic composition of juveniles should of course match that of their parents. However, most salmonid fishes spawn in distinct spawning redds. When the fry emerge from the redds they tend to establish territories in the close vicinity, and it may take one or more years before they move any significant distances up- or downstream in the river. Consequently, if a large number of fry are sampled from a limited stretch of the river, e.g., 50 m, it is possible that they represent just a few families, which is likely to impose a strong bias on estimates of allele frequencies of the population as a whole. This problem of 'family sampling' in salmonid fishes was first described by Allendorf and Phelps (1981b) and has been empirically demonstrated by Hansen *et al.* (1997). Apparently there are no corresponding problems in relation to marine fishes. At least, Herbinger *et al.* (1997) did not find any evidence of family structure in samples of cod larvae.

The solution to 'family sampling' problems in salmonid fishes is obviously to sample spawners instead of their offspring. However, as described previously there may be practical difficulties with that. In addition, it is important to be aware of what actually constitutes a 'spawning population'. Mature male parr are present in large numbers in most salmonid fish populations and probably make a significant contribution to the spawning (Jordan and Youngson, 1992; Moran *et al.*, 1995). Consequently, mature male parr should also be included in the samples. Another alternative consists in sampling juveniles of several age classes (for instance 0+, 1+ and 2+), and make sure that sampling takes place over longer river stretches (several hundred meters or more). The sampling could be adjusted in accordance with biological information such as the observed number of spawning redds. It is also important not to sample across obvious physical barriers separating individual populations, such as taking one sample covering more than one tributary. This could result in the inclusion of more than one population in a sample.

There is, however, an even more fundamental problem in sampling of salmonid populations. Genetic differentiation among tributary populations within river systems is often observed (Ferguson, 1989). The significance of this microgeographical differentiation in relation to evolutionary biology and conservation remains unresolved, but it is necessary to take it into account in the sampling of populations. Thus, one single sample from a tributary population is not necessarily representative for the river system as a whole, and more than one tributary population should be sampled.

Finally, confirmation of the temporal stability of allele frequencies is a basic feature, which is unfortunately often ignored both in salmonid and marine fishes. Temporal stability may be addressed in two different ways, i.e., by taking samples in different years or by dividing samples into cohorts, for instance 1+ and 2+ fish and test for differences in allele frequencies. In some cases, spawning may take place more than once within a spawning season on a specific locality, and it is important to study whether there is genetic heterogeneity among the different groups of spawning fish. In conclusion, WGAGFM recommends the following:

Summary of recommendations on sampling strategies

1) The issues of required sample sizes in relation to number of alleles, the power of statistical tests, and which statistical framework should be applied have become crucial issues with the development of highly variable DNA markers. WGAGFM needs to clarify a number of topics related to this issue, and wants to include it in its Terms of Reference for 1999 (cf. Section 3.3, point i).
2) Generally for experimental designs, WGAGFM recommends that preliminary studies based on 3–4 samples with sample sizes of ca. 50 be undertaken before the start of the main study. The results may serve as a basis for planning sample sizes and sampling design in the main study.
3) In most cases, both for marine and salmonid fishes, sampling at the spawning grounds at the time of fertilisation will be the optimal sampling strategy. In particular, in the case of marine fishes this requires precise information

from fisheries biologists regarding the reproductive biology, the geographical location of spawning grounds, migratory behaviour, etc., of the species studied.

4) In the case of marine fishes, biological information should be recorded on the individual fish sampled. The minimum information required includes age, sex, length, weight, maturity, precise location and date of sampling.

5) If studies of salmonid populations are based on sampling of juveniles, it is important to ensure that the individuals sampled do not represent just a few families. As many age classes as possible should be included in the samples, and samples should be taken over larger river stretches (i.e., several hundred metres). Publications reporting results based on sampling of juveniles should include information on the circumstances of sampling (i.e., age classes included and extent of the sampled area).

6) In salmonid fishes, genetic differentiation is often observed among tributary populations within a river system. A single sample from one tributary is not necessarily representative of the whole river system, and more than one tributary population should be sampled.

7) The temporal stability of allele frequencies should be tested. This could be done by analysing samples taken in different years or by comparing allele frequencies of different cohorts.

REFERENCES

Allendorf, F.W. and Phelps, S.R. (1981a). Use of allelic frequencies to describe population structure. Ecol. Bull., 34, 37-52.

Allendorf, F.W. and Phelps, S.R. (1981b). Use of allelic frequencies to describe population structure. Can. J. Fish. Aquat. Sci., 38, 1507–1514.

Arnason, E. and Palsson, S. (1996). Mitochondrial cytochrome b DNA sequence variation of Atlantic cod *Gadus morhua*, from Norway. Mol. Ecol., 5, 715-724.

Dahle, G. (1991). Cod, *Gadus morhua* L., populations identified by mitochondrial DNA. J. Fish. Biol., 38, 295-303.

Ferguson, A. (1989). Genetic differences among brown trout (*Salmo trutta*) stocks and their importance for the conservation and management of the species. Freshw. Biol., 21, 35–46.

Ferguson, A., Taggart, J.B., Prodöhl, P.A., McMeel, O., Thompson, C., Stone, C., McGinnity, P. and Hynes, R. A. (1995). The application of molecular markers to the study and conservation of fish populations, with special reference to *Salmo*. J. Fish Biol., 47(Supplement A): 103–126.

Hansen, M.M., Nielsen, E.E. and Mensberg, K.-L.D. (1997). The problem of sampling families rather than populations: Relatedness among individuals in samples of juvenile brown trout (*Salmo trutta* L.). Mol. Ecol., 6: 469-474.

Herbinger, C.M., Doyle, R.W., Taggart, C.T., Lochmann, S.E., Brooker, A.L., Wright, J.M. and Cook, D. (1997). Family relationships and effective population size in a natural cohort of Atlantic cod (*Gadus morhua*) larvae. Can. J. Fish. Aquat. Sci., 54 (Suppl. 1): 11–18.

Jordan, W.C. and Youngson, A.F. (1992). The use of genetic marking to assess the reproductive success of mature male Atlantic salmon parr (*Salmo salar* L.) under natural spawning conditions. J. Fish Biol., 41: 613-618.

Moran, P., Pendas, A.M., Beall,E. and Garcia-Vazquez, E. (1996). Genetic assessment of the reproductive success of Atlantic salmon precocious parr by means of VNTR loci. Heredity, 77: 655-660.

Mork, J., Ryman, N., Ståhl, G., Utter,F. and Sundnes, G. (1985). Genetic variation in Atlantic cod (*Gadus morhua*) throughout its range. Can. J. Fish. Aquat. Sci., 42: 1580–1587.

Pemberton, J.M., Slate, J., Bancroft, D.R. and Barrett, J.A. (1995) Non-amplifying alleles at microsatellite loci: a caution for parentage and population studies. Mol. Ecol., 4: 249-252.

Rico, C., Ibrahim, K.M., Rico, I. and Hewitt, G.M. (1997). Stock composition in North Atlantic populations of whiting using microsatellite markers. J. Fish. Biol., 51: 462-475.

3 WORKING GROUP BUSINESS

3.1 Comments on Working Group Functions

The establishment of pre-prepared position papers and specific responsibilities for chairing sessions and thematic colloquia have enhanced the efficiency of the annual meetings substantially. Likewise, the possibility to communicate by e-mail with most members, and the establishment of an Internet Homepage have greatly simplified the administration of the WGAGFM.

From a geographic point of view, the engagement by U.S. members in the WGAGFM activities is still low, and scientifically, the representation of scientists with a quantitative genetics background should be increased.

However, the general attendance at the WGAGFM meetings has been steadily increasing each year since 1994. Currently, 51 persons are appointed as members of WGAGFM.

At this year's meeting in Cork, 19 members from 12 different countries took part. In addition, 10 observers from Ireland attended parts of the meeting.

3.2 Comments on Travel Funds for WG Members

For some members the situation has improved, but lack of travel funds continues to be a major obstacle for many members to attend the annual WG meeting. WGAGFM noted this problem in the 1994, 1995, 1996, and 1997 WGAGFM Reports. WGAGFM once more recommended that ICES Member Countries follow up their appointment of members to the Working Groups with some responsibility that travel funds are made available.

3.3 Suggestions for WG ToR and Meeting Place in 1999

During discussions on meeting place in 1999, the WGAGFM responded positively to a generous invitation from Professor Jakob Jakobsson, the Director of the Marine Research Institute in Reykjavik, Iceland, to host the 1999 WGAGFM meeting in week 15 (12–15 April).

Concerning Terms of Reference and meeting place for 1999, it was decided to recommend that:

The Working Group on the Application of Genetics in Fisheries and Mariculture (New Chairman to be appointed at the 1998 ICES ASC in Lisbon, Portugal) will meet at the Marine Research Institute, Reykjavik, Iceland, 12–15April 1999, to:

a) continue the review of general population genetics topics in fisheries and mariculture, with emphasis on the utilisation of possibilities arising from the combination of qualitative and quantitative genetics;
b) review the potential of molecular markers as tools in breeding programmes;
c) review and discuss the status and future development of triploidy in aquaculture species;
d) review and evaluate measures used for protecting marine genetic diversity;
e) review the use of genetic tags in the study and management of wild stocks;
f) review problems and potential remedies concerning the gender of fish;
g) review patenting of technology as a potential problem in genetic research on marine species;
h) review genetic tissue authentication for forensic purposes;
i) review basic experimental design and statistical framework when using highly variable genetic markers in various species;
j) prepare updated protocols of fishery and mariculture genetics research in ICES Member Countries, and identify scopes for enhanced international cooperation.

3.4 Justifications for the Suggested 1999 ToRs:

a) WGAGFM currently acts as a relatively informal forum where members can feel free to discuss and update each other on practical and theoretical problems related to genetics of marine species. Experience has shown that there is a need for an open scientific session at the annual meetings, where topics that are not necessarily listed in the

Terms of Reference can be enlightened by the competence and experience existing in the WGAGFM. Not least have those topics which need competent input from both qualitative and quantitative genetics benefited from these discussions.

b) In terrestrial farming, the production in many sectors, e.g., poultry, dairy cattle, is based largely on selectively bred individuals as selection programmes have been demonstrated to cost-effectively increase production. Indeed, they are now a competitive necessity. In contrast, less than 1 % of aquaculture production is based on genetically improved strains.

In spite of the obvious benefits of selective breeding, efforts in the aquaculture industry have largely been restricted to the improvement of rearing conditions. This is unfortunate as the high fecundity of fish and most other aquatic animals provides tremendous scope for rapid genetic improvement of production traits and a concomitantly high return on investment in a selective breeding programme. Various factors lie behind the limited exploitation of selective breeding to increase production, but one of the biggest obstacles is the cost and difficulty of monitoring fish pedigrees and of evaluating the breeding value of individuals. At present, selective breeding of fish species requires specialized facilities to rear families in isolation until they can be physically tagged and estimates of breeding values are estimated statistically using phenotypic assessments of performance traits based on family variation. Recent advances in molecular biology offer the possibility of developing and applying molecular markers which can, at least in part, overcome these obstacles and, thereby, facilitate a more widespread implementation of selective breeding programmes in the aquaculture industry. The extent to which molecular markers can be used to facilitate the selective breeding of aquaculture species is uncertain and needs to be considered. *Position paper: Eric Verspoor.*

c) The induction of triploidy has been reported in many aquaculture species (fish: salmonids, seabass, seabream, turbot..., shellfish: mussels, oysters, scallops, clams...). In fish, triploidy is generally induced by pressure or thermal shocks while in shellfish, it is induced by the suppression of polar body formation in fertilised eggs. An alternative method is based on the mating of tetraploids and diploids to obtain all-triploid stocks. Tetraploids have been obtained in several species (rainbow trout, carp, oysters, mussels).

As triploidy induces sterility (or strongly reduces gametogenesis), better performance of triploids over diploids can be explained by two main factors:

1) The energy normally allocated to reproduction in diploids can be reallocated to growth in triploids. Consequently, triploidy will be of greater interest in species with high allocation to reproduction. Additionally, triploidy potentially induces higher mean heterozygosity, which has been shown to be positively correlated with growth in many shellfish species.

2) As sexually mature animals can be of lower quality for consumption than immature animals (e.g., oysters), sterility (or reduced gametogenesis) improves the marketing value of the products.

Additionally, the recent need for sterility to ensure a genetic confinement of transgenic organisms has led to new interest in triploidy.

The present status of triploidy and its potential for future development in aquaculture species will be reviewed by the WGAGFM to establish specific recommendations. *Position paper: Pierre Boudry.*

d) The decline and extinction of many populations of wild fish and shellfish, the emergence of enhancement programmes using cultured stock, and the establishment of closed [to fishing] areas by many countries will have impacts on marine genetic diversity. The Working Group proposes to review and evaluate measures used to protect marine genetic diversity with a view to the development of guidelines that are suitable for protecting genetic diversity in different types of species. *Position paper: Ellen Kenchington.*

e) The development of minisatellite and microsatellite DNA profiling over the past five years has made it possible to identify individuals, families and parentage of fish. This is opening up many new areas of investigation both in respect of natural populations and experimental studies under realistic field conditions. Important areas of study in this respect include: detailed study of breeding behaviour; individual relatedness within shoals/geographical areas/ spawning aggregations; identification of the origin of captured fish; genetic stock identification in mixed stock fisheries; monitoring success of supplemental stocking; experiments on the impact of deliberate and inadvertent introductions of non-native fish. It is now timely to review this area and examine further ways in which genetic tagging can be applied to the study and management of fish stocks. *Position paper: Andy Ferguson.*

f) Unlike mammals, the genotypic and phenotypic sex of bony fish varies among species. Halibut, seabass, European eel and turbot among others show variable sex ratios in natural and cultured populations, presumably in response to environmental and population dynamics factors. So far, no common sex determining system has been observed, but the aromatase gene seems to play a pivotal role in sex differentiation. Only 10 % of all fish species have been

reported to carry sex chromosomes, but few have been verified by breeding. The few sex probes developed (rainbow trout, chinook salmon, guppy and medaka amongst others) have been proven to be species-specific. Consequently, the determination of the genetic sex remains problematic and causes major economic losses in aquaculture. WGAGFM wants to review current knowledge and discuss future ways to proceed to solve these problems, with a view to giving recommendations to ICES as to the kind of fundamental and applied research (e.g., genetics, molecular biology and endocrinology) needed. *Position paper: Filip Volckaert.*

g) On the basis of known cases where current patenting practices in different countries have created real and/or potential problems for carrying out research, or to implement research results in aquacultural production, WGAGFM wants to review this field in order to identify to what degree this can be a problem for ICES Member Countries. *Position paper: Willie Davidson.*

h) Several of the laboratories represented in the Working Group have been engaged in work with the identification of animal tissues for forensic purposes, and there is a growing need to coordinate research and methodology in this field. WGAGFM feels that it is time to establish the status of this field, and to look into the possibilities for a better international network/coordination. *Position paper: Geir Dahle and Willie Davidson.*

i) The issues of required sample sizes in relation to number of alleles, the power of statistical tests, and which statistical framework should be applied to highly variable genetic markers in marine fishes are so important and basic that members of the Working Group will try to organize an EU-funded workshop in association with the next WGAGFM meeting. The workshop should involve experts in the statistical treatment of population genetic data along with fish population genetics. The output from the workshop should form the basis for a general discussion in a specific session at the next WGAGFM meeting, built around case studies of species from different ends of the spectre of genetic differentiation (e.g., tuna, herring, cod, squid, mussel and brown trout). *Position paper: Michael Møller Hansen and Andy Ferguson.*

j) The national activity reports which are compiled and updated each year by WGAGFM serve as a useful information base for geneticists in ICES Member Countries who are seeking cooperation or information on specific species or specific methodologies. This information base also makes it possible to monitor potential changes in research focus within finfish and shellfish genetics throughout the Member Countries. *Responsible for compilation: Anna Danielsdottir.*

ANNEX 1

TERMS OF REFERENCE FOR THE 1998 WGAGFM MEETING IN CORK, IRELAND

ICES C.Res.1997/2:23

The Working Group on the Application of Genetics in Fisheries and Mariculture (Chairman: Prof. J. Mork, Norway) will meet at the University College, Cork, Ireland, March 30 to April 2, 1998, to:

a) continue the review of population genetic topics in fisheries and mariculture, including the questions of selective fisheries and GMOs (Genetically Modified Organisms), with emphasis on a combination of qualitative and quantitative genetics;

b) treat the question of genetic management of new species in mariculture, including the application of breeding programs to increase production, with a view to give recommendations on the topic;

c) discuss genetic aspects in the management of pelagic marine species;

d) treat the question of practical sampling strategies in studies of genetic population structure of marine and anadromous fish species, with a view to give recommendations on the topic;

e) prepare updated protocols of fishery and mariculture genetic research in the member countries, and identify scopes for enhanced international cooperation.

Justifications:

a) In the long term management of marine resources the issue of selective fisheries is a very important one which deserves broad attention. The complexity of the problem suggests that it should be attacked on a broad front in ICES, e.g., as a joint approach by geneticists, fishery statisticians, biologists and modellers. In 1996, WGAGFM initiated a cooperation between geneticists and modellers which proved very fruitful for both parts and which clearly must be continued. This year, WGAGFM has produced an updated review and literature survey on the topic which concerned both qualitative and quantitative works. WGAGFM wants to keep this topic on its agenda also in 1998, with a view to establish the basis for a broad approach to the problem.

b) The science of applied selective breeding and genetics has contributed greatly to the steadily increasing productivity of terrestrial agriculture. The rate of change has been particularly rapid in the last 2-3 decades and today nobody will think of utilizing wild stocks for milk, meat, egg and wool production and animal husbandry without selection programmes. Today the high yields of land animal products are depending totally on genetically improved domesticated breeds and some level of controlled input. This has not been true for aquaculture where only one percentage of aquaculture production is based on improved stocks. Aquatic species are therefore, in the genetic sense, still much closer to the wild state than are the major terrestrial animals and food crops. Thus there is a great disparity between the need for increased aquaculture production and the genetic quality of the stocks available to meet that need. Moreover, full benefits of the investments in management improvements can only be obtained through a genetically improved fish that is able to respond on these improvements in an optimal way.

   Effective breeding programs are scarce in aquaculture. However, during the last two decades the prospects for genetic improvement have been well documented in several species, like Atlantic salmon, rainbow trout, Nile tilapia and rohu carp. As a result, there is a growing interest to start selective breeding programmes for other fish- and shellfish species. In Europe there is a growing production in many fish- and shellfish species among which Seabass, Seabream, Turbot, Carps, Halibut, Flat oyster, Scallop, Lobster are the most important. Genetic improvement programmes are not applied in any of these species. WGAGFM feels it is necessary to discuss the application of, and to spread information about the benefits of using selective breeding to improve production traits for various marine species.

c) Stocks of important pelagic marine resource species like herring, capelin, blue whiting, squids, and not least the large cosmopolitic tunas and swordfishes often have an international oceanic distribution and perform extensive oceanic migrations across national borders and economic zones. Their nursery areas are often in the mesopelagic water layers, meaning that their geographic distributions are not so restricted by bottom topography, local sea-bed production and water depths as for bottom-dwelling species. They therefore tend to have more continuous oceanic distributions in which temperature fronts and ocean current systems appear to be the major physical cohesion factors. It may be hypothesized that this has an effect on the genetic structure of the species in question. WGAGFM feels it is appropriate to investigate this question, and to formulate testable hypothesis about it. The answers may provide valuable insight in the evolutionary factors currently moulding the genetic structure of species, for the gain of basic population genetic science as well as for the practical management of marine resources.

d) Geographical variation in fish species is typically studied by taking samples of fish from different localities. However, different populations may occur sympatrically outside the spawning season, but migrate to distinct spawning areas during the spawning season. It can be argued that ideally, if the aim is to study genetic differentiation among populations, sampling should preferably take place during the spawning season and on the spawning sites, but this is often difficult for practical reasons. Also, in the case of e.g., the Arcto-Norwegian and the Norwegian coastal cod stocks which spawn simultaneously in the Lofoten area, such a sampling strategy would actually introduce representativity problems instead of solving them.

For anadromous species (salmonids) population sampling is often based on juveniles. Since juveniles do not disperse much from the spawning redds, there is a risk of including only few families in samples assumed to represent the whole population.

Consequently, well-design sampling strategies must also take the biological characteristics of the species into consideration. WGAGFM wants to review this topic in general as well as for specific cases, with a view to present practical recommendations for management as well as for workers in the field.

ANNEX 2

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ANNEX 3

NATIONAL ACTIVITY REPORTS FOR 1998

BELGIUM

Study 1

LABORATORY/RESEARCHER	Royal Belgian Institute of Natural Sciences (RBINSc), Brussels / T. Backeljau, B. Winnepenninckx and H. De Wolf. Joint program with University of the Azores, Portugal / A.M. Frias Martins, C. Brito and R. Medeiros. The Natural History Museum London / D. Reid. University of Leeds, U.K. /J. Grahame and P.J. Mill. Regional Technical College, Galway, Ireland / E. Gosling.
SPECIES	*Littorinidae* (periwinkles), particularly *Littorina striata* (Mollusca, Gastropoda and Prosobranchia).
PROJECT FUNDING	EU MAST-III program; PRAXIS (Portugal); graduate student grant by the IWT (Belgium); Joint Basic Research Project grant by the Belgian National Science Foundation.
OBJECTIVE	Integrating population genetic and morphological variation over the entire geographical range of the species; separating genetic and phenotypic elements in shell polymorphisms and determining their biological significance in order to investigate what factors/mechanisms are responsible for the macro- and microgeographic maintenance of shell polymorphisms in the presence of extensive gene flow (i.e., selection versus phenotypic plasticity). Phylogenetic analysis of littorinid genera and family levels.
DESIGN	The whole geographic range of *L. striata* (Macaronesian archipelagos Azores, Madeira, Canary Islands, Cape Verde) has been intensively sampled; at several places, sampling involved detailed microgeographic patterns; as such several thousands of individuals have been (and still are) analyzed for morphometric and genetic variation. Field transplantation experiments are being performed. Radular myoglobins in several littorinids are being studied for taxonomic and population genetic purposes.
METHODOLOGY	Electrophoresis of allozymes and radular myoglobins; random amplified polymorphic DNA; development of microsatellite DNA markers and Single Strand Conformation Polymorphisms (SSCP); DNA sequencing; morphometrics of shell features
STATUS	License degree theses; ongoing program within MAST-III.

Study 2

LABORATORY/RESEARCHER	Royal Belgian Institute of Natural Sciences, Brussels / T. Backeljau. In collaboration with the University of Vigo, ES / T. Willems, J. Troncoso and A. Sanjuan and University of the Azores, P / A.M. Frias Martins and C. Brito.
SPECIES	*Artemia salina,* Rotifera.
PROJECT FUNDING	Own funding.
OBJECTIVE	Genetic characterisation of strains of *Artemia salina* and Rotifera.
DESIGN	Sampling at various locations.
METHODOLOGY	DNA fingerprinting (RAPD and AFLP).
STATUS	Under development.
COMMENTS	Funding requested.

Study 3

LABORATORY/RESEARCHER	Katholieke Universiteit Leuven, Zoological Institute, Leuven, Belgium / F. Volckaert and E. Daemen.
SPECIES	European eel (*Anguilla anguilla*).
PROJECT FUNDING	Ph.D. fellowship and University grants.
OBJECTIVE	Characterisation of the population genetics of the European eel, including genetic structure, gene flow and selection.
DESIGN	Comparative spatial analysis of five glass eel populations along the European continental shelf.
METHODOLOGY	DNA microsatellites and mitochondrial DNA sequence variation.
STATUS	Ph.D. thesis in progress; several publications in progress. DNA microsatellite primer paper published in Animal Genetics (1997).

COMMENTS	We are looking for collaboration with eel biologists for future application at EU-FAIR (fifth framework).

Study 4

LABORATORY/RESEARCHER	Katholieke Universiteit Leuven, Zoological Institute, Leuven / F. Volckaert and E. Gysels.
SPECIES	Gobies (*Pomatoschistus minutus* and *P. lozanoi*).
PROJECT FUNDING	IWT (Flemish research fund for applied research) and Belgian Ministry of Science Affairs (project "Sustainable development of the North Sea").
OBJECTIVE	Characterisation of the population genetics of two sympatric populations of gobies along the European continental shelf, including genetic structure, gene flow and selection.
DESIGN	Various samples collected by benthic sledge and beam trawl at various scales and time patterns in the North Sea.
METHODOLOGY	Allozyme electrophoresis and mitochondrial DNA sequence variation and polymorphisms.
STATUS	Ph.D. project in progress as well as a graduate thesis; project is open ended; we welcome collaboration.
COMMENTS	

Study 5

LABORATORY/RESEARCHER	Katholieke Universiteit Leuven, Zoological Institute, Leuven, Belgium / F. Volckaert.
SPECIES	European eel (*Anguilla anguilla*) and African catfish (*Clarias gariepinus*).
PROJECT FUNDING	Own funding.
OBJECTIVE	The isolation of sex-specific molecular markers in European eel and catfish.
DESIGN	Molecular markers are isolated in model species with known sex determining systems; this expertise is translated to catfish and eel.
METHODOLOGY	Various techniques to isolate sex-specific DNA sequences such as AFLP, microsatellite DNA fingerprinting, SOX and Smcy genes and selective breeding.
STATUS	In progress.
COMMENTS	Review paper in preparation. Project continues at low intensity with national funds. Future funding under DGXIV (fifth framework) envisaged.

Study 6

LABORATORY/RESEARCH	Katholieke Universiteit Leuven, Zoological Institute, Leuven / F. Volckaert and M. Zietara.
SPECIES	Monogenea *Gyrodactylus* sp..
PROJECT FUNDING	Belgian Ministry of Scientific Affairs (project "Sustainable development of the North Sea).
OBJECTIVE	The isolation of molecular markers in *Gyrodactylus* for population genetic studies.
DESIGN	Samples of various *Gyrodactylus* species on various hosts (gobies and sticklebacks) are collected at 4 sites on the European continental shelf.
METHODOLOGY	Characterisation of ITS nuclear region and ND2/3 mitochondrial locus by means of sequencing.
STATUS	In progress.

Study 7

LABORATORY/RESEARCH	Katholieke Universiteit Leuven, Zoological Institute, Leuven / F. Volckaert and 11 other teams (Coordinator B. Chatain, IFREMER, Palavas-les-Flots, France).
SPECIES	Sea bass (*Dicentrarchus labrax*).
PROJECT FUNDING	EU -Concerted Action (DGXIV).
OBJECTIVE	To establish a programme for strain testing of sea bass.
DESIGN	Four working groups review population genetics, strain characterisation, sex determination and selection.
METHODOLOGY	Informal and formal meetings, litterature review, book and CD-ROM
STATUS	In progress since 01.01.98.
COMMENTS	

Study 8

LABORATORY/RESEARCH	Katholieke Universiteit Leuven, Zoological Institute, Leuven / F. Volckaert.
SPECIES	Dover Sole (*Solea solea*).
PROJECT FUNDING	Belgian Ministry of Scientific Affairs (project "Sustainable development of the North Sea).
OBJECTIVE	To detail the gene flow of a coastal spawner.
DESIGN	Samples of brood stock all along the European continental shelf.
METHODOLOGY	Characterisation of the mitochondrial DNA genome by means of RFLP analysis and sequencing.
STATUS	In progress.
COMMENTS	Biopsies of 20 mature fish from several spawning grounds are welcome.

Study 9

LABORATORY/RESEARCH	Agricultural Research Centre-Ghent, Department of Sea Fisheries, Oostende.
SPECIES	Flatfishes initially, later on all seafood or seafood products.
PROJECT FUNDING	Own funding.
OBJECTIVE	To develop DNA -based methods for authentication of commercially important species (unprocessed and processed).
METHODOLOGY	RAPD, SSCP and AFLP.
STATUS	Project started in September 1997.
COMMENTS	The project aims at the development of reliable, reproducible, cheap and easy DNA techniques suitable to construct a database.

Study 10

LABORATORY/RESEARCH	Department Sea Fisheries, Ankerstraat 1, B-8400 Oostende, / D. Delbare and R. De Clerck. Joint project with CEFAS Fisheries Laboratory, Lowestoft, United Kingdom / R. Millner, and the Danish Institute for Fisheries Research (DIFMAR), Charlottenlund, DenM. / M. Winter.
SPECIES	*Scophthalmus rhombus* (brill) (Pisces, Pleuronectiformes).
PROJECT FUNDING	European Commission, Directorate General XIV Fisheries.
OBJECTIVE	To describe the stock structure of the brill in relation to fisheries, by comparison of biological parameters (growth, sex ratio, age of maturity, stage of maturity, and gonadosomatic index) and genetic variation.
DESIGN	A two year research programme was started to collect biological data which are required for fishery assessment. In addition DNA analysis on sequence variation will provide a view on population differentiation, necessary to rational for genetic management of the different brill stocks.
METHODOLOGY	Mitochondrial DNA sequencing of the control region.
STATUS	Project in progress.

Study 11

LABORATORY/RESEARCH	RIVO-DLO, IJmuiden, The Netherlands / H. Heesen. Joint project with Department Sea Fisheries / E. Ongenae, D. Delbare and R. De Clerck.
SPECIES	*Psetta maxima* (turbot) and *Scophthalmus rhombus* (brill) (Pisces, Pleuronectiformes).
PROJECT FUNDING	European Commisson, Directorate General XIV Fisheries.
OBJECTIVE	Preliminary assessments of two important by-catch species to provide data on mortality and recruitment combined with the precise identification of unity stocks.
DESIGN	Additional age- and length compositions of landings, together with biological parameters will be compared with the results obtained in EU-projects 95/039 and 96/001 (by Department Sea Fisheries), to estimate recruitment, spawning stock biomass and mortality rates for the species concerned.
METHODOLOGY	Mitochondrial DNA sequencing of the control region, SSCP and AFLP.
STATUS	Project started on 01.01.98.

CANADA

Study 1

LABORATORY/RESEARCHER	NRC Institute for Marine Biosciences, Halifax /Dr S. Douglas project leader, in collaboration with Mr D. Cook, Marine Gene Probe Laboratory, Dalhousie University.
SPECIES	*Pleuronectes americanus*, winter flounder.
PROJECT FUNDING	NRC core budget.
OBJECTIVE	Microsatellite DNA markers are being obtained to assist in estimating genetic divergence of wild stocks and to aid in future broodstock selection in aquaculture.
DESIGN	Fish were sampled from various fishing areas around Nova Scotia, Canada.
METHODOLOGY	Microsatellites are being cloned and sequenced from genomic DNA and primers are designed to amplify specific microsatellites by PCR. The products are resolved on an automated DNA sequencer.
STATUS	In progress.

Study 2

LABORATORY/RESEARCHER	NRC Institute for Marine Biosciences, Halifax / Dr S. Douglas and Dr M. Reith, project leaders.
SPECIES	*Pleuronectes americanus*, winter flounder.
PROJECT FUNDING	NRC core budget.
OBJECTIVE	Establishment of an EST database from winter flounder.
DESIGN	DNA libraries established from a number of tissues of a winter flounder.
METHODOLOGY	Random cDNA clones from several winter flounder libraries (pyloric caeca, intestine and spleen) are being sequenced and identified by comparing against the genetic databases. This identifies clones containing sequences of highly expressed genes and gives valuable information regarding codon usage (necessary for design of PCR primers). The EST data provides a starting point for isolating genes and for genome mapping in this organism.
STATUS	Currently approximately 1000 ESTs in the database from the following libraries: stomach, pyloric caeca, spleen, intestine and pancreas.

Study 3

LABORATORY/RESEARCHER	NRC Institute for Marine Biosciences, Halifax / Dr S. Douglas project leader.
SPECIES	*Pleuronectes americanus*, winter flounder.
PROJECT FUNDING	NRC core budget.
OBJECTIVE	Ontogeny of digestive enzyme activity in teleost fish.
DESIGN	cDNA clones are isolated from gut tissues of winter flounder and used to probe expression patterns.
METHODOLOGY	Portions of genes are amplified by PCR using primers based on conserved motifs. Genes are sequenced and used to develop assays for gene expression (molecular and microscopic).
STATUS	Genes cloned for amylase, trypsin, pepsin, aminopeptidase and elastase. Assays underway.

Study 4

LABORATORY/RESEARCHER	NRC Institute for Marine Biosciences, Halifax / Dr S. Douglas project leader.
SPECIES	*Pleuronectes americanus*, winter flounder.
PROJECT FUNDING	NRC core budget.
OBJECTIVE	Investigation of malpigmentation in flatfish using molecular biological techniques.
DESIGN	The gene for a critical enzyme in the biochemical pathway leading to melanin is being cloned.
METHODOLOGY	Portions of the gene are being amplified by PCR using primers based on conserved motifs. The gene will be sequenced and used to assay expression at the molecular level.
STATUS	In progress.

Study 5

LABORATORY/RESEARCHER	NRC Institute for Marine Biosciences, Halifax and Department of Fisheries and Oceans / C.J. Bird (NRC) and E. Kenchington (DFO) project leaders.
SPECIES	*Placopecten magellanicus* (sea scallop), other scallops (*Chlamys, Pecten, Argopecten, Crassodoma*), oysters (*Ostrea edulis* (European Oyster), *Crassostrea*), mussels (*Mytilus* sp.), clams (*Mactromeris*).
PROJECT FUNDING	NRC and DFO core budget.
OBJECTIVE	Genetic discriminants and markers for bivalves.
DESIGN	DNA samples are being examined for animals from a number of locations.
METHODOLOGY	Microsatellites and nucleotide sequences of ribosomal RNA genes (including their internal transcribed spacers) are being evaluated as discriminants of taxa and populations. Microsatellites in particular are valuable for labeling pedigreed broodstock and checking the provenance of aquacultured stocks. DNA nucleotide sequence is less sensitive intraspecifically but provides a measure of species relatedness.
STATUS	Largely finished; preparing for publication.

Study 6

LABORATORY/RESEARCHER	Dept. Biology, Dalhousie University, Halifax, /Dr E. Zouros; Dept. Fisheries and Oceans, Dartmouth, N.S / Dr E. Kenchington; NRC Institute for Marine Biosciences, Halifax / C. Bird.
SPECIES	*Placopecten magellanicus* (sea scallop).
PROJECT FUNDING	Natural Sciences and Engineering Research Council (NSERC) of Canada; DFO core funds, NRC core funds.
OBJECTIVES	Use of nuclear (microsatellite, cDNA) and mitochondrial DNA markers to measure genetic differentiation among commercial scallop beds and to produce superior strains for aquaculture or sea-ranching. Microsatellites in particular are valuable for labeling pedigreed broodstock and checking the provenance of aquacultured stocks.
METHODOLOGY	Scallops have been collected from all of the commercial scallop beds on the Scotian Shelf, from St. Pierre Bank, Nfld, the Gulf of St. Lawrence and the Virginia Capes (US). Separate year classes have been analyzed from one bed to determine cohort effect.
STATUS	A publication on the microsatellite markers appeared in the Journal of Shellfish Research (December 1997). Final year.

Study 7

LABORATORY/RESEARCHER	Dept. Biology, Dalhousie University, Halifax, /Dr E. Zouros (project leader), L. Cao, Y. Shi. and DFO, Dartmouth, N.S., Canada / Dr E. Kenchington.
SPECIES	Blue mussel (*Mytilus edulis*), *Mytilus trossulus, Placopecten magellanicus.*
PROJECT FUNDING	Natural Sciences and Engineering Research Council (NSERC) of Canada; DFO.
OBJECTIVES	Genetics of natural and contained populations (stock discrimination, population structure, hybridization and dispersal-genetic improvement of stocks used in aquaculture).
METHODOLOGY	Molecular markers of nuclear and mitochondrial DNA, induction of triploidy, sex manipulation.
STATUS	Ongoing. A large number of publications in primary research journals detailing mtDNA inheritance and sex determination in mussels, molecular genetics of natural populations of scallops and significance of enzyme variation for growth and viability in oysters and scallops.

Study 8

LABORATORY/RESEARCHER	Dept. Fisheries and Oceans, Aquaculture Division, Gulf Fisheries Centre, Moncton / T. Landry. Institut Maurice Lamontagne, Ministere des peches et Oceans, Mont-Joli, PQ / J.M. Sevigny, R. Tremblay. MAPAQ, Iles de la Madeleine / B. Myran.
SPECIES	Blue mussel (*Mytilus edulis*).
PROJECT FUNDING	AFRI Can / P.E.I. Cooperative Agreement.
OBJECTIVE	Compare genetic characteristics of mussels stocks from bay with and without aquaculture activities (preliminary investigation).
DESIGN	Genetic variation of wild and cultured mussel is evaluated in two bays with aquaculture activity and two bays without aquaculture activity in PEI.

METHODOLOGY	Allozyme.
STATUS	Ongoing.

Study 9

LABORATORY/RESEARCHER	Dept. Fisheries and Oceans, Aquaculture Division, Gulf Fisheries Centre, Moncton / T. Landry (project leader) and T.W. Sephton.
SPECIES	*Mercenaria mercenaria* (Bay quahaug), local wild species and aquaculture "notata" variety.
PROJECT FUNDING	Can/NB/PEI Cooperation Agreement for Alternative Species Research.
OBJECTIVES	Evaluate the growth, survival and production of seedstock from two sources of broodstock 1) F three broodstock from PEI, and 2) "notata" variety broodstock.
DESIGN	Two source of seed are being compared in side by side replicated field trials at three locations in the southern Gulf of St. Lawrence for a two year growth experiment.
STATUS	Second and final year.

Study 10

LABORATORY/RESEARCHER	Department of Anatomy and Cell Biology, University of Saskatchewan, Health Sciences Building, Saskatoon / Dr P. Krone.
SPECIES	zebrafish (*Danio rerio*).
PROJECT FUNDING	NSERC.
OBJECTIVE	Regulation and role of heat shock proteins (hsps) during normal embryonic development. Regulation and role of heat shock proteins in following exposure to environmental stress.
DESIGN	Embryos at different stages of embryonic development are treated with the environmental stressors (heat shock, etc.) and the corresponding morphological and molecular changes are assessed. Overexpression of hsps and dominant negative forms hsps and examination of subsequent effects on development as above. Pharmacological inhibition of hsp function and examination of subsequent effects on development as above.
METHODOLOGY	Recombinant DNA techniques (cDNA cloning, etc; whole mount in situ hybridization for the examination of tissue specific patterns of gene expression; Northern and Southern blot analysis; microscopy (stereo, compound and compound w/ DIC).
STATUS	Project ongoing.

Study 11

LABORATORY/RESEARCHER	Department of Anatomy and Cell Biology, University of Saskatchewan, Health Sciences Building, Saskatoon, Saskatchewan / Dr P. Krone.
SPECIES	Zebrafish (*Danio rerio*).
PROJECT FUNDING	Canadian Network of Toxicology Centres.
OBJECTIVE	Assessment of molecular and cellular effects of endocrine-disrupting compounds on embryonic development.
DESIGN	Treatment of embryos with putative endocrine disrupting compounds and examination of subsequent (morphological and molecular) impact on development.
METHODOLOGY	Recombinant DNA techniques (cDNA cloning, etc; whole mount in situ hybridization for the examination of tissue specific patterns of gene expression; Northern and Southern blot analysis; microscopy (stereo, compound and compound w/ DIC).
STATUS	Project ongoing.

Study 12

LABORATORY/RESEARCHER	Simon Fraser University, Dept. of Biological Sciences, Burnaby / B. McKeown and S. Tang.
SPECIES	Rainbow trout.
PROJECT FUNDING	NSERC funded.
OBJECTIVE	To characterize the SPARC (secreted protein acidic and rich in cystine) and PLP (proteolipid protein) genes.
DESIGN	Gene cloning and controls of expression.

METHODOLOGY	Gene cloning.
STATUS	Project ongoing.
COMMENTS	These genes have been identified and sequenced. Expression in various tissues and conditions have been found.

Study 13

LABORATORY/RESEARCHER	Simon Fraser University, Dept. of Biological Sciences, Burnaby / B. McKeown and K. Poon.
SPECIES	Rainbow trout.
PROJECT FUNDING	NSERC funded.
OBJECTIVE	To characterize the ras oncogene.
DESIGN	Gene cloning and controls of expression.
METHODOLOGY	Gene cloning.
STATUS	Project ongoing.
COMMENTS	This gene has been isolated and sequenced. Work is now continuing on controls of expression.

Study 14

LABORATORY/RESEARCHER	Simon Fraser University, Dept. of Biological Sciences, Burnaby / B. McKeown and K. Poon.
SPECIES	Rainbow trout.
PROJECT FUNDING	NSERC funded.
OBJECTIVE	To identify the growth hormone receptor gene.
METHODOLOGY	Gene cloning.
STATUS	Project ongoing.
COMMENTS	We are presently in the process of trying to clone this gene.

Study 15

LABORATORY/RESEARCHER	Zoology Dept., University of Guelph, Guelph, Ontario / J.S. Ballantyne (project leader) with P.D.N. Hebert, E. Boulding, P. Wright.
SPECIES	Arctic charr.
PROJECT FUNDING	NSERC Strategic Grant.
OBJECTIVE	Enhancement of Arctic charr aquaculture in Canada.
STATUS	Project ongoing.

Study 16

LABORATORY/RESEARCHER	Sciences and Technology Dept., Laval University, Quebec / J. de la Noue (project leader) with S.L. Scott.
PROJECT FUNDING	NSERC Strategic Grant.
OBJECTIVE	Enhanced oral delivery of microbial phytase.
METHODOLOGY	Using novel pH-sensitive polymers to improve fish growth performance and reduce phosphorus discharge from aquaculture production.
STATUS	Project ongoing.

Study 17

LABORATORY/RESEARCHER	Oceanography Dept., University of Quebec, Rimouski / H.I. Browman (project leader).
PROJECT FUNDING	NSERC Research Grant.
SPECIES	Cod (*Gadus morhua*).
OBJECTIVE	Effects of solar ultraviolet radiation, maternal condition, quality and temperature on survivorship, growth and feeding performance of cod larvae.
STATUS	Project ongoing.

Study 18

LABORATORY/RESEARCHER	Biology Dept., University of Ottawa, Ottawa, Ontario / F. Chapleau (project leader).
PROJECT FUNDING	NSERC Research Grant.
SPECIES	Flatfish.
OBJECTIVE	Phylogeny and the evolution of life history traits in flatfishes.
STATUS	Project ongoing.

Study 19

LABORATORY/RESEARCHER	Zoology Dept., University of Guelph, Guelph, Ontario / R.G. Danzmann (project leader).
PROJECT FUNDING	NSERC Research Grant.
SPECIES	Salmonids.
OBJECTIVE	Genetics of development, fitness and life-history variability salmonid fishes.
STATUS	Project ongoing.

Study 20

LABORATORY/RESEARCHER	Zoology Dept., University of Toronto, Toronto, Ontario / H.H. Harvey (project leader).
PROJECT FUNDING	NSERC Research Grant.
SPECIES	White sucker (*Catostomus commersoni*).
OBJECTIVE	Phenotypic plasticity and genetic polymorphism in the white sucker.
STATUS	Project ongoing.

Study 21

LABORATORY/RESEARCHER	Zoology Dept., University of British Columbia, Vancouver, B.C. / J.D. McPhail (project leader).
PROJECT FUNDING	NSERC Research Grant.
SPECIES	Sticklebacks and charr.
OBJECTIVE	Hybridization, natural selection and genetic divergence in sticklebacks and charr.
STATUS	Project ongoing.

Study 22

LABORATORY/RESEARCHER	Faculty of Medicine, University of Ottawa, Ottawa, Ontario / M. Ekker (project leader).
PROJECT FUNDING	NSERC Research Grant.
SPECIES	Zebrafish (*Danio rerio*).
OBJECTIVE	Functional analysis of dlx homeoproteins in transgenic zebrafish embryos.
STATUS	Project ongoing.

Study 23

LABORATORY/RESEARCHER	Department of Biological Sciences, University of Calgary, Calgary, Alberta / L. Gedamu (project leader).
PROJECT FUNDING	NSERC Research Grant.
SPECIES	Rainbow trout.
OBJECTIVE	Rainbow trout metallothionein gene regulation.
STATUS	Project ongoing.

Study 24

LABORATORY/RESEARCHER	Zoology Department, University of Manitoba, Winnipeg, Manitoba / R.A. McGowan (project leader).
PROJECT FUNDING	NSERC Research Grant.
SPECIES	Zebrafish (*Danio rerio*).
OBJECTIVE	Dominance modification and genome imprinting in zebrafish; to investigate the role of

	methylation and the DNA methyltrasferase gene in zebrafish development.
DESIGN	Breeding of transgenetic zebrafish to non-trangenetic mates and track the methylation and expression status of a variety of loci during early developmental stages of the zebrafish in order to produce a developmental profile of methylation changes. Isolation of homologue of the DNA methyltransferase gene from zebrafish in order to investigate its role in early developmental decision making processes.
METHODOLOGY	Variety of molecular techniques. Methylation is assayed with the use of methylation-sensitive restriction enzymes and Southern blotting techniques. the isolation of genes is accomplished by using already cloned sequences from other species to identify homologous sequences in zebrafish cDNA libraries.
STATUS	Project ongoing.
COMMENTS	We have been able to establish that a parent-of-origin effect is evident in these fish at the level of a transgene locus. We are now attempting to evaluate endogenous loci. The methylation analyses are fairly preliminary but results are very promising.

Study 25

LABORATORY/RESEARCHER	Department of Biology, University of New Brunswick / T. J. Benfey (project leader).
PROJECT FUNDING	NSERC Strategic Grant, Department of Fisheries and Oceans, NRC Institute of Marine Biosciences, Marine Mariculture Inc., RandR Finfish Developments Ltd., Stolt SeaFarm Inc.
SPECIES	Halibut.
OBJECTIVE	Development of all-female halibut stocks for aquaculture.
METHODOLOGY	Genetic and hormonal manipulations used successfully to develop all-female stocks of salmon and trout for aquaculture will be tested for halibut.
STATUS	Project ongoing.

Study 26

LABORATORY/RESEARCHER	Dept. Fisheries and Oceans, Canada, Northwest Atlantic Fisheries Centre, St. John's, Newfoundland / R. Penney (project leader).
SPECIES	Blue mussel (*Mytilus edulis*), *M. trossulus.*
PROJECT FUNDING	DFO core funding.
OBJECTIVES	1) Delineate existing *edulis* - *trossulus* proportions on commercial mussel culture sites 2) Survey populations of edulis and trossulus for allelic variation.
DESIGN	Twenty-five wild and fifteen cultured populations have been sampled since 1994.
METHODOLOGY	Laboratory analysis using electrophoretic techniques is still underway. Four isozymes are being used, MPI, GPI, PGM and LAP. Samples are being classified to species using the MPI.
STATUS	The results thus far indicate *trossulus* mussels are widespread in occurrence throughout Newfoundland. Typically, most sites are a mix of edulis and trossulus types. Proportionally, edulis usually is the dominant species at most sites. There does not appear to be any geographic separation of species nor are any other distribution patterns apparent. Sites in close (< 5 km.) proximity have similar scales of genetic variation as was found over the entire study area, a coastline of over 9600 km. Culture sites generally have proportionally more *M. trossulus* compared to wild sites. The relative proportions of the two species at individual sites was not related to any of a suite of topographic or hydrographic features used to characterize inlets.

Study 27

LABORATORY/RESEARCHER	Dept. Fisheries and Oceans, Canada, Northwest Atlantic Fisheries Centre, St. John's, Newfoundland / R. Penney (project leader).
SPECIES	*Modiolus modiolus.*
PROJECT FUNDING	DFO core funding.
OBJECTIVES	To determine the allozyme patterns of the horse mussel for comparison with *Mytilus edulis* and *M. trossulus.*
DESIGN	Four sites were sampled where *M. modiolus* grows either mixed with *Mytilus* species or immediately adjacent to *Mytilus* beds.
METHODOLOGY	A series of enzymes are under consideration, including MPI, GPI, PGM, LAP, EST, AAP, AP.
STATUS	Initial sampling of four sample site has been completed. Allozyme variation in Modiolis

	is now being compared to M. edulis and M. trossulus to determine which, if any, may be used to discriminate between species.

Study 28

LABORATORY/RESEARCHER	NRC Institute for Marine Biosciences, Halifax / Dr M. Ragan, project leader. With Atlantic Veterinary College, Charlottetown / Dr R. Cawthorn, St Mary's University, Halifax / T. Rand and DFO Nanaimo M. Kent.
SPECIES	Various protists parasitic in salmon and other marine fish, and in lobsters.
PROJECT FUNDING	NRC core budget for the most part, grant funding is received by collaborators.
OBJECTIVE	Characterization of protistan parasites of fish and shellfish using sequence data from nuclear ssu-rRNA genes; consideration is being given to genomic sequencing of a selected parasite if funding can be obtained.
DESIGN	DNA samples are being obtained for a variety of protist parasites.
METHODOLOGY	Characterization of protistan parasites of fish and shellfish using sequence data from nuclear ssu-rRNA genes. Design and application of oligonucleotide probes for detection of protistan parasites. Molecular (DNA- and protein-level) characterization of protistan parasites of economic importance in aquaculture.
STATUS	Largely complete; priority now is to publish results.

Study 29

LABORATORY/RESEARCHER	NRC Institute for Marine Biosciences, Halifax / Dr M. Reith, project leader.
SPECIES	*Pleuronectes americanus*, winter flounder, other marine fish species.
PROJECT FUNDING	NRC core budget.
OBJECTIVE	To undertake a search for sex-linked DNA markers in flatfish.
DESIGN	Various cDNA Markers isolated from reproductive and other tissues of male and female flounder are being isolated and compared for expression and for segregation in male and female fish.
METHODOLOGY	Molecular biology tools are being used to obtain probes that reveal polymorphic loci and to examine segregation of these alleles in male and female fish (test for linkage with sex-determining locus).
STATUS	In progress.

Study 30

LABORATORY/RESEARCHER	NRC Institute for Marine Biosciences, Halifax / Dr M. Ragan, project leader, with DFO Nanaimo / Dr M. Kent.
SPECIES	Selected microsporidian protists parasitic in salmon and other marine fish.
PROJECT FUNDING	NRC and DFO core budgets.
OBJECTIVE	Characterization of protistan parasites of fish and shellfish using sequence data from random genomic fragments; funding for more extensive genomic sequencing of a selected parasite is being sought.
DESIGN	DNA samples are being obtained for target parasites.
METHODOLOGY	Partial characterization of protistan parasites of fish using DNA sequence data. Design and application of oligonucleotide probes for detection of protistan parasites and search for potential targets for therapeutants.
STATUS	Recently initiated, initial challenge has been to get sufficient, clean, parasite DNA.

Study 31

LABORATORY/RESEARCHER	NRC Institute for Marine Biosciences, Halifax / Dr M. Ragan, project leader, with Natural History Museum, London / Dr M. Embley.
SPECIES	Selected microsporidian protist parasitic in marine fish.
PROJECT FUNDING	NRC core budgets and European grant money (for Embley).
OBJECTIVE	Characterization of the parasite using sequence data from ESTs; funding for more extensive genomic sequencing of a selected parasite is being sought.
DESIGN	cDNA samples are being sent to Halifax for sequencing.
METHODOLOGY	Partial characterization of protistan parasites of fish using DNA sequence data. Design and application of oligonucleotide probes for detection of protistan parasites and search for potential targets for therapeutants.

STATUS	Recently initiated.

Study 32

LABORATORY/RESEARCHER	NRC Institute for Marine Biosciences, Halifax / Dr M. Reith project leader, in collaboration with University of New Brunswick / Drs C. McGowan and T. Benfy.
SPECIES	Atlantic halibut.
PROJECT FUNDING	NRC core budget and strategic NSERC grant.
OBJECTIVE	Microsatellite DNA markers are being obtained to assist in estimating genetic divergence of wild stocks and to aid in future broodstock selection in aquaculture.
DESIGN	Initial fish were taken from broodstock at DFO St. Andrews; others to be added.
METHODOLOGY	Microsatellites are being cloned and sequenced from genomic DNA and primers are designed to amplify specific microsatellites by PCR. The products are resolved on an automated DNA sequencer.
STATUS	In progress.

Study 33

LABORATORY/RESEARCHER	Institut Maurice-Lamontagne, Ministère des Pêches et des Océans, Mont-Joli / B. Sainte-Marie and J.-M. Sévigny. Département des Sciences Animales, Université McGill, Sainte-Anne-de-Bellevue / N. Urbani, A. Rondeau and U. Kuhnlein.
SPECIES	Snow crab (*Chionoecetes opilio*).
PROJECT FUNDING	Department of Fisheries and Oceans.
OBJECTIVES	(1) Description of the population structure in the Northwest Atlantic; (2) Description of inter-cohort genetic variability; (2) Study of the snow crab mating system.
DESIGN	(1) Mature males of different size classes were sampled at several sites in the Gulf of St. Lawrence and the Atlantic. (2) Several cohorts collected at the same sampling site are being analyzed. (3) Mating system is being studied under laboratory and field conditions. Experiments describing the behavior of males mated noncompetitively and competitively are carried out. Paternity is also determined under laboratory and field condition.
METHODOLOGY	Morphometry, allozyme, mtDNA, microsatellite DNA.
STATUS	Description of the population structure will be completed in 1998 as well as the description of inter-cohort variability. Study of the mating system is ongoing. Allozyme analyses of the progenies obtained in controlled mating experiments carried out over two female breeding cycles support the last-male sperm precedence hypothesis. The field study of paternity insurance using microsatellite is ongoing.

Study 34

LABORATORY/RESEARCHER	Institut Maurice-Lamontagne, Ministère des Pêches et des Océans, Mont-Joli / J.-M. Sévigny and M. Black. Département de Biologie, Université Laval, Québec / L. Bernatchez and S. Roques.
SPECIES	*Sebastes* sp.
PROJECT FUNDING	Department of Fisheries and Oceans.
OBJECTIVES	Species and stock discrimination in the Northwest Atlantic.
DESIGN	Redfish samples are being collected at several sites from the Gulf of Maine to Labrador Sea. Molecular markers are being developed for species and stock discrimination. The project is carried out in collaboration with Dr G. Naevdal of Bergen University.
METHODOLOGY	Allozyme, mtDNA, rDNA and microsatellite DNA.
STATUS	Ongoing.

Study 35

LABORATORY/RESEARCHER	Ministère l'Agriculture, des Pêcheries et de l'Alimentation du Québec / B. Myrand. Département de Biologie, Université Laval, Québec / R. Tremblay. Institut Maurice Lamontagne, Ministère des Pêches et des Océans, Mont-Joli / J.-M. Sévigny.
SPECIES	Blue mussel (*Mytilus edulis*).
PROJECT FUNDING	Ministère l'Agriculture, des Pêcheries et de l'Alimentation du Québec.
OBJECTIVES	Assess the impacts of mussel farming practices on wild mussel populations in Magdalen Island lagoons.

DESIGN	Genetic variation of wild stocks is described in different lagoons of the Magdalen Islands and compared with the variability detected in mussel populations cultivated under various regimes of density.
METHODOLOGY	Allozyme.
STATUS	Ongoing.

Study 36

LABORATORY/RESEARCHER	Dept. Fisheries and Oceans, Aquaculture Division, Gulf Fisheries Centre, Moncton, N.B / Mr T. Landry (project leader), R. Tremblay and B. Gillis.
SPECIES	*Mytilus edulis* (Mussel), PEI wild and cultured stocks.
PROJECT FUNDING	DFO and PEI AFRI.
OBJECTIVES	Evaluate the qualitative contribution of the wild stock versus the cultured stocks and the interaction of mussel mariculture and wild mussel fisheries.
DESIGN	Four bays in PEI (sites) are being investigated. Enzymes polymorphism are analyzed with electrophoretic techniques to describe the genetic variability of wild and cultured mussels.
STATUS	Ongoing.

Study 37

LABORATORY/RESEARCHER	Biology Dept., Dalhousie University, Halifax / Dr J. Wright (project leader).
SPECIES	Pacific herring (*Clupea harengus*).
PROJECT FUNDING	Alaska Dept. Fish and Game.
OBJECTIVES	To examine population differentiation of this species and temporal stability of allele frequencies in Prince William Sound, Alaska.
METHODOLOGY	Microsatellite markers.
STATUS	Ongoing. A manuscript is in press in the Journal of Fish Biology by O'Connell *et al.*

Study 38

LABORATORY/RESEARCHER	Biology Dept., Dalhousie University, Halifax / Dr J. Wright (project leader).
SPECIES	Atlantic salmon.
PROJECT FUNDING	Department of Fisheries and Oceans.
OBJECTIVES	To evaluate stocking and enhancement of Atlantic salmon in NS.
DESIGN	We have examined stocking practices with endogenous fish and fish from other rivers on the Grand R. and LaHave R., NS.
METHODOLOGY	Microsatellite markers have been developed for use on scale samples as old as 50 years. Also, dinucleotide and tetranucleotide microsatellites have been co-amplified in the same reaction and exhibit nonoverlapping allele length distributions. A paper on this rapid analysis method has been published (*CJFAS* 532292-2298).
STATUS	Ongoing. We have had great success with reading scale samples.

Study 39

LABORATORY/RESEARCHER	Biology Dept., Dalhousie University, Halifax / Dr J. Wright (project leader).
SPECIES	Atlantic salmon.
PROJECT FUNDING	Private.
OBJECTIVES	Use of microsatellite markers for pedigree analysis and breeding programmes for a Scottish aquaculture company.
METHODOLOGY	Microsatellite markers.
STATUS	Ongoing.

Study 40

LABORATORY/RESEARCHER	Biology Dept., Dalhousie University, Halifax / Dr J. Wright (project leader).
SPECIES	*Tilapia.*
PROJECT FUNDING	NSERC.
OBJECTIVES	Development of various genetic markers (e.g., repetitive DNAs such as SINEs and

	expressed sequence tags from brain, heart and liver) for use in genome mapping, and aquaculture breeding programs, etc.
STATUS	Ongoing.

Study 41

LABORATORY/RESEARCHER	Biology Dept., Dalhousie University, Halifax / Dr J. Wright. NRC Institute for Marine Biosciences, Halifax / Dr J. Wright.
SPECIES	Various toxin producing strains of dinoflagellates.
PROJECT FUNDING	NRC core budget, pending.
OBJECTIVES	Development of various genetic markers to detect toxin-producing strains of dinoflagellates. Toxic algae are a major concern in the shellfish mariculture industry.
STATUS	New project in preliminary stages of development.

Study 42

LABORATORY/RESEARCHER	Marine Gene Probe Lab., Dalhousie University, Halifax / Dr C. Taggart, Dr D. Ruzzante, D. Cook and S. Lang.
SPECIES	*Gadus morhua* (Atlantic cod).
PROJECT FUNDING	Canadian Dept. of Fisheries and Oceans.
OBJECTIVES	To define discrete stocks in the Gulf of St. Lawrence and approaches, and to determine relative contributions of the individual stocks to the mixed populations on feeding grounds.
DESIGN	Samples collected from areas suspected to be discrete stocks throughout Gulf of St. Lawrence and approaches during spawning aggregation and samples collected from several areas on feeding grounds.
METHODOLOGY	Blood samples collected from fish and preserved in alcohol, all information regarding collection area and individual fish collected. DNA extracted from preserved blood and assayed for six microsatellite loci as described
	CJFAS 51 1959–1966, 1994. Analysis of results of microsatellite assays for spawning aggregations similar to methods described
	CJFAS 53 634-645, and samples from feeding grounds assayed at the same loci. Based on results from these assays attempts will be made to assign specific components of the mixed stock to previously characterized spawning groups.
STATUS	First phase of project complete all samples have been collected and lab work 90 % complete. At this stage several stocks have been defined however at this time analysis of mixed stocks has not been carried out. A further study has been proposed to determine temporal stability of mixed stocks.

Study 43

LABORATORY/RESEARCHER	Marine Gene Probe Lab., Dalhousie University, Halifax / D. Cook, Dr D. Ruzzante, S. Lang, and Dr C. Taggart.
SPECIES	*Gadus morhua* (Atlantic cod); *Gadus ogac* (Greenland cod) *Boreogadus saida* (Arctic cod).
PROJECT FUNDING	Canadian Dept. Fisheries and Oceans.
OBJECTIVES	To determine the utility of microsatellites to identify listed species larvae in areas which may be mixed spawning grounds.
DESIGN	Identified adults of listed species assayed on 14 candidate microsatellites to determine allelic distributions and accuracy of identification of various species based on allele size.
METHODOLOGY	Blood of fin clip samples as available collected from three species, DNA extracted and run on all microsatellites available.
STATUS	Data has been collected from approximately 5,000 adult Atlantic cod (for other purposes) data collection complete for Arctic cod and is presently being collected for Greenland cod. To date results have shown identification of Arctic cod will be 100 % accurate, results for Greenland cod unknown.

Study 44

LABORATORY/RESEARCHER	Marine Gene Probe Lab., Dalhousie University, Halifax / Dr D. Ruzzante, D. Cook, and

	Dr C. Taggart.
SPECIES	*Gadus morhua* (Atlantic cod).
PROJECT FUNDING	Canadian Dept. Fisheries and Oceans.
OBJECTIVES	To determine whether or not there is evidence of genetic differentiation between Spring and Fall spawning cod populations on the Scotian Shelf.
STATUS	In progress.

Study 45

LABORATORY/RESEARCHER	Marine Gene Probe Lab., Dalhousie University, Halifax / Dr D. Ruzzante, D. Cook, and Dr C. Taggart.
SPECIES	*Gadus morhua* (Atlantic cod).
PROJECT FUNDING	Canadian Dept. Fisheries and Oceans.
OBJECTIVES	Larval cod aggregations on the Scotian Shelf and off Newfoundl and source-sink populations
STATUS	In progress.

Study 46

LABORATORY/RESEARCHER	Marine Gene Probe Lab., Dalhousie University, Halifax / Dr D. Ruzzante, D. Cook, and Dr C. Taggart; with D. Townsend and I. Kornfield (US).
SPECIES	*Gadus morhua* (Atlantic cod).
PROJECT FUNDING	NSF.
OBJECTIVES	Larval exchange between Georges Bank and Browns Bank.
STATUS	In progress.

Study 47

LABORATORY/RESEARCHER	Marine Gene Probe Lab., Dalhousie University, Halifax / Dr D. Ruzzante, D. Cook, and Dr C. Taggart.
SPECIES	*Gadus morhua* (Atlantic cod).
PROJECT FUNDING	Canadian Dept. Fisheries and Oceans.
OBJECTIVES	Assessment of historical DNA from cod populations in the NW Atlantic.
METHODOLOGY	DNA is extracted from archived otolith collections.
STATUS	In progress.

Study 48

LABORATORY/RESEARCHER	Marine Gene Probe Lab., Dalhousie University, Halifax / D. Cook, Arran Macpherson and Dr C. Taggart.
SPECIES	Capelin.
PROJECT FUNDING	Canadian Dept. Fisheries and Oceans.
OBJECTIVES	Development of tetranucleotide probes for capelin.
STATUS	New project.

Study 49

LABORATORY/RESEARCHER	Marine Gene Probe Lab., Dalhousie University, Halifax / D. Cook and Dr C. Taggart.
SPECIES	Shark species.
PROJECT FUNDING	FAO.
OBJECTIVES	Development of species identification markers for shark species.
STATUS	New project.

Study 50

LABORATORY/RESEARCHER	Marine Gene Probe Lab., Dalhousie University, Halifax / Dr D. Ruzzante, D. Cook, and Dr C. Taggart. Memorial University of Newfoundland / S. Goddard.
SPECIES	*Gadus morhua* (Atlantic cod).

PROJECT FUNDING	Canadian Dept. Fisheries and Oceans.
OBJECTIVES	Genetic differences between inshore and offshore Atlantic cod (Gadus morhua) off Newfoundland.
METHODOLOGY	Microsatellite markers, blood antifreeze level (to assign overwintering location as inshore or offshore).
STATUS	There are two papers published on this subject The first describes evidence of genetic structure between inshore and offshore cod off Newfoundland (Ruzzante *et al.* 1996 *CJFAS* 53634-645). The second provides evidence of temporal stability of the genetic structure at the scale of 2 to 4 years (Ruzzante *et al.* 1997 *CJFAS* 542700-2708).

Study 51

LABORATORY/RESEARCHER	Marine Gene Probe Lab., Dalhousie University, Halifax / Dr D. Ruzzante, D. Cook, and Dr C. Taggart.
SPECIES	*Gadus morhua* (Atlantic cod).
PROJECT FUNDING	Canadian Dept. Fisheries and Oceans.
OBJECTIVES	The genetics of a larval cod aggregation and genetic identification of a larval cohort in relation to some oceanographic features (Gyre-like eddies) are determined.
METHODOLOGY	Six microsatellite DNA loci were assessed for polymorphism.
DESIGN	Cod larvae were sampled repeatedly over a 3-week period from an aggregation on Western Bank.
STATUS	There is one paper published on this subject (Ruzzante *et al.* 1996 *CJFAS* 532695-2705).

Study 52

LABORATORY/RESEARCHER	Marine Gene Probe Lab., Dalhousie University, Halifax / Dr D. Ruzzante, D. Cook, and Dr C. Taggart.
SPECIES	*Gadus morhua* (Atlantic cod).
PROJECT FUNDING	Canadian Dept. Fisheries and Oceans.
OBJECTIVES	To describe broad and fine-scale genetic structure among cod populations in the NW Atlantic.
METHODOLOGY	Microsatellite markers.
STATUS	There are two papers on this topic the first (Bentzen *et al.* 1996 *CJFAS* 53 2706-2721) describes evidence of genetic structure at ocean basin and continental shelf scales. Another manuscript has been provisionally accepted in Molecular Ecology (Ruzzante *et al.*) describing emerging evidence of genetic structure among cod populations from throughout the species range in the NW Atlantic in relation to oceanographic features (gyre-like circulations or eddies that might act as retention mechanisms for eggs and/or larvae) and spatio-temporal differences in peak spawning time.

Study 53

LABORATORY/RESEARCHER	Marine Gene Probe Lab., Dalhousie University, Halifax / Dr D. Ruzzante.
SPECIES	*Gadus morhua* (Atlantic cod).
PROJECT FUNDING	Canadian Dept. Fisheries and Oceans.
OBJECTIVES	A comparison of several measures of genetic distance and population structure with microsatellites.
METHODOLOGY	Microsatellite markers.
STATUS	There is currently a paper in press in *CJFAS* by D. Ruzzante (1998) on this topic (*CJFAS* 55(1)).

Study 54

LABORATORY/RESEARCHER	Dept. Fisheries and Oceans, Canada, West Vancouver Laboratory, West Vancouver, British Columbia / I.I. Solar (project leader), E.M. Donaldson.
SPECIES	Salmonids.
PROJECT FUNDING	NRC-IRAP, DFO core funding.
OBJECTIVES	Develop one generation technique for production of Atlantic female milt based on

	gynogenesis and masculinization. Extend production of monosex females from chinook to Atlantics and coho. Production of monosex males by direct masculinization or indirectly (YY males produced by androgenesis). Monosex production objectives will also be approached by current and future work linked with the Genetic Engineering Program. The long term development of identical cloned salmon will enable the future replication of superior fish. Studies with aromatase inhibitors, antiestrogens and antiandrogens on sex differentiation are expected to reveal valuable information on the mechanism of sex differentiation and lead to new ways to produce monosex salmon stocks.
STATUS	Success has been achieved in the use of an effective sperm extender and the establishment of suitable protocols for UV radiation of extended sperm and the induction of gynogenetic Atlantic, chinook and coho salmon. Studies are underway in collaboration with the Genetics Program for further production and testing of gynogenetic groups and this subsequent sex reversal (masculinization) of these using a high potency non aromatizable androgen. We are cooperating with the MAFF and MELP Province of B.C. and the Salmon Farmers Association to produce trial groups of monosex female Atlantic salmon. Success has been achieved in the production of monosex female Atlantic salmon and coho sperm by masculinization of gynogenes.

Study 55

LABORATORY/RESEARCHER	University of Guelph, Ontario Agricultural College, Guelph, Ontario / I. McMillan (project leader).
SPECIES	Salmonids.
OBJECTIVES	Genetic improvement of commercial stocks of salmonids in the province. Development of a spring-spawning commercial rainbow trout strain for industry. (1) Comparison of growth, mortality, maturation rates and reproductive traits among four strains of spring-spawned rainbow trout and two management groups (1991 year class). (2) Comparison of early growth, maturation and mortality in crosses of three of four strains from (1) (1994 year class). (3) Initiation of additional crosses of three of the four strains in (1) (95/96 year class). (4) Development of computer models to compare inbreeding and rates of genetic progress under different genetic improvement strategies.
DESIGN	Characterisation of four pure strains and crosses between 95/96 year classes.
METHODOLOGY	Measurements of growth, mortality, maturation rates and reproductive traits. Development of computer models.
STATUS	On going.

Study 56

LABORATORY/RESEARCHER	Department of Fisheries and Oceans, Canada, Vancouver / T. Beacham, K. Miller, R. Withler (project contact).
SPECIES	Pacific Salmonids.
OBJECTIVES	To isolate MHC genes in Pacific salmonid species and determine levels of genetic variation at these loci within and among species. To determine if specific MHC genotypes are resistant to BKD (bacterial kidney disease).
STATUS	One Class II and two Class I genes have been isolated from seven Pacific salmonids. PCR assays have revealed high levels of genetic variation both among and within species. Analysis of coho salmon families resistant and susceptible to BKD is underway to examine possible correlation with MHC genotype. A BKD challenge of Chinook salmon is underway.

Study 57

LABORATORY/RESEARCHER	Science Branch, Department of Fisheries and Oceans, St. John's /V. Pepper (project leader), T. Nicholls.
SPECIES	Atlantic salmon (Salmo salar).
PROJECT FUNDING	Project implemented in 1989. Present funding SCB Fisheries Limited. 1995 Department of Fisheries and Oceans, 1994 Atlantic Fisheries Adjustment Program, 1989 Newfoundland Inshore Fisheries Development Agreement.
OBJECTIVES	To develop a native Newfoundland salmon stock for application to salmon farming under local industry conditions and evaluate the performance of this stock relative to the industry standard strain (Saint J. River) of Atlantic salmon.

DESIGN	Parallel grow-out (GCR vs. SJR). Monthly sampling to document growth (G); mortality (Z); biomass elaboration (G-Z); and Food Conversion Ratio.
METHODOLOGY	Insufficient funding to date to address genetic markers. Expected to take part as one component of a breeding program if planned facilities are available in time for the 1997 spawning season.
STATUS	Grand Codroy performance inferior for first generation aquaculture salmon. - Grand Codroy strain outperformed industry standard strain during second generation on-growing. Industry has set aside 1000 of the best performers of the GCR strain as brood stock for 1997.

Study 58

LABORATORY/RESEARCHER	Science Branch, Department of Fisheries and Oceans, St. John's / V. Pepper (project leader), T. Nicholls.
SPECIES	Atlantic salmon (Salmo salar).
PROJECT FUNDING	Project implemented in 1991. Present funding SCB Fisheries Limited, 1995. Atlantic Fisheries Adjustment Program, 1994 Atlantic Fisheries Adjustment Program. Department of Fisheries and Oceans, 1991
OBJECTIVE	To transfer, adapt and demonstrate procedures for development of non-maturing Atlantic salmon for use by the Newfoundland salmon farming industry and to quantify the relative merits of non-maturing salmon relative to the industry standard strain (Saint J. River) of Atlantic salmon.
DESIGN	Parallel grow-out (all-female, triploid salmon vs. SJR). Monthly sampling to document growth (G); mortality (Z); biomass elaboration (G-Z); and Food Conversion Ratio.
METHODOLOGY	Blood sampling, flow cytometry.
STATUS	Through the first summer of estuarine on-growing, all-female triploid salmon out performed all other salmon in the industry net-pens. However, a bimodal size distribution developed in the experimental groups in August of 1996. The experiment will not be complete until the fall of 1997.

Study 59

LABORATORY/RESEARCHER	Biology Department and Ocean Science Centre, Memorial University of Newfoundland, St. John's, Nfld. / Dr D. Innes (project leader), Dr R. J. Thompson, J. E. Toro, Ph.D. Student.
SPECIES	*Mytilus edulis*, *M. trossulus* (mussels).
OBJECTIVE	Physiology, ecology and genetics of the hybridizing marine bivalve molluscs *Mytilus trossulus* and *Mytilus edulis* in Eastern Newfoundland.
DESIGN	Dynamic and static cohort analysis will be carried out. Reproductive cycles and physiological variables determined in both species and their hybrids.
METHODOLOGY	Mussels are collected subtidaly by SCUBA at four locations, located in Trinity Bay, eastern coast of Newfoundland, from November 1995 (after the spawning season). At each location, two sites will be sampled, one exposed to wave action and another protected and sheltered. Species M.er A PCR-based nuclear species Marker developed by Heath *et al.* (1995), based on the internal transcribed spacer (ITS) regions between the 18S and 28S nuclear rDNA coding regions is applied in the present study.
STATUS	In progress

Study 60

LABORATORY/RESEARCHER	Marine Gene Probe Lab., Dalhousie University, Halifax / D. Ruzzante, C. Taggard, D. Cook in collaboration with University of Iceland / E. Arnason.
SPECIES	*Gadus morhua* (Atlantic cod).
PROJECT FUNDING	NATO.
OBJECTIVES	Examination of cod stocks from around Iceland with microsatellite markers.
METHODOLOGY	Microsatellite markers.
STATUS	Ongoing.

Study 61

LABORATORY/RESEARCHER	Marine Gene Probe Lab., Dalhousie University, Halifax / D. Ruzzante, C. Taggard, D. Cook in collaboration with P. Galvin (Ireland) and J. Mork (Norway).
SPECIES	Various gadoid species.
PROJECT FUNDING	EU.
OBJECTIVES	European Union Gadoid Program.
METHODOLOGY	Microsatellite markers.
STATUS	Ongoing. MGPL sent samples of various species, fresh tissue. We also sent cod samples from NFLD and Scotian Shelf that we did microsatellites on for their use as an outgroup and for comparison of techniques.

Study 62

LABORATORY/RESEARCHER	Dept. of Fisheries and Oceans, West Vancouver, B.C / R. Devlin.
SPECIES	Salmon.
OBJECTIVES	Production of transgenic salmon with enhanced growth and altered reproductive capability using "all-salmon" gene constructs.
STATUS	Ongoing.

Study 63

LABORATORY/RESEARCHER	Dept. of Fisheries and Oceans, West Vancouver, B.C / R. Devlin.
SPECIES	Salmon.
OBJECTIVES	Characterization of Y-chromosomal DNA probes from salmon for use in monosex all-female culture.
STATUS	Ongoing.

Study 64

LABORATORY/RESEARCHER	Dept. of Fisheries and Oceans, West Vancouver, B.C / R. Devlin.
SPECIES	Salmon.
OBJECTIVES	Development of DNA based diagnostics for several Microsporean and Myxosporean parasites to assist with management of infection in sea-farm facilities.
STATUS	Ongoing.

Study 65

LABORATORY/RESEARCHER	Dept. of Fisheries and Oceans, West Vancouver, B.C / R. Devlin.
SPECIES	Salmon.
OBJECTIVES	Examination of the potential for hybridisation between Atlantic and Pacific salmon with regard to the possible reproductive interaction between escaped farmed Atlantic salmon and wild Pacific salmon stocks.
STATUS	Ongoing.

Study 66

LABORATORY/RESEARCHER	Dept. of Fisheries and Oceans, West Vancouver, B.C / R. Devlin.
SPECIES	Chinook Salmon.
OBJECTIVES	Development of a RAPD linkage map for Chinook salmon.
STATUS	Ongoing.

Study 67

LABORATORY/RESEARCHER	Dept. of Fisheries and Oceans, West Vancouver, B.C / R. Devlin.
SPECIES	Salmon.
OBJECTIVES	Development of a sensitive PCR-based assay for CYPIA 1 gene expression to evaluate the biological effects of xenobiotic exposure.
STATUS	Ongoing.

Study 68

LABORATORY/RESEARCHER	Applied Breeding Technology, St. Andrew's, New Brunswick / Dr J. Bailey (project leader).
SPECIES	Atlantic salmon (*Salmo salar*).
FUNDING	Atlantic Canada Opportunities Agency, Canadian Institute of Biotechnology, Department of Fisheries and Oceans, National Research Council, New Brunswick Department of Fisheries and Aquaculture, New Brunswick Salmon Growers Association.
OBJECTIVE	To establish four Atlantic salmon strains for aquaculture.
DESIGN	Growth and developmental traits are monitored in both fresh and sea water for each year class of each strain. Selection is carried out when the fish have spent 18 months in sea water and the broodstock population is reduced from approximately 5000 to 800. Spawning takes place the following year with a population of approximately 400 fish.
METHODOLOGY	Selection is based on an index to increase percent 1+ smolts, percent non-grilse, M.et length and resistance to bacterial kidney disease. In one of the strains, selection was based on truncated mass selection for market length.
STATUS	Ongoing.
COMMENTS	Substantial genetic gains of significant economic value to salmon farmers have been made.

Study 69

LABORATORY/RESEARCHER	Salmon Genetics Research Program, Atlantic Salmon Federation, St. Andrews, New Brunswick / G. Friars, J. Bailey and F. O'Flynn. University of New Brunswick / T. Benfey and A. McGeachy.
SPECIES	Atlantic salmon (*Salmo salar*).
PROJECT FUNDING	Atlantic Canada Opportunities Agency, Canadian Institute of Biotechnology, Department of Fisheries and Oceans, National Research Council, New Brunswick Department of Fisheries and Aquaculture, New Brunswick Salmon Growers Association.
OBJECTIVE	To compare the aquacultural performance of diploid and triploid Atlantic salmon.
DESIGN	Mixed-sex triploid groups of Atlantic salmon were made in all SGRP aquaculture strains. All-female triploid groups were made in two SGRP strains and have all-female diploid contemporaries. Growth and survival is being monitored in both fresh and sea water.
METHODOLOGY	A 2.7 litre pressure vessel was used to produce triploid salmon. Fertilisation with mono-milt produced all-female groups. At the parr stage blood samples were taken to test the ploidy level of the fish by flow cytometry.
STATUS	Completed.

Study 70

LABORATORY/RESEARCHER	Salmon Genetics Research Program, Atlantic Salmon Federation, St. Andrews, New Brunswick / G. Friars, J. Bailey and F. O'Flynn. Research and Productivity Council / S. Griffiths.
SPECIES	Atlantic salmon (*Salmo salar*).
PROJECT FUNDING	Atlantic Canada Opportunities Agency, Canadian Institute of Biotechnology, Department of Fisheries and Oceans, National Research Council, New Brunswick Department of Fisheries and Aquaculture, New Brunswick Salmon Growers Association.
OBJECTIVE	To investigate genetic variation in resistance to Bacterial Kidney Disease (BKD).
DESIGN	Samples of parr and smolt from three SGRP strains were challenged with *Renibacterium salmonimum*.
METHODOLOGY	Heritability values were estimated, based on full-sib families, for survival and time to death.
STATUS	Project Completed.
COMMENTS	The information obtained from this study was used to include resistance to BKD as an index trait in the selection of broodstock.

Study 71

LABORATORY/RESEARCHER	Institute of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby / B. P. Brandhorst, G. Corley-Smith and J. Chinten Lim.
SPECIES	*Danio rerio* (zebrafish).
PROJECT FUNDING	NSERC.
OBJECTIVE	The production of diploid androgenetic fish and their use as a genetic tool.
DESIGN	The female genome is eliminated by X-ray irradiation, and the first cleavage is inhibited by heat shock. Polymorphic DNA markers are used to assess transmission from the female and male parents.
METHODOLOGY	DNA markers.
STATUS	Numerous diploid androgenotes have been produced with a success rate of 1-2 %. These have a normal appearance and have been bred. A manuscript has been submitted. Haploid androgenotes have been produced with an efficiency of up 30-50 %. This should allow their use in haploid genetic mutational screens, and production of a male meiotic cross-over map in collaboration with J. Postlethwait (U. Oregon) is near completion. Currently, the focus is on improving the efficiency of production of androgenotes and assessing the sex of androgenotes and their progeny, which may be informative about sex determination, another interest of the laboratory.
COMMENTS	The extensive DNA Marker data provides compelling evidence for the production of androgenotes with little or no leakage of maternal genes. The methods may be adaptable to other fish. A paper on this work was published in *Genetics* **142** (1996) 1265–1276.

Study 72

LABORATORY/RESEARCHER	Institute of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby / B. P. Brandhorst, G. Corley-Smith and J. Chinten Lim.
SPECIES	*Oncorhynchus nerka* (sockeye salmon).
PROJECT FUNDING	None at present.
OBJECTIVE	Development of a method for the rapid identification of stock specific DNA markers.
DESIGN	Random amplified polymorphic DNA (RAPD) analysis using fluorescent primers is being applied to bulked DNA samples of spawning sockeye salmon from adjacent and distant geographic regions, in an effort to establish the efficacy of a method for quickly identifying stock specific markers. Sequence analysis of distinctive amplification products, if any, should result in the production of highly specific PCR primers allowing for rapid DNA typing on small amounts of material.
METHODOLOGY	RAPD analysis using fluorescent primers and an ABI automated DNA sequencer, running GeneScan software.
STATUS	The sensitivity and reliability of RAPD analysis is considerably improved with the use of fluorescent primers and high resolution polyacrylamide gel electrophoresis. Application to identification of stock specific DNA markers is just beginning.
COMMENTS	This is a proof of concept project, not part of a planned long term program.

Study 73

LABORATORY/RESEARCHER	Departments of Clinical Biochemistry and Biochemistry, University of Toronto / C.L. Hew.
SPECIES	Winter flounder (*Pleuronectes americanus*), ocean pout (*Macrozoarces americanus*).
PROJECT FUNDING	Medical Research Council of Canada.
OBJECTIVE	To investigate the molecular mechanisms controlling the seasonal and hormonal regulated synthesis of fish antifreeze proteins, and to explore the use of antifreeze protein genes in conferring freeze resistance to other fish species.
DESIGN	These include gene cloning, promoter analysis in tissue culture cells, characterization of transcription factors, and the development of transgenic fish.
METHODOLOGY	Gene cloning, transcripted factors and transgenics.
STATUS	We have demonstrated that the winter flounder contains both extracellular and intracellular AFPs. These have raised further questions on the structure and function, regulation and evolution of AFPs (Gong *et al.*, 1996. Skin antifreeze protein genes of the winter flounder, *Pleuronectes americanus*, encode distinct and active polypeptides without the secretory signal sequences. *J. Biol. Chem.* In Press).

Study 74

LABORATORY/RESEARCHER	Departments of Clinical Biochemistry and Biochemistry, University of Toronto / C.L. Hew.
SPECIES	Chinook salmon (*Oncorhynchus tschawytscha*), rainbow trout (*Oncorhynchus mykiss*).
FUNDING	Medical Research Council of Canada.
OBJECTIVE	Using salmon as a model, we are studying the molecular events controlling fish reproduction. The genetic mechanism(s) for gonadotropin gene expression is examined.
DESIGN	The cis-acting and transcription factors important in gonadotropin gene expression are characterised by a wide variety of biochemical and molecular biological techniques.
METHODOLOGY	Gene cloning, promoter analysis, characterization of transcription factors, etc.
STATUS	We have demonstrated for the first time in the gonadotropin gene that both steroidogenic factor and estrogen receptor act in synergism for the gonadotrope-specific expression of the salmon gonadotropin IIß subunit gene (Le Drean *et al.*, 1996, Steroidogenic factor 1 and estradiol receptor act in synergism to regulate the expression of the salmon gonadotropin IIß subunit gene. *Mol. Endocrinol.* In press).

Study 75

LABORATORY/RESEARCHER	Departments of Clinical Biochemistry and Biochemistry, University of Toronto / C.L. Hew.
SPECIES	Atlantic salmon (*Salmo salar*).
PROJECT FUNDING	Natural Sciences and Engineering Research Council of Canada.
OBJECTIVE	The objective is the development of transgenic salmon beneficial to aquaculture. these include (i) the transfer of antifreeze protein gene (AFP) for freeze resistance; (ii) the transfer of growth hormone gene (GH) for growth enhancement; and (iii) the transfer of lysozyme gene (LYZ) for disease resistance.
DESIGN	These genes (AFP, GH, LYZ) were injected separately into salmon eggs by gene transfer. The inheritance and expression of the transgene is being studied.
METHODOLOGY	Transgenetics.
STATUS	Positive transgenic fish have been accomplished for AFP and GH gene transfer. GH transgenic fish grow 5 to 10 times faster than the control and the inheritance of transgenes to F2 generation is established (See Gong and Hew 1995), Transgenic fish in aquaculture and developmental biology. Current Topics in *Developmental Biology* **30** 177-214.

Study 76

LABORATORY/RESEARCHER	Departments of Clinical Biochemistry and Biochemistry, University of Toronto / C.L. Hew.
SPECIES	Chinook salmon (*Oncorhynchus tschawytscha*) and zebrafish (*Danio rerio*).
PROJECT FUNDING	Natural Sciences and Engineering Research Council of Canada
OBJECTIVE	To investigate the structure, function and regulation of Isl–1 and related gene family in the neuroendocrine cell and motor neuron development.
DESIGNS	Isl–1, Isl-2 and Isl-3 are LIM domain homeodomain transcription factors. They are detected in brain, pituitary and other organs. However, the role of these proteins is unclear. Biochemical, molecular biological and cell biology techniques are used to examine the role of these proteins.
METHODOLOGY	In situ hybridisation, DNA binding assay and others.
STATUS	The genes are cloned and their ontogeny established. In situ hybridisation indicates that the transcripts of all three genes are localised in subsets of neurons in the brain and spinal cord (Gong *et al.*, 1995. Presence of isl–1-related LIM domain homeobox genes in teleost and their similar patterns of expression in brain and spinal cord. *J. Biol. Chem.* **270** 3335-3345

Study 77

LABORATORY/RESEARCHER	Magaguadavic Watershed Management Association, General Delivery, St. George, New Brunswick and Marine Gene Probe Laboratory, Dalhousie University, Halifax, Nova Scotia / J. Carr, G. Hammond, A.J.D. Ambali and J. Anderson.

SPECIES	Atlantic salmon (*Salmo salar*).
PROJECT FUNDING	Magaguadavic Watershed Management Association, Atlantic Salmon Federation, Canada \ N.B. Coop. Agreement on Rec. Fisheries Salmon Growers Association, N.B. Depart. of Fisheries and Aquaculture, Depart. of Fisheries and Oceans Salmon Council.
OBJECTIVE	To establish if genetic introgression is occurring between wild and aquaculture escapees in the Magaguadavic River, New Brunswick.
DESIGN	Scale and blood samples were collected from wild salmon of the Magaguadavic River, and from aquaculture salmon that escaped from the N.B. industry. Samples included wild salmon scales collected from 1975-77, wild salmon scale and blood samples from 1992-94, and scales from aquaculture escapees from 1994. The 1975-77 samples represented the original Magaguadavic River strain before the development of the N.B. salmon aquaculture industry in 1979.
METHODOLOGY	Population polymorphisms at four microsatelite loci (Omy 27,38,105, and Ssa 4) were examined in Atlantic salmon from 7 year-classes by extracting DNA from scale and blood samples.
STATUS	The wild 1970's strain was genetically distinct from the wild 1990's strain. The 1994 escapees were genetically distinct from both year classes of wild salmon, but were closer to the 1990's strain of wild salmon.

Study 78

LABORATORY/RESEARCHER	University of New Brunswick / T.J. Benfey.
SPECIES	Various salmonids (incl. Brook trout, Arctic charr, Atlantic salmon and rainbow trout).
PROJECT FUNDING	Natural Sciences and Engineering Research Council of Canada, Canada Department of Fisheries and Oceans, New Brunswick salmon Growers Association, Canada/New Brunswick Subsidiary Agreement on Industrial Innovation and Technology Development, University of New Brunswick, Atlantic Veterinary College (University of Prince Edward Island).
OBJECTIVE	To examine the basic physiology and behaviour of triploid salmonids.
DESIGN	Experimental assessment of physiological and behavioural characteristics under controlled laboratory conditions.
METHODOLOGY	Currently concentrating in the following areas (1) respiratory physiology - haematology, oxygen consumption rate, opercular pumping and swimming efficiency, and aerobic capacity; (2) competitive abilities - feeding hierarchies and growth rates for triploids cohabitating at different densities with diploids; (3) ovarian development - histological examination of ovarian development in triploids beyond the normal age of reproduction; (4) thermal optima - development and growth at various temperatures, acute and chronic tolerance of high temperatures; and (5) stress response - endocrinological and haematological responses to stress.
STATUS	Ongoing.
COMMENTS	There is growing pressure from various sources for Canadian fish farmers to use triploid fish, in order to prevent spawning in the wild of any escaping farmed fish. Optimal rearing conditions for triploids, based on a better understanding of their basic biology, must be determined before advocating their widespread use in commercial culture.

Study 79

LABORATORY/RESEARCHER	Memorial University of Newfoundland / C. McGowan and W.S. Davidson.
SPECIES	Atlantic salmon and brown trout.
PROJECT FUNDING	Natural Sciences and Engineering Research Council of Canada.
OBJECTIVE	To develop a method to distinguish normal XY males from sex-reversed XX males.
DESIGN	Screening a library of primers for any that show differences in DNA fragment sizes after PCR amplification.
METHODOLOGY	RAPD technique - randomly amplified polymorphic DNA based on screening a library of oligonucleotide primers (each 10 base pair long) on DNA purified from male and female Alantic salmon and brown trout.
STATUS	Ongoing
COMMENTS	The RAPD technique has been used successfully to determine the sex of birds and plants. 300 to 400 markers were tested on Arctic charr and brook trout but no sex specific Marker was found.

Study 80

LABORATORY/RESEARCHER	Dept. of Biochemistry, Memorial University, St. John's, Newfoundland / C. McGowan and W. Davidson.
SPECIES	Brown trout and Atlantic salmon.
PROJECT FUNDING	NSERC.
OBJECTIVE	Genome mapping of Salmo species.
DESIGN	Hybrid families have been produced and segregation of alleles at different loci is being examined.
METHODOLOGY	Genetic markers being examined include RAPD, microsatellites and expressed sequence tags (cDNA's).
STATUS	Six linkage groups have been identified to date for brown trout and five for Atlantic salmon This is an Ongoing project.

Study 81

LABORATORY/RESEARCHER	Dept. Fisheries and Oceans, Canada, West Vancouver Laboratory, West Vancouver, British Columbia / I.I. Solar, E.M. Donaldson.
SPECIES	Salmonids.
PROJECT FUNDING	NBS and Province of BC.
OBJECTIVE	Develop one generation technique for production of Atlantic female milt based on gynogenesis and masculinization. Extend production of monosex females from chinook to Atlantics and coho. Studies with aromatase inhibitors, antiestrogens and antiandrogens on sex differentiation are expected to reveal valuable information on the mechanism of sex differentiation and lead to new ways to produce monosex salmon stocks.
STATUS	Success has been achieved in the use of an effective sperm extender and the establishment of suitable protocols for UV radiation of extended sperm and the induction of gynogenetic Atlantic, chinook and coho salmon. Studies are underway in collaboration with the Genetics Program for further production and testing of gynogenetic groups and the subsequent sex reversal (masculinization) of these using a high potency non aromatizable androgen. We are cooperating with the MAFF and MELP Province of B.C. and the Salmon Farmers Association in a farmscale trial of regular, monosex female and monosex female triploid Atlantic salmon. Success has been achieved in the production of monosex female Atlantic and coho salmon sperm by masculinization of gynogenes.

Study 82

LABORATORY/RESEARCHER	Dept. of Biology, Memorial University / S. Carr. Dept. of Biochemistry, Memorial University / W. Davidson. Department of Fisheries and Oceans, St. John's, Newfoundland / R. Bowering.
SPECIES	Greenland halibut (turbot).
PROJECT FUNDING	CCFI.
OBJECTIVE	Population structure of Greenland halibut in the North Atlantic.
DESIGN	40 turbot from 7 sampling sites across the North Atlantic were examined for genetic variation within and between samples.
METHODOLOGY	Sequence analysis of a 400 bp region of the cytochrome b mitochondrial DNA was examined.
STATUS	Completed. No evidence for genetic substructuring of turbot in the North Atlantic from as far apart as Norway and the Gulf of St. Lawrence. (Published in *CJFAS* Vis *et al.* 1997).

Study 83

LABORATORY/RESEARCHER	Dept. of Biology, Memorial University, St. John's, Newfoundland / S. Carr.
SPECIES	Atlantic cod.
PROJECT FUNDING	NSERC.
OBJECTIVE	Population structure of Atlantic cod.
DESIGN	Many samples of cod from all over the North Atlantic have been examined for evidence of population structuring.

METHODOLOGY	Mitochondrial DNA and microsatellites.
STATUS	Ongoing project.

Study 84

LABORATORY/RESEARCHER	Stocks Assessment and Genetics Unit, Ontario Ministry of Natural Resources, Maple / P.E. Ihssen and G.Wm. Martin.
SPECIES	Atlantic salmon, aurora trout, brook trout, brown trout, Chinook salmon, trout, lake whitefish, coho salmon and rainbow trout.
PROJECT FUNDING	Ontario Ministry of Natural Resources (OMNR).
OBJECTIVE	Monitoring of OMNR hatchery stocks for maintenance of genetic variability.
DESIGN	Successive year classes of hatchery-reared fish of the above species are monitored for approximately 50 allozyme loci. In cases where the phenotypes of the original parents were determined, comparison is made with succeeding year classes to determine if there has been a loss of genetic variability. In the case of Atlantic salmon, Chinook salmon and lake whitefish, gametes are collected from wild fish. For all other species, gametes are collected from hatchery brood stock.
METHODOLOGY	Starch gel and cellulose acetate electrophoresis of cathodal and general muscle protein and 23 allozyme systems.
STATUS	Ongoing.

Study 85

LABORATORY/RESEARCHER	Dept. of Biochemistry, Memorial University, St. John´s, Newfoundland / J. Johansen and W.S. Davidson.
SPECIES	Arctic charr.
PROJECT FUNDING	NSERC.
OBJECTIVE	Marker assisted selection of broodstock.
DESIGN	Families have been produced and association genetics is being carried out to relate microsatellite alleles with growth.
METHODOLOGY	Genetic markers being examined are microsatellites.
STATUS	In progress.

DENMARK

Study 1

LABORATORY/RESEARCHER	National Institute of Animal Sciences / L.-E. Holm.
SPECIES	Rainbow Trout.
PROJECT FUNDING	In house/ Agricultural Science Research Council.
OBJECTIVE	Development and use of genetic markers to be used for identification of hatchery strains and for markers of commercially important traits.
DESIGN	Screening of rainbow trout from a number of Danish hatchery strains.
METHODOLOGY	Microsatellites.
STATUS	Ongoing.

Study 2

LABORATORY/RESEARCHER	Danish Institute for Fisheries Research, Dept. of Inland Fisheries, Silkeborg / E.E. Nielsen.
SPECIES	Atlantic salmon.
PROJECT FUNDING	In house.
OBJECTIVE	Studies of long-term temporal changes in allele frequencies at loci that are possibly affected by selection.
DESIGN	Geographically distinct populations are analysed. Variation is analysed on a temporal scale by amplifying DNA from old scale samples.
METHODOLOGY	ScnDNA.
STATUS	Ongoing. Started 1998, due to end by 2000.

Study 3

LABORATORY/RESEARCHER	Danish Institute for Fisheries Research, Dept. of Inland Fisheries, Silkeborg / M.M. Hansen, University of Aarhus / H.B.H. Jørgensen, S. Østergaard, V. Loeschcke.
SPECIES	Brown trout.
PROJECT FUNDING	In house.
OBJECTIVE	Estimation of genetic variability and differentiation in and among Danish brown trout populations and hatchery strains. Analysis of metapopulation dynamics (extinction-recolonisation). Analysis of relationship between fluctuating asymmetry and heterozygosity (at microsatellite loci).
DESIGN	Sampling of trout from various localities.
METHODOLOGY	Microsatellites (from tissue and old scale samples), mtDNA, analysis of metric and meristic morphological traits, modelling.
STATUS	Ongoing.

Study 4

LABORATORY/RESEARCHER	Danish Institute for Fisheries Research, Dept. of Inland Fisheries, Silkeborg / M.M. Hansen.
SPECIES	Brown trout.
PROJECT FUNDING	In house.
OBJECTIVE	Estimation of the impact of stocking activity (using non-native hatchery trout) on natural brown trout populations.
DESIGN	Hatchery trout are stocked into wild populations. Reproductive performance and interbreeding between stocked and wild trout is monitored, using genetic markers. The development in the stocked populations will be followed through more generations.
METHODOLOGY	Microsatellites and mtDNA.
STATUS	Ongoing.

Study 5

LABORATORY/RESEARCHER	Danish Institute for Fisheries Research, Dept. of Inland Fisheries, Silkeborg / M.M. Hansen. Pike microsatellites developed by J. Taggart, University of Stirling, U.K.
SPECIES	*Coregonus lavaretus, C. "oxyrhynchus", Esox lucius, Thymallus thymallus.*
PROJECT FUNDING	In house.
OBJECTIVE	Estimation of phylogeographic patterns and genetic differentiation.
DESIGN	Screening of samples from geographically distinct and morphologically divergent populations.
METHODOLOGY	Microsatellites and mtDNA. New tetranucleotide microsatellites have been developed for pike.
STATUS	Ongoing. The study of coregonid fishes show that postglacial recolonisation of Denmark probably has taken place via the postglacial Elbe River system, whereas populations from the Baltic Sea appear to be the result of another recolonisation event.

Study 6

LABORATORY/RESEARCHER	Danish Institute for Fisheries Research, Dept. of Inland Fisheries, Silkeborg / E.E. Nielsen and M.M. Hansen. Several collaborators from the Danish Institute for Fisheries Research, Dept. of Marine Fisheries, Copenhagen. University of Aarhus / P. Grønkjær and V. Loeschcke.
SPECIES	Cod.
PROJECT FUNDING	The Danish Ministry of Agriculture and Fisheries.
OBJECTIVE	Studies of the genetic population structure of cod in the South-eastern part of Kattegat, the Danish Belt Sea and in the Baltic Sea area. Estimation of the possible drift of juvenile cod into the Baltic Sea and the contribution of Belt Sea cod to the fishery in the Baltic Sea area.
DESIGN	Sampling of cod larvae and adult spawners from various localities.
METHODOLOGY	Microsatellites and other molecular markers.
STATUS	Started 1998, due to end by 2000.

Study 7

LABORATORY/RESEARCHER	Danish Institute for Fisheries Research, Dept. of Inland Fisheries, Silkeborg / M.M. Hansen (coordinator) + 22 other participants from laboratories in Europe and Canada.
SPECIES	Brown trout.
PROJECT FUNDING	EU FAIR.
OBJECTIVE	Concerted Action on brown trout population genetics (TROUTCONCERT). The objectives are to promote collaboration among laboratories that are active in research on population genetics of brown trout, to harmonise the use of genetic markers, to give recommendations for a European strategy for management and conservation of the species, and to establish databases on relevant literature, available genetic markers and data from published and unpublished studies. The databases will be made publicly accessible on the World Wide Web (WWW).
DESIGN	Concerted action, i.e., network among laboratories.
METHODOLOGY	Workshops, exchange visits among laboratories, common databases and WWW facilities.
STATUS	Two-year project (1998–1999).

RECENT DANISH PUBLICATIONS:

Nielsen, E.E., Hansen, M.M. and Loeschcke, V. (1997). Analysis of microsatellite DNA from old scale samples of Atlantic salmon: A comparison of genetic composition over sixty years. *Molecular Ecology* **6**: 487-492.

Hansen, M.M., Nielsen, E.E. and Mensberg, K.-L.D (1997). The problem of sampling families rather than populations: Relatedness among individuals in samples of juvenile brown trout (*Salmo trutta L.*). *Molecular Ecology* **6**, 469-474.

Hansen, M.M., Mensberg, K.-L. D., Rasmussen, G. and Simonsen, V. (1997). Genetic variation within and among Danish brown trout (*Salmo trutta L.*) hatchery strains, assessed by PCR-RFLP analysis of mitochondrial DNA segments. *Aquaculture* **153:** 15-29.

Hansen, M.M. and Mensberg, K.-L.D. (1998). Genetic differentiation and relationship between genetic and geographical distance in Danish sea trout (*Salmo trutta* L.) populations. *Heredity*. In press.

Nielsen, E.E., Hansen, M.M. and Mensberg, K.-L.D. (1998). Improved primer sequences for the mitochondrial ND1, ND3/4 and ND5/6 segments in salmonid fishes. Application to RFLP analysis of Atlantic salmon. *Journal of Fish Biology*. In press.

ESTONIA

Study 1

LABORATORY/RESEARCHER	Dept. of Fish Farming, Institute of Animal Husbandry, Estonian Agricultural University, Tartu / R. Gross, T. Paaver, A. Vasemägi.
SPECIES	Sea trout and Atlantic salmon.
PROJECT FUNDING	Estonian Science Foundation, Estonian Fisheries Foundation.
OBJECTIVE	To reveal genetic differentiation and structure of natural and hatchery salmon and trout populations, estimate the influence of stocking on gene pools of natural populations, reveal frequency of salmon x trout hybrids in salmon rivers.
DESIGN	Samples are taken from parr, caught by electrofishing in five salmon rivers or collected from juveniles, reared in two hatcheries of Estonia.
METHODOLOGY	PCR-amplified DNA markers (microsatellites, growth hormone genes, mtDNA genes), allozymes.
STATUS	Ongoing (1997–1999)

FINLAND

Study 1

LABORATORY/RESEARCHER	Finnish Game and Fisheries Research Institute, Helsinki / M.-L. Koljonen. University of Helsinki, Department of Animal Science / J. Tähtinen and M. Säisä.
SPECIES	Atlantic salmon, brown trout.
PROJECT FUNDING	In house, Academy of Finland.

OBJECTIVE	Estimate stock composition of salmon catches and proportion of wild stocks in the catches.
METHODOLOGY	Genetic stock identification (GSI), allozymes, microsatellites.
STATUS	Ongoing.

Study 2

LABORATORY/RESEARCHER	Agricultural Research Centre, Department of Animal Breeding, Jokioinen / L. Siitonen.
SPECIES	Rainbow trout.
PROJECT FUNDING	In house.
OBJECTIVE	Develop rainbow trout stocks with better growth rate.
METHODOLOGY	Selective breeding.
STATUS	Ongoing.

Study 3

LABORATORY/RESEARCHER	University of Joensuu, Department of Biology / J. Vuorinen.
SPECIES	Coregonids.
PROJECT FUNDING	In house.
OBJECTIVE	Evolution and taxonomy of Holarctic Coregonids.
DESIGN	Mapping of gene frequencies.
METHODOLOGY	Enzyme electrophoresis, mtDNA, sequencing (collaboration), SINEs (collaboration).
STATUS	Ongoing.

Study 4

LABORATORY/RESEARCHER	University of Kuopio, Department of Applied Zoology and Veterinary Medicine / H. Mölsä, T. Pitkänen, M. Reinisalo and A. Krasnov.
SPECIES	Rainbow trout.
PROJECT FUNDING	Ministry of Agriculture and Forestry, Ministry of Interior Affairs, Academy of Finland, In house.
OBJECTIVE	Enhanced growth and metabolism of rainbow trout via gene transfer technology.
DESIGN	Micro injections and integration assays, gene expression
METHODOLOGY	Micro injections, mRNA, RT-PCR.
STATUS	Ongoing

Study 5

LABORATORY/RESEARCHER	University of Oulu, Department of Biology / J. Lumme.
SPECIES	Atlantic salmon
PROJECT FUNDING	Academy of Finland, mostly open.
OBJECTIVE	Origin and evolution of Baltic salmon.
METHODOLOGY	MtDNA sequencing, microsatellite variation.
STATUS	Preliminary results D-loop unsuitable; ND1 and micros wait funding.

Study 6

LABORATORY/RESEARCHER	University of Joensuu, Department of Biology / L. Kuusipalo.
SPECIES	Salmo trutta, Ciclids, clupeids and Nile perch in lakes Malawi and Tanganyika.
PROJECT FUNDING	FAO, Culture Fund of Finland, Women's Science Foundation, The Foundation of Research of Natural Resources in Finland.
OBJECTIVE	Speciation, stock identification.
METHODOLOGY	Allozymes and RAPD, microsatellites.
STATUS	Ongoing.

FRANCE

Study 1

LABORATORY/RESEARCHER	Laboratoire de Génétique, Aquaculture et Pathologie, IFREMER – BP133 – 17390 La Tremblade / A. Gérard.
SPECIES	Pacific oyster (*Crassostrea gigas*).
PROJECT FUNDING	IFREMER.
OBJECTIVE	Development and optimization of triploidy and tetraploidy induction in C. gigas. Triploidy induces sterility or reduced gametogenesis. As the energy allocated to gametogenesis is large in oysters, triploidy leads to better growth and better quality during the reproductive period.
DESIGN	Fertilized eggs of diploid and triploid oysters are treated to induce triploid and tetraploid offspring respectively.
METHODOLOGY	Cytochalasin B or 6-dimethylaminopurine treatments are used to suppress polar body I or II formation in fertilized eggs of diploid and triploid. Ploidy level is estimated by image analysis techniques.
STATUS	Triploidy induction is routine work and efforts are dedicated to tetraploidy and mating tetraploids and diploids to obtain all-triploid stocks.
COMMENTS	S.K. Allen Jr currently applies for a patent on tetraploid oysters and related techniques.

Study 2

LABORATORY/RESEARCHER	Laboratoire de Génétique, Aquaculture et Pathologie, IFREMER – BP133 – 17390 La Tremblade / P. Boudry.
SPECIES	Pacific oyster (*Crassostrea gigas*), Portuguese oyster (*Crassostrea angulata*).
PROJECT FUNDING	IFREMER, Conseil Général de Charente-Maritime.
OBJECTIVE	Study of genetic resources and genetic differentiation of Pacific oyster stocks.
DESIGN	Pacific oysters originated from various origins have been sampled both for DNA and live animals. Live oysters are maintained under strict quarantine conditions according to ICES recommendations. Sampled populations will be bred in the hatchery and progenies will be compared to the French stock of *C. gigas*.
METHODOLOGY	MtDNA PCR-RFLP, microsatellites, comparative breeding.
STATUS	In progress.
COMMENTS	

Study 3

LABORATORY/RESEARCHER	Laboratoire de Génétique, Aquaculture et Pathologie, IFREMER – BP133 – 17390 La Tremblade / A. Gérard, E. Goyard, S. Lapègue, J. P. Baud and P. Boudry.
SPECIES	European flat oyster (*Ostrea edulis*).
PROJECT FUNDING	IFREMER.
OBJECTIVE	Selective breeding of the flat oyster *O. edulis* for resistance against bonamiosis (*Bonamia ostreae*).
DESIGN	Breeding was formerly based on mass selection and mass spawning. Selection is now based on full-sib families in order to control effective population size and inbreeding.
METHODOLOGY	Resistance to bonamiosis is assessed both under field conditions and laboratory infection with the parasite. Microsatellites are used to estimate genetic diversity and inbreeding in the 3 populations under selection.
STATUS	In progress.
COMMENTS	This project was started in 1985. Growth performance has now been included as a selection criteria.

Study 4

LABORATORY/RESEARCHER	Laboratoire de Génétique, Aquaculture et Pathologie, IFREMER – BP133 – 17390 La Tremblade / Y. Naciri-Graven and F. Bonhomme.
SPECIES	European flat oyster (*Ostrea edulis*).
PROJECT FUNDING	IFREMER, BRG (Bureau des Ressources Génétiques, Paris).
OBJECTIVE	Assessment of genetic differentiation and genetic structure of European flat oyster populations. Samples were collected from 13 European wild populations and studied

	for five microsatellite loci.
METHODOLOGY	Microsatellites.
STATUS	Achieved.
COMMENTS	This study should be extended using mitochondrial DNA markers on the same samples.

Study 5

LABORATORY/RESEARCHER	Laboratoire de Génétique, Aquaculture et Pathologie, IFREMER – BP133 – 17390 La Tremblade / A. Gérard, M. Héral, P. Boudry, S. Bougrier, J. and J.F. Samain.
SPECIES	Pacific oyster (*Crassostrea gigas*).
PROJECT FUNDING	IFREMER, EC (FAIR Project).
OBJECTIVE	To establish relationships between growth, genetics and physiology in *C. gigas*.
DESIGN	Three populations, based on factorial crosses of five males and five females from three French sites, were established in 1996. Rearing practices were designed to maximize genetic and phenotypic variability. A multi-disciplinary approach is favored by studying the same material from growth, physiological and genetical parameters.
METHODOLOGY	Individual growth recording, allozymes, aneuploidy, microsatellites, physiological studies (metabolism, digestive enzymes, protein turn-over).
STATUS	In progress (started in 1996, to be concluded in 2000).
COMMENTS	This is a FAIR project coordinated by A. Gérard. European partners are T. Hawkins (U.K.), C. Thiriot (France), F. Bonhomme (France), N. Wilkins (Ireland) and E. Zouros (Greece).

Study 6

LABORATORY/RESEARCHER	Mediterranean laboratory on marine pisciculture research, IFREMER 34250 Palavas-les-flots / C. Fauvel.
SPECIES	Sea bass, *Dicentrarchus labrax*.
PROJECT FUNDING	IFREMER.
OBJECTIVE	Optimization of gamete quality, artificial fertilization and cryopreservation.
DESIGN	Fertility, fertilization and early development are being studied under different conditions in order to identify and control variation factors. A conservation medium is being used without interfering with the quality of gametes.
METHODOLOGY	Reproduction physiology, analytical rearing techniques.
STATUS	To be reevaluated and reoriented next year.
COMMENTS	Basic work for genetic purposes. It already allows to limit the environmental and physiological interaction on phenotype.

Study 7

LABORATORY/RESEARCHER	Mediterranean laboratory on marine pisciculture research, IFREMER 34250 Palavas-les-flots / B. Menu and B. Chatain.
SPECIES	Sea bass, *Dicentrarchus labrax*.
PROJECT FUNDING	IFREMER, the French Agriculture Council, the French Research Council, the French Syndicate of poultry and fish breeders (SYSAAF).
OBJECTIVE	Study of sex determining mechanisms in the European sea bass in order to produce monosex female populations.
METHODOLOGY	Gynogenesis, study of sex-ratios of sex inverted genitors' offspring, caryotype analysis.
STATUS	The project has begun in 1995.
COMMENTS	The project is finished by the end of 1999. Collaborations with the National Institute of Agronomical Research (INRA Rennes), the Scientific Research National Center (CNRS Montpellier), the Natural History Museum (Paris).

Study 8

LABORATORY/RESEARCHER	Mediterranean laboratory on marine pisciculture research, IFREMER 34250 Palavas-les-flots / J.C. Falguière and B. Chatain.
SPECIES	Sea bass, *Dicentrarchus labrax*.
PROJECT FUNDING	IFREMER.
OBJECTIVE	Control of the maturation by polyploidy.

DESIGN	Production of triploids and evaluation of sex ratio, morphological and weight characteristics. Comparison of performances between communal and separate rearing.
METHODOLOGY	Polyploidisation by pressure and temperature shocks on eggs.
STATUS	Analysis in progress.

Study 9

LABORATORY/RESEARCHER	Mediterranean laboratory on marine pisciculture research, IFREMER 34250 Palavas-les-flots / B. Chatain and F. Bonhomme.
SPECIES	Sea bass, *Dicentrarchus labrax*.
PROJECT FUNDING	IFREMER, European Community (FAIR), industries.
OBJECTIVE	Identification of wild and domestic populations and evaluation of their zootechnical performances
DESIGN	Study of genetic polymorphism in wild and domestic populations in collaboration with the Scientific Research National Center (CNRS Montpellier). Besides, IFREMER is coordinator of a concerted action aiming at assessing procedures for the development of a European standardized multisite testing program. This work is conducted in partnership with 13 European laboratories.
METHODOLOGY	PCR and microsatellites markers.
STATUS	The polymorphism study ended in 1997. The concerted action, started in 1998, will last 2 years and proposals will be done to the EC through a final report.
COMMENTS	A study on the estimation of genetic parameters in sea bass with low common environmental effects is in project for the coming year.

Study 10

LABORATORY/RESEARCHER	Laboratoire " Flux de Matière et Réponse du vivant " UMR CNRS 6539, Institut Universitaire Européen de la Mer Université de Bretagne Occidentale Place Copernic, technopôle Brest-Iroise, 29280 Plouzané / D. Moraga.
SPECIES	Manila cam (*Ruditapes philippinarum*), Pacific oyster (*Crassostrea gigas*).
PROJECT FUNDING	CNRS.
OBJECTIVE	Genetic differentiation and of invertebrate populations to environmental variation.
DESIGN	Sampling of populations exposed to different natural or experimental environmental factors (temperature, salinity, pollutants) to identify genes associated to adaptive response of populations.
METHODOLOGY	Allozymes, cloning of genes involved in heavy metal resistance.
STATUS	In progress.

Study 11

LABORATORY/RESEARCHER	Laboratoire Génome et Populations UPR 9060 CNRS, Station Méditerranéenne de l'Environnement Littoral, Sète / P. Borsa and F. Bonhomme.
SPECIES	The blue mussel species complex (*Mytilus edulis, M. galloprovincialis, M. trossulus*).
PROJECT FUNDING	CNRS.
OBJECTIVE	Intron length polymorphism and phylogeography in the mussels from *M. edulis* species complex.
DESIGN	Genetic characterization of blue mussels populations using intron length polymorphism and reconstitution of the genus *Mytilus* biogeographical history using nuclear gene genealogies.
METHODOLOGY	PCR amplification in intron nuclear genes, sequencing.
STATUS	Analysis in progress.

Study 12

LABORATORY/RESEARCHER	Laboratoire Génome et Populations UPR 9060 CNRS, Station Méditerranéenne de l'Environnement Littoral, 1 Quai de la Daurade, 34200 Sète / F. Bonhomme.
SPECIES	*Dicentrarchus labrax* and *D. punctatus*.
PROJECT FUNDING	CNRS.
OBJECTIVE	Study of the populations of *D.labrax* and *D.punctatus* using six hypervariable microsatellite markers.

DESIGN	Genetic characterization of seabass wild populations, comparison with reared stocks.
METHODOLOGY	PCR amplification of microsatellites loci.
STATUS	Analysis in progress.

Study 13

LABORATORY/RESEARCHER	Laboratoire Génome et Populations UPR 9060 CNRS, Station Méditerranéenne de l'Environnement Littoral, 1 Quai de la Daurade, 34200 Sète / F. Bonhomme
SPECIES	Sea bass (*Dicentrarchus labrax*).
PROJECT FUNDING	CNRS.
OBJECTIVE	Genetic analysis of the response to the environmental stress in the mediterranean sea bass (*Dicentrarchus labrax*).
DESIGN	Identification of genes responsible for the differentiation between sea and lagoon stocks. Analysis of the effects of the selection on the genes fluxes between sea and lagoon.
METHODOLOGY	Bulk Segregant Analysis on multiloci amplification methods. RNA differential display. Sequencing.
STATUS	Beginning of the study.

Study 14

LABORATORY/RESEARCHER	Laboratoire Génome et Populations UPR 9060 CNRS, Station Méditerranéenne de l'Environnement Littoral, 1 Quai de la Daurade, 34200 Sète; Laboratoire de Zoogéographie, Université Paul Valéry Montpellier / F. Blanc and F. Bonhomme.
SPECIES	Pearl oysters (Pinctada mazatlanica, Pteria sterna).
PROJECT FUNDING	
OBJECTIVE	Study of the genetic differentiation and population genetic structure along the mexican coasts using mitochondrial and DNA nuclear markers.
METHODOLOGY	MtDNA, PCR-RFLP, SSCP.
STATUS	In progress.

Study 15

LABORATORY/RESEARCHER	Laboratoire Génome et Populations UPR 9060 CNRS, Station Méditerranéenne de l'Environnement Littoral, 1 Quai de la Daurade, 34200 Sète / J.-J. Versini and F. Bonhomme.
SPECIES	Giant tiger prawn (*Penaeus monodon*).
PROJECT FUNDING	IFREMER, CNRS.
OBJECTIVE	Population genetical structure and stock identification using microsatellites loci.
METHODOLOGY	Microsatellites.
STATUS	In progress.

Study 16

LABORATORY/RESEARCHER:	Laboratoire d'Aquaculture Tropicale, Centre Océanologique du Pacifique - IFREMER, BP 7004 TARVAO, TAHITI, French Polynesia / JL Martin and E. Bedier.
SPECIES:	*Penaeus stylirostris.*
PROJECT FUNDING:	IFREMER.
OBJECTIVE:	Study of genetic variability of tahitian and caledonian hatchery stocks using microsatellite loci. Parental analysis of family selected for growth and IHHN virus resistance. The project is first emphasized on markers (microsatellites) associated heterosis as an explanation for genetic variability in small controlled breeding populations.
METHODOLOGY:	Microsatellites, amplification and revelation using silver staining system.
STATUS:	In progress

GERMANY

Study 1

LABORATORY/RESEARCHER	Institute of Animal Husbandry and Genetics (IAG), University of Göttingen, Göttingen / G. Hörstgen-Schwark and A. Müller-Belecke.
SPECIES	*Oreochromis niloticus.*
PROJECT FUNDING	Deutsche Forschungsgemeinschaft (DFG).
OBJECTIVE	Development and performance testing of homozygous tilapia lines and their crosses.
DESIGN	Mitotic and meiotic gynogenesis, test cross diallels including separate and communal testing of genetic groups.
METHOLOGY	UV treatment of sperm, heat treatment of activated tilapia eggs.
STATUS	Ongoing project.

Study 2

LABORATORY/RESEARCHER	Institute of Animal Husbandry and Genetics (IAG), University of Göttingen, Göttingen/G. Hörstgen-Schwark and S. Huang.
SPECIES	*Brachydanio rerio.*
PROJECT FUNDING	IAG and Friedrich Naumann Stiftung.
OBJECTIVE	Development of test fish populations of defined genetic variability for ecotoxicological studies.
DESIGN	Mitotic and meiotic gynogenesis, test cross diallels between homozygous lines, performance testing under unpolluted water conditions and reference tests according to the German Chemical Control Act.
METHOLOGY	UV treatment of sperm, heat-, cold- and pressure treatments of activated zebra fish eggs for suppression of first mitosis.
STATUS	Ongoing project.

Study 3

LABORATORY/RESEARCHER	Institute of Animal Husbandry and Genetics (IAG), University of Göttingen, Göttingen / G. Hörstgen-Schwark, J.-N. Meyer. Institute of Inland Fisheries (inc.) (IIF), 14476 Groß Glienicke / H. Wedekind. Research Center for Animal Production and Technology of the Faculty of Agriculture (RCAPT), University of Göttingen / H.-J. Langholz and K. Eder.
SPECIES	*Oncorhynchus mykiss.*
PROJECT FUNDING	IAG, IFF, RCAPT.
OBJECTIVE	Comparison of growth, carcass- and meat quality of heat-shocked and tetraploid-derived triploid and diploid rainbow trout.
DESIGN	Paternal half sib families, consisting of heat-shocked and tetraploid derived triploid rainbow trout and diploid controls have been raised under the same environmental conditions till fish were slaughtered at 2.5 years (at the beginning and the end of spawning season).
METHOLOGY	Measurements and classifications of the outer product quality (growth, body proportions, carcass composition) and the inner product quality (physical-technological-, chemical- and sensorial criteria).
STATUS	Ongoing project.

Study 4

LABORATORY/RESEARCHER	Institute of Animal Husbandry and Genetics (IAG), University of Göttingen, Göttingen / J.-N. Meyer, G. Hörstgen-Schwark, I. Jenneckens and A. Müller-Belecke. Institute of Inland Fisheries (inc.) (IIF), 14476 Groß Glienicke / H. Wedekind.
SPECIES	*Acipenser* spp., *Oreochromis* spp.
PROJECT FUNDING	DFG.
OBJECTIVE	Identification of species, populations (lines) within species and hybrids between species by the use of gene markers.
DESIGN	Collection of adequate samples from different origins
METHOLOGY	Enzyme electrophoresis and DNA analyses (multilocus DNA fingerprinting, RAPD, SSRa-PCR, AFLP).

STATUS	Ongoing project.

Study 5

LABORATORY/RESEARCHER	Institute of Fishbiology and Institute of animal breeding and genetics, Technical University of Munich –Weihenstephan / M Baars and Prof. O, Rottmann
SPECIES	*Thymallus thymallus.*
PROJECT FUNDING	Landesfischereiverband Bayern e.V.
OBJECTIVE	In an ecological work on grayling differences in growthrate and maximal growth were found in Bavarian grayling populations. These differences are to be correlated to DNA polymorphism.
DESIGN	Populations from three Bavarian river-systems will be sample and analyzed.
METHODOLOGY	DNA analyses.
STATUS	Just started.

Study 6

LABORATORY/RESEARCHER	Bundesforschungsanstalt fhr Fischerei, Institut for Fisheries Ecology / J. Trautner. University of Hamburg, Institut for Hydrobiology and Fisheries Research / W. Nellen.
SPECIES	*Oncorhynchus mykiss* and *Zoarces viviparus.*
PROJECT FUNDING	Ministry of agriculture.
OBJECTIVE	Population structure of wild populations and hatchery strains of *O. mykiss* and wild populations of *Z. viviparus*. Estimation of intraspecific biodiversity.
DESIGN	*O. mykiss* species have been sampled from hatcheries and Canadian lakes and Rivers and *Z. viviparus* from the North Sea. DNA analyses are performed.
METHODOLOGY	RFLP-, RAPD- AFLP- and mtDNA -analyses.
STATUS	Ongoing project.

Study 7

LABORATORY/RESEARCHER	Northrhine-Westfalian Agency for Ecology, Land and Forestry/Northrhine-Westfalian Office for Agriculture Developement in Recklinghausen (LÖBF NRW) / J. Lehmann and F.-J. Stürenberg.
SPECIES	*Salmo salar* and *Salom trutta trutta.*
PROJECT FUNDING	Land Northrhine-Westfalia/NRW.
OJECTIVE	Genetic identification and characterisation of wild Atlantic salmon and Salmo trutta trutta in the Rhenanian drainage and Weser system of NRW. Eyed eggs and fingerlings from eight wild populations used for reintroduction for the Rhine were reared up to analyses at LÖBF.
METHODOLOGY	Enzyme electrophoresis (allozyme genotyping) and flow-catofluorometic determination of relative DNA contents of cell nuclei (relative genome sizes).
STATUS	Ongoing project.

Study 8

LABORATORY/RESEARCHER	Institute of Freshwater Ecology and Inland Fisheries (IGB), Depart. of Fish Culture and Fish Pathology, Berlin / K. Kohlmann.
SPECIES	*Salmo salar.*
PROJECT FUNDING	IGB.
OBJECTIVE	Genetic identification of wild Atlantic salmon used for reintroduction into r. Elbe, Germany.
DESIGN	Eyed eggs from three wild populations (two Irish and one Swedish) used for reintroduction were incubated and fingerlings were reared up to analyses at IGB. Enzyme and DNA analyses were performed.
METHODOLOGY	Enzyme electrophoresis, RFLP and microsatellite analyses of DNA.
STATUS	Ongoing project.

Study 9

LABORATORY/RESEARCHER	Institute of Freshwater Ecology and Inland Fisheries (IGB), Depart. of Fish Culture and Fish Pathology, Berlin / K. Kohlmann. Inland Fisheries Institute Olsztyn, Salmonid Research Laboratory Rutki, 83-330 Zukowo (Poland) / S. Dobosz and K. Goryczko.
PROJECT FUNDING	IGB (German part) and State Committee for Scientific Research Poland (Polish part).
SPECIES	*Oncorhynchus mykiss*.
OBJECTIVE	Genetic improvement of rainbow trout growth.
DESIGN	Family selection based on mixed half and full sib families. Estimation of heritabilities for body weight and length. Influence of parental body weight on progeny performance has been studied.
METHODOLOGY	Performance tests (separate incubation and rearing until tagging as one summer old fingerlings, communal rearing later on) with control measurements of growth (at half year intervals) and sexual maturation.
STATUS	Ongoing project.

Study 10

LABORATORY/RESEARCHER	Institute of Freshwater Ecology and Inland Fisheries (IGB), Depart. of Fish Culture and Fish Pathology, Berlin / K. Kohlmann. University of South Bohemia, Research Institute of Fish Culture and Hydrobiology, Vodnany (Czech Republic) / M. Flajšhans. Academy of Sciences of Czech Republic, Institute of Animal Physiology and Genetics, Department of Genetics, Libechov (Czech Republic) / V. Slechtova and V. Slechta.
PROJECT FUNDING	IGB (German part) and Ministry of Agriculture (Czech part).
SPECIES	*Tinca tinca*.
OBJECTIVE	Genetic characterisation of wild and cultured populations; genetic improvement of cultured strains.
DESIGN	Tench from wild and cultured populations were collected in Germany and Czech Republic. The examination of growth rate, food conversion efficiency, survival and product quality in the different populations will be accompanied by investigations on their genetic structure.
METHODOLOGY	Enzyme electrophoresis is performed, DNA analysis will be introduced. Performance tests are carried out under warm water conditions in a closed recirculating system.
STATUS	Ongoing project.

Study 11

LABORATORY/RESEARCHER	Institute of Freshwater Ecology and Inland Fisheries (IGB), Depart. of Biology and Ecology of Fishes, Berlin / A. Ludwig and C. Wolter.
SPECIES	Different species of *Cyprinids*.
PROJECT FUNDING	Bundesministerium für Forschung und Technologie.
OBJECTIVE	Investigation of population as well as sub-population structure. Estimation of intraspecific and interspecific biodiversity.
DESIGN	Development of marker systems for geneflow within different sampling points.
METHODOLOGY	Sequence and microsatellites analyses.
STATUS	Ongoing project.

Study 12

LABORATORY/RESEARCHER	Institute of Freshwater Ecology and Inland Fisheries (IGB), Depart. of Biology and Ecology of Fishes, Berlin, Germany/ A. Ludwig and F. Kirschbaum.
SPECIES	*Acipenser sturio*.
PROJECT FUNDING	Deutsche Forschungsgemeinschaft, KI 189/11–1.
OBJECTIVE	Genetic characterisation of historical stocks of Acipenser sturio originated in German waterways. Comparison with samples from other sturgeon catches of European waters, especially the Gironde population with respect to the reestablishment of Acipenser sturio in German waterways.
DESIGN	Museum samples of Acipenser sturio were collected originating from different River systems and from the North Sea. DNA analyses are performed.
METHODOLOGY	Sequence analyses.

STATUS	Starting project.

Study 13

LABORATORY/RESEARCHER	Zoologisches Institut, LMU of Munich, Prof. Tautz, U. Schliewen, Dr K. Rassmann and C. Englbrecht.
SPECIES	*Salmo trutta* and *Salvelinus alpinus*.
PROJECT FUNDING	Federal Ministry of environment (UBA).
OBJECTIVE	1) to study changes in genetic variability (connected with changes in behavior, morphology, etc.) of autochthonous species resulting from crossing with non-autochthonous species (e.g., stocking). 2) To establish a model to estimate the risk of releasing genetically manipulated organisms.
DESIGN	Salmo trutta species have been sampled in many brooks within Bavaria. Salvelinus alpinus samples were obtained from major prealpine lakes and small alpine lakes of Germany. Morphological and genetic analyses are performed.
METHODOLOGY	Sequencing of mtDNA, Microsatellite-analyses.
STATUS	Ongoing project.

Study 14

LABORATORY/RESEARCHER	Zoological Institute I, University of Heidelberg, Heidelberg / A. Schreiber.
SPECIES	*Salmo trutta, Thymallus, Cottus, Gobio, Chondrostoma* and *Lampetra*.
PROJECT FUNDING	Fisheries authorities.
OBJECTIVE	Genetic population structure as basis for conservation management.
DESIGN	Population samples from wild stocks in different river basins are investigated.
METHODOLOGY	Enzyme electrophoresis, RAPDs, morphometry.
STATUS	Ongoing project.

Study 15

LABORATORY/RESEARCHER	UFZ-Centre for Environmental Research, Dept. Community Ecology / B. Lauchstaedt.
SPECIES	*Rutilus rutilus*.
PROJECT FUNDING	UFZ-Centre for Environmental Research.
OBJECTIVE	Habitat fragmentation in riverside forest waters of the "Biosphaerenreservat Mittlere Elbe".
DESIGN	Sampling of 24 sites from the Elbe river and backwaters at different stage of isolation.
METHODOLOGY	Allozyme analyses.
STATUS	Ongoing project.

Study 16

LABORATORY/RESEARCHER:	UFZ-Centre for Environmental Research, Dept. Community Ecology / B. Lauchstaedt and B. Haenfling
SPECIES:	24 Central European *Cyprinid* species
PROJECT FUNDING:	UFZ-Centre for Environmental Research.
OBJECTIVE:	Phylogenetic relationship among and within *cyprinid* subfamilies.
DESIGN:	Sampling of 24 cyprinid species of German waters, including all genera occurring in Central Europe
METHODOLOGY:	Allozyme analyses.
STATUS:	Ongoing project.

ICELAND

Study 1

LABORATORY/RESEARCHER	Holar Agricultural College, Saudarkrokur / E. Svavarsson.
SPECIES	Arctic charr.
PROJECT FUNDING	The National Research Council and the Agricultural Productivity Fund in Iceland.
OBJECTIVE	To determine genetic parameters, i.e., heritability and genetic correlation of

	economically important traits of Arctic charr in Aquaculture. The results will be utilised in a national breeding program of Arctic charr.
DESIGN AND METHODOLOGY	Data are collected from charr in the first 2 or 3 year classes of the Arctic charr breeding program. Each year class is made up of 100 - 120 full sib families, with two or three families pr. sire. The families are reared for two and a half year from hatching. Data are collected on growth, sexual maturity at different life stages, flesh coloration and possibly fat content of fish. Data are analysed after standard methods in animal breeding.
STATUS	The project started in 1993 and is planned for four years. Preliminary results for the first year class have been published in Iceland. A revised project plan has been sent to the Research Council for the years 1996 - 1998. Continued work according to the revised plan will depend on funding.
COMMENTS	The project is in co-operation between The Agricultural school at Hólar in North Iceland, that is in charge of the actual breeding program for Arctic charr, The Institute of Freshwater Fisheries and The Agricultural Research Institute. The breeding program is funded by the Agricultural Productivity Fund in Iceland.

Study 2

LABORATORY/RESEARCHER	Marine Research Institute (MRI), c/o Biotechnology House, Reykjavik / A. K. Danielsdottir, O.D. Jonsdottir and O.Y. Atladottir. An EU-FAIR project in collaboration with University of Trondheim, Norway / J. Mork; University College Cork, Ireland / T. Cross; University of East Anglia, U.K. / G. M. Hewitt and C. Rico; Directorate of Fisheries Research, MAFF, U.K. / R. S. Millner and M. Nicholson.
SPECIES	Cod (*Gadus Morhua*), hake (*Merluccius merluccius*), blue whiting (*Micromesistius poutassou*) and poor cod (*Trisopterus minututs*).
PROJECT FUNDING	MRI and EU FAIR.
OBJECTIVE	Cod stock structure in Icelandic waters and calibration of different molecular Markers for use in discrimination and management of cod, blue whiting, hake and poor cod.
METHODOLOGY	Haemoglobin's, allozymes and anonymous cDNA RFLP.
STATUS	Four year project. Cod sampling has started, analysis of samples started in October 1996. (1996-2000).

Study 3

LABORATORY/RESEARCHER	Marine Research Institute (MRI), c/o Biotechnology House, Reykjavik / A.K. Danielsdottir (project leader).
SPECIES	Redfish (*Sebastes mentella*).
PROJECT FUNDING	MRI, The National Research Council of Iceland and various trawlers.
OBJECTIVE	Study the genetic population structure of oceanic and deep-sea *S. mentella* in Irminger sea and Icelandic waters.
DESIGN	Redfish samples from different locations Southwest of Iceland and the Irminger Sea.
METHODOLOGY	Allozymes, haemoglobin's and anonymous cDNA RFLP.
STATUS	Three to five year project. Redfish sampling and analyses started summer 1995.
COMMENTS	The project is in collaboration with University of Bergen, Norway / T. Johansen and G. Naevdal.

Study 4

LABORATORY/RESEARCHER	Institute of Freshwater Fisheries, c/o Biotechnology House, Reykjavik / A.K. Danielsdottir and S. Gudjonsson. Danish Institute for Fisheries Research, Dept. of Inland Fisheries, Silkeborg / M.M. Hansen (coordinator) + 21 other participants from laboratories in Europe and Canada.
SPECIES	Brown trout (*Salmo trutta*).
PROJECT FUNDING	In house, the Icelandic Science fund and EU FAIR
OBJECTIVE	Genetic variation in wild populations of landlocked and anandromous brown trout in Iceland. Concerted Action on brown trout population genetics (TROUTCONCERT). The objectives are to promote collaboration among laboratories that are active in research on population genetics of brown trout, to harmonise the use of genetic markers, to give recommendations for a European strategy for management and conservation of the species, and to establish databases on relevant literature, available genetic markers and data from published and unpublished studies. The databases will be made publicly accessible on the World Wide Web (WWW).

DESIGN	Mapping of gene frequencies. Concerted action, i.e., network among laboratories.
METHODOLOGY	Allozymes. Workshops, exchange visits among laboratories, common databases and WWW facilities.
STATUS	Samples from 13 locations have been analysed and the study is ongoing. Two-year project (1998–1999).

Study 5

LABORATORY/RESEARCHER	University of Iceland, Department of Biology, Reykjavik / E. Arnason.
SPECIES	Cod, salmon, brown trout and Arctic charr.
PROJECT FUNDING	In house and the Icelandic Science fund.
OBJECTIVE	Genetic population structure and species variation.
DESIGN	Mapping of gene frequencies and sequence variation.
METHODOLOGY	RFLP of mtDNA, mtDNA cytochrome b sequencing and microsatellite markers.
STATUS	Ongoing.

Study 6

LABORATORY/RESEARCHER	Stofnfiskur ltd., private fishfarmers / J. Jonasson.
SPECIES	Atlantic salmon.
PROJECT FUNDING	Icelandic Government, private.
OBJECTIVE	Use selective breeding to improve economically important traits in rearing of salmon in landbased units and net pens.
DESIGN	Produce 100-200 families a year for selection.
STATUS	Started in 1991, ongoing.

Study 7

LABORATORY/RESEARCHER	Stofnfiskur Ltd., private fishfarmers / J. Jonasson.
SPECIES	Atlantic salmon.
PROJECT FUNDING	Icelandic Research Council.
OBJECTIVE	Establish rearing methods by using geothermal heat and light regimes to accelerate growth and age at maturity to shorten the generation interval to increase response to selection.
DESIGN	Produce 100–150 families a year.
STATUS	Started in 1993–1997.

Study 8

LABORATORY/RESEARCHER	Stofnfiskur Ltd., private fishfarmers / J. Jonasson.
SPECIES	Atlantic salmon.
PROJECT FUNDING	Icelandic Research Council.
OBJECTIVE	Salmon quality. Estimate heritabilities for fat content and genetic correlation between fat content and other life history traits in salmon farming.
METHODOLOGY	Use Tory-fish fat meter to measure fat content.
DESIGN	Produce 100–150 families a year.
STATUS	Started in 1995–1997.

Study 9

LABORATORY/RESEARCHER	Stofnfiskur Ltd. / J. Jonasson. Saebyli ltd. / S. E. Stefansson. Institute of Freshwater Fisheries / A. Gudnason. The Marine Research Institute,, A. Steinarsson.
SPECIES	Red Abalone.
PROJECT FUNDING	Icelandic Research Council.
OBJECTIVE	Estimate genetic parameters for body weight, survival and shell and meat proportion, in the attempt to plan a breeding program for red abalone culture in Iceland to reduce production cost for coming years.
METHODOLOGY	Classic selective breeding programs.

DESIGN	Produce 100–150 full- and half-sib families a year.
STATUS	Started in 1996–1999.

Study 10

LABORATORY/RESEARCHER	University of Iceland, Holar Agricultural College, Saudarkrokur / S. Skulason and D. Gislason. Joint population genetic laboratory of the Marine Research Institute and Institute of Freshwater Fisheries, c/o Biotechnology House, IS–112 Reykjavik / A.K. Danielsdottir. Also one partner in Ireland, one in Sweden and one in Scotland. In collaboration with the University of Guelph / M. Ferguson.
SPECIES	Arctic charr, *Salvelinus alpinus*.
PROJECT FUNDING	EU FAIR-CT-96–1981.
OBJECTIVE	Development of sustainable aquaculture of Arctic charr.
DESIGN	Multidisciplinary approach involving fish-farmers, ecologists, brood stock managers, fish husbandry experts and molecular biologists. Holistic approach encompassing a variety of techniques to provide a sound scientific basis for the development of this species for aquaculture.
METHODOLOGY	Genetic variation in wild populations and domesticated stains from Iceland, Scotland Ireland and Sweden by the use of Microsatellites.
STATUS	Project will start in September 1997, to continue for 2 years.

RECENT ICELANDIC PUBLICATIONS:

Daníelsdóttir, A.K., Marteinsdóttir, G., Árnason, F. and Guðjónsson S. 1997. Genetic structure of wild and reared Atlantic salmon (*Salmo salar* L.) populations in Iceland. *ICES Jounal of Marine Science* **54(6)**:986-997.

IRELAND

Study 1

LABORATORY/RESEARCHER	National Diagnostics Centre, University College, Galway / T. Smith, S.A.M.Martin, H. Husbye and M. Johnson.
SPECIES	Salmon (Galway), trout (Rennes), tilapia (Southampton) and zebrafish (Oslo and Southampton).
PROJECT FUNDING	EU Biotech programme.
OBJECTIVE	The use of transgenesis to render fish sterile and to evaluate the effectiveness of the induced sterility.
TITLE	Biological containment of transgenic fish and risk assessment of interspecies gene transfer.
DESIGN	Antisense and ribozyme technology is being used to inhibit the synthesis of gonadotropin releasing hormone (GnRH). This is expected to render fish sterile as has been shown previously in the mouse. Injections of GnRH will be used to return some fish to fertility and provide brood stocks. As part of the studies further insights into fish reproductive physiology will be achieved. Reporter genes will be co-injected into fish to monitor transgenesis and possible gene flow. Ultimately the aim would be to introduce sterility in conjunction with a valuable trait (e.g., disease resistance).
METHODOLOGY	The Galway group is involved in the isolation of strong all tissue expressing promoters from salmon which will be used to drive expression of antisense GnRH and reporter genes. In parallel brain specific cDNAs and their promoters are being isolated.
STATUS	The current situation is that antisense and reporter gene expression constructs have been made and are being tested *in vitro* and being microinjected into fish for *in vivo* analysis. Duration three years from Dec.1997.

Study 2

LABORATORY/RESEARCHER	National Diagnostics Centre, University College, Galway / T. Smith, S.A.M. Martin, J. Davidson and P. O'Dea.
SPECIES	Rainbow trout (Galway), trout and medaka (INRA, Paris) and medaka (Wurzburg).
PROJECT FUNDING	EU FAIR programme.
OBJECTIVE	Identification of genes involved in fish immunity. Generation of molecular markers to predict fish immunity and use identified genes to protect fish from pathogen infection.

TITLE	Molecular basis of fish immunity for disease resistance.
DESIGN	a) Cloning of cellular and humoral factors involved in immune response using a variety of approaches, b) Isolation and culture of fish cells involved in immune response, c) Combination of a) and b) above to establish functional relationship. Transfer of genes identified into fish.
METHODOLOGY	The Galway group is involved in the isolation of genes whose expression is up or down-regulated as a result of infection. Differential cloning and differential display RT-PCR will be used to identify such genes.
STATUS	Differential display RT-PCR and subtractive hybridisation experiments are ongoing in an attempt to isolate novel genes. Duration three years (Jan. 96-Dec 98).

Study 3

LABORATORY/RESEARCHER	National Diagnostics Centre, University College, Galway / T. Smith, S.A.M. Martin and O. McMeel.
SPECIES	Rainbow trout.
FUNDING	EU FAIR Grant.
OBJECTIVE	Basis of sex determination and gonadal sex differentiation for sex control in aquaculture.
DESIGN	Isolation cDNAs expressed in a male / female specific pattern. Genes are isolated from developing gonads isolated from genetically male and female line of trout.
METHODOLOGY	Differential display rt-PCR and subtractive cloning to isolate cDNAs expressed in male or female gonads prior to differentiation. In parallel homology cloning of genes know in other species will be performed.
STATUS	Three year project (JAN 98-2000).

Study 4

LABORATORY/RESEARCHER	National Diagnostics Centre, University College, Galway / S.A.M. Martin.
SPECIES	Atlantic salmon.
FUNDING	Funding applied for, presently in house funding.
OBJECTIVE	Molecular biology of Thyroid Stimulating Hormone and its role in smoltification in Atlantic salmon (*Salmo salar*).
DESIGN	Clone and characterise the Atlantic salmon TSH b-subunit. Study the expression during key stages of the life cycle.
METHODOLOGY	Sequence analysis. Determine expression pattern by Northern blot analysis of pituitary RNA samples. Analyses of feedback mechanism of thyroid hormones on TSH gene expression.
STATUS	Funding applied for.

Study 5

LABORATORY/RESEARCHER	Biochemistry Department, National University of Ireland, Galway/ L. Byrnes and K. Gately.
SPECIES	Atlantic salmon, *Salmo salar*.
PROJECT FUNDING	BioResearch Ireland.
OBJECTIVE	To examine the regulation of salmon transferrin gene expression, particularly during smoltification.
DESIGN	Promoter of salmon transferrin gene has been isolated.
METHODOLOGY	DNA sequence analysis, electrophoretic mobility shift assays, DNase footprinting, functional assays of promoter activity in cell lines.
STATUS	Final year of project.

Study 6

LABORATORY/RESEARCHER	Biochemistry Department, National University of Ireland, Galway / L. Byrnes, J. Hill and A. Kelly. Also two partners in Iceland, one partner in Sweden and one partner in Scotland.
SPECIES	Arctic charr, *Salvelinus alpinus*.
PROJECT FUNDING	EU FAIR-CT-96–1981.

OBJECTIVE	Development of sustainable aquaculture of Arctic charr.
DESIGN	Multi-disciplinary approach involving fish-farmers, ecologists, brood stock managers, fish husbandry experts and molecular biologists
METHODOLOGY	Holistic approach encompassing a variety of techniques to provide a sound scientific basis for the development of this species for aquaculture.
STATUS	Project started in December, 1996, to continue for three years.

Study 7

LABORATORY/RESEARCHER:	Recombinant DNA Group, Department of Microbiology, National University of Ireland, Galway / R. Powell, E. Powell and G. Cloherty.
SPECIES:	Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*).
PROJECT FUNDING:	E.U. FAIR Programme 1996–1999.
OBJECTIVE:	Generation of highly informative DNA markers and genetic marker maps of salmonid fishes (SALMAP).
STATUS:	Research involves the cloning, isolation and design of PCR assays targeting repetitive microsatellite DNA sequences in the genome of the selected salmonids. The objectives are to define low density genetic maps for the three selected salmonid species.

Study 8

LABORATORY/RESEARCHER	Recombinant DNA Group, Department of Microbiology, National University of Ireland, Galway / R. Powell, N.P. Wilkins, J.A. Houghton and G. Rafferty.
SPECIES	Pacific oyster (*Crassostrea gigas*).
PROJECT FUNDING	E.U. FAIR Programme 1995–1999.
OBJECTIVE	Development of a molecular karyotype system for Pacific oyster.
STATUS	Research involves the construction of large-insert genomic DNA libraries of Pacific oyster using *E. coli* cosmid vectors. The aim is to define clones that mark specific chromosome pairs and develop a chromosomal karyotype system based on such molecular markers.

Study 9

LABORATORY/RESEARCHER	Recombinant DNA Group, Department of Microbiology, National University of Ireland, Galway / R. Powell and G. Davey.
SPECIES	Atlantic salmon (*Salmo salar*).
PROJECT FUNDING	Forbairt Basic Science Programme 1997-2000.
OBJECTIVE	Generation of expressed sequence tags for Atlantic salmon.
STATUS	Research involves the construction of cDNA libraries from four tissues of Atlantic salmon. Partial DNA sequencing will be used to generate ESTs and define the major abundant messenger RNA transcripts in the selected tissues.

Study 10

LABORATORY/RESEARCHER	Recombinant DNA Group, Department of Microbiology, National University of Ireland, Galway / R. Powell, D. Nolan, T. Smith and Sam Martin.
SPECIES	Sea lice (*Lepeophtheirus salmonis*).
PROJECT FUNDING	Marine Institute Operational Programme 1997–1999.
OBJECTIVE	Cloning and characterisation of *Lepeophtheirus salmonis* microsatellite genetic elements as useful tools in sea lice ecology studies.
STATUS	Research involves the cloning and design of PCR assays targeting sea lice microsatellite elements. The aim is to examine whether such microsatellite assays can be used to provide useful data tracing sea lice populations with respect to their impact on cultured and wild fish systems.

Study 11

LABORATORY/RESEARCHER	Recombinant DNA Group, Department of Microbiology, National University of Ireland, Galway / R. Powell and J. Thornton.

SPECIES	*Aeromonas salmonicida*, Salmonid and non-salmonid species.
PROJECT FUNDING	E.U. AIR Programme 1994–1997.
OBJECTIVE	Improved identification and taxonomy of atypical isolates of the fish pathogen *Aeromonas salmonicida.*
STATUS	Research is underway on a genetic, biochemical and immunological analysis of new isolates of 'atypical' *Aeromonas salmonicida* presently being isolated from a large range of diseased sea- and fresh-water fish species. The objectives are (i) to develop definitive diagnostic procedures for the identification of this bacterial group, and (ii) to quantify the detrimental effect of this group on native fish resources.

Study 12

LABORATORY/RESEARCHER:	Department of Genetics, Trinity College, Dublin / A. Norris.
SPECIES:	Atlantic salmon.
PROJECT FUNDING:	Forbairt, Hydro Seafood Fanad.
OBJECTIVE:	1. To investigate levels of heterozygosity and allelic variation among Fanad's stocks and the hypothesis that inbreeding may be a cause of declining fertility and other physiological problems. 2. To investigate methods for increasing genetic improvement for certain traits by selection procedures.
DESIGN:	Samples have been collected from 5 years of Fanad's stocks, Fanad's original broodstock (archived), wild Salmon from 3 Irish rivers and 1 Norwegian river. Samples from a number of full-sib groups and their parents are also being used in a parentage study.
METHODOLOGY:	Microsatellite DNA for the first stage of the project, quantitative genetics procedures for the second stage.
STATUS:	Two year project finishing in October 1998.

Study 13

LABORATORY/RESEARCHERS	Department of Zoology, University College, Dublin / E. J. Duke, J.J. Bracken, M. Sutton and M. Kelly-Quinn.
SPECIES	Brown trout, *Salmo trutta.*
PROJECT FUNDING	Zoology, University College, Dublin.
OBJECTIVE	Examination of the molecular genetics of isolated brown trout populations.
DESIGN	a) fish farm, b) isolated river above impassable waterfall, i.e., no upstream movement of fish to this system.
METHODOLOGY	MtDNA, RAPDs, genetic fingerprinting.
STATUS	One year project which started in August 1996.

Study 14

LABORATORIES/RESEARCHERS:	School of Science, Regional Technical College, Galway / E. Gosling. Also one UK, one Belgian and one Portuguese partner.
SPECIES:	Periwinkle species, *Littorina* (*L. saxatilis* group, *L. littorea*, *L. striata*).
PROJECT FUNDING:	EC MAST III CT95-0042.
OBJECTIVES:	Using periwinkles as model organisms, to determine the interrelationships between the physical properties of ecosystems and the ecology of organisms in the generation of biodiversity, to measure the resultant diversity, and to produce operational concepts of biodiversity which are of general applicability and importance.
DESIGN:	Sample collection over a wide geographic range in Western Europe and the Azores.
STATUS:	Three year project finishing in January 1999

Study 15

LABORATORY/RESEARCHER	Salmon Research Agency of Ireland / P. McGinnity. Queens University Belfast / P. Prodohl and A. Ferguson. National University of Ireland, Cork / T. Cross.
SPECIES	Atlantic salmon.
PROJECT FUNDING	In house, external funding being sought.
OBJECTIVE	Determination of relative ocean survival of wild and farmed salmon and their F1 hybrids, also the freshwater survival of F2 hybrids and backcrosses relative to wild and

	farmed parents
DESIGN	Use of experimental stream and hatchery controls, with salmon where parents typed for several microsatellite loci to allow identification of progeny.
METHODOLOGY	Field studies using microsatellites as molecular tags.
STATUS	Four year project started 1997.

Study 16

LABORATORY/RESEARCHER	Salmon Research Agency of Ireland / P. McGinnity.
SPECIES	Atlantic salmon.
PROJECT FUNDING	Salmon Research Agency of Ireland.
OBJECTIVE	To determine the genetic impact of Ocean Ranch Atlantic salmon on natural populations.
DESIGN	Two scenario's are being studied where (a) the ocean ranch population has originated from the recipient wild population and (b) where there is no relationship between the ocean ranch population and the recipient population.
METHODOLOGY	Allozyme analysis.
STATUS	Ongoing study.

Study 17

LABORATORY/RESEARCHER	Salmon Research Agency of Ireland / R. Poole. With one Norwegian and two UK partners.
SPECIES	Atlantic salmon, anadromous and resident brown trout.
PROJECT FUNDING	AIR3 PL94 2484.
OBJECTIVE	The goal of the research project is to quantify and understand the effects of hybridisation between Atlantic salmon and brown trout, particularly as it relates to escapes from aquaculture.
DESIGN	Quantify interspecific hybridisation and introgression in unspoiled and genetically compromised rivers
METHODOLOGY	Application of mini-satellite and mitochondrial DNA identification techniques.
STATUS	Two year project finished in March 1997.

Study 18

LABORATORY/RESEARCHER	Salmon Research Agency of Ireland / D. Cotter. With one Irish, two Scottish and one Norwegian partner.
SPECIES	Atlantic salmon.
PROJECT FUNDING	EC AIR Programme.
OBJECTIVE	A comprehensive evaluation of the use of sterile triploid Atlantic salmon in reducing the interaction between wild and farm stocks.
DESIGN	Characterisation of the performance of triploids in culture.
METHODOLOGY	Setting up experimental population, control population, ocean ranching, rearing experiments, tagging, simulation of escapes from sea cages.
STATUS	Four year programme to be completed October 1998.

Study 19

LABORATORY/RESEARCHERS	Aquaculture Development Centre, National University of Ireland, Cork / T. Cross, P. Galvin and E. Dillane. With two UK and several other European partners.
SPECIES	Ommastrepid squid, *Illex coindetii* and *Todaropsis eblanae*.
PROJECT FUNDING	EC FAIR.CT96.1520.
OBJECTIVE	To develop microsatellite primers for these squid species, and to use them to search for inter population variability throughout the range.
METHODOLOGY	Microsatellite DNA loci.
STATUS	Three year project started 1997.

Study 20

LABORATORY/RESEARCHERS	Aquaculture Development Centre, National University of Ireland, Cork T. Cross, R. FitzGerald, J. Coughlan and P. Galvin. With one Irish turbot farming company.
SPECIES	Turbot, *Scophthalmus maximus*.
PROJECT FUNDING	Irish Marine Operational Programme.
OBJECTIVE	To compare genetic variability in wild and farmed turbot.
DESIGN	Four microsatellite loci were developed for turbot and tested for Mendelian inheritance. Farmed and wild samples have been compared from Ireland and Norway.
METHODOLOGY	Microsatellite loci.
STATUS	Two year project finished in October 1997.

Study 21

LABORATORY/RESEARCHERS	Aquaculture Development Centre, National University of Ireland, Cork / T. Cross, R. FitzGerald, J. Coughlan and M. O. Stefansson. With Norwegian and Dutch groups.
SPECIES	Turbot, *Scophthalmus maximus*, halibut, *Hippoglossus hippoglossus*.
PROJECT FUNDING	FAIR CT97-3544.
OBJECTIVE	To quantify genetic variability in wild turbot and halibut and to compare it with levels in natural populations. Also, to assess the extent of geographic variability in wild populations.
METHODOLOGY	Microsatellite loci.
STATUS	Three year project started January 1998.

Study 22

LABORATORY/RESEARCHERS	Aquaculture Development Centre, National University of Ireland, Cork / T. Cross, P. Galvin, J. Coughlan, L. Bourke. With two UK, one Norwegian and one Icelandic partner.
SPECIES	Cod, hake, blue whiting and poor cod.
PROJECT FUNDING	EC FAIR CT95.0282.
OBJECTIVE	To investigate population structure on macro and micro-geographic scales.
DESIGN	Samples are being screened for macrogeographic variation and additional loci are being characterised.
METHODOLOGY	Minisatellite DNA loci, transcribed sequences.
STATUS	Four year project from April 1996.

Study 23

LABORATORY/RESEARCHERS	Aquaculture Development Centre, National University of Ireland, Cork / T. Cross, L. Bourke and P. Galvin.
SPECIES	Atlantic salmon, *Salmo salar*.
PROJECT FUNDING	Electricity Supply Board.
OBJECTIVE	To assist the breeding programmes on the rivers Shannon and Lee by carrying out molecular studies, also to collect baseline data for GSI.
DESIGN	Several hatchery and wild samples have been screened.
METHODOLOGY	Minisatellite DNA loci.
STATUS	One year project from March 1996.

Study 24

LABORATORY/RESEARCHERS	Aquaculture Development Centre, National University of Ireland, Cork / T. Cross, P. Galvin, M. Cross and M. Aherne.
SPECIES	Atlantic salmon, *Salmo salar*.
PROJECT FUNDING	EC Interreg.
OBJECTIVE	To study genetic variability in the hatchery strain in the river Erne and wild populations from nearby rivers.
DESIGN	Samples from the hatchery and four nearby rivers have been screened for minisatellites and are now being assayed for microsatellites.

METHODOLOGY	Minisatellite and microsatellite DNA loci.
STATUS	Four year project from November 1996.

LATVIA

Study 1

LABORATORY/RESEARCHER:	Latvian Fisheries Research Institute / O.P. Vasin.
SPECIES:	Atlantic salmon.
PROJECT FUNDING: In house.	**OBJECTIVE:** Survey of genetic variation. Genetic monitoring of main hatchery stock.
DESIGN:	To study the genetic structure and it's temporal diversity in order to prevent reduction and conserve the genetic structure in hatchery reared population.
METHODOLOGY:	Allozymes (in Polyacrilamide).
STATUS:	Long term study, since 1984.

NORWAY

Study 1

LABORATORY/RESEARCHER	Department of Fisheries and Marine Biology, University of Bergen (DFMB) / G. Nævdal.
SPECIES	Sandeels (*Ammodytidae*).
PROJECT FUNDING	The Norwegian Research Council/University of Bergen.
OBJECTIVE	Study the genetic variation between morphological similar species, and the population structure within the most aboundant species of sandeels.
DESIGN	Samples from localities from the North Sea, Iceland and Scotland, the Faeroe Islands and Denmark are being analysed.
METHODOLOGY	Gel electrophoresis and isoelectric focusing (allozymes).
STATUS	Three year project started in January 1995 and was terminated in 1997 except for data treatment.
COMMENTS	Cooperation has been established with several fisheries research institutes around the North Sea and Icland. With a very few exceptions all samples collected from Norwegian waters have been identified as *Ammodytes marinus*.

Study 2

LABORATORY/RESEARCHER	Department of Fisheries and Marine Biology, University of Bergen (DFMB) / G. Nævdal. In collaboration with Institute of Marine Research (IMR) Bergen, and Møreforskning, Ålesund.
SPECIES	Redfish, Genus *Sebastes*.
PROJECT FUNDING	The Norwegian Research Council, IMR and the University of Bergen.
OBJECTIVE	Study the genetic variation between morphologically similar species, and the population structure within the species.
DESIGN	Extensive sampling has taken place throughout the distibution areas of the redfish species, with main emphasise on Icelandic and Greenland waters in collaboration with Institute of Marine Research, Reykjavik, and Møreforsking, Ålesund. Samples have also been exchanges with Canadian researchers.
METHODOLOGY	Gel electrophoresis and isoelectric focusing (allozymes). RAPD in collaboration with IMR
STATUS	Studies on haemoglobins and allozymes by electrophoresis and isoelectric focusing have been going on since 1987; the last years with main emphasize on Icelandic and Greenland waters. From 1995 DNA-analyses have been included with the main emphasize of studying the oceanic and deep sea *S. mentella*. A "new" three year project started in January 1995.
COMMENTS	The project has revealed a relative simple species and population structure for redfish species in the eastern North Atlantic, while the picture seems very complicated in Greenland waters. The Giant redfish at the Reykjanes Ridge deviate from the other redfish groups.

Study 3

LABORATORY/RESEARCHER	Department of Fisheries and Marine Biology, University of Bergen (DFMB) / G. Nævdal.
SPECIES	Mesopelagic fish species.
PROJECT FUNDING	Own funding and partly the Norwegian Research Council.
OBJECTIVE	Identify morphologically similar species and study the structure of the more common mesopelagic fishes (*Maurolicus mülleri, Benthosema glaciale, Notolepis rissoi kroyeri*).
DESIGN	Samples from Norwegian fjords and offshore waters are being analysed.
METHODOLOGY	At the moment SGE of allozymes. (DNA analyses are planned, but not yet funded).
STATUS	Several polymorphic systems are identified in the three main species. Comparisons of frequency distributions are under way.
COMMENTS	Designed as doctor thesis.

Study 4

LABORATORY/RESEARCHER	Institute of Marine Research (IMR), Bergen, Øystein Skaala.
SPECIES	Atlantic salmon (*Salmo salar L.*).
PROJECT FUNDING	The Norwegian Research Council.
OBJECTIVE	1. To study the genetic implications of transgenic fish by using genetically marked multigeneration cultivated salmon as a model organism. 2. To quantify gene flow from the model species to wild salmon populations. 3. To estimate growth and survival of different genotypes (wild, introduced and heterozygotes). 4. To investigate the extent of genetic introgression from the model organism to sympatric salmonid species, i.e., brown trout (*Salmo trutta L.*).
DESIGN	Release of genetically marked multigeneration farmed salmon in a river with salmon and trout stocks.
METHODOLOGY	Allozymes and minisatellite DNA.
STATUS	Spawners with genetic markers returned to spawn in 1995 and 1996, and F1 individuals, in 1996 and 1997 year class, with marker detected. Further minisatellite typing necessary to improve resolution of material.
COMMENTS	Collaborative work on minisatellites with John B. Taggart, University of Stirling. The study does not include transgenic fish, but employ multigeneration farmed salmon as a model to investigate impacts from trangenic fish potensially used in fish farming in the future.

Study 5

LABORATORY/RESEARCHER	Institute of Marine Research (IMR), Bergen / Øystein Skaala.
SPECIES	Atlantic salmon.
PROJECT FUNDING	The Directorate for nature management.
OBJECTIVE	Studies of temporal stability of gene frequencies in R. Vosso salmon.
DESIGN	Screening of naturally spawned year classes between 1983 and 1996, including spawners classified as "wild" and "farmed" type by morphology and scales.
METHODOLOGY	starch gel electrophoresis with emphasis on the MEP-2* locus, where the fast allel is close to fixation in one of the major brood stocks of farmed salmon, and elevated in "farmed" type spawners.
STATUS	Baseline samples of wild Vosso salmon and farmed salmon analysed, all together some 800 individuals from at least 8 year classes. Gradual increase over year classes in frequency of *125 allele from 0.49 to 0.65 which corresponds to frequency of the allele in "escaped" spawners in the river. Significant differences between wild and farmed spawners at MEP-2*.
COMMENTS	Collaborative work with Dr K. Hindar at NINA

Study 6

LABORATORY/RESEARCHER	Institute of Marine Research (IMR), Bergen / Øystein Skaala.
SPECIES	Atlantic salmon and Brown trout.
PROJECT FUNDING	The Norwegian Sea ranching programme (PUSH).
OBJECTIVE	Genetic comparison of three salmon stocks employed under the ranching programme.
DESIGN	Genotyping by polymorphic isozyme loci.

METHODOLOGY	Isozyme loci AAT-4*, IDDH-2*, IDHP-3*, MDH-3,4*, MEP-2*, TPI-3*.
STATUS	All three stocks sampled, genotyped and compared. Pairwise comparisons revealed significant differences between all stocks at several loci.

Study 7

LABORATORY/RESEARCHER	Institute of Marine Research (IMR), Bergen / K.E. Jørstad.
SPECIES	European lobster (*Hommarus gammarus*).
PROJECT FUNDING	IMR, Norwegian Research Council.
OBJECTIVE	Genetic comparison of cultured and wild lobsters.
DESIGN	Sampling of wild and recaptured cultured lobsters. Comparison with samples of wild stock at Kvitsøy and nearby regions.
METHODOLOGY	Starch gel electrophoresis, polymorphic enzymes.
STATUS	Preliminary report 1997.
COMMENTS	The work is part of a large-scale lobster enhancement project.

Study 8

LABORATORY/RESEARCHER	Institute of Marine Research (IMR), Bergen / K.E. Jørstad.
SPECIES	Mainly herring (*Clupea harengus*).
PROJECT FUNDING	IMR, Ministry of Foreign Affairs, Norway.
OBJECTIVE	Yearclass study of herring fjord stocks; identification methods of different herring stocks in Barents Sea and Russian coastal areas.
DESIGN	Sampling by research vessel surveys; analyses carried out on board.
METHODOLOGY	Starch gel electrophoresis/allozyme variation.
STATUS	First report 1997.
COMMENTS	Part of the study is a joint work with Russian institutions (Moscow State University; SevPINRO (Arkhangelsk) and PINRO (Murmansk).

Study 9

LABORATORY/RESEARCHER	Institute of Marine Research (IMR), Bergen / K.E. Jørstad.
SPECIES	European lobster (*Hommarus gammarus*).
PROJECT FUNDING	IMR, Norwegian Research Council.
OBJECTIVE	Estimate genetic impact from Scottish lobster/import.
DESIGN	Collection of lobster samples from Scotland and compare with samples from recipient areas in Norway.
METHODOLOGY	allozyme and microsatellite DNA analyses.
STATUS	Initiated spring 1998.
COMMENTS	Partly in co-operation with prof Ferguson, Belfast.

Study 10

LABORATORY/RESEARCHER	Department of Aquaculture, Institute of Marine Research (IMR), Bergen / G. Dahle.
SPECIES	Halibut (Hippoglossus hippoglossus).
PROJECT FUNDING	Norwegian Research Council.
OBJECTIVE	Produce genetic markers in the aquaculture species halibut.
DESIGN	Clone restriction digested DNA into plasmid vector, and search in the DNA "library" for repeated sequences which can be used as microsatellite loci.
METHODOLOGY	Cloning, sequencing and extensive testing of possible microsatellite primers.
STATUS	Three year project started in 1996, and will terminate in 1998.
COMMENTS	A DNA library has been established and is being screened for possible microsatellite regions.

Study 11

LABORATORY/RESEARCHER	Department of Aquaculture, Institute of Marine Research (IMR), Bergen / G. Dahle.
SPECIES	Mackerel (Scomber scombrus).

PROJECT FUNDING	Norwegian Research Council.
OBJECTIVE	Develop and use new species specific mtDNA markers in order to study the population structure in mackerel.
DESIGN	Sample individuals from three different locations in the North East Atlantic, isolated mtDNA, and clone and sequence selected fragments.
METHODOLOGY	Isolation and sequencing of mtDNA fragments to identify possible primer sites.
STATUS	Two year project started in 1998.
COMMENTS	

Study 12

LABORATORY/RESEARCHER	Department of Aquaculture, Institute of Marine Research (IMR), Bergen / G. Dahle.
SPECIES	Different marine species.
PROJECT FUNDING	Norwegian Research Council.
OBJECTIVE	Describe two different DNA techniques for identification of seafood on a species level. Determine the error in each of the methods based on limits for identification of different species in mixed products.
DESIGN	Isolate DNA from a variety of different marine species (fish, mussels, scallops, crabs etc). Create a database based on RAPD and RFLP of selected mtDNA fragments. Use this database to determine the content of different mixed products.
METHODOLOGY	RFLP of different mtDNA amplified fragments and RAPD based on approximately 60 different primers.
STATUS	Three year project started in 1997. Several species have been characterised with 40 - 60 different primers (RAPD) and RFLP of selected PCR amplified mtDNA regions.
COMMENTS	

Study 13

LABORATORY RESEARCHER	The Norwegian College of Fishery Science, University of Tromsø / S.E. Fevolden. In collaboration with Norwegian Institute of Fisheries and Aquaculture (NIFA), Tromsø.
SPECIES	Deep water shrimp, (*Pendulous borealis*).
PROJECT FUNDING	The Norwegian Research Council.
OBJECTIVE	To study the population structure of deep water shrimp in the Barents Sea and fjords of Northern Norway.
DESIGN	Shrimps are sampled north (Spitsbergen), east and west in the Barents Sea plus in various fjords in Northern Norway.
METHODOLOGY	Allozyme variation plus RAPDs (NIFA).
STATUS	Three years project starting in 1995.
COMMENTS	One allozyme locus (*MDH*) shows highly significant allele frequency differences when Barents sea shrimps are compared to shrimps sampled in fjords in Northern Norway.

Study 14

LABORATORY/RESEARCHER	University of Tromsø, Norwegian College of Fishery Science / S.E. Fevolden.
SPECIES	Atlantic cod.
PROJECT FUNDING	Norwegian Research Council.
OBJECTIVE	To study possible genetic differentiation between the North-East Arctic cod and coastal cod in Northern Norway.
DESIGN	Samples of spawning cod and of 0-group cod from the Barents sea and from various fjords in Northern Norway are compared for DNA variation over consecutive years.
METHODOLOGY	RFLP at a single copy nuclear DNA polymorphism.
STATUS	Three years project commenced 1995.
COMMENTS	The results so far have revealed highly significant allele frequency differences between the NE Arctic cod and Norwegian coastal cod.

Study 15

LABORATORY RESEARCHER	The Norwegian College of Fishery Science, University of Tromsø / S.E. Fevolden (Norwegian partner in a joint EU-project coordinated by Institute of Freshwater Ecology, The Windermere Laboratory).

SPECIES	Rainbow trout, *Oncorhynchus mykiss.*
PROJECT FUNDING	EU.
OBJECTIVE	To establish a protocol for the selective breeding of finfish for increased tolerance to stress and to assess whether stress tolerance is at an advantage under aquaculture conditions in terms of growth, disease resistance and reproductive performance.
DESIGN	The heritability, or genetic components of stress-related traits will be determined in progenies groups from parents selected among 50 families being tested for stress tolerance. The performance of each progeny group (growth, adaptability and disease resistance)will be assessed.
METHODOLOGY	The selection scheme will be based on stress response of individuals within families. The selection traits are post-stress plasma cortisol levels and post-stress lysozyme levels.
STATUS	Four year project started in 1996.

Study 16

LABORATORY/RESEARCHER	Norwegian Institute for Nature Research (NINA) / K. Hindar.
SPECIES	Atlantic salmon.
PROJECT FUNDING	Directorate for Nature Management, Norway and NINA.
OBJECTIVE	Establish baseline information about the population genetic structure of Atlantic salmon in Norway.
DESIGN	Samples from all over Norway to analyse spatial and temporal variation in gene frequencies.
METHODOLOGY	Allozymes.
STATUS	Ten-year project to be completed 1998.

Study 17

LABORATORY/RESEARCHER	Norwegian Institute for Nature Research (NINA) / K. Hindar. In collaboration with two UK and one Irish group.
SPECIES	Atlantic salmon and brown trout.
PROJECT FUNDING	EU AIR3 94 2484.
OBJECTIVE	Quantify and understand hybridisation between Atlantic salmon and brown trout, especially in the light of an increasing tendency of escaped farmed salmon to hybridise with trout.
DESIGN	Index samples from Ireland, Scotland and Norway including undisturbed and "genetically polluted" rivers; behavioural studies of spawning; estimates of fitness components in artificially produced hybrids.
METHODOLOGY	Genetic markers (allozymes, nuclear and mitochondrial DNA); feeding history markers (natural and synthetic pigments); constructed spawning arenas; rearing and release studies.
STATUS	27 month study completed December 1996.

Study 18

LABORATORY/RESEARCHER	Norwegian Institute for Nature Research (NINA) / I. Fleming, B. Jonsson and K. Hindar.
SPECIES	Atlantic salmon.
PROJECT FUNDING	Research Council of Norway.
OBJECTIVE	Quantify reproductive success of farmed and sea ranched fish relative to wild fish.
DESIGN	Behavioural-ecological analysis of reproduction in artificial spawning arenas; release of genetically marked wild and farmed fish into a river.
METHODOLOGY	Video recording and direct observation of spawning; ecological and genetic analysis of spawners and their offspring.
STATUS	Ongoing project to be completed 1999.

Study 19

LABORATORY/RESEARCHER	Norwegian Institute for Nature Research (NINA) / K. Hindar and K. Kvaløy.
SPECIES	Atlantic salmon.

PROJECT FUNDING	Research Council of Norway.
OBJECTIVE	Analyse genetic variation in extinct and re-established populations based on microsatellite DNA isolated from dried scales.
DESIGN	Study of populations for which good scale samples exist.
METHODOLOGY	PCR able microsatellite DNA.
STATUS	Four-year project started in 1998.

Study 20

LABORATORY/RESEARCHER	University of Trondheim, Biological Station / J. Mork.
SPECIES	Atlantic salmon.
PROJECT FUNDING	Research Council of Norway..
OBJECTIVE	General, interactive PC simulation program for, e.g., prediction and analysis of genetic effects of interaction between cultured and wild populations.
DESIGN	Simultaneous handling of combined genetic effects from random genetic drift, gene flow (model-independent), and selection (additive effects) at multiple loci on a genetically pre-characterized set of populations. Any number of generations can be run.
METHODOLOGY	Theoretical population genetics, mathematical modelling, computer, Monte Carlo simulations.
STATUS	Functional version in use at several sites.

Study 21

LABORATORY/RESEARCHER	University of Trondheim, Biological Station / J. Mork.
SPECIES	Indifferent.
PROJECT FUNDING	Institutional.
OBJECTIVE	General, interactive PC simulation program for, e.g., prediction and analysis of genetic effects of interaction between cultured and wild populations.
DESIGN	Simultaneous handling of combined genetic effects from random genetic drift, gene flow (model-independent), and selection (additive effects) at multiple loci on a genetically pre-characterized set of populations. Any number of generations can be run.
METHODOLOGY	Theoretical population genetics, mathematical modelling, computer, Monte Carlo simulations.
STATUS	Functional version in use at several sites.

Study 22

LABORATORY/RESEARCHER	University of Trondheim, Biological Station / M. Giæver.
SPECIES	Blue whiting (*Micromesistius poutassou*).
PROJECT FUNDING	The Norwegian Research Council, grant NF 113606/122.
OBJECTIVE	To enlighten the genetic population structure in the blue whiting, with special emphasis on the north-eastern parts of its distribution range (the Norwegian Sea and the Barents Sea).
DESIGN	Genotyping of a large number of individuals from a tight sampling net in the relevant areas, during and outside the spawning season.
METHODOLOGY	Allozymes and minisatellites.
STATUS	Project to be end reported in 1998.
COMMENTS	Allozyme allele frequencies in a previous study indicated a separate stock in the north-east part of the blue whiting distribution area. This study has supported those results and enabled a more detailed delineation and genetic characterization of the north-eastern blue whiting.

Study 23

LABORATORY/RESEARCHER	University of Trondheim, Biological Station / J. Mork.
SPECIES	Cod (*Gadus morhua*).
PROJECT FUNDING	Institutional.
OBJECTIVE	Study of the long term stability of haemoglobin, allozyme and DNA markers allele frequencies in a local population of cod, and test for correlations between genotype and growth/survival.

DESIGN	Bi-annual sampling (research vessel) of about 200 specimens from a local cod population with no commercial exploitation.
METHODOLOGY	Collection of biological data (length, age, sex etc.), and genotyping for polymorphic haemoglobins and tissue enzymes.
STATUS	Haemoglobin analysis started in 1974 and is ongoing; allozymes from 1980 and ongoing.
COMMENTS	DNA mini- and microsatellites included from 1998 (back-tracking analyses possible as well).

Study 24

LABORATORY/RESEARCHER	Biological Station, University of Trondheim / J. Mork. In collaboration with University College, Cork, Ireland / T. Cross and P. Galvin, University of Wales, Swansea, U.K. / G. Carvalho and C. Turan and the Norwegian Institute of Fisheries and Aquaculture, Tromsø, Norway / J.E. Eliassen.
SPECIES	Cod, haddock, whiting, saithe, blue whiting, Norway pout, capelin and herring.
PROJECT FUNDING	The Norwegian Research Council and The Directorate for Nature Management.
OBJECTIVE	Baseline studies of genetic population structures in Norwegian coastal waters.
DESIGN	Collection of ~100 specimens from most Norwegian fjords from the Kola peninsula to Aalesund (totalling about 40 locations), storing tissue samples at -84 °C, and analysing them using allozymes and various others techniques when such become available. Sample collection during intensive research vessel cruises along the Norwegian coast 6-7 weeks each year 1992–1994.
METHODOLOGY	Allozymes, haemoglobins, DNA mini- and micro-satellites.
STATUS	Haddock, cod and blue whiting allozyme analyses are a jour (>3000 specimens each). DNA minisatellite analyses ongoing for whiting and (as pilot studies) some other species.
COMMENTS	The genetic studies are coordinated with biological studies on the same material by The Norwegian Institute of Fisheries and Aquaculture, Tromsø, in its Coastal Resource Program. All specimens are biologically characterised (sex, length, age etc.). Tissue samples have been made available for colleagues with interesting projects.

Study 25

LABORATORY/RESEARCHER	Biological Station, University of Trondheim / J. Mork. In collaboration with University College, Cork, Ireland / T. Cross, Marine Research Institute, Iceland / A.K. Danielsdottir, University of East England / H. Godfrey (co-ordinator) and C. Rico and MAFF, Lowestoft, UK.
SPECIES	Cod, hake, blue whiting and poor cod.
PROJECT FUNDING	EU FAIR CT95 0282 (4 years1996-2000).
OBJECTIVE	To develop and calibrate a set of molecular markers for use in detection and characterization of stocks of commercially important marine fish species in the north Atlantic.
DESIGN	Collection of ~100 specimens from each node in a macro-geographic sampling net throughout the species' distribution ranges. Thereafter a micro-geographic sampling schedule in areas of special interest. Use of traditional as well as development of new genetic markers which are tested for usability. Exploring potential general patterns and correlations between genetic structures and species-specific traits in biology.
METHODOLOGY	Allozymes, haemoglobins, DNA mini- and micro-satellites, cDNA, transcribed sequences, mtDNA.
STATUS	Haddock, cod and blue whiting allozyme analyses are a jour (>3000 specimens each). DNA minisatellite analyses are ongoing for whiting, blue whiting and cod.
COMMENTS	For all samples, individual biological records (sex, length, weight, maturity stage, age) as well as sampling information (vessel, date, gear, fishing depth) are collected.

POLAND

Study 1

LABORATORY/RESEARCHER	Sea Fisheries Institute, Gdynia / E. Wlodarczyk and R. Wenne
SPECIES	Sea trout (*Salmo trutta*).
PROJECT FUNDING	Institutional.

OBJECTIVE	To study population genetic structure of the sea trout in Poland.
DESIGN	Collection of eight samples (40 specimens each) from Polish rivers. Fin clippings are stored in ethanol.
METDOLOGY	RFLP analysis of PCR amplified mtDNA segments (ND–1 and ND-5/6).
STATUS	Ongoing.

Study 2

LABORATORY/RESEARCHER	Sea Fisheries Institute, Gdynia / A. Was and R. Wenne.
SPECIES	Sea trout (*Salmo trutta*).
PROJECT FUNDING	Institutional.
OBJECTIVE	To study population genetic structure of the sea trout in Poland.
DESIGN	Collection of eight samples (40 specimens each) from Polish rivers. Fin clippings are stored in ethanol.
METHODOLOGY	Tetranucleotide microsatellites, PCR, silver staining.
STATUS	Ongoing.

Study 3

LABORATORY/RESEARCHER	Marine Biology Center, Polish Academy of Sciences, Gdynia / M. Pempera and R. Wenne. In collaboration with School of Biological Sciences, University of Wales, Swansea, UK / D.O.F. Skibinski and S. Bell.
SPECIES	Mussel, *Mytilus trossulus,*
PROJECT FUNDING	Committee for Scientific Research 6P04C 004 11 and 6P04C 065 09.
OBJECTIVE	To characterise length heteroplasmy of mitochondrial DNA and population variation in Poland.
DESIGN	11 samples (50 specimens each) were collected and analysed.
METHODOLOGY	PCR amplification and restriction analysis of two regions of mtDNA, sequencing of the major noncoding region.
STATUS	Ongoing.

Study 4

LABORATORY/RESEARCHER	Marine Biology Center, Polish Academy of Sciences, Gdynia / B. Smietanka and R. Wenne. In collaboration with Zoological Museum, Helsinki University, Finland / R. Vainola.
SPECIES	Mussels, *Mytilus.*
PROJECT FUNDING	Institutional, Committee for Scientific Research 6P04C 004 11.
OBJECTIVE	To compare European populations.
DESIGN	Eleven samples representing populations in Europe were collected and are analysed.
METHODOLOGY	PCR amplification of mtDNA, restriction analysis.
STATUS	Ongoing.

Study 5

LABORATORY/RESEARCHER	Inland Fisheries Institute, Salmonid Research Department, Rutki / K. Goryczko and S. Dobosz. In collaboration with Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany / K. Kohlmann and Warsaw University of Agriculture / A. Zynczynski.
SPECIES	Rainbow trout.
PROJECT FUNDING	Committee for Scientific Research and institutional.
OBJECTIVE	To improve the breeding value of rainbow trout.
DESIGN	Family selection from outbred broodstock. The 100 F1 families were started in 1991. In 1994 from the 10 selected families the 100 F2 families were produced and reared during 1995. Growth and morphology were monitored.
METHODOLOGY	Each family is reared separately until the end of the first season, then the fishes are tagged (PIT tags), number of families culled is 60, fishes are reared in one pond until

	sexual maturity.
Study 6	
LABORATORY/RESEARCHER	Inland Fisheries Institute, Salmonid Research Department, Rutki / K. Goryczko, S. Dobosz and H. Kuzminski.
SPECIES	Rainbow trout.
PROJECT FUNDING	Institutional.
OBJECTIVE	To protect genetic diversity in a valuable strain maintained by stocking (Vistula sea trout gene bank).
DESIGN	Freshwater broodstock is produced from representative group of river ascending sea trout.
METHODOLOGY	Samples of 50 g of fertilised eggs from each wild female spawned were taken, incubated and reared at SRL. Random samples of 1991 and 1993 year generations presmolts were PIT tagged (1200 and 600 fish respectively). Smoltification, growth and age at first, maturity are monitored.
Study 7	
LABORATORY/RESEARCHER	Inland Fisheries Institute, Salmonid Research Department, Rutki / K. Goryczko and S. Dobosz. In collaboration with University of Agriculture and Technology, Olsztyn / M. Luczynski.
SPECIES	Whitefish.
PROJECT FUNDING	Committee for Scientific Research.
OBJECTIVE	Enhancement of endangered stock of Baltic whitefish.
DESIGN	Freshwater broodstock produced from eggs obtained during 3 consecutive years from wild spawners.
METHODOLOGY	Using the trout farming methods the stocking material (Summer fingerlings) and brood fishes were produced. The biochemical genetic studies of farmed whitefish were realised.
Study 8	
LABORATORY/RESEARCHER	Institute of Oceanography, University of Gdansk, Gdynia / K. Blicharska and M. Wolowicz. In collaboration with Observatoire Oceanologique, Universite Pierre et Marie Curie, CNRS-INSU, Villefranche sur Mer, France / C. Thiriot-Quievreux.
SPECIES	Bivalves *Mytilus trossulus, Macoma balthica, Cerastoderma glaucum* and *Mya arenaria.*
PROJECT FUNDING	Institutional.
OBJECTIVE	To characterise karyotypes of Baltic bivalve species.
DESIGN	
METHODOLOGY	Analysis of colhicin-treated mitotic chromosomes from somatic tissue, silver staining.
Study 9	
LABORATORY/RESEARCHER	Chair of Genetics and Cytology, University of Gdansk, Gdansk / A. Wysocka and T. Sywula.
SPECIES	Ostracod, *Candona neglecta.*
PROJECT FUNDING	Committee for Scientific Research and institutional.
OBJECTIVE	Genetic comparison of populations from fresh and sea waters.
DESIGN	Nine populations from lakes and the Gulf of Gdansk were sampled.
METHODOLOGY	Allozymes.
Study 10	
LABORATORY/RESEARCHER	Chair of Genetics and Cytology, University of Gdansk, Gdansk / J. Laszczuk and T. Sywula.
SPECIES	Marenzelleria viridis (Polychaeta), Palaemonetes variants and Rhithropanopeus harrisii (Decapoda), Neogobius melanostomus (Gobiidae).
PROJECT FUNDING	Institutional.

OBJECTIVE	To characterise genetic polymorphism in recently established populations in Baltic.
DESIGN	
METHODOLOGY	20-25 allozyme loci for each species were studied.

Study 11

LABORATORY/RESEARCHER	Biological Station, University of Gdansk, Gorki Wschodnie / E. Mulkiewicz and E.F. Skorkowski.
SPECIES	*Saduria enthomon* (Isopoda)
PROJECT FUNDING	Institutional.
OBJECTIVE	To characterise genetic polymorphism of LDH.
DESIGN	Expression of 3 tetrameric LDH (A, B and C) loci in different tissues was studied.
METHODOLOGY	

Study 12

LABORATORY/RESEARCHER	Institute of Maritime and Tropical Medicine, Gdynia / B. Szostakowska and P. Myjak. In collaboration with Gdansk Technical University, Dept. of Microbiology / J. Kur.
SPECIES	Anisakis, Pseudoterranova, Contracaecum.
PROJECT FUNDING	Institutional.
OBJECTIVE	To construct molecular diagnostic markers for species identification.
DESIGN	
METHODOLOGY	Allozymes, nuclear DNA, PCR, RFLP.

Study 13

LABORATORY/RESEARCHER	Chair of Biochemistry, University of Gdansk, Gdansk / M. Zmijewski, G. Klein and B. Lipinska.
SPECIES	Sea bacteria *Vibrio harveyi*.
PROJECT FUNDING	Institutional, Committee for Scientific Research.
OBJECTIVE	To characterise gene coding heat shock protein HSP - DnaK and DnaJ and to study role of its products.
DESIGN	
METHODOLOGY	Molecular cloning, Northern blotting, sequencing, transcription analysis.

Study 14

LABORATORY/RESEARCHER	Olsztyn University of Agriculture and Technology, Department of Basic Fishery Sciences / M. Luczynski and collaborators. In collaboration with Sea Fisheries Institute / M. Wyszynski.
SPECIES	Whitefish (*Coregonus lavaretus*), bream (*Abramis brama*), pikeperch (*Stizostedion lucioperca*), river lamprey (*Lampetra fluviatilis*), herring (*Clupea harrengus*) and other species.
PROJECT FUNDING	Committee for Scientific Research; Institutional.
OBJECTIVE	Baseline studies of genetic population structures in the Polish Baltic Sea coastal waters.
DESIGN	Collection of ~100 specimens from different locations, analysing them using allozymes Sample collection during the spawning season.
METHODOLOGY	Allozymes.
STATUS:	Ongoing

Study 15

LABORATORY/RESEARCHER	Olsztyn University of Agriculture and Technology, Department of Basic Fishery Sciences / M. Luczynski and collaborators. In collaboration with Sea Fisheries Institute in Gdynia / R. Bartel and Fish Farm "Aquamar" / Marczynski.
SPECIES	Salmon (*Salmo salar*).
PROJECT FUNDING	Institutional, Polish Committee for Scientific Research.
OBJECTIVE	To assess genetic polymorphism in hatchery population.
DESIGN	

METHODOLOGY	Allozymes.
STATUS	Ongoing.

PORTUGAL (1997)

Study 1

LABORATORY/RESEARCHER	UCTRA, Universidade do Algarve, Portugal; Biology Department. University of Padova; Department of Genetics. Institute of Marine Biology of Crete (coordinator).
SPECIES	Anchovy (*Engraulis encrasicolus*).
PROJECT FUNDING	DG XIV FAIR.
OBJECTIVE	**Study** of the stock structure in the Mediterranean and adjacent seas.
DESIGN	Adult and larval samples from Black, Aegean, Adriatic, Tyrrhenian, Alboran Seas and from the Atlantic coast of Portugal will be analysed with the proposed methodology. All laboratories will standardise the procedure with a central depository of samples and data in Crete.
METHODOLOGY	RFLPs, mtDNA and microsatellites.
STATUS	Started December 1996.

Study 2

LABORATORY/RESEARCHER	UCTRA, Universidade do Algarve (coordinator). School of Sciences, University of Birmingham, UK.
SPECIES	Norway lobster (*Nephrops norvegicus*).
PROJECT FUNDING	DG XIV Biological studies.
OBJECTIVE	Estimation of population sizes in Norway lobster, a new methodology.
DESIGN	Collection of specimens in 2 consecutive years. Development of a genomic library for microsatellites. Screening of samples to estimate genetic variation. Data analysis.
METHODOLOGY	Use of molecular genetic techniques to estimate genetic variation and its relationship to population breeding size. Genetic variability will be estimated as the expected proportion of heterozygotes assuming Hardy-Weinberg equilibrium. This statistic will be used as a basis for the estimation of effective population size.
STATUS	Starts March 1997.

Study 3

LABORATORY/RESEARCHER	Lab. de Citogenetica, ICBAS / Prof. I. Malheiro. University of Porto / Dr C. Thiriot. Observatoire Oceanologique de Villefarnce-sur-Mer, University P. et M. Curie, CNRS-INSU.
SPECIES	*Ostrea edulis, Crassostrea angulata, C. gigas, C. virginica* and *C. sikama.*
OBJECTIVE	Chromosome analysis to study the cytogenetic organisation of different species of oyster; detection of the response of aneuploidy and the possible transmission of this phenomenon to the next generation; relationships between the presence of aneuploidy and development.
METHODOLOGY	Karyotyping from branchial tissue, morphometric analysis of the chromosome, C, G, and NOR chromosome banding.
STATUS	Ph. D. thesis project in progress, in collaboration with France (thesis in co-tutela).
COMMENTS	This project opens the door to a special cooperation between the two countries. The thesis that is involved in this project will be simultaneously recognised in Portugal and France without an extra evaluation.

Study 4

LABORATORY/RESEARCHER	Dept. of Fisheries Technology, IPIMAR / Dr A.M. teia dos Santos. Instituto de Investigaciones Marinas (S - Head of project). Federal Research Centre of Fisheries (D). University de Santiago de Compostela (S). Rowett Research Institute (UK).
SPECIES	Sardine and squid.
PROJECT FUNDING	EU-FAIR (accepted).
OBJECTIVE	To develop DNA-based diagnostic techniques adequate to identify species of aquatic organisms (fish, shellfish and molluscs) in products of which other techniques, such as protein methods are inappropriate.

METHODOLOGY	Various techniques to isolate and distinguish DNA sequences such as RFLP, SSCP, specific probes and sequencing.
COMMENTS	This project has the aim to set up a DNA computer data base for the identification of fishery products.

Study 5

LABORATORY/RESEARCHER	Dept. of Fisheries technology, IPIMAR / Dr A.M. Teia dos Santos. INETI (IBQTA/DB/BQII).
SPECIES	Sardine (*Sardinia pilchardus*).
PROJECT FUNDING	PRAXIS XXI (submitted).
OBJECTIVE	Intra- and inter-specific genetic variability **Study** of sardine from the Portuguese continental coast.
METHODOLOGY	Various techniques to isolate and distinguish DNA sequences such as RFLP, RAPD, SSCP, microsatellite DNA fingerprint and sequencing.
COMMENTS	This project has two principal aims to know what kind of behaviour the species shows in this area in order to allow an ordered and rational management of this resource, and the maintenance of Portuguese sardine fisheries derived products quality in order to guarantee the competitiveness of these products in the internal and external markets.

SPAIN (1997)

Study 1

LABORATORY/RESEARCHER	Department of Genetics, Faculty of Science, University of Vigo / A. Sanjuan López.
SPECIES	*Cephalopod.*
PROJECT FUNDING	AMB94-0371. CICYT.
PROJECT TITLE	Genetic variation in *cephalopod* species of commercial importance by mean of mtDNA sequence and allozyme polymorphism.

Study 2

LABORATORY/RESEARCHER	Department of Genetics, Faculty of Biology, University of Granada / M. Ruiz Rejón.
SPECIES	*Sparidae.*
PROJECT FUNDING	PB92-0964. DGICYT.
PROJECT TITLE	Study of phylogenetic relationships between *Sparidae* species using ribosomal and satellite DNA analysis.

Study 3

LABORATORY/RESEARCHER	Department of Genetics, Faculty of Sciences, University of Málaga / M. C. Alvarez Herrero.
PROJECT FUNDING	BIO93–1461-CE. CICYT.
PROJECT TITLE	Identification of genes involved in early development of fish.

Study 4

LABORATORY/RESEARCHER	Instituto de Acuicultura de Torre de Sal. IARS, CSIC / S. Zanuy Doste.
PROJECT FUNDING	AGF94–1321-CE. CICYT.
PROJECT TITLE	Development of genetic DNA Markers for sex determination in farmed fish.

Study 5

LABORATORY/RESEARCHER	Department of Genetics, Faculty of Medicine, University of Oviedo. E. García Vázquez.
SPECIES	Atlantic salmon.
PROJECT FUNDING	AIR1-CT-92-0719. UE.
PROJECT TITLE	An assessment of the genetic consequences of deliberate or inadvertent introduction of non-native Atlantic salmon into natural populations.

Study 6

LABORATORY/RESEARCHER	Department of Genetics. Faculty of Medicine. University of Oviedo / E. García Vázquez.
SPECIES	Atlantic salmon and brown trout.
PROJECT FUNDING	DGICYT.
PROJECT TITLE	Contribution of precocious mature Atlantic salmon male to hybridisation with brown trout.

Study 7

LABORATORY/RESEARCHER	Department of Genetics, Faculty of Medicine, University of Oviedo / J.A. Sánchez Prado.
SPECIES	Atlantic salmon and brown trout.
PROJECT FUNDING	AQ-2.493. UE.
PROJECT TITLE	Selective breeding and genetic management through genome marking and inbred clones.

Study 8

LABORATORY/RESEARCHER	Department of Genetics, Faculty of Medicine, University of Oviedo / J.A. Sánchez Prado.
SPECIES	Atlantic salmon.
PROJECT FUNDING	PB-92-0992. DGICYT.
PROJECT TITLE	Development of molecular genetic Markers to identify natural populations of Atlantic salmon.

Study 9

LABORATORY/RESEARCHER	Department of Genetics, Faculty of Medicine, University of Oviedo / J.A. Sánchez Prado.
SPECIES	Turbot.
PROJECT FUNDING	PB-94–1348. DGICYT.
PROJECT TITLE	Use of chromosome manipulation and molecular technique s in genetic improvement of turbot.

Study 10

LABORATORY/RESEARCHER	Department of Genetics, Faculty of Medicine, University of Oviedo / J.A. Sánchez Prado.
SPECIES	Brown trout and Atlantic salmon.
PROJECT FUNDING	Institutional and regional funds of Navarra, Guipúzcoa and León Governments.
PROJECT TITLE	Genetics studies of brown trout and/or Atlantic salmon restocking programs in rivers of Navarra, Guipúzcoa and León.

Study 11

LABORATORY/RESEARCHER	Department of Genetics, Faculty of Medicine, University of Oviedo / J.A. Sánchez Prado.
SPECIES	Brown trout, rainbow trout, Atlantic salmon, Pacific salmon.
PROJECT FUNDING	ICI, Spain, FONDEF PI–10, Chile.
PROJECT TITLE	Genetic analysis of Chilean salmonid species.

Study 12

LABORATORY/RESEARCHER	Department of Genetics, Faculty of Veterinaria, University of Santiago de Compostela, Campus de Lugo / L. Sánchez Piñón.
SPECIES	Brown trout.
PROJECT FUNDING	PB-93-0648. DGICYT.

PROJECT TITLE	Chromosomal distribution of DNA tandem repeats in salmonids.

Study 13

LABORATORY/RESEARCHER	Department of Genetics, Faculty of Veterinaria, University of Santiago de Compostela, Campus de Lugo / L. Sánchez Piñón.
SPECIES	Eel..
PROJECT FUNDING	XUGA-26109B95. Xunta de Galicia.
PROJECT TITLE	Molecular analysis and chromosomal location of satellite sequences in eel species.

Study 14

LABORATORY/RESEARCHER	Department of Genetics, Faculty of Veterinaria, University of Santiago de Compostela, Campus de Lugo / P. Martínez Portela.
SPECIES	Brown trout.
PROJECT FUNDING	XUGA 26201A94. Xunta de Galicia.
PROJECT TITLE	Polymorphism of ribosomal genes of brown trout.

Study 15

LABORATORY/RESEARCHER	Department of Genetics, Faculty of Veterinaria, University of Santiago de Compostela, Campus de Lugo / P. Martínez Portela.
SPECIES	Turbot.
PROJECT FUNDING	MAR95-1855. CICYT.
PROJECT TITLE	Use of chromosomal techniques and genetic diversity analysis in the improvement of turbot.

Study 16

LABORATORY/RESEARCHER	Department of Genetics, Faculty of Veterinaria, University of Santiago de Compostela, Campus de Lugo / L. Sánchez Piñón and P. Martínez Portela.
SPECIES	Brown trout.
PROJECT FUNDING	SC95/005. INIA.
PROJECT TITLE	Ecological and genetic variation in brown trout

SWEDEN

Study 1

LABORATORY/RESEARCHER	Salmon Research Institute / H. Jansson.
SPECIES	Atlantic salmon.
PROJECT FUNDING	National funds.
OBJECTIVE	National survey of genetic variation in Atlantic salmon.
DESIGN	Gene frequencies are used to describe spatial and temporal genetic diversity among salmon populations.
METHODOLOGY	Allozymes and DNA.
STATUS	Long term study.

Study 2

LABORATORY/RESEARCHER	SLU- Dept of Aquaculture / Jan Nilsson.
SPECIES	Arctic charr.
PROJECT FUNDING	EC, Swedish Council for Forestry and Agricultural Research.
OBJECTIVE	Develop sustainable aquaculture of Arctic charr, develop breeding plan for Arctic char in European aquaculture.
DESIGN	Genotype- environment interactions are studied using family structured breeding populations replicated and reared in different fish-farms. Importance of variation in genes with potential effects on economically important traits are studied in domesticated strains as an attempt to obtain useful genetic markers for breeding.

METHODOLOGY	Quantitative and molecular genetics.
STATUS	Second year.

Study 3

LABORATORY/RESEARCHER	Institute of Freshwater Research, Fisheries Board of Sweden / L.Edsman and B. Ekstrand.
SPECIES	Noble crayfish (*Astacus astacus*), Signal crayfish (*Pacifastacus leniusculus*).
PROJECT FUNDING	Carl Tryggers Foundation.
OBJECTIVE	Mapping genetic variation in the native noble crayfish-genotypical diversity for biologically relevant crayfish management and policy.
DESIGN	Samples of noble crayfish populations from different geografical regions in Sweden are collected and tested for genetic differences in the laboratory.
METHODOLOGY	PCR, RFLP in mtDNA, microsatellites.
STATUS	Just started, first report discribing methodology for sampling and for getting a PCR product ready.

Study 4

LABORATORY/RESEARCHER	National Board of Fisheries, Institute of Freshwater Research, Laboratory of Fish Genetics / T. Järvi (Prof.), B. Ekstrand (Res. ass.), L. Laikre (Scientist 20 %).
SPECIES	Atlantic salmon, Brown trout.
PROJECT FUNDING	N. Bd. Fish., Swedish Council for Forestry and Agricultural Research, EC.
OBJECTIVE	Reveal any ecological effect of releasing domesticated salmon and trout on wild conspecifics. The study include quantitative genetics (QTL) and paternity studies based on micro satellites.
DESIGN	
METHODOLOGY	RFLP/mtDNA Microsatellites.
STATUS	Just started.

Study 5

LABORATORY/RESEARCHER	Salmon Research Institute / H. Jansson.
SPECIES	Atlantic salmon and brown trout.
PROJECT FUNDING	National funds.
OBJECTIVE	Genetic moitoring of hatchery stocks.
DESIGN	Hatchery stocks are monitored at regular intervals in order to prevent reduction of genetic variability.
METHODOLOGY	Allozymes and DNA.
STATUS	Long term study.

Study 6

LABORATORY/RESEARCHER	Salmon Research Institute / H. Jansson. Department of Genetics, Uppsala University / K. Fredga and H. Tegelström.
SPECIES	Atlantic salmon x brown trout hybrids.
PROJECT FUNDING	Swedish Council for Forestry and Agricultural Research.
OBJECTIVE	To study 1) the incidence and direction of hybridisation between Atlantic salmon and brown trout in Sweden, 2) factors that promote hybridisation, and 3) genotypes, survival and fertility of progeny from hybrids backcrossed to parental species.
DESIGN	Hybrid frequencies are assessed annually in different habitats. The maternal species of the hybrids is determined. Experiments with different types and numbers of spawners are performed in controlled environment. First generation hybrids and backcross individuals are used in crossing experiments. Parts of the project are performed in cooperation with T. Järvi, E. Petersson and B. Ragnarsson / National Board of Fisheries.
METHODOLOGY	Allozymes and mitochondrial DNA.
STATUS	Three year study 1997–1999.

Study 7

LABORATORY/RESEARCHER	Department of Zoology, Uppsala University / J. Dannewitz. Institute of Freshwater Research, National Board of Fisheries / E. Petersson.
SPECIES	Atlantic salmon (*Salmo salar*).
PROJECT FUNDING	Institutional funding and private funding.
OBJECTIVE	To investigate the influence of egg size on future growth, survival and life history adoption in Atlantic salmon. To test new methods for releasing hatchery produced Atlantic salmon.
DESIGN	The experiments will be conducted in natural and semi-natural streams.
METHODOLOGY	Microsatellites will be used as markers in the parentage-studies.
STATUS	One field experiment has been initiated. The laboratory work will start in autumn 1998.

Study 8

LABORATORY/RESEARCHER	Division of Population Genetics, Stockholm University / N.Ryman.
SPECIES	Brown trout (model organism).
PROJECT FUNDING	Swedish Natural Science Research Council (NFR).
OBJECTIVE	Long-term genetic/ecological study of natural brown trout populations in a protected area in northern Sweden. The aim is to illuminate how natural populations function genetically. Several issues have been addressed using the data collected so far, and the results will be of practical significance for fish conservation in general. For instance, theory developed at the Division for estimating effective population size when generations are overlapping has been applied to empirical data accumulated within the project.
DESIGN	The same natural and introduced populations are sampled annually. Data on age, sex, length, weight are collected for every individual. Tissue samples (muscle, liver, eye) is collected for every individual.
METHODOLOGY	Allozyme and, partly, mtDNA analyses. Theory development, statistical analyses.
STATUS	Ongoing, long term study.

Study 9

LABORATORY/RESEARCHER	Division of Population Genetics, Stockholm University / N.Ryman and L. Laikre
SPECIES	Brown trout (model organism).
PROJECT FUNDING	Foundation for Strategic Environmental Research (MISTRA).
OBJECTIVE	The release of hatchery fish into the wild (stocking) is practised extensively within the field of fishery management. Stocking may result in a series of genetic interactions between the hatchery-bred fish and natural populations. The genetic integrity of wild populations is threatened not only when releasing fish with an exotic genetic background - loss of genetic variation may occur also when the released fish originate from, or belong to, the recipient population (so-called supportive breeding). Nevertheless, the genetic effects of breeding-release activities on the genetic composition of natural populations are poorly understood. The aim of the project is to produce information that makes it possible to reduce or eliminate the harmful effects on biodiversity on the gene level that are potentially inherent to stocking activities. Anadromous brown trout populations from the Baltic Sea (Gotland) will be used as a model system.
DESIGN	Theory development and computer simulations. Biochemical analyses of brown trout tissues samples collected from populations at Gotland.
METHODOLOGY	Theory development, primarily allozyme analysis.
STATUS	Ongoing.

Study 10

LABORATORY/RESEARCHER	Division of Population Genetics, Stockholm University / N.Ryman.
SPECIES	Brown trout (model organism).
PROJECT FUNDING	Swedish Natural Science Research Council (NFR).
OBJECTIVE	Studies of molecular genetic markers have added relatively little to the understanding of the genetic basis for variation in phenotypic traits. Here the existence of genetically determined phenotypic differences between populations of brown trout that are divergent at electrophoretically detectable protein loci is investigated.

DESIGN	Genetically tagged individuals from two stocks exhibiting behavioral and ecological differences have been introduced into a drainage system previously void of brown trout. In the common environment the presence of phenotypic differences among different groups of offspring is expected to reflect genetically determined dissimilarities between the original stocks.
METHODOLOGY	Allozyme analyses, statistical evaluation of genetic and morphological/ecological data.
STATUS	Ongoing, long term study.

Study 11

LABORATORY/RESEARCHER	Division of Population Genetics, Stockholm University / N.Ryman.
SPECIES	Brown trout (model organism).
PROJECT FUNDING	Swedish Natural Science Research Council (NFR).
OBJECTIVE	Release of genetically modified organisms poses a potential threat to wild populations. Important information on the spread of genes can be obtained through the study of gene introgression via organisms which are not genetically altered. By not using "real" transgenic organisms risks are avoided and costs minimized.
DESIGN	Two genetically different stocks of brown trout have been translocated into a natural lake system. The introgression of genes from these stocks to naturally occurring brown trout populations is studied.
METHODOLOGY	Allozyme analyses, computer simulations, statistical evaluations.
STATUS	Ongoing, long term study.

Study 12

LABORATORY/RESEARCHER	Division of Population Genetics, Stockholm University / L. Laikre.
SPECIES	Brown trout (model organism).
PROJECT FUNDING	County administrative board of Värmland (Länsstyrelsen, Värmland).
OBJECTIVE	To address the problems of monitoring biological diversity at the gene level using natural brown trout populations in the Province of Värmland.
DESIGN	Biochemical analyses of tissue samples collected from selected brown trout populations.
METHODOLOGY	Primarily allozyme analysis, statistical evaluations, computer simulations.
STATUS	Ongoing.

Study 13

LABORATORY/RESEARCHER	Division of Population Genetics, Stockholm University / L. Laikre.
SPECIES	Brown trout (model organism).
PROJECT FUNDING	Erik Philip-Sörensens Foundation.
OBJECTIVE	Information regarding the temporal dynamics of alleles at genetic marker loci in natural populations is exceedingly sparse. Typically, population genetic investigations include sampling at one particular occasion only. This fairly limited knowledge of the extent of temporal variation of DNA markers influences the interpretation of observed spatial patterns; it is largely unclear if they are stable over time. In this project temporal shifts of mtDNA haplotypes in natural brown trout populations in the Province of Jämtland is studied. The amount of genetic drift over several consecutive cohorts (year classes) is quantified and provides the basis for estimating female effective size in these populations. The extent of mtDNA haplotype frequency change is compared with the corresponding allele frequency changes at allozyme loci for the same populations and cohorts.
DESIGN	Tissue sample collections from natural brown trout populations over several years. Biochemical analyses of the samples followed by data analyses involving application of theoretical developments provided by other projects at the Division.
METHODOLOGY	Primarily mtDNA analyses using PCR and restriction enzyme analysis, statistical evaluations, computer simulations.
STATUS	Ongoing.

UNITED KINGDOM, NORTHERN IRELAND

Study 1

LABORATORY/RESEARCHER	School of Biology and Biochemistry, The Queen's University of Belfast / O. McMeel, L. Hoey and A. Ferguson.
SPECIES	Brown trout (*Salmo trutta*).
PROJECT FUNDING	Natural Environment Research Council.
OBJECTIVE	To obtain cDNA nucleotide sequences of the *LDH-C1* 100* and **90* alleles for brown trout and, based on the sequence difference, to develop a method for genotyping *LDH-C1** after PCR amplification of genomic DNA from tissue biopsies and archive material.
DESIGN	Produce cDNA by reverse transcriptase PCR of RNA isolated from retinal tissue and sequence.
METHODOLOGY	PCR, automated sequencing.
STATUS	Completed March 1998.

Study 2

LABORATORY/RESEARCHER	School of Biology and Biochemistry, The Queen's University of Belfast / A. Duguid, P.Prodöhl and A. Ferguson.
SPECIES	Brown trout (*Salmo trutta*).
PROJECT FUNDING	Dept of Education for NI until September 1998; Fisheries Society of the British Isles Studentship 1998 - 2001.
OBJECTIVE	To determine the extent of population structuring within and among brown trout in large freshwater lakes in Scotland.
DESIGN	Population sampling of major lake systems. Population genetic analysis.
METHODOLOGY	Allozymes, mtDNA RFLPs, microsatellites, specific nuclear gene RFLPs and sequencing.
STATUS	Started October 1997.

Study 3

LABORATORY/RESEARCHER	School of Biology and Biochemistry, The Queen's University of Belfast / R. Hynes, P. Prodöhl and A. Ferguson.
SPECIES	Brown trout (*Salmo trutta*).
PROJECT FUNDING	Internal University funds.
OBJECTIVE	To determine the extent of population structuring and postglacial colonisation patterns for brown trout in Britain and Ireland.
DESIGN	Sampling of unstocked freshwater and anadromous populations. Population genetic analysis.
METHODOLOGY	MtDNA RFLPs and sequencing; transferrin and LDH sequencing.
STATUS	Ongoing.
COMMENTS	

Study 4

LABORATORY/RESEARCHER	School of Biology and Biochemistry, The Queen's University of Belfast / P. Prodöhl, R. Hynes and A. Ferguson. In collaboration with the Salmon Research Agency of Ireland / P. McGinnity.
SPECIES	Atlantic salmon (*Salmo salar*).
PROJECT FUNDING	Internal funding.
OBJECTIVE	To determine the survival at sea and homing abilities of Atlantic salmon of native, farmed and hybrid parentage.
DESIGN	Four groups were reared in common environment in hatchery and released to sea at smolt stage. Adults sampled in coastal drift nets and on return to freshwater in fixed traps. Parentage is being determined by DNA profiling.
METHODOLOGY	Standard fisheries measurements, microsatellite and minisatellite DNA profiling.
STATUS	Due for completion June 1998.
COMMENTS	Follow on from juvenile freshwater project which was funded by EU-FAIR.

Study 5

LABORATORY/RESEARCHER	School of Biology and Biochemistry, The Queen's University of Belfast / P. Prodöhl and A. Ferguson. In collaboration with Salmon Research Agency of Ireland / P. McGinnity. Stofnfiskur Ltd., Iceland / J. Jonasson. National University of Ireland Cork / T. Cross.
SPECIES	Atlantic salmon (*Salmo salar*).
PROJECT FUNDING	Internal funds used to support initial work.
OBJECTIVE	To determine the genetic impact of hybridisation between wild and farmed Atlantic salmon on native populations through an assessment of the performance of second generation hybrids and backcrosses.
DESIGN	The freshwater and marine performance of F2 hybrids and backcrosses of F1 hybrids to both wild and farmed stocks is being assessed.
METHODOLOGY	Standard fisheries measurements, microsatellite DNA profiling.
STATUS	Ongoing.
COMMENTS	EU FAIR funding applied for.

Study 6

LABORATORY/RESEARCHER	School of Biology and Biochemistry, The Queen's University of Belfast / P. Prodöhl and A. Ferguson. In collaboration with IMR, Bergen, Norway / K. Jorstad. Shellfish Research Laboratory, Galway, Ireland / J. Mercer. Aristotle University of Thessaloniki, Greece / C. Triantaphyllidis.
SPECIES	European lobster (*Homarus gammarus*).
PROJECT FUNDING	Internal funding; Norwegian Research Council.
OBJECTIVE	To develop microsatellite and mitochondrial DNA markers and to optimise screening conditions to enable high-resolution studies of European lobster genetics. To determine the contribution of ranched individuals in mixed wild and ranched harvests and the potential genetic impact of stock management and enhancement on natural populations. To elucidate the breeding structure in various European lobster populations.
DESIGN	Population samples are being obtained from throughout the native range. Eggs from "berried" females are being examined for parentage. Genetic impact of ranching is being assessed using genetic tags.
METHODOLOGY	Standard fishery measurements, microsatellites, mtDNA RFLPs.
STATUS	Ongoing.
COMMENTS	EU-FAIR funding applied for.

Study 7

LABORATORY/RESEARCHER	School of Biology and Biochemistry, The Queen's University of Belfast / P. Prodöhl and A. Ferguson. In collaboration with Danube Delta Institute, Romania / R. Suciu.
SPECIES	Sturgeons (*Acipenser, Huso*).
PROJECT FUNDING	Royal Society.
OBJECTIVE	To determine the genetic population structure of endangered sturgeon species of the Lower Danube.
DESIGN	Biopsy tissue samples will be obtained from ascending individuls in the lower part of the river. Individuals will be given ultrasonic tags to determine final spawning locations.
METHODOLOGY	Microsatellites, mtDNA RFLPs.
STATUS	Ongoing.

RECENT NORTHERN IRELAND PUBLICATIONS:

Prodöhl, P.A., Walker, A., Hynes, R., Taggart, J.B. and Ferguson, A. (1997). Genetically monomorphic brown trout (Salmo trutta L.) populations, as revealed by mitochondrial DNA, multilocus and single locus minisatellite (VNTR) analyses. *Heredity* **79**: 208-213.

Stone, C.E., Taggart, J.B. and Ferguson, A. (1997). Single locus minisatellite DNA variation in European populations of Atlantic salmon (*Salmo salar* L.). *Hereditas* **126.** 269-275.

McGinnity, P., Stone, C., Taggart, J.B., Cooke, D., Cotter, D., Hynes, R., McCamley, C., Cross, T. and Ferguson, A. (1997). Genetic impact of escaped farm Atlantic salmon (*Salmo salar* L.) on native populations: use of DNA profiling to assess freshwater performance of wild, farm and hybrid progeny in a natural river environment. *ICES Journal of Marine Science.* **54**: 998–1008.

Clifford, S.L., McGinnity, P. and Ferguson, A. (1998). Genetic changes in an Atlantic salmon (*Salmo salar* L.) population resulting from escaped juvenile farm salmon. *J. Fish Biol.* **52:** 118–127.

Clifford, S.L., McGinnity, P. and Ferguson, A. Genetic changes in Atlantic salmon (*Salmo salar* L.) populations of NW Irish rivers resulting from escapes of adult farm salmon. *Can. J. Fish. Aquat. Sci.* (in press)

Thompson, C., Poole, R., Matthews, M. and Ferguson, A. Genetic assessment, using minisatellite DNA profiling, of secondary male contribution in the fertilisation of wild and ranched Atlantic salmon (*Salmo salar* L.) ova. *Can. J. Fish. Aquat. Sci.* (in press).

UNITED KINGDOM, SCOTLAND

Study 1

LABORATORY/RESEARCHER	Molecular Genetics Section, Fish Cultivation Team, FRS Marine Laboratory, Aberdeen / E. Verspoor (project leader). In collaboration with Department of Zoology, University of Aberdeen and Scottish Agricultural College, Edinburgh.
SPECIES	*Salmo salar.*
PROJECT FUNDING	Scottish Office, NERC, Scottish Salmon Growers Association.
OBJECTIVE	To develop the scientific basis for the application of molecular markers to the selective breeding of Atlantic salmon.
DESIGN	Research into three areas of molecular marker development and application pedigree analysis, assessemnt of genetic diversity, and assessment of breeding merit using QTLs.
METHODOLOGY	Microsatellites; allozymes, mtDNA and minisatellites.
STATUS	Ongoing.

Study 2

LABORATORY/RESEARCHER	Molecular Genetics Section, Fish Cultivation Team, FRS Marine Laboratory, Aberdeen / E. Verspoor. In collaboration with Department of Cell and Molecular Biology, University of Aberdeen / P.J. Wright and N. Haites.
SPECIES	*Ammodytes marinus; Melanogrammus aeglefinus*
PROJECT FUNDING	Scottish Office.
OBJECTIVE	To identify optimal molecular markers for marine fish population structure studies.
DESIGN	To identify variation in the coding and non-coding regions of the DNA of growth hormone and tranferrin genes and compare their utility in resolving population subdivisions.
METHODOLOGY	Minisatellites, cDNA libraries, DNA sequencing.
STATUS	Ongoing.

Study 3

LABORATORY/RESEARCHER	Molecular Genetics Section, Fish Cultivation Team, FRS Marine Laboratory, Aberdeen / E. Verspoor. In collaboration with the Centro Ictiológico de Arredondo, Spain / C. Garcia de Leaniz.
SPECIES	*Salmo salar.*
PROJECT FUNDING	EU, Scottish Office, British Council.
OBJECTIVE	To gain insight into the genetic consequences of deliberate or inadvertent transfers of salmon from one river to another.
DESIGN	Transplantation and monitoring of genetically markered groups of fish using common garden experiments.
METHODOLOGY	MtDNA, allozymes, minisatellites.
STATUS	Ongoing.

Study 4

LABORATORY/RESEARCHER	Molecular Genetics Section, Fish Cultivation Team, FRS Marine Laboratory, Aberdeen / E. Verspoor. In collaboration with others.
SPECIES	*Salmo salar.*
PROJECT FUNDING	Scottish Office, Atlantic Salmon Trust, INTAS (pending).
OBJECTIVE	To investigate into the phylogenetics and phylogeography of Atlantic salmon across the species range.
DESIGN	Collation of published and unpublished genetic data; selected sampling of new locations; synthetic analysis of data.
METHODOLOGY	Microsatellites, mtDNA, allozymes.
STATUS	Ongoing.

Study 5

LABORATORY/RESEARCHER	Molecular Genetics Section, Fish Cultivation Team, FRS Marine Laboratory, Aberdeen / C. Cunningham (project leader).
SPECIES	*Gyrodactylus salaris.*
PROJECT FUNDING	EU, Scottish Office.
OBJECTIVE	To resolve taxanomic groups at the specific and intraspecific level to facilitate pathogen detection.
DESIGN	Analysis of DNA sequence variation among parasites associated with different hosts and the same hosts in different geographical regions focusing on ribosomal and mitochondrial DNA.
METHODOLOGY	DNA sequencing, RFLP analysis.
STATUS	Ongoing.

Study 6

LABORATORY/RESEARCHER	Molecular Genetics Section, Fish Cultivation Team, FRS Marine Laboratory, Aberdeen / C. Cunningham.
SPECIES	Various.
PROJECT FUNDING	Scottish Office.
OBJECTIVE	To develop novel, rapid, sensitive methods for the detection of pathogens in fish tissues.
DESIGN	Sequencing of pathogen DNA and development of species specific PCR detection method.
METHODOLOGY	DNA sequencing, RFLP analysis.
STATUS	Ongoing.

Study 7

LABORATORY/RESEARCHER	Molecular Genetics Section, Fish Cultivation Team, FRS Marine Laboratory, Aberdeen / M. Snow and C. Cunningham.
SPECIES	*Rhabdovirus.*
PROJECT FUNDING	Scottish Office, EU.
OBJECTIVE	To determine if different species specific or geographically distinct straints of Rhabdoviruses exist in relation to VHS outbreaks in marine species.
DESIGN	Culture of Rhabdoviruses from different species and locations and sequencing of genes.
METHODOLOGY	DNA sequencing.
STATUS	Ongoing.

Study 8

LABORATORY/RESEARCHER	Gatty Marine Laboratory, University of St. Andrews, St. Andrews / I. Johnstone. Molecular Genetics Section, Fish Cultivation Team, FRS Marine Laboratory, Aberdeen / A. McLay.
SPECIES	*Salmo salar, Salmo trutta.*
PROJECT FUNDING	NERC,Scottish Office, British Council.
OBJECTIVE	To determine the effect of incubation temperature on early growth and muscle

	development.
DESIGN	Comparison of parameters among genetically tagged families and populations reared under controled hatchery conditions and under ambient conditions in the wild.
METHODOLOGY	Microsatellites.
STATUS	Ongoing.

Study 9

LABORATORY/RESEARCHER	FRS Marine Laboratory, Aberdeen, Scotland / A. McLay.
SPECIES	*Salmo salar.*
PROJECT FUNDING	Scottish Office.
OBJECTIVE	To assess family and population variation in maturation timing.
DESIGN	Comparison of two synchronously spawned, genetically tagged stocks of salmon in a common controlled rearing environment.
METHODOLOGY	Minisatellites; hormone assays.
STATUS	Ongoing.

Study 10

LABORATORY/RESEARCHER	Gatty Marine Laboratory, School of Environmental and Evolutionary Biology, University of St Andrews, St Andrews / C.D. Todd, K. Wolff and M.G. Ritchie.
SPECIES	*Lepeophtheirus salmonis* (*Salmo trutta, S. salar*).
PROJECT FUNDING	NERC, U.K. (1997-2000).
OBJECTIVE	(1) Development of molecular markers for population analyses of sea lice infesting wild and farmed salmonids around the Scottish coasts. (2) Quantification of interactions between wild and farmed stocks in terms of infestation dynamics.
DESIGN	Confirmation of marker heritability in laboratory cultures. Screening of parasites from wild and farmed stocks. Time-series analyses of specified populations.
METHODOLOGY	DNA sequencing, RAPD, SCAR, microsatellites.
STATUS	Ongoing.

Study 11

LABORATORY/RESEARCHER	Behaviour, Speciation and Genetics Research Group, Bute Building, University of St Andrews etc. / M. G. Ritchie, J. Graves and A. E. Magurran
SPECIES	Various Mexican Goodeid species.
PROJECT FUNDING	NERC, UK.
OBJECTIVE	(1) To determine levels of genetic diifferentiation among lineages of goodeid which differ in the extent of female-controlled sexual selection (2) To determine the genetic heterogeneity of captive populations of endangered species.
DESIGN	Collection of samples from the wild and captive populations. Deveopment of microsatellite DNA markers and DNA sequencing for genetic analysis.
METHODOLOGY	DNA sequencing, microsatellites, behavioural analysis.
STATUS	Ongoing.

Study 12

LABORATORY/RESEARCHER	Fish Muscle Research Laboratory, Gatty Marine Laboratory, School of Enivronmental and Evolutionary Biology, University of St. Andrews, St. Andrews / I.A. Johnston. Part of larger project involving Marine Laboratory, Scottish Office Agriculture and Fisheries Laboratory, Aberdeen; Department of Zoology, University College, Galway, Ireland; Matre Aquaculture Research Station, Havforskingsinstitutett, Norway.
SPECIES	*Salmo salar.*
PROJECT FUNDING	ECU.
OBJECTIVE	Minimising the interaction of cultured and wild fish a comprehensive evaluation of the use of sterile, triploid, Atlantic salmon.
DESIGN	Sampling of fish to assess muscle growth throughout development.
METHODOLOGY	Histology, microscopy and image analysis.
STATUS	Ongoing.

Study 13

LABORATORY/RESEARCHER	Department of Zoology, University of Aberdeen, Aberdeen. In collaboration with others / P. Boyle and E. Greatorex
SPECIES	*Loligo forbesi* plus others.
PROJECT FUNDING	EU.
OBJECTIVE	To identify molecular markers which can resolve population structure.
DESIGN	development of microsatellite loci; screening of wild samples from different geographical areas.
METHODOLOGY	Microsatellite cloning, DNA sequencing, PCR primer development.
STATUS	Ongoing.

Study 14

LABORATORY/RESEARCHER	Department of Zoology, University of Aberdeen and FRS Marine Laboratory, Aberdeen / N. Bailey, P. Boyle and L. Noble.
SPECIES	*Nephrops norvegicus.*
PROJECT FUNDING	Scottish Office, Aberdeen University.
OBJECTIVE	To identify molecular markers which can resolve population structure.
DESIGN	development of microsatellite loci; screening of wild samples from different geographical areas.
METHODOLOGY	Microsatellite cloning, DNA sequencing, PCR primer development, mtDNA RFLPs.
STATUS	Ongoing.

Study 15

LABORATORY/RESEARCHER	FRS Freshwater Fisheries Laboratory, Pitlochry / A. Youngson, J. Taggart and others.
SPECIES	*Salmo salar.*
PROJECT FUNDING	Scottish Office, MAFF.
OBJECTIVE	To assess within population variation in spawning success, offspring survival and distribution in fish ascending the Girnock Burn, Scotland.
DESIGN	Biopsy of mature fish passing through the Girnock trap, sampling of spawning redds above the trap, electrofishing of post-hatch juveniles.
METHODOLOGY	Minisatellite DNA fingerprinting.
STATUS	Ongoing.

Study 16

LABORATORY/RESEARCHER	Atlantic Salmon Trust, Pitlochry, Scotland; FRS Freshwater Fisheries Laboratory, Pitlochry/ J. Webb, A. Youngson, J. Taggart
SPECIES	*Salmo salar*
PROJECT FUNDING	Atlantic Salmon Trust, Scottish Office
OBJECTIVE	to study competition among families in relation to parentage, redd location, fry densities and patterns of dispersal and survival in the Baddoch burn, Scotland
DESIGN	planting out of families at the eyed ova stage into the burn in artificial incubatiors followed by sampling of fry and juveniles by electrofishing and in a downstream trap; monitoring of returning adults.
METHODOLOGY	Minisatellite DNA fingerprinting
STATUS	Ongoing.

Study 17

LABORATORY/RESEARCHER	School of Environmental and Evolutionary Biology; Behaviour, Speciation and Genetics Group, University of St Andrews, St Andrews / A. Magurran, J. Graves and J. Evans.
SPECIES	*Poecilia reticulata.*
FUNDING	PhD studentship University of St Andrews.
OBJECTIVE	The development of microsatellites for the analysis of paternity and for population structure.

DESIGN	Use enriched technique to isolate microsatellite sequences, design primers and test for polymorphism.
STATUS	Ongoing.

Study 18

LABORATORY/RESEARCHER	Genetics and Reproduction Research Group, Institute of Aquaculture University of Stirling, Stirling / B.McAndrew;Adams.
SPECIES	*Salmo salar.*
PROJECT FUNDING	Commercial/NERC.
OBJECTIVE	Inheritance of disease resistance.
DESIGN	200 families will be monitored under commercial conditions and resistant and susceptible individuals will be identified to family level. Full sib families will be challenged and results correlated with commercial results.
METHODOLOGY	Parentage analysis using microsatellites and controlled disease challenges.
STATUS	Ongoing.

Study 19

LABORATORY/RESEARCHER	Genetics and Reproduction Research Group, Institute of Aquaculture University of Stirling, Stirling / B. McAndrew and N. Bromage.
SPECIES	Atlantic halibut.
PROJECT FUNDING	Private/BBSRC.
OBJECTIVE	To describe differences in growth and other features in male and female halibut. Identify sex-determination mechanism and develop microsatellite markers for broodstock management.
DESIGN	Compare growth and performance under controlled conditions of farm produced halibut fry.
METHODOLOGY	Chromosome set manipulation, microsatellites.
STATUS	Ongoing.

Study 20

LABORATORY/RESEARCHER	Genetics and Reproduction Research Group, Institute of Aquaculture University of Stirling, Stirling / N. Bromage.
SPECIES	Tilapia / rainbow trout.
PROJECT FUNDING	EC Training and Mobility Grant.
OBJECTIVE	To follow chromosome pairing during meiosis in triploid fish to identify possible sites of sex specific markers.
DESIGN	Follow gonadal maturation in experimentally derived populations during multiple (tilapia) and single (rainbow trout) gonadal maturation cycles.
METHODOLOGY	Supranemal chromosome complex and dna probing.
STATUS	Ongoing.

Study 21

LABORATORY/RESEARCHER	Genetics and Reproduction Research Group, Institute of Aquaculture University of Stirling, Stirling / D. Penman and B. McAndrew.
SPECIES	*Puntius gonionotus.*
PROJECT FUNDING	DFID Fish Genetics Programme.
OBJECTIVE	Development of monosex culture in Puntius species.
DESIGN	Investigation of the sex determination systems of Puntius species; production and evaluation of monosex female *P. gonionotus*.
METHODOLOGY	Chromosome set manipulation, DNA fingerprinting.
STATUS	Ongoing.

Study 22

LABORATORY/RESEARCHER	Genetics and Reproduction Research Group, Institute of Aquaculture University of

	Stirling, Stirling / D. Penman.
SPECIES	Common and major carp species.
PROJECT FUNDING	DFID Fish Genetics Programme.
OBJECTIVE	Genetic improvement of Indian and common carp for aquaculture.
DESIGN	Investigation of the present status of Catla catla in Karnataka state and development of a genetic improvement programme. Investigation of early sexual maturation and unwanted reproduction of common carp in Karnataka state and development of solutions.
METHODOLOGY	MtDNA, allozymes, microsatellites, chromosome set manipulation.
STATUS	Ongoing.

UNITED KINGDOM (1997)

Study 1

LABORATORY/RESEARCHER	School of Ocean Sciences, University of Wales, Bangor / A. Beaumont and M.D.R. Portilla.
SPECIES	*Mytilus edulis.*
PROJECT FUNDING	CONACYT (Mexico) and UWB (PhD programme).
OBJECTIVE	To investigate the potential genetic effects of the artificial selection of fast growing larvae in hatchery culture.
DESIGN	Series of laboratory matings (mass matings and single family crosses) with subsequent selection for fast and slow growing larvae and eventual allozyme electrophoresis of juveniles.
METHODOLOGY	Larval rearing, allozyme electrophoresis and some DNA analysis.
STATUS	Ongoing - preparation of papers.

Study 2

LABORATORY/RESEARCHER	School of Ocean Sciences, University of Wales, Bangor / A. Altun, A. Beaumont and J. Latchford.
SPECIES	*Mytilus edulis* and *Crassostrea gigas.*
PROJECT FUNDING	Mustafa Kemal University, Turkey and UWB (PhD programme).
OBJECTIVE	To develop gene transfer technologies suitable for bivalves.
DESIGN	Development of suitable insert DNA and its transfection by electroporation of eggs.
METHODOLOGY	Electroporation, cloning, genomic DNA library.
STATUS	Ongoing.

Study 3

LABORATORY/RESEARCHER	School of Ocean Sciences, University of Wales, Bangor / F. Carissan and A. Beaumont. Plymouth Marine Laboratory / R. Pipe. ZENECA Laboratories, Brixham / T. Hutchinson.
SPECIES	*Mytilus edulis* and *Hediste* (Nereis) diversicolor.
PROJECT FUNDING	Welcome Trust Ecotoxicology Studentship (PhD programme).
OBJECTIVE	To investigate genetic variability in relation to immunocompetence.
DESIGN	Individuals characterised on the basis of their immunocompetence and correlated to allozyme genotype at enzyme loci.
METHODOLOGY	Immunocompetence measured on the basis of variation in numbers of different blood cell types and their phagocyte capacity in the face of challenge. Allozyme electrophoresis at enzyme loci.
STATUS	Ongoing.

Study 4

LABORATORY/RESEARCHER	School of Ocean Sciences, University of Wales, Bangor / K. Abey, A. Beaumont and J. Latchford.
SPECIES	*Cerastoderm edule*, the cockle.
PROJECT FUNDING	NERC and UWB (PhD studentship).
OBJECTIVE	To investigate population genetic variation over species range.

DESIGN	Develop microsatellite Markers and test on samples from various populations.
METHODOLOGY	Create DNA library, search for and sequence suitable microsatellite Markers. Develop primers and use with PCR to investigate population genetic variation.
STATUS	Ongoing.

Study 5

LABORATORY/RESEARCHER	Molecular Ecology and Fisheries Genetics Laboratory, Department of Biological Sciences, University of Hull, HULL, HU6 7RX, / G.R. Carvalho and W.H. Hutchinson) and CEFAS. Fisheries Laboratory, Lowestoft / S. Rogers.
SPECIES	Cod, herring and plaice.
PROJECT FUNDING	Hull University Scholarship and in-house funding.
OBJECTIVE	To determine whether changes in the intensity and nature of exploitation have influenced genetic population structure in North Sea exploited fishes
DESIGN	Examine genetic structure in past and present-day populations using archived otoliths and fish scales
METHODOLOGY	Techniques will be developed to extract DNA from archived material (otoliths and scales) for microsatellite and mitochondrial DNA analysis from north sea fishes collected over the past 30-40 years. Data will examine changes in levels and distribution of genetic diversity, as well as investigation of relationships between documented shifts in phenotypic characters (e.g., reduction in size and age at maturity) and genotypic structure.
STATUS	Started in October 1997, and will continue for 3 years. At early stage of methodological development and sample collection.
COMMENTS	The study will form the basis for a Ph.D. thesis and part of an Ongoing programme of studies designed to assess the impact of selective fishing on levels of population biodiversity.

Study 6

LABORATORY/RESEARCHER	Molecular Ecology and Fisheries Genetics Laboratory, Department of Biological Sciences, University of Hull, HULL, HU6 7RX, / G.R. Carvalho and G. Adcock). British Antarctic Survey, Cambridge / P.G. Rodhouse.
SPECIES	Squid (*Illex argentinus*).
PROJECT FUNDING	Natural Environment Research Council, UK.
OBJECTIVE	To determine the impact of fishing intensity on genetic diversity.
DESIGN	Preserved samples of *I. argentinus* collected from Falkland waters between 1988–1996 will be examined to compare genetic structure over a period that the intensity of fishing has changed markedly, and there have been documented crashes in population size.
METHODOLOGY	Microsatellite analysis of preserved samples will be undertaken to assess levels of genetic diversity and temporal patterns of allele frequencies. Genetic data will be compared with information on the intensity of exploitation, catches landed and estimates of population size.
STATUS	The project will commence in April 1997, and continue for an initial 9 month period.
COMMENTS	This Study will provide one of the first to compare genetic structure in an exploited fishery over a period of major change in the intensity of harvesting. Data will provide some indication of whether the current low levels of genetic diversity are characteristic of relatively unexploited populations, or related to fishery-induced reductions in population size.

Study 7

LABORATORY/RESEARCHER	Molecular Ecology and Fisheries Genetics Laboratory, Department of Biological Sciences, University of Hull, HULL, HU6 7RX, / G.R. Carvalho and C. Turan.
SPECIES	Atlantic herring (*Clupea harengus*).
PROJECT FUNDING	Overseas post-graduate studentship (Turkey) + in-house funding.
OBJECTIVE	To develop novel molecular Markers for stock discrimination of herring.
DESIGN	To develop novel genetic Markers in widely-separated populations of herring from the North Sea (esp. Norwegian fjords), Baltic and Canadian waters using novel approaches (Polymerase chain reaction (PCR) based analysis of mitochondrial and nuclear DNA.
METHODOLOGY	PCR-based analysis of mtDNA (ND genes), allozymes and microsatellites, morphometrics and meristics.

STATUS	April 1994 - April 1997.
COMMENTS	All practical studies are now complete, and final stages of analysis are underway. Genetic differentiation (allozymes) between Norwegian fjord herring and coastal stocks, and all samples and Baltic herring. Significant genetic differentiation detected between previously genetically homogeneous samples using microsatellites (e.g., Icelandic herring). Paper in press in *J. Mar. Biol. Assoc. U.K.* (late 1997).

Study 8

LABORATORY/RESEARCHER	School of Biological Sciences, University of Wales Swansea, Singleton Park, Swansea / Dr D.O.F. Skibinski.
SPECIES	Mussels (*Mytilus*).
PROJECT FUNDING	NERC.
OBJECTIVE	To analyse growth and gene flow in mussel populations.
DESIGN	Allozyme, nuclear DNA and mitochondrial DNA analysis of diverse populations and species.
METHODOLOGY	As above.
STATUS	Ongoing.

Study 9

LABORATORY/RESEARCHER	School of Biological Sciences, University of Wales Swansea, Singleton Park, Swansea / Dr D O F Skibinski.
SPECIES	Aquatic animals.
PROJECT FUNDING	NERC.
OBJECTIVE	To analyse causes of genetic diversity in aquatic animals.
DESIGN	Use of allozyme database.
METHODOLOGY	Statistical and simulation analyses of database.
STATUS	Ongoing.

Study 10

LABORATORY/RESEARCHER	School of Biological Sciences, University of Wales Swansea, Singleton Park, Swansea / Dr D O F Skibinski.
SPECIES	Tilapia.
PROJECT FUNDING	ODA.
OBJECTIVE	To produce improved strains for aquaculture in Africa and the Far East.
DESIGN	Selective breeding and chromosome manipulation.
METHODOLOGY	DNA and transgenic technology.
STATUS	Ongoing.