Mariculture Committee

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REPORT OF THE

WORKING GROUP ON THE APPLICATION OF GENETICS IN FISHERIES AND MARICULTURE

Gdynia, Poland
17–21 February 1997

International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

Palægade 2–4 DK–1261 Copenhagen K Denmark

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1 INTRODUCTION

Accordant with C.Res. 2:31 adopted at the 1996 Annual Science Conference in Reykjavik, Iceland, the Working Group on the Application of Genetics in Fisheries and Mariculture (WGAGFM; Chairman J. Mork, Norway) met at the Sea Fisheries Institute in Gdynia, Poland, Feb. 17-21, 1997 to deal with its Terms of Reference (Appendix 2).

1.1 Attendance

There are currently 40 appointed members and observers in the WGAGFM (Appendix 4). Fifteen of these, from eleven different countries, attended the 1997 WG meeting in Gdynia, Poland (Appendix 3). Countries represented (number of persons in parenthesis) were Belgium (1), Canada (1), Denmark (1), Germany (1), Finland (1), Iceland (2), Ireland (1), New Zealand (1), Norway (2), Poland (3), and Portugal (1). The composition of the qualitative and quantitative sub-groups during the meeting was as follows:

Qualitative genetics sub-group: **T. Cross,** (leader), R. Castilho, G. Dahle, A.K. Danielsdottir, M. M. Hansen, E. Kenchington, M. Luczynski, J. Trautner, F. Volckaert, E. R. Wenne, and E. Wlodarczyk.

Quantitative genetics sub-group: **J. Jonasson** (leader), P. Smith, and L. Siitonen,

1.2 Working form

Prior to the meeting, specific members agreed to prepare position papers related to specific issues in the Terms of Reference, 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 final plenary discussion and inclusion in the WG Report.

Peter Smith chaired «Selective fisheries» Terms of Reference (ToR) point A1.
Ellen Kenchington chaired «Genetically modified organisms (GMO)). ToR point A2.
Michael Møller Hansen chaired «Review of computer software» (position paper co-authored by Paul Galvin, University College Cork, Ireland). ToR point B.
Geir Dahle chaired «Review of DNA molecular markers». ToR point C.
Tom Cross chaired «Genetic aspects of impact cultured ---> wild fish». ToR point E.

The session chairmen were also responsible for leading the respective colloquia 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 with them (on diskette) to Gdynia. A preliminary report on national activities could thus be compiled during the meeting.

The Working Group decided that, like in 1994–1996, the preparation of the WG Report should mainly be done by the members present at the meeting.

2 TERMS OF REFERENCE 1997 (CF. APPENDIX 2)

2.A1 Selective Fisheries

[Based on position paper by Peter Smith, New Zealand. Adopted by WGAGFM in Gdynia]

Summary

Evidence for the genetic effects of selective fishing from empirical, experimental and modelling studies is briefly reviewed. For some demersal and salmonid fisheries there are long term declines in mean size, mean age at first maturity, and even change in spawning period. Data from Atlantic salmon ranching show a significant additive genetic effect on variation as well as genetic linkage (correlation) between life history traits. Experimental studies have shown that selection against large fish results in a reduction in growth rate. In general modelling studies have shown that size selection favours slow-growing and late-maturing fish, although modelling trawl fisheries for North Sea cod indicated that the fishery selects for fast growing early-maturing fish. In the short term modelling may provide the best opportunity to estimate the effects of selective fishing, but will require input on key biological parameters such as size at maturity and the selective parameters such as size range/frequencies of captured fish. Future modelling may have to account for different selective pressures by different fishing gear on the same stock. Without realistic biological parameters it is difficult to estimate the genetic effects of selective fishing gear and propose realistic mitigation methods.

1 Introduction

Genetic change occurs in natural populations when there is a selective mortality on genotypes, or populations are severely reduced in size. Fishing is a selective agent and has the potential to change populations: different fishing methods catch different size fish in the same area, in addition fishers often target specific size classes of fish. In spite of this potential for selection evidence for genetic changes due to fishing has been limited. In an early review Miller (1957) concluded that there was little evidence for any heritable changes due to exploitation or introductions in freshwater fishes. Since then genetic changes due to exploitation have been reported in a wide variety of fisheries from the Arctic Ocean (Borisov 1979) to tropical lakes (Gwahaba 1973). Many genetic responses to exploitation were reported before direct methods for measuring genetic diversity were developed, and were based on long term changes in life history traits, such as growth rate and size/age at first maturity. Life history traits are notoriously plastic and can change in response to changes in the environment, making it difficult to determine the genetic effects of fishing.

To determine the genetic effects of fishing gear we need to demonstrate:

A. Genetic variation in commercial fish stocks. There is ample evidence for genetic variation in fish populations based on life history traits, morphometrics, behaviour and genetic markers such as allozymes, mtDNA, and nDNA. Genetic markers have been applied as stock identification tools on the premise that the markers are selectively neutral and therefore not influenced by fishing pressure, although some allozyme loci are under selection in marine species (eg Mitton & Koehn 1975, DiMichele & Powers 1982). Ranching studies on Atlantic salmon in Iceland have produced heritability estimates for various life history traits (Jonasson *et al* 1997). The estimated across year-class heritability

for return rate was 0.12 for grilse, 0.04 for two-sea-winter salmon and 0.08 for total return rate; for body weight of grilse it was 0.36±0.11 and 0.00±0.15 for body weight of two-sea-winter salmon. Genetic correlation between return rate of grilse (one-sea-winter salmon) and grilse body weight was estimated to be 0.16±0.16, between return rate of grilse and two-sea-winter salmon was -0.29±0.16, and between freshwater survival and total return rate was 0.33±0.14. Even though not all of these correlations were significant they indicate that changing one life history trait by selection might have a positive or negative effect on another trait. This shows that variation in life history traits in Atlantic salmon ranching are to a certain extent controlled by additive gene effects and they are genetically correlated (Jonasson *et al.* 1997).

B. The selective agent. Many fishing methods are size selective, plus fishers often target size specific fish. Genetic traits that are linked with growth rate are likely to be under selection (eg onset of sexual maturity). However fishing regulations are rarely constant, so that the selective pressure changes over time; fluctuating selection makes the selective effects more difficult to detect in natural populations. In addition migratory stocks may be under different selection pressures in different parts of their range due to different fishing methods (eg trawl and longline fisheries catch different size range).

C. Measurement of change in population over time. Change can be brought about by dissimilar mechanisms but produce similar short term results, particularly for life history traits. Not all changes in fish stocks are genetic, and agents other than fishing may produce genetic change.

Non-genetic changes. Fishing theory predicts that an increase in fishing mortality will produce an increase in growth and recruitment. The complex and often poorly understood relationships between the genetic components of growth rate and the size and age at first maturity, and the non-genetic responses of these traits to changes in population density and other environmental parameters, such as water temperature, make it difficult to separate the genetic and non genetic impact of fishing on natural populations.

In the cod *Gadus morhua* the growth rate increased and the age at maturity decreased when fish were reared under favourable conditions (Godo & Moksness 1987). Cod stocks from two Norwegian fisheries, with different growth and age at maturity traits, grew and matured at similar rates when grown under similar and favourable (excess food) conditions, indicating that the differences in the fisheries are probably not of genetic origin (Godo & Moksness 1987). Weight at age in cod varies greatly throughout its range and is strongly linked with temperature (Brander 1995). An increase in growth rate in the North Sea sole *Solea solea* was related to increased food availability from trawling activities rather than a reduction in stock density (de Veen 1976).

Genetic drift. Stocks of commercial fish species are rarely reduced to such a small size that drift might be operating. However changes may be produced in small populations when large numbers of individuals are introduced from a different gene pool. This situation is rare in natural populations, but can occur in enhancement programmes and has been recorded with escapes of farmed salmonids (eg Heggberget *et al.* 1993, Hindar *et al.* 1991, Skaala *et al.* 1990).

Directional selection due to environmental change. Genetic changes occur in fish populations in response to selective agents other than fishing. Extreme fluctuations in water temperature have produced changes in allozyme frequencies in freshwater and estuarine fishes (Mitton & Koehn 1975, Smith *et al.* 1983). The haemoglobin polymorphism in cod *Gadus morhua* appears to be under selection (Karpov & Novikov 1980, Mork *et al.* 1983) and juveniles show different

survival rates with respect to the *Gpi-1** and *Ldh-3** loci (Mork & Sundnes 1985a).Year class differences have been reported in several population studies (eg Mork & Sundnes 1985b, Lacson & Morizot 1991).

Geographical variation. Numerous genetic studies have shown regional variation in allele frequencies at allozymes (eg Mork *et al.* 1985), mtDNA haplotypes (eg Dahle 1991), and nDNA markers (eg Galvin *et al.* 1995). Size and age at sexual maturity varies between intraspecific stocks in skate *Raja radiata* (Templeman 1987), American plaice *Hippoglossoides platessoides* (Roff 1982) and cod *Gadus morhua* (Garrod & Horwood 1984). Herring *Clupea harengus* and European plaice *Pleuronectes platessa* show geographic variations in fecundity (Mann & Mills 1979). The genetic basis for regional differences in life history traits is unknown, but some studies indicate a high environmental component (eg Godo & Moksness 1987).

Differential mortality by fishing gear. There is evidence from empirical, experimental and modelling studies for genetic changes in heavily exploited stocks. The empirical evidence is often inconclusive, as it has not been possible to show that the observed changes were due to genetic or nongenetic responses to exploitation.

2 Evidence for a differential genetic mortality in fish stocks

A. Empirical evidence

Evidence for genetic changes in fish are listed by species and area in Table 1. The types of genetic change can be summarised under four categories:

i. Selection for early or small size at maturity

A common observation in the North Atlantic demersal fisheries has been a decline in mean size and age at maturity in gadoid and flatfish species; similar observations have been made in Atlantic and Pacific salmon and in lake fisheries (see Table 1). Life history traits such as size and age at maturity have a genetic base, but are phenotypically plastic and respond to environmental changes. At reduced densities fish grow faster and reach maturity at an earlier age and smaller size.

- Declines in age at maturity in cod *Gadus morhua* have been attributed to a selective removal of late maturing cod from the population (Beacham 1983a, Borisov 1979, Rowell 1993). Cod that matured at smaller or younger sizes would have a selective advantage under heavy fishing pressure as the larger and older maturing cod would be captured before the onset of sexual maturity (Beacham 1983a).

- In the coho salmon *Oncorynchus kisutch* males mature after 6 months in the ocean as jacks or after 18 months as hooknose. These two alternative life history strategies are maintained by disruptive (natural) selection favouring small "sneaky" males and large "fighting" males (Gross 1985). The fishery has selectively harvested the larger fish increasing the relative frequency of jacks (Gross 1991). However anthropogenic changes at other stages of the life cycle also influence the ratio of jack to hooknose males. Stream clearance may reduce the available refuges for jacks, favouring hooknose fish on the spawning grounds; eutrophication may increase fry growth rate leading to an increase in the proportion of juveniles maturing as jacks (Gross 1991).

- The protandrous shrimp *Pandalus borealis* fishery in the Skaggerak showed a reduction in size of females over a period of heavy exploitation (Jensen 1965, 1967). Furthermore small females (<75mm) were not present in 1953, but by 1961-62 formed 21-30% of the catch

(Jensen 1965). Increased fishing mortality on the large females has selected for individuals that mature as female at the age of first breeding (Charnov 1981).

ii. Selection for small size.

Many species have shown a reduction in mean size over the past few decades of heavy fishing, but there is uncertainty if the changes are due to selective removal of larger fish, to selective depletion of stocks with large body size, or to changes in oceanographic conditions (eg Ricker *et al.* 1978, McAllister & Peterman 1992). Some key examples are:

- In Pacific salmon three species exhibited a decrease in mean size at age following years of size selective fishing (Ricker 1981). The gill net and troll fisheries for coho *Oncorynchus kisutch* and pink salmon *O. gorbuscha* tend to catch the larger fish and these species exhibited the greatest declines in mean sizes (Ricker 1981). The chinook salmon *O. tshawytscha* decreased in both mean size and age between 1951 and 1975 (Ricker 1980). This decrease in size and age may be due to the troll fishery which captures both maturing and non-maturing fish and selects against late maturing individuals. However the fishing methods and mesh sizes have changed over the sampling period (Ricker 1981).

- In the sockeye salmon *Oncorhynchus nerka* disruptive selection in the gillnet fishery has favoured the survival of small 3 year- and large 4 year-old ocean fish. As a result the 3 year old fish have become smaller and the 4 year old fish larger, the difference between them increasing by about 500g (Ricker 1982).

- In the cod *Gadus morhua,* modern hook caught fish off Nova Scotia are much smaller than cod caught during the 1750's, although cod of similar maximum size are occasionally caught, based on measurements of cleithra bones from the pectoral girdle (Kenchington & Kenchington 1993).

iii. Selection for spawning period/season

There is a genetic component to spawning time in salmonids as shown by hatchery studies (Siitonen & Gall 1989, Gharret & Smoker 1993) and release experiments (Hansen & Jonsson 1991). Transplant experiments have demonstrated a genetic component to spawning period and intra-stock differences for spawning time in scallop *Pecten maximus* (Cochard & Devauchelle 1993, Mackie & Ansell 1993). Therefore for a species with a long spawning season, unequal fishing pressure over the season may produce a selective mortality.

- The first arrival and subsequent spawning of herring *Clupea harengus* on the Norwegian coast has changed over a sixty year period (Devold 1963). Around the turn of the century herring returned to the spawning grounds in September-October, but this return date was delayed progressively so that by the 1950's herring did not appear on the spawning grounds until January (Devold 1963). Mathisen (1989) interpreted this delay as due to the effects of fishing which has selectively harvested the first returning sub groups of herring, so that with time these contributed less to the fishery and were replaced serially by later returning sub groups. However changes in the spawning time of other groups of herring have been explained by changes in environmental conditions. In the Baltic herring the less fecund spawn in the spring and the more fecund in the autumn (Anokhina 1971). The disappearance of autumn spawners has been linked to improved feeding conditions, due to eutrophication, whereby adults have sufficient food reserves to spawn in the spring (Aneer 1985).

- In the Columbia River chinook salmon *Oncorhynchus tsawytscha* the spawning run lasted from April to August with a peak in June-July when the fishery developed last century (Thompson 1951). The fishery operated mostly over the summer and heavy fishing pressure

on the peak run reduced catches; by 1938 the spawning run peaked in May and in August with few fish caught in the original peak period of June-July (Thompson 1951). Mathisen (1989) has interpreted these observations as disruptive selection, selecting for both early and late spawners, which have not replaced the middle peak period spawners. In an extension of this selective spawning-fishery hypothesis Mathisen (1989) has suggested that the decline of the Peruvian anchoveta *Engraulis ringens* was accelerated by disruptive selective fishing on the "best reproductive units" leaving only the marginal groups to rebuild the stocks.

iv. Changes in genetic diversity

Changes in genetic diversity, measured with allozymes, have been reported in heavily exploited populations (Altukhov 1990,1993; Kirpichnikov *et al.* 1990; Smith *et al.* 1991). Allozyme markers provide a rapid method to estimate genetic diversity; although much of this diversity may be selectively neutral, some, but not all, studies have shown that heterozygosity is positively associated with growth rate (eg Zouros & Foltz 1987). Kirpichnokov *et al.* (1990) showed that during the fry stages of sockeye salmon *Oncorynchus nerka,* slow growing individuals were less heterozygous than fast growing individuals. The loss of genetic diversity reported with the initial exploitation of orange roughy around New Zealand (Smith *et al.* 1991) has not been been maintained over a longer time period (Smith & Benson 1997).

B. Experimental evidence

If evidence for selective effects in wild fisheries is weak and compounded by unknown environmental changes and absence of control samples, then stronger evidence for selective effects of fishing comes from experimental studies:

- Two populations of *Oreochromi mossambicus*, a mouth breeder used in tropical pond aquaculture, were established and, after 39 months, harvested at two monthly intervals by removing about 10% of the fish (Silliman 1975). One population was selectively fished by removing only fish that could not pass through a grid placed in the tank. The second control population was unselectively fished by removing a similar number of fish but from all size classes. After 63 months the fishing pressure was increased to 20% and continued for a further 12 months. In both the selected and unselected populations males grew faster than females, but the males in the unselected population grew more rapidly than males in the selected population (Silliman 1975). Selective fishing produced a decline in growth rate in males in only three generations. It is possible that females were less affected by the size selection due to their slower growth rate than males.

- Two contrasting harvesting regimes, removing small or large individuals, were compared in laboratory populations of the water flea *Daphnia magna* (Edley & Law 1988). Yields declined under both harvesting regimes, but were greatest in populations harvested for large individuals. Under the harvesting regime of removing small individuals the mean size at age increased as did the size at first reproduction, while under the harvesting regime removing large individuals, the reverse occurred with a decline in size at age and decline in size at first reproduction (Edley & Law 1988).

- Populations of guppies *Poecilia reticulata* transferred from river systems with cichlid predators, that prey on large guppies, to rivers with killifish, that prey on small guppies, resulted in larger offspring and females that reproduced at larger size in the transplanted populations (Reznick *et al.* 1990). Rearing groups of descendants from the two populations

under similar laboratory conditions showed that the observed life history differences had a genetic basis (Reznick *et al.* 1990).

C. Evidence from modelling studies on selective effects of fishing

Modelling provides the quickest method for determining genetic responses to selective fishing, and perhaps the only method for stocks that have been selected. Models are dependent on the use of realistic biological parameters; several models have used a biallelic approach when life history traits are likely to be polygenic, while others have taken a generalist approach (eg Bergh and Getz 1989, Kapuscinski & Lannan 1984,1986). Even models which consider additive effects do not take account of traits that are effected by epistasis and pleiotropy. The most useful models have been those developed for specific fisheries:

-Menshutkin *et al.* (1989) produced a simulation model of the sockeye salmon *Oncorhyncus nerka* fishery in Kamchatka. When growth was controlled by a single gene with two alleles the population became unstable with loss of one allele, supporting observations that growth is under polygenic control. When growth was polygenic and multiallelic then the percentage of "fast growth", which equate to early maturity, alleles increased in the selected population and the proportion of non migratory fish increased. These results are supported by observations in the fishery where there has been an increase in the proportion of jacks and lake-resident fish (Altukhov 1990).

- Favro *et al.* (1979, 1982) used a multilocus model to estimate the magnitude of genetic effects in a trout fishery subject to minimum size limits. Results showed that mean size and total numbers decreased with moderate levels of fishing pressure and were in agreement with observations in a brown trout *Salmo trutta* fishery in Michigan. Extending the model to select for fish from a specific size range, whereby a minimum and a maximum size limit was set in the fishery, then the model showed that the double-size limit produced a similar decrease in larger fish as did conventional minimum size limits (Favro *et al.* 1980).

- Law & Grey (1989) used an age specific model to describe exploitation acting on life history traits. Selective fishing lead to changes in life histories that resulted in changes in yield. Applying this model to the Arcto-Norwegian cod *Gadus morhua*, it was shown that the optimum reproductive life history strategy, measured as an individuals total egg production, changed between the spawning fishery at the Lofoten Islands and the feeding fishery based on mature and immature fish in the Barents Sea. Fishing on the spawning population selected for females maturing at 6-8 years, but on the feeding population selected for females maturing at 4 years (Law & Grey 1989).

- Desharnais *et al.* (1985) used a single locus model in which heterozygotes had a 5% advantage in growth rate to estimate the effects of fishing with a.) a constant mortality above a minimum size and b.) mortality a linear function of size. Both models gave similar results showing that in order to maintain the polymorphism a low rate of fishing or a legal minimum size close to the maximum realised size was required.

- Thompson and Stokes (1996) used two models (a bi-allelic Mendelian model and a quantitative model) to show that trawl-fisheries for North Sea cod, which catch fish >20/30 cm, favour fast growing fish. Although fast growing fish suffer a higher annual mortality than slow growing fish they mature earlier producing more eggs and consequently leave more offspring for the next generation. Only at a high minimum size does fishing selectively favour slow growing fish. It was pointed out that under reduced density, due to high fishing pressure, growth rate is

optimised so that genes coding for growth have maximum effects (Thompson & Stokes 1996). Under a quantitative genetics model, increased fishing mortality might be expected to favour slow growing fish, as they are less likely to be caught. Indeed experimental studies suggest that selection favours slow-growing fish (Silliman 1975, Edley & Law 1988). However selection pressures on the experimental populations were different to those in the cod trawl fishery: the experimental studies removed the largest fish, whereas the trawl fishery with knife edge selection removed cod greater than 30 cm. The two opposing results: selection for slow-growing and fast-growing fish, show that it is premature to make general conclusions about the selective effects of fisheries. The genetic consequences are likely to be dependent upon the selection differentials of different gear types and the heritability of the onset of sexual maturity and its relationship to the size and age of individuals.

In the short term modelling provides the best option for estimating the genetic effects of selective fishing; but will need to be species and fishery specific. Clearly the size at onset of sexual maturity and the size selectivity of the fishing gear are critical for determining the genetic outcomes of selective fishing. Estimates of the heritability of age/size at onset of sexual maturity are needed, for marine species; although selection will occur even when heritability is low, the time scale of genetic change will be longer.

3. Long term effects of selective fishing

"Rome is burning and you (geneticists) worry about the building materials. We (fishery managers) need to put out the fire".

Selective fishing potentially has two impacts on natural populations: a short term decline in yields and a longer term loss of genetic diversity. If slow growing fish are selected by fishing then productivity will be reduced. However some modelling indicates that the reverse could occur with selective fishing favouring fast growing individuals (Thomson & Stokes 1996).

It is often stated that species with low, or reduced, genetic diversity are more vulnerable to changes in the environment and long term extinction. However some species survive with low genetic diversity as measured with allozymes (eg teleosts, Fujio & Kato 1979, Johnson & Utter 1976). There is little evidence from natural populations that loss of genetic diversity (as opposed to low effective population size) makes a species more vulnerable to extinction, although measuring such effects is beyond the working life span of researchers. For polygenic traits, such as life history traits, the loss of diversity will be low when the trait is affected by a large number of loci (Bentsen 1994).

4. How can any negative genetic impacts of fishing be reduced?

Negative impacts can only be reduced once there is good evidence for, and understanding of, the genetic response due to selective fishing. The evidence summarised above suggests that selective fishing may favour fast or slow growing fish and the direction of the genetic change depends on the size at sexual maturity and the size range captured by the gear. Modelling studies suggest that the impact may be different to the expected impact (Thompson & Stokes 1996). It is likely that the genetic impact will differ between fisheries due to different selection pressures of the gear and the biological traits of the fish. Therefore no universal method can be proposed to reduce the selective effects of fisheries. Some specific suggestions have been:

- Law & Grey (1989) suggested that cod fisheries on the younger 4 year old "feeding stocks" in the Barents Sea should be restricted and fishing concentrated on the larger spawning fish on the

Norwegian coast. This fishing pattern would select for late maturing individuals and give an increase in yield. Mortality on immature fish selects for early maturity and results in reduced long term yield due to reduced input into somatic growth. In principle the fishery manager opts for the harvest pattern that will select for an optimum life history producing the greatest long term yield: the "evolutionary stable optimal harvesting strategy" (Law & Grey 1989).

- Brown & Parman (1993) have extended the concept of the "evolutionary enlightened manager" who considers the effects of harvesting on prey size and compared it with an "ecologoically enlightened manager" who considers the effects of harvesting on population size. The evolutionary manager choses a lower harvest rate and selects for larger size fish to preserve yield in evolutionary time, whereas the ecological manager maintains a higher harvest rate which selects for smaller adults.

- A large scale experimental management approach to test the heritability of growth rate and the effects of size selective fishing on a natural population of pink salmon *Oncorhynchus gorbuscha* was proposed by McAllister & Peterman (1992) with the overall aim of increasing catch biomass. *O. gorbuscha* has shown a reduction in mean size over the past twenty years (Ricker *et al.* 1978); controlled selective harvesting of small fish would permit a test of the heritability of growth rate and, provided that growth rate is inherited, lead to an increase in mean size in the fishery. A decision analysis showed that this experimental approach to management of pink salmon stocks was likely to produce a higher harvest value than the current management practice (McAllister & Peterman 1992). Unfortunately this option has not been taken up (McAllister pers comm).

Finally all of the empirical examples of genetic effects of fishing have been with stocks that have been overfished. Tighter management controls may help to reduce any selective effects of fisheries.

Summary of

WGAGFM recommandations concerning selective fisheries

1. Further modelling studies should be undertaken for fisheries exploiting different species to estimate changes in life history traits over time with different gear types (eg trawl, set net, line fishing, etc).

2. Heritabilities of life history traits in marine species, especially survival, growth rate and age at maturation, should be determined through enhancement (ranching) and aquaculture studies. The impact of changing growth rate on survival and age at maturity need to be determined.

3. Studies should be undertaken to estimate genetic change over time in heavily exploited stocks by comparing genetic diversity in historical samples (dried otolith and scale samples and midden samples) and current samples, although some of the markers recovered in preserved samples may be selectively neutral. Additional long term comparisons could be undertaken in marine mammals that were heavily exploited by comparing genetic diversity in dried skin samples with current samples.

4. Given the possible collapse of the North Sea cod stocks then consideration should be given to recovering archived frozen tissue samples from laboratory collections, for genetic comparison with current samples. Genetic techniques should include allozymes, mtDNA, and micro- and mini- satellites to provide a range of selected and neutral markers.

Acknowledgments. Kevin Stokes, MAFF Lowestoft is hereby thanked for making available unpublished results from modelling selective effects on the North Sea cod fishery.

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Temporal changes in life-history traits of fish stocks; possible evidence for genetic change due to selective fishing.

Species (Area)	Changes	Reference
Atlantic cod,	***Gadus morhua***	
Scotian shelf	reduction in mean size and age; decline in mean age at maturity >5yrs (1960's) to <3yrs (1978)	Beacham 1983a
West Greenland	decline in mean age at maturity from 9.9yrs (1917) to 6.4 yrs (1936) in northern stocks and 9.3yrs (1922) to 7.6 yrs (1936) in southern stocks	Hansen 1949
Arcto-Nowegian	decline in age at maturity from 8/10yrs in 1930 to 6 yrs in 1970's	Borisov 1979
North Sea	decline in size at maturity between 1893-1974	Oosthuizen & Daan 1974 Rowell 1993
NW Atlantic	reduction in size between 18-20 centuries	Kenchington & Kenchington 1993
Haddock,	***Melanogrammus aeglefinus***	
NW Atlantic	decline in mean age at maturity from 4.6 to 3.3 yrs in males and 5.9 to 4.3 yrs in females between 1948/51 and 1969/75	Templeman & Bishop 1979b
Scotian shelf	mean length at sexual maturity declined from 3.9 to 2.7 yrs in males & 4.4 to 3.0 yrs in females between 1959/64 and 1975/79	Beacham 1983c
Plaice,	***Hippoglossoides platessoides***	
Scotian shelf	decline in mean length and age at sexual maturity	Beacham 1983b
Grand Banks		Pitt 1975
North Sea	decline in mean size and age at sexual maturity females mature at 31-43 cm and age 5-8 yrs in 1900's and 30-35 cm and 4-5 years in 1985/86; males mature at 30-37 cm and age 5-6 yrs in 1900's and 20-24 cm and 2-3yrs in 1985/86.	Rijnsdrop 1993
Yellowtail flounder,	***Limanda ferruginea***	
	decline in mean age at sexual maturity	Beacham 1983b
Yellowfin sole,	***Limanda espersa***	
USSR	decline in age at 50% maturity of females from 8.5 to 7.2 yrs between 1961-69.	Tikhonov 1977
Atlantic herring,	***Clupea harengus***	
Norway	seasonal delay in return to spawning grounds from Sep/Oct at turn of century to Jan in 1950's	Devold 1963 Mathisen 1989

Species (Area)	Changes	Reference
Chinook salmon, *Oncorynchus tshawytscha*		
British Columbia 1981	decrease in mean size and age of returning fish 1951-75	Ricker 1980,
Columbia River	seasonal change in peak runs from Jun/Jul at turn of century to May/ August 1938	Thompson 1951 Mathieson 1989
Coho salmon, *Oncorynchus kisutch*		
	increased frquency of jacks decerease in mean size	Gross 1991 Ricker 1981
Sockeye salmon, *Oncorynchus nerka*		
	increase in proportion of returning jacks, increase in resident dwarf males	Altukhov 1990
	smaller 3 year olds and larger 4 year olds due to disruptive selection by gill nets	Ricker 1982
Pink salmon, *Oncorynchus gorbuscha*		
	decrease in mean size	Ricker 1981
Atlantic salmon, *Salmo salar*		
	decline in age of first returning fish	Schaffer & Elson 1975
whitefish, *Coregonus clupeaformis*		
Canadian lakes	fish matured at a younger age & smaller size in exploited than unexploited populations	Healey 1975
Alberta	decline in length & weight at given age, 1940-70	Hanford *et al.* 1977
whitefish, *Coregonus lavaretus*		
Lake Femund, Norway	reduction in size at age and proportional decline in pelagic morph	Sandlund & Naesje 1989
cichlid, *Tilapia nilotica*		
Lake George, Uganda	decline in mean size from 0.9 to 0.4kg and decline in size at maturity from 20/29 to 18/24 cm between 1950-70.	Gwahaba 1973
shrimp, *Pandalus borealis*		
Norway	large shrimps, predominately females, declined from 44 to 14% between 1944-61. Small females (<75mm) not present in 1953 but formed 21-30% of catch 1960/61	Jensen 1965, 1967 Charnov 1979, 1981
spiny lobster, *Panulirus marginatus*		
Hawaii	decline in size at onset of egg production	Polovina 1989

2.A2 Genetically Modified Organisms (GMOs)

[Based on position paper by Ellen Kenchington, Canada. Adopted by WGAGFM in Gdynia.]

Background

Genetically modified fish and shellfish research is now sufficiently advanced (cf. Warmbrodt and Stone 1993) that commercial trials for some species have commenced. The greatest interest in the marine environment is with respect to the application to mariculture for food production and for bioreactors, although there is some interest for the use of sterile transgenic fish for release into the wild to contribute to public fisheries (e.g., trophy fish). Because escape from commercial mariculture cages and net pens has been significant (e.g., Hindar *et al.*. 1991), there is particular concern that transgenic organisms should not have an impact on wild populations. Some countries have advanced policies on the use of GMO in fisheries and mariculture with differing levels of constraints depending upon the perspective of the country toward the risks and benefits associated with GMO. For example, the Council of European Communities Directive (90/220/EEC) on the deliberate release of GMO binds the European Community member states to adopt provisions necessary to ensure that notification is given by any person planning release of any transgenic organism. Norway has developed an "Act Relating to the Production and Use of Genetically Modified Organisms" (Gene Technology Act, 2 April, 1993) which applies to all organisms, limits research to approved closed systems, and sets out stringent conditions for consideration of use outside the laboratory. More specific recommendations concerning aquatic organisms are presently being considered. Similarly, Finland has legislated a Gene Technology Act for the contained use and manufacture of GMO and to their deliberate release to the environment. Supervision of the gene technology is based on a permit and notification system and subsequent control. Canada has produced a draft policy on "Research with, and Rearing of, Transgenic Aquatic Organisms" (DFO Aquaculture and Oceans Science Branch) which is designed to permit research and the use of transgenic organisms while ensuring wild resources are protected. This policy differentiates between rearing inside a laboratory and rearing outside a laboratory, in that unless otherwise authorized, rearing of transgenic organisms outside a laboratory may be made only with functionally sterile organisms. The United States has developed "Performance Standards for Safely Conducting Research with Genetically Modified Fish and Shellfish" (US Dept. Agriculture, Office of Agricultural Biotechnology, Doc Nos. 95-04,05). However, policies and regulations on the utilization of GMO are still lacking in many countries (Bartley and Hallerman 1995). In addition some industry organizations, for example the International Salmon Farmers Association, have developed policies rejecting the use of transgenics.

The International Council for the Exploration of the Seas (ICES) Standing Committee on Mariculture directed its Working Group on Genetics and its Working Group on Introductions and Transfers of Marine Organisms (WGITMO) jointly to develop guidelines for research to evaluate ecological effects of the release of transgenic organisms. The ICES Code of Practice (1994) has been amended to specifically address transgenic organisms and member countries are bound to that code. In 1995, the Working Group on the Application of Genetics to Fisheries and Mariculture (WGAGFM) was asked by the Mariculture Committee to make comments on how ICES member countries should or could go about assessing a GMO release. The working group undertook to give advice on risk analysis of the spread of transgenes from GMO to wild populations. The 1996 WGAGFM Report (ICES CM1996/F:2) discussed the WGITMO definition of a GMO and clarified the definition of a transgenic organism. The WGAGFM considers that a transgenic organism is an organism bearing within its genome a copy or copies of novel genetic constructs produced through recombinant DNA technology. This definition includes organisms manipulated with their own genetic material.

Current Status of GMO Fish Research with Mariculture Applications

Growth hormone genes have been used amongst others in common carp (Zhang *et al.*. 1990), rainbow trout (Penman *et al.*. 1990), Pacific salmon (Devlin *et al.*. 1994) and Atlantic salmon (Hew *et al.*. 1995). In transgenic coho salmon, dramatic growth increases have been demonstrated producing fish 37 times heavier than the average control weight after 14 months in freshwater. However, Devlin *et al.*. (1995) observed profound morphological abnormalities in the first generation as well as the increased growth. These had disproportionate growth of the head and operculum cartilage giving a deformed appearance and leading ultimately to respiration problems.

Anti-freeze protein genes have been isolated from winter flounder, ocean pout and wolfish which allow these species to survive sea temperatures below -1°C by producing antifreeze proteins (Gong and Hew 1993). These genes have been transferred to Atlantic salmon in an effort to increase tolerance to freezing conditions (Hew *et al.*. 1995), however, while the genes are active and can be passed to progeny, the introduced gene does not induce sufficient freeze tolerance.

Other genes which have been transferred in fish or are being discussed include metallothionein genes (Olsson 1993), esterase genes, which could make farmed salmon more resistant to the organophosphates used to treat for sea lice (Maclean and Penman 1990), disease resistance genes and non-functional segments of DNA, which could serve as a heritable internal marker for reared fish.

GMO shellfish have not been experimented with to the same extent. The small size of most shellfish eggs do not make them readily amenable to nuclear injection. One abstract has been reported on the production of transgenic abalone (*Haliotis rufescens*) with growth hormone (Powers *et al.*. 1994), and transgenic clams (*Mulinia lateralis*) have been produced with pantropic retroviral vectors (Kan-Lu *et al.*. 1988). Transgenic oysters have been produced in France under laboratory conditions, and commercial GMO shellfish are probably not far in the future.

Similarly, GMO production of seaweed is complicated by very small egg size and complex and in some cases poorly understood life histories, at least in the commercially important red algae. Transgenic research is reported for *Porphyra* protoplasts (Kuebler *et al.*. 1994).

The longevity of the gene insert will be a factor in determining the genetic impact of escapees. Presently, our experience with recombinant DNA plasmids is that the gene inserts are not always stable. There are many cases of stable integrations, but the number of labile integrations might be under-reported. An introduced tyrosinase gene producing a black colouration in medaka (*Oryzias latipes*) has been observed to be lost after 10 generations. Improvements in site integration will lead to more persistent incorporation of transgenic genes over time.

Concerns Associated with the Gene Construct

For the introduced gene to be functional in the target fish the regulatory sequence must be included, as well as a termination sequence. Early transgenic work used viral and mammalian regulatory sequences, but now fish sequences are available which alleviate some concerns over the introduction of unrelated genes. It may be advantageous to use a mixture of gene and regulatory sequence of separate function, where the latter can be switched on at will or at a time when or in a tissue where, the target gene is not normally active. For example, a metallothionein regulator can be induced by the action of heavy metal ions, and cause a gene of alternative function to become active.

In the only commercially available GMO (see below), an ocean pout antifreeze regulatory sequence is spliced to a salmonid growth hormone gene and inserted into Atlantic salmon. Since antifreeze proteins are produced in the liver, growth hormone will be produced there by this construct, as well as in its normal site of production, the pituitary gland. Growth hormone production from the pituitary gland is inhibited by low temperature, but this will not be the case in the liver, so growth will continue throughout the winter. Thus, higher levels of growth hormone will be produced during the summer, because it is being produced in two tissues and production will continue during the winter. This is

considered to be the advantageous aspect of the GMO. In fish where an antifreeze gene is introduced there is potential for the GMO to extend its natural range should it escape (Chan *et al.*. 1993, Davies *et al.*. 1993).

Recommendations:
The first priority for research relating to constructs of transgenic fish should be to investigate the secondary effects of the insert utilized. This should be associated with a marker sequence that would enable identification of the fish containing the construct. In this way, the potential secondary effects could be evaluated, by measuring phenotypic changes induced by the insert.

"All-(shell)fish" regulatory sequences are recommended. Research to increase the available number of regulatory sequences for mariculture applications is encouraged.

Concerns and Impacts Associated with the Release of GMO

Specific to the GMO organism is the presence of introduced genes in its genome. The GMO therefore has the potential for large impacts on the wild population, in addition to those contributed by the farmed nature of the product which has potential impacts of its own. As with other introductions, if a transgenic organism with modified characters were to interbreed with locally adapted populations, the genetic structure of the population could be affected. A transgenic organism could also have an impact on members of other species and could cause environmental change. The difficulty in evaluating the impact of a GMO release is that each modification creates a new line of organism with a specific phenotype for which we cannot address *a priori* the behaviour or impacts in the natural environment. The GMO will also bear characteristics acquired through their culture process alone which must be evaluated (e.g., Table 1, Hindar *et al.*. 1991). Given that there is difficulty in predicting the effects of influxes of maricultured organisms on local populations in general, it may be that precise models to assess the general impact of GMO are an unrealistic goal.

Some countries have adopted risk assessment and risk management processes to reach a decision on the environmental release of aquatic GMO; 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 which 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 GMO and the application is rated accordingly. Software is also available for Risk Assessment (e.g., PC at Risk).

Recommendation:
All countries within ICES should consider risk assessment protocols for GMO management.

In order to properly undertake risk assessment associated with GMO culture in the sea, research may be required to determine the GMO response to contact with unmodified conspecifics and other species. Strategies for minimizing the genetic impact on natural populations can also be incorporated into proposals for the culture protocols of GMO animals. These include closed culture, sterilization (cf. Devlin and Donaldson 1992), and careful site selection. The WG had concerns over the assumption in the policy literature that sterilization of GMO could facilitate open water culture. For this to be done successfully using current technology, a two generation process is involved. All-

female fish are produced by sex reversing chromosomally female parents to functional males then using these to fertilize normal eggs. The resulting zygotes are then pressure or temperature shocked to yield sterile triploid all-females. The use of sterilization through triploidy should be regarded with caution as triploid induction is never guaranteed (i.e., 100% effective). Furthermore, the number of sex determining systems in fish and invertebrates are very diverse and often labile, and sterilization techniques on species which are known to have sex reversal (e.g., sea bream *Sparus aurata*), for example, are more complex. Generalizations regarding sterilization procedures should be viewed with caution. Work with antisense mRNA GtH (gonadotropin hormone) or other expression factors which interfere directly with reproduction may be more direct, however, public concern over the use of hormone inhibitors is likely to be strong. Suicide genes, which make the fish dependent on nutrients or metabolites supplemented by feeding, may provide a future alternative to sterilization in that eventual escapees are unable to survive at all if the supplement is rare or non-existent in the wild. Much work on the molecular physiology of fish needs to be done before these techniques can throroughly be understood. Other alternatives such as surgery, radiation treatment, hybrid sterility and autoimmune sterility have been considered but have not been implemented for various reasons.

A mass release of sterile fish could still negatively impact a wild population through fitness differences and competition. Further, some sterilization protocols advance production of single sex GMO for open culture. The WG drew attention to the fact that many fish species occur in populations with stable sex ratios (e.g., 1:3, 1:1) which relate to the mating behaviour of the species. Influx of large numbers of single sex GMO have the potential to disrupt the reproductive cycle in the short term.

Recommendation:
Until there is a technique to produce 100% sterilization effectiveness, GMO should not be held in or in connection with open water systems.

It was felt by the WG group that the GMO should be evaluated in field studies where possible, and that phenotypic traits associated with the GMO should be reported. For the development of research in transgenic fish, an important consideration from an environmental perspective will be to discourage research that results in large phenotypic changes in the organism. The degree of risk associated with the release of GMO is in part related to the degree to which the phenotype differs from the natural strain. Field trials using secure systems to prevent escape of the fish from the experimental area at any stage of the life-cycle are a necessary evil. Through simulation of an escape into such an enclosed ecosystem, it should be possible to evaluate:

a) the degree to which the transgenic fish are capable of mating with a native population;
b) the success of that mating as defined by fertilized eggs;
c) the relative fitness of juveniles of pure transgenic crosses, hybrids between native and transgenic crosses, and the pure native crosses;
d) the competitive advantages/disadvantages of transgenic fish;
e) the behaviour of transgenic fish in the wild.

A controlled release of GMO salmon may be practical in Ireland, where an experimental river system is available in Burrishoole. This system is 1.7 km long with a concrete base upstream from a concrete trap. This system has been used successfully to study the genetic effects of farmed salmon on wild stock through manipulated experiments in an EU project (Dr. T. Cross, pers. comm.). While the system would not be secure to poaching, adapting this system to facilitate manipulated experiments with salmon GMO might be proactive as opposed to waiting for an accidental GMO release from which to evaluate genetic and environmental impacts.

Presently, GMO are produced from only a few females. A small number of broodstock dams would cause a genetic bottleneck and some of the genetic variability present in the original population would be lost in the GMO offspring. If transgenic individuals are subsequently used as broodstock there is a high probability of inbreeding defects in subsequent generations. Ultimately, the GMO should be

subjected to a traditional quantitative genetic breeding program so that the mariculture product incorporates the most desirable traits of the whole genome and not just the presence or absence of the construct gene(s).

Recommendations:
The possibilities of constructing closed systems for field testing of GMO should be explored.

GMO production should be considered as the alteration of a particular trait while maintaining a uniform genetic background. If sufficient protective measures are taken and if sufficient background knowledge exists about the novel phenotype, then GMO offspring should be subjected to quantitative genetic breeding programs so that the mariculture product incorporates desirable traits of the whole genome.

National Reports on Transgenic Research of Marine and Anadromous Species

Canada: To date research has been limited on transgenic aquatic organisms in Canada. There are at least 6 laboratories conducting transgenic research, all on fish. One product for Atlantic salmon is currently available. This is a construct, which combines an ocean pout antifreeze promoter with a salmonid growth hormone gene. This construct is marketed by Aqua Bounty Farms, a subsidiary of AF Proteins Inc., who have offices in Massachusetts, USA and Newfoundland, Canada. In Prince Edward Island, Aqua Bounty Farms is rearing eggs of Atlantic salmon from GMO parents in a land-based hatchery.

Finland: Transgenic research on rainbow trout to enhance growth and metabolism is being conducted at the University of Kuopio, Kuopio, Finland.

*France:*Research on transgenic rainbow trout is conducted at INRA in Paris, and Rennes. Work on the anti-sense mRNA in GNRH is in progress.

Ireland: The only reported work on transgenics in Ireland is being conducted at the National Diagnostics Center, University College, Galway (see National Acitivity Reports below). Salmon transgenics are being studied. Recently, the introduction of the Aqua Bounty Farms salmon GMO was considered, but given that all salmon culture in Ireland is pen culture, importation of GMO was thought to be too high a risk at the present time.

Poland: Transgenic research is being conducted at a single laboratory in Golysz, Poland (Polish Academy of Sciences). Transgenic carp with human growth hormone genes have been produced. These fish caused considerably controversy when they were stolen from the outdoor ponds.

Scotland: The salmon GMO marketed by Aqua Bounty Farms, a subsidiary of AF Proteins Inc.(see above) is also being farmed in freshwater in a land-based operation in Scotland. These fish will eventually be transferred to seawater tanks on the same site.

Spain: Transgenic research on sea bass and sea bream is being conducted in the Mediterranean Basin.

New Zealand (Observer Status): Transgenic work on Pacific salmon (*Oncorhynchus*) and abalone is being conducted at the University of Canterbury (Project leader Dr. Frank Sinn).

Summary of

WGAGFM recommendations concerning GMO

1. The first priority for research relating to constructs of transgenic fish should be to investigate the secondary effects of the insert utilized. This should be associated with a marker sequence that would enable identification of the fish containing the construct. In this way, the potential secondary effects could be evaluated, by measuring phenotypic changes induced by the insert.

2. "All-(shell)fish" regulatory sequences are recommended. Research to increase the available number of regulatory sequences for mariculture applications is encouraged.

3. All countries within ICES should consider risk assessment protocols for GMO management.

4. ***Until there is a technique to produce 100% sterilization effectiveness, GMO should not be held in or in connection with open water systems.***

5. ***The possibilities of constructing closed systems for field testing of GMO should be explored.***

6. GMO production should be considered as the alteration of a particular trait while maintaining a uniform genetic background. If sufficient protective measures are taken and if sufficient background knowledge exists about the novel phenotype, then GMO offspring should be subjected to quantitative genetic breeding programs so that the mariculture product incorporates desirable traits of the whole genome.

Suggestions for Topics to be Addressed at the ASC Minisymposium on GMO, 1998

The WG considered topics for theme sessions at the ASC minisymposium (approximately 1/2 day) on GMO to be held at Lisboa, Portugal in 1998. In light of the above report and discussions held during the meeting, the following ideas were put forward:

- Current Status of GMO Production For Mariculture Applications
- The Use of Risk Assessment and Flow Charts for GMO Management

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2.B Review and evaluation of available computer software for population genetic analyses in fisheries and aquaculture

[Based on position papers by Dr. Paul Galvin, University College Cork, Ireland, and Michael Møller Hansen, Denmark. Adopted by WAGFM in Gdynia.]

Introduction

There are two primary considerations when choosing between the various software packages for analysis of genetic data:
(1) What are the relevant evolutionary models used and which of the packages base their analyses on those models?
(2) Which packages are the most "user friendly"?

It is clear from many reports and publications produced in the past that the latter consideration ranked highest. By the standards of that time BIOSYS-1 facilitated a relatively easy but comprehensive analysis of data generated from allozymes, and it is evident from the literature that an "acceptable standard analysis" of such data was as follows:

(a) calculation of levels of polymorphism and allele frequencies for each locus,
(b) χ^2 test for conformance of each of the taxonomic units at each locus with Hardy-Weinberg expected proportions,
(c) heterogeneity χ^2 analysis to test for significant differences between allele frequencies in different taxonomic units, analysis of F-statistics (including hierarchical analyses) to determine the proportion of genetic diversity within and among sub-units (F_{ST}), to estimate the rate of gene flow (from F_{ST}), and to determine if instances of non-conformance with HW expectations were due to excesses or deficiencies of heterozygotes (F_{IS}),
(e) calculation of Nei's 1972 or 1978 genetic distances, and
(f) generation of an UPGMA dendrogram from the genetic distances.

While this approach addresses many aspects relating to genetic variability in fish populations, its acceptance as a "standard" analysis, applicable to all situations, and the related presumption that the use of this kind of analysis enabled results from different studies to be easily compared, was an over simplification. Before beginning to discuss the merits of some of the more recently developed software packages, it is important to outline the issues that need to be considered.

Points to be considered in statistical analyses of data from biochemical and molecular markers

With the development of DNA techniques, and particularly the isolation of mini- and microsatellite DNA loci, many difficulties that have been noted with analysis of allozyme data, have become obstacles to analysis of the data resulting from studies involving these highly variable loci. The following points need to be considered:

(a) The basic aims of the study need to be defined at an early stage, in order to determine the most appropriate analyses to undertake. Discrimination between taxonomic units can be carried out using loci which are under the influence of selection, provided that the allele frequencies are stable over time. Separation of taxonomic units can be defined by significant results from a heterogeneity χ^2 analysis, by principle component analysis or using genetic stock identification algorithms. Studies aiming to describe population structure need to be based on selectively neutral loci, where there is at least some impression of the mutation rate: high mutation rates result in homoplasies and thus tend to underestimate differentiation.

(b) Low frequency alleles, and particularly genotypes have become more common as the number of detectable alleles increase. This may result in an increased risk of sampling error and hence,

difficulties associated with implementation of the various tests, due to the inappropriateness of the conventional contingency χ^2 test. Some of the new exact tests are partly the solution to this problem. However, it must at the same time be stressed that in order not to decrease the power of tests sample sizes should not be lower than those that would have been used for studies based on allozymes. In some of the early literature on mtDNA analysis, the potentially increased resolving power of the technique was suggested to reduce the need for large sample sizes. In fact, however, since mtDNA analyses involve sampling only n genes (as opposed to 2n genes for nuclear loci) it could be argued that sample sizes should be twice as large as for studies based on nuclear markers.

(c) Although the mutation rate of allozyme loci is generally regarded as being low (e.g. 10^{-6}), the rate of mutation of DNA loci can be considerably higher and may vary depending on the marker. Mutations rates of 10^{-1} have been estimated for some minisatellite loci in humans while rates of less than 10^{-6} have been estimated for some coding sequences. In the case of microsatellites mutation rates in the range of 10^{-3} to 10^{-4} are often assumed, but it must be stressed that mutation rates may vary considerable even among loci within the same class of marker. In general, very little is known about mutation rates in fish. Also, the mode of mutation varies among different DNA loci, and to date the mechanism by which mutations arise in VNTR (Variable Number of Tandem Repeats) loci is not clearly defined and thus it is impossible to determine which model of evolution can be applied to the interpretations of the data.

(d) While F_{ST} analysis can be used to determine structuring of genetic diversity within a species, in most cases the statistical significance of this structuring is left untested. The importance of such structuring is generally accepted as critical to management and conservation considerations, and thus it would seem imperative that appropriate testing of this aspect should be undertaken.

(e) The study of genetic population structure is a demanding task which, among other things, involves estimation of genetic differentiation and gene flow. For such studies great caution is required. Many studies, due to limited resources or availability of polymorphic loci are restricted in this respect. In studies where only the most discriminating loci are screened, there is a risk of any single locus being under the influence of either balancing or directional selection (i.e. non neutral), and biasing the conclusions as a consequence. What is generally required is that as many loci as possible are screened and preferably a consensus-type dendrogram should be used for interpretation, where the data has been subjected to numerical resampling. The genetic distance calculated together with algorithm used to generate the dendrogram, are also of great importance, as these reflect the assumptions that are being made regarding the evolutionary processes.

f) It is important to be aware of deviations from expected Hardy-Weinberg proportions. When the genotypic frequencies do not conform with HW equilibrium, some models and tests may not be valid.

g) Estimates of homozygosity and heterozygosity have taken on a new importance with PCR'able VNTR loci due to problems with "null alleles" (in particular due to mutations in the primer-binding region of the template DNA). Reporting of excesses or deficits of homozygotes / heterozygotes should be accompanied by data detailing how many (if any) of the individuals were not scored for a particular locus. Failure to get data from an individual may reflect the presence of a "null homozygote".

h) As stated earlier, the conventional χ^2 test is not appropriate for most VNTR data, due to the problems with very small expected numbers in many of the categories. The preferred alternative is based on Fisher's exact test. Computational difficulties associated with the implementation of the exact test with a large number of alleles has been overcome using a pseudo-random method of testing subsets of the data as described by Guo and Thompson (1992). HW assumes that genes are distributed independently into genotypes. Therefore, this test for HW conformance involves simulation that assume independent distribution of the genotypes, using the same allele frequencies as

in the original sample. By repeating the process several times (permutations), it is possible to build up a distribution of the genotype frequencies that would be expected based on HW expectations. The original genotypic frequencies can then be compared with the permutation results to determine the probability of the observed frequencies conforming with HW expected proportions. This overcomes the difficulty associated with small expected numbers for individual classes.

i) The conventional homogeneity χ^2 test of allele frequencies is also of limited value with respect to VNTR loci, due to difficulties with large numbers of alleles with low expected numbers in many of the classes. This problem can be overcome by numerical resampling techniques. A relatively simple example of this is the test decribed by Roff and Bentzen (1989) which is often applied to mtDNA data; the null hypothesis is that the samples are simply random subsets of a larger population. The test therefore determines if the haplotype frequencies reflect haplotypes distributed independently into samples. This is done by calculating a χ^2 value on the basis of the original data set. Next, the haplotypes from all samples are pooled, haplotypes are sampled randomly equivalent to those of original population sizes from the poled data set, and a new χ^2 value is calculated from this new data set. This is repeated a number of times, and the p-value is determined as the relative proportion of resampled χ^2 values that exceeded the original χ^2 value.

Some widely used numerical resampling techniques are permutation, bootstrapping and jackknifing which operate on a slightly different basis. Permutation involves permuting, for instance, alleles among individuals; bootstrapping involves randomly resampling subsets of the original data to provide new samples of equivalent size as the original (sampling with replacement); jackknifing eliminates one observation of the data (e.g. individual / locus) at a time, so that the number of new data sets is equal to the number of observations. The latter two techniques allows for calculating variance within each data set (95% CIs from bootstrapping; SDs from jackknifing), and are thus applicable in a number of test situations.

j) Wright (1931) introduced the statistic F_{ST} which he defined as the proportion of the total genetic variability which was due to differences among populations. There is an increasing range of algorithms for the same purpose. Various authors use other assumptions about the evolutionary model and consequent modifications to the algorithm. These include θ, β_{ST}, G_{ST}, and R_{ST}. The two most commonly used algorithms until recently were those of θ, and G_{ST}, however while the latter has the advantage of not showing negative values when sub-populations appear similar, it is also biased in those circumstances and results in an overestimate of the degree of sub-structuring. Over the last two years, some of the newer software packages have included calculations of R_{ST}, which assumes that microsatellite evolution is primarily driven by the stepwise mutation model. However, due to the difficulties involved in testing the mutation process of microsatellites, because of the fact that repeat units do not usually vary across a repeat region, this mutation model cannot be assumed at present. While early models assumed that mutations at microsatellite loci took the form of the gain or loss of a single repeat unit (conforming to a strict stepwise mutation model), available empirical evidence has demonstrated that this is certainly not the case (some of the latest models are therefore not based on a strict one-step mutation model). In fact, while recent studies by Jeffreys and co-workers have shown that a completely different mutation process (gene conversion type) dominates in minisatellite loci, there is no evidence to suggest that the same process is not also dominant in microsatellite loci. Therefore, any models which assume that there is a relationship between the number of repeat units and the divergence time of any two VNTR alleles, need to be interpreted with great caution. Such analysis should only be conducted alongside analysis based on alternative models, where any differences between the results can be noted. In other words, estimates of genetic differentiation should not be based only on calculations of R_{ST}, but F_{ST} should be calculated as well.

k) Until recently much of the literature relating to fish and shellfish population studies was based on Nei's 1972 and 1978 genetic distance measures. For situations where the population(s) have been stable over a long time, these are well defined measures. However, Nei's genetic distances depend on

mutation rates. In the case of many aquatic species, the extent of founder effects and genetic drift can be considerable and much more important than divergence due to different mutations accummulated in separate populations. Therefore the meaning of Nei's genetic distances in these situations is less clear. Two alternative measures are often recommended where genetic drift is an important factor. I.e. Cavalli-Sforza and Edwards (1967) chord distance, and Reynolds, Weir and Cockerham (1983) co-ancestry coefficient. Both these measures are based on F_{ST} values, where the relationship between genetic distance and time is approximately linear in instances of recent divergence. The major difference between the genetic distances of Nei (including the more recent D_A measure) and the F_{ST} based measures is that the former are absolute distance measures while the latter are relative measures (i.e. reflects the relative differentiation among populations). Also, Nei's distances weights intermediate frequency differences highly and differences between extreme frequencies much less (i.e. a much greater genetic distance would appear from differences between 0.55 and 0.45 than from 0.00 and 0.10). While the same occurs in the case of the F_{ST} based measures, the "problem" appears to be much less. Therefore, further research is required to determine the "optimal" genetic distance measure.

It should also be noted that where the genetic distances measured are very low (e.g. < 0.005), there is likely to be a large error associated with the distance (unless it has been derived from a very large number of loci).

l) UPGMA dendrograms have tended to predominate in past literature. While this method of phylogeny reconstruction has some favourable attributes, its underlying assumptions are rarely met. There are several "additive" type dendrograms that can be used which should be preferred. An example of one of these is the Neighbour-Joining method of Saitou & Nei (1987). Whatever algorithm is used for generating the dendrogram, bootstrapping procedures should be used followed by construction of a consensus dendrogram to enable the reliability of the nodes to be assessed. This involves bootstrapping over loci, which assumes that each of the loci represent independent data sources. As with all measures of genetic relatedness, generation of a dendrogram relies on the data that has been collected. Therefore, it is essential to have as many loci as possible included in the study, to enable the dendrogram to be interpreted as an "organism tree" rather than a "gene tree" (in the case of single locus data).

Software review

Diploid data

It would be beyond the scope of this document to provide a comprehensive listing of the capabilities of all available software packages and the document is more intended to promote discussion in this area. However, the performance of some of the more commonly used packages for analysing diploid data has been evaluated according to the following criteria:

A. User-friendliness (for instance the presence of well-structured menus and, in particular, good documentation/good manuals.
B. Easy and flexible input.
C. Compatibility with/output to other programs
D. Is the program regularly updated and are new analyses included?
E. Is the program Windows based.

In addition, the presence or absence of a number of features has been recorded. The importance of most of these has been described previously in the paper. The features considered are:

1. Calculation of allele frequencies
2. Calculation of average expected heterozygosity
3. Tests for fit to expected Hardy-Weinberg proportions (preferably exact)

4. Tests for gametic phase disequilibria (not only useful for testing for linkage between loci but also for detecting admixture populations, among other things)
5. Tests for differences of allele frequencies among populations
6. F_{ST}/G_{ST}, preferably unbiased, such as Weir & Cockerham's (1984) estimators.
7. Mantel test (test for correlation between two matrices, e.g., genetic and geographical distances between populations)
8. Isolation by distance analyses
9. Calculation of genetic distances and/or output to software doing this + constructing trees/dendrograms

In addition to these tests and statistics which are very useful for analyses of allozyme data the following features may be useful for analyses of microsatellite and other VNTR loci:
10. Calculation of distances between individuals, which can be used for constructing neighbor-joining dendrograms based on individuals.
11. Calculation of distances incorporating sizes of alleles (BUT assuming a step-wise mutation model).
12. Calculation of R_{ST} (assumes a step-wise mutation model)
13. Assignment test (Paetkau *et al..*, 1995; individuals are assigned to one of a defined set of baseline samples according to the likelihood of the individual's multilocus genotype being derived from any particular sample [based on the allele frequencies of the sample]).

The following packages have been evaluated:

Biosys (by Swofford & Selander, 1981)
Genepop 3.0 (by Raymond & Rousset, 1997)
Microsat (by Minch *et al.*, 1995)
GENETIX 3.0 (by Belkhir *et al..*, 1996)
G-STAT 5.0 (by Siegismund, 1996)
It must be stressed that the list is far from complete. For instance, programs such as Arlequin (by Excoffier *et al..*, 1996) and FSTAT (by Goudet, 1995) have not been considered.

Summary of
Evaluation of some genetic software packages

Features:	Biosys	Genepop	Microsat	GENETIX	G-STAT
A	*	***	*	***	***
B	*	***	*	***	**
C	**	**	**	**	***
D	*	***	?	***	***
E	no	no	no	yes	no
1	+	+	+	+	+
2	+	+	(?)	+	+
3	(+)	+	-	+	+
4	?	+	-	+	+
5	(+)	+	-	+	+
6	(+)	+	+	+	+
7	-	+	-	+	+
8	-	+	-	-	-
9	+	-	+	+	+
10	-	-	+	-	-
11	-	-	+	-	-
12	-	+	+	-	-
13	-	-	-	-	+

* = insufficient, ** = satisfactory, *** = very good
(+) The feature is included, but not highly sophisticated (e.g., not calculation of unbiased estimates)

It is evident that no single package contains all features but, overall, the GENEPOP and GENETIX packages must at present be considered the best available platforms for analysing diploid data. At the other end of the scale the BIOSYS package must be regarded as largely outdated, though it should be noted that this package allows for performing hierarchical analyses of genetic differentiation (F-statistics). This feature is missing from most new packages. Some other shortcomings of the packages include lack of possibilities for doing multivariate analyses, and lack of tests for neutrality. Multivariate analyses can be done with general software for statistical analyses, but the inclusion of this feature in commonly used software for analyses of diploid data might ensure a more widespread use. A test for neutrality (Watterson, 1978) is included in the G-STAT package, but it should be recommended to include this or other tests for neutrality in other packages as well.

mtDNA data
In addition to software for analyses of diploid data a few packages for analysing mtDNA RFLP data should be mentioned (mtDNA sequence data have not been considered).

REAP (by McElroy *et al.*., 1991). Performs Roff & Bentzen (1989) "Monte Carlo" tests, calculates molecular distances among haplotypes as well as among populations, and writes output to PHYLIP and other programs.

RESTSITE (by Miller, 1990). Calculates molecular distances, incl. bootstrapping and jackknifing. Draws Neighbor-Joining dendrograms.

AMOVA 1.53 (by Excoffier *et al.*., 1992). Performs "analysis of molecular variance", i.e. estimation of genetic differentiation. The program calculates Φ-statistics, related to Weir & Cockerham's F-statistics, but for haploid data, and molecular distances between haplotypes are/may be included. The statistics are tested, using a permutation approach.

Genetic distances and dendrograms

The most commonly used package for calculating genetic distances and drawing trees and dendrograms is the PHYLIP package (by Felsenstein, 1993). It covers almost everything, for instance Neighbor-Joining, UPGMA, maximum-likelihood trees, different sorts of parsimony etc., etc. Microsatellite distances and distances based on RFLP data are not included. However, distances can be generated by other programs and imported.

A number of other packages are available, but none of these are as universal as PHYLIP. A listing of these packages may be found in the documentation for PHYLIP.

In conclusion, there are now numerous packages available for analyses of genetic marker data. Many of the programs can be downloaded from the World Wide Web, and for listing of genetics related software it would be useful to check out the following sites:
http://phylogeny.arizona.edu/tree/programs/programs.html.
http://www.ucmp.berkeley.edu/subway/phylo/phylosoft.html
http://evolution.genetics.washington.edu/phylip/software.html
Some specific addresses for software:
GENEPOP (ftp.cefe.cnrs-mop.fr)
F-STAT (ftp.oracle.bangor.ac.uk, login: anonymous, go to cd pub/fstat)
PHYLIP (http://evolution.genetics.washington.edu/phylip/getme.html)
DISPAN (ftp.bio.indiana.edu/molbio in directory molbio/ibmpc as files dispan.*)
ARLEQUIN (http://anthropologie.unige.ch/arlequin)
GENETIX (K.Belkhir, Laboratoire Genome et Populations, CNRS URA 1493, Montpellier, France).

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2.C Review of DNA molecular markers

[Based on position paper by Geir Dahle, Norway. Adopted by WGAGFM in Gdynia]

Introduction

Marine fish species populations are normally numerous, and experience no geographical or physical barrier that could prevent migration and interaction between different groups. They show on average much less population structuring than anadromous or freshwater fishes (Ward *et al.* 1994), and the degree of gene flow among marine subpopulations (N_e*m) is considerable greater than among freshwater populations.

In mariculture the increasing importance of marine fishes prompts the need for genetic markers to assist in selective breeding, both in the identification of families in selective breeding programmes, and with the aim of identifying economically important trait loci, e.g. increased disease resistance, higher growth performance, delayed maturation etc. Marine fish eggs and larvae are very small compared to salmonide, and experience a very high mortality through metamorphosis. The size of eggs and fry/larvae and high mortality through metamorphosis makes it virtually impossible to discriminate between families in marine species.

Hundreds of protein coding loci and thousands of nucleotide sequences exist, any of which may be potentially useful in identifying populations, family groups, or individuals.

Genetic markers

"Any population of a species that are distinguishable from other populations by significant differences in their allele frequencies at one or more detectable loci are genetically marked" (Utter and Seeb, 1990).

Stock or population separation questions usually arise when small but consistent differences appear in some characteristic of the species (morphology, life history, growth rate, feeding habitats etc.) between two or more areas. Only in rare instances however, are these small differences indicative of fixed genetic characteristics.

Genetic techniques may be made more useful in situations of low natural genetic variation by one of two procedures:

a) increase sample size to several hundred
b) more powerful techniques

Analyses of the DNA molecule can provide better resolution of genetic variation than does protein electrophoresis, but the number of loci that can be analysed are currently limited in most species.

Allozymes

Electrophoretic data suggests that pelagic species show little genetic differentiation at allozyme loci over wide sea areas. A comparison of genetic subdivision within freshwater, anadromous and marine species, shows that the average proportion of the total genetic diversity allocated between areas is approx 30% for freshwater, 4% for anadromous and 1.5% for marine fish. The limited genetic differences between many marine stocks could either reflect a larger gene flow in the marine environment, or too little time for genetic differentiation to occur after the retreat of the last glacial. Nevertheless, the allozyme markers have shown quite useful for stock identification and characterization. Fish geneticists have used starch gel allozyme electrophoresis as their primary tool to charaterize genetic variation at the population level in various fish species. In mariculture, however, (e.g.family studies) the allozyme markers are normally not very useful as natural genetic markers. Since many mutations do not alter the electric charge of proteins, only 30 % of the genetic variation in allozymes can be detected by electrophoresis.

Southern blotting/hybridisation

DNA separated by agarose gel electrophoresis is transferred to a membrane (nitrocellulose or most common nylon). The DNA is then fixed to the membrane by baking it, and the membrane containing the DNA can then be hybridised with various probes. Probes are normally labelled with radioactivity (32P), and in the following autoradiography, an X-ray film will indicate the position of the fragments/sequences complementary to the radioactive labelled probe. The technique is quite expensive, and requires good quality DNA.

PCR (Polymerase Chain Reaction) -technique

The Polymerase Chain Reaction - PCR is a new tool for molecular biology that has revolutionised molecular biology and molecular genetics in particular. The technique is so sensitive that a single DNA molecule can be amplified, and single copy genes extracted out of a complex mixture of genomic sequences, and visualised as distinct bands on agarose gels. Numerous applications have evolved since the discovery of this technique, but each technique and often each study requires an optimisation of the PCR conditions, makes it possible to isolate sections of DNA by identifying the flanking regions of the section, and constructing primers complimentary to these regions.

mtDNA - RFLP

The restriction-fragment-length-polymorphism of mtDNA was the first of the DNA related methods used in fish population genetics. Little variation has been found in most marine species studied, even on the population level, and it might not stand up as one of the candidates of a future genetic marker method.

Species in the North East Atlantic have obtained their current distribution within the past 8 - 12.000 years, following the retreat of the last glacial. In such a short time we might expect the mtDNA of small populations to diverge, but large populations which constitute a sustainable fishery are not likely to exhibit large differences, unless the fishing is selective.

According to Brown (1983) mitochondrial DNA evolve at a rate of 2% per million year, or 330 base pairs assuming a molecule with the size of fish mtDNA (16.500 bp). Considering the time since the last glacial period (approx. 10.000 years) this should correspond to 3.3 bp substitutions in the entire mitochondrial chromosome. Combined with the small percentage of the mitochondrial genome normally actually analysed with restriction enzymes (1-4%), the possibility of finding any postglacial divergence is very small. Fortunately the discrimination between stocks does not necessarily rest only upon postglacial divergence, but might be based on pre-existing mtDNA polymorphism.

Johansen *et al.*. (1989) showed that the homology in the aminoacid sequence at several mtDNA genes is much higher between cod and salmonids than among mammals. This would indicate that amino acid substitutions in fish mtDNA encoded proteins is about 5 times lower than among mammals, reducing the potential use of fish mtDNA in population genetic studies.

The discovery of the PCR technique has however uncovered new possible applications of the mtDNA genome. Amplification of specific parts of the mtDNA combined with restriction digestion (of larger amplified fragments) or sequencing of 300 to 500 base pair fragments has revealed new possible applications of this molecule. The PCR technique has made the mitochondrial genome accessible for large scale population studies. One of the most variable regions in the mtDNA chromosome of mammals, the D-loop, has however shown less or no variation (e.g. eel) in marine species. Furthermore the existence of pseudogenes would interfer with interpretation of results unless primersets are made from clean mtDNA.

VNTR's - Variable Number of Tandem Repeats

Nucleotide sequence divergence can be useful in describing phylogenetic relationships of haplotypes, but not necessarily relationships of populations or species. The number of haplotypes and their spatial distribution, on the other hand, may be more useful for assessment of structure and gene flow among populations.

General classification of the genome differentiate between coding (unique) and non-coding regions. Only about 1% of the billions of nucleotides in the genome regulate or codes for essential proteins, and of the coding regions only those identifiable with histochemical stain can be used in allozyme analyses.

Most of the non-coding region is made up of variant sized stretches, often tandemly repeated sequences. The size of the repeated sequence and consequently the size of the allele is the only difference between mini- and microsatellites. The different alleles at each loci is determined by the number of repeats. The evolution of new alleles is believed to be a stochastic process not influenced by selection, but by mutation and genetic drift. Different alleles can primarily occur due to differences in the number of repeat units which make up the allele, or due to mutations at the restriction sites.

Minisatellites

Minisatellite DNA fingerprinting studies of several species have revealed an alternative source of highly polymorphic markers. Due to their complexity however, multilocus profiles are only of limited value for studies at the population level.

The minisatellite regions giving multilocus profiles, are very complex, have a high mutation rate and heterozygosity, and should therefore be ideal for pedigree and parenthood testing. Due to the high mutation rate at these minisatellite loci, approx. 5% per gamete for the most unstable human minisatellites isolated, occasional novel alleles, i.e. size mutations, have been observed in other pedigree analyses of minisatellite loci. Although studies so far indicate relatively low mutation rate in Atlantic salmon, this germline instability must therefore be taken into account when using hypervariable loci as genetic markers particularly in pedigree and parenthood testing.

The locus-specific minisatellite probes, single locus probes (SLP's), provide a more practical approach to population genetic studies. SLP's provides a set of highly informative genetic markers for both individual and family identification. Some loci, particularly the less variable ones should prove to be valuable in population studies as well. A single-locus is a better candidate for a population comparison than the complex fingerprint provided by the universally used 33.15, 33.6, and other similar probes.

Screening of variation with SLP's involves digesting the genomic DNA with a restriction enzyme, separating the fragments by agarose electrophoresis, transfer the DNA to a nylon membrane by Southern blotting and hybridisation of the membrane bound DNA to the SLP (labelled with 32P). Allelic variation is then revealed by autoradiography, and approx 1500 individuals could be analysed per man year.

Screening of VNTR loci will provide many additional genetic markers for more accurate assessment of inbreeding effects. Single locus VNTR probes normally reveal from a few to more than 20 alleles per. locus, and rare alleles are likely to be lost in a hatchery facility due to inbreeding.

An alternative method of identifying a minisatellite region is by identifying the flanking region of the minisatellite locus, produce a primer, and amplify the minisatellite region by PCR.

cDNA

Single locus nuclear restriction fragment length polymorphism's (RFLPs) detected by anonymous cDNA probes.

The cDNA method is based on restriction site polymorphism in the flanking regions of single complimentary DNA (cDNA). cDNA probes are made from mature polyA mRNA from liver tissue cDNA library, and therefore represent a transcribed functional gene, like Actin and Myosin. The samples are treated basically like minisatellite DNA, with blotting and hybridisation. The most common alleles arise from presence or absence of restriction sites in the vicinity of the single-copy gene probed.

Like mtDNA-RFLP, multilocus and SLP minisatellite, anonymous cDNA markers all have in common the need to first cut the DNA with a restriction enzyme, transfer it to a nylon membrane, and then probe the membrane by hybridising a labelled probe to the membrane-bound DNA. This is a very laborious method compared with the PCR-technique, and it is also recommended that the cDNA probes are sequenced, to identify the probes.

As with other RFLP methods, resolution of alleles can be poor resulting in data being "binned" for analysis. This information loss may result in homogenization of the populations.

Microsatellites

Microsatellites are small non-coding repetitive loci in the DNA, that can be amplified with the PCR-technique. The nature of the microsatellites makes them very well suited as markers in different genetic studies. Microsatellites have been used with success in species like cod, salmon and tilapia both in population genetic studies and monitoring of family groups. The microsatellite analysis offers a very fast and accurate technique, much faster than "normal" DNA fingerprinting. The method is however, dependent on species specific primers, although some microsatellites may be used across closely related species.

Microsatellite loci can be found to have low, medium or high variability. According to the purpose of the investigation (i.e. studies of families or populations), it would be possible to choose the suitable microsatellite loci (primer set which recognises the loci) displaying the right number of alleles. This implies that although primers primarily made for one type of study, monitoring of offspring/family groups, easily could be used for other purposes as well (population genetic studies of wild populations, and screening of broodstock in different countries).

Microsatellites normally are advantageous compared with minisatellites since the alleles are easily amplified from minute amounts of DNA by the PCR technique. This in combination with species specific primers and the fact that the amount of variation can be tremendous in these loci, makes it at

present one of the most promising methods in population genetic studies. Variation at these VNTR loci is typically much greater than allozyme and mtDNA.

Microsatellite primers for studies in several different species are being produced constantly, and the results are yet too new to drawn any major conclusions. To get a good evaluation of the microsatellite loci questions about temporal stability and mutation rate should be addressed, as should possible linkage between the different alleles. A heterozygosity deficiency is also experienced in some studies. This could be caused by so-called "null-alleles", a condition to be studied for each species and primer set.

Using an automated sequencer in combination with multipexing, approx. 40.000 individulas could be analysed per man year. Large differences in allele size, would however require separate gel conditions to be scored.

RAPD

Amplification of random polymorphic DNA (RAPD) by using a small (normally 10 base pair) unspecific primer, is a very fast method, but the RAPD markers are usually dominant since polymorphisms are detected as either presence or absence of bands after PCR amplification. Multilocus polymorphic markers like RAPDs generated by PCR, allow the examination of genomic variation without prior knowledge of DNA sequences (Williams *et al.*., 1990, 1993).

Intrapopulation RAPD variation has been detected with different primers in tilapia (Bardaki and Skibinski, 1994). This suggests that RAPD analysis might be more sensitive than mtDNA analysis which failed to reveal variation within tilapia populations. The technique also offers the possibility of carrying out compatibility analysis with unlimited number of primers each detecting variation at several regions of the genome.

There has been performed limited RAPD structure analysis of fish populations, although the technique has been used extensively in studies of plants and microbes. Based on results from other species in particular plant and microbes, RAPD seem to be a fast and possible method for population genetic studies. The method is however, very fragile in both replication, interpretation and production of data.

AFLP

A combination of RAPD- and RFLP- analyses named AFLP-analyses (amplified fragment length polymorphism) is used to generate genetic markers in recent genetic population studys. Total DNA is cut with two restriction enzymes with recognition sites of six and four base pairs respectively, and adapters are ligated to the resulting protruding ends. A preamplification with primers matching to the adapters and posessing an additional selective nucleotide at the 3' end reduce the amount of restriction fragments. The PCR-products of the preamplification are used as a template for the final PCR. For this PCR the primer matching to the six basepair restriction site is end-labelled so that only fragments with at least one six-cutter adaptor end are detected ether in the autoradiography of a PAGE or by an automated sequencer. By adding selective nucleotides to the 3'end of the primers, the amount of bands, and to a certain extend the amount of polymorphisms on the gel, can be adjusted.

AFLP markers have various advantages over RAPD markers. Better reproduceability due to the higher annealing temperatures used in the PCR reaction, more fragments and potential polymorphisms can be discriminated because of the high resolution of PAGE. The method can easly be adjusted to different species and diversity levels.

Transcribed sequences

Transcribed sequences are generally used in phylogenetic studies as the sequence of genes are often conserved at the species level. Several genes have been particularly well studied, e.g., ruBISCO, while data on many common genes are available, e.g., actin, myosin. More often the inter gene spacers regions (IGS) or internally transcribed spacer regions (UTS) are used in population studies as they are variable. However, it is usually necessary to sequence the areas if they are small, which is expensive, and time consuming (see Sequencing below).

Sequencing

Although this could be a method for identifying individuals and families, it does not seem like a practical method in mariculture. The technique is not particularly fast, demands skilled workers and creates a large amount of «unnecessary» information. The new method of cycle sequencing adapted through the PCR-technique, has greatly enhanced efficiency, and increased the speed with which to analyse new sequences.

The mtDNA evolves rapidly, and does not undergo recombination. The molecule is therefore an excellent marker for studies of both long and short term genetic changes. Population specific differences or genotype frequency differences may be observed, depending on the region surveyed. Beckenbach *et al.*. (1990) sequenced a 2214 bp fragment of the mtDNA from six different rainbow trout. Two fish from the same river displayed identical DNA sequences, but differences were observed which distinguished the rainbow trout from five locations.

PCR-band examination of mtDNA (Carr and Marshall 1991) displayed significant differences in mtDNA genotypes of cod from Newfoundland and Norway. McVeigh *et al.* (1991) sequenced part of the mtDNA (cytb) in Atlantic salmon from 8 North American and 11 European populations. Compared to Atlantic cod (Carr and Marshall, 1991), they found a very low number of variants, only 3 genotypes. All European samples did however, contain the same cytb gene sequence (genotype B), while all 3 genotypes were found in North American Atlantic salmon. 38 of the fishes were then examined for both mtDNA-RFLP and cytb sequence: All "European" on the basis of RFLP corresponded to genotype B, while the North American genotype according to Bermingham comprised of the A and C genotype.

Conclusions

The various options of molecular genetic markers in fish species, has given both the scientists, fisheries managers and farmers new ways to identify and discriminate between populations, individuals and families, and the PCR technique has increased the speed and accuracy by which these analyses can be performed.

Identification of families and individuals is probably best done today by various VNTR methods, although the RAPD technique has not been fully investigated neither in large scale population surveys nor in extensive pedigree studies.

At the molecular level, which method to use depends not only on the discriminating power that is needed, but on the technical skill of the staff. The possibilities of the PCR technique will also make the different molecular techniques more easily available.

Knowledge about the intraspecific genetic structure and a thorough understanding of the evolutionary relationships among naturally reproducing populations are essential prerequisites for planning and carrying out biological sound management. In marine fish, with high effective population size, a few

hundred animals exchanging every generation, will prevent genetic differentiation. "Recent" separation is not enough to accumulate genetic differences.
RFLP and fingerprinting (micro- and minisatellites) offers the promise of being able to determine genetic relations between spawning groups by using DNA sequences which evolve more rapidly than conventional allozyme polymorphisms identified by gel electrophoresis. The PCR technique has also made the mtDNA more available for large population surveys.

Despite the growing amount of genetic data generated and the increasing use of DNA methodologies in population structure analyses, there will still be considerable disagreement about whether such information will be useful to fisheries management or the mariculture industry. Molecular genetic research is still in its infancy, and until more data have been accumulated we must extrapolate the information from other organisms. Present and future available DNA related techniques makes it important to choose the right method. The user must be prepared to make several attempts with various probes, different parts of the genome, different primers etc. to find a suitable genetic marker. He/she must make a decision:

mtDNA or nDNA
VNTR's or specific, unique genes
PCR or RFLP or a combination

In combination with further advanced biological and behavioural studies, the new molecular techniques could improve fisheries management, but it is important to remember that no single technique is optimal for every question, problem or species (Table 1). And even though we manage to find systems to identify populations or stock, we also have to know how to translate the data obtained into fishery practice.

Summary of

Evaluation of DNA molecular markers

Table 1. Description and evaluation of different genetic markers and techniques

	Allozymes	mtDNA*			scnDNA*			VNTR's			Microsat.	RAPDs	AFLPs	Transcribed seq.
		1	2	3	1	2	3	Single	PCR-able minisat	Multi				
Tissue preservation[1]	F/Fr	F/Fr/E	F/Fr/E/D	F/Fr/E/D	F/Fr/E	F/Fr/E/D	F/Fr/E/D	F/Fr/E	F/Fr/E/D	F/Fr/E/D	F/Fr/E/D	F/Fr/E	F/Fr/E/D	F/Fr/E/D
Tissue quality[2]	H	H	L	L	H	L	L	H	M	H	L	H	L	L
Sacrifice of specimens[3]	O	O	N	N	O	N	N	O	N	O	N	N	N	N
Number of loci	10^2-10^3	1			$> 10^6$			10^4			10^4-10^5	Unknown	Unknown	1
Cost (labor/cons.)[2]	L	M	L	L	H	M	M	H	L	H	M	L	M	M
Pedigree analysis	NS	NS			Good			Excellent			Good	Fair	Excellent	NS
Population genetics	Good	Good			Good			Good		NS	Good	Fair	Good	Good
Hybid zones	Good	Good/Excellent			Good			NS			NS	Fair	Fair	NS
Phylogenetics	NS	Good			Good			NS			NS	NS	NS	Excellent

* 1, RFLP analysis; 2, PCR/RFLP; 3, PCR/sequencing

[1] Preservation; **F**(resh), **Fr**(ozen), **E**(tanol), **D**(ried)

[2] Quality and cost; **H**(igh), **M**(edium), **L**(ow)

[3] **O**(ften), **N**(o).

2.D National activity reports / international cooperation

The list of projects going on in the European countries and in Canada has been steadily increasing during the last four years, and now represents a valuable reference list for researchers seeking information of, or cooperation with, specific research milieus in the European countries and Canada. Still, however, it is a drawback that information from the U.S members of WGAGFM about relevant activities in U.S.A is lacking.

It is a general impression, from recent years' discussions and activities in WGAGFM, that the interest in international cooperation has been increasing. This is seen in many areas, ranging from an increase in the exchange of samples, to joint studies and transfer of laboratory technology. Some ongoing EU projects are run by partners from WGAGFM and were initiated during annual meetings in the Working Group. The National Activity Reports are listed in Appendix 1.

2.E Genetic aspects of interactions between farmed escaped salmonids and wild populations

[Based on position paper by Tom Cross, Ireland. Adopted by WGAGFM in Gdynia.]

Introduction

The original terms of reference for this item suggested that attempts should be made to quantify the scale of escapes from sea cages and to discuss the possible interactions that might occur with wild salmonid populations. These queries were addressed to several working groups and it was then decided that the WGAGFM would only deal with the genetic aspects.

In this document, the possible effects on intra population variation are first considered and the effects on inter population variation are then discussed. Finally, genetic methods of minimising effects are considered.

Throughout this document the convention of referring to wild catchment groups as "populations" and farmed escapes as "strains" is adopted. The majority of this discussion document refers to Atlantic salmon, *Salmo salar*, which has a farmed production of more than 400,000 t around the North Atlantic. We are aware of one case where brown trout, *Salmo trutta,* are farmed in sea cages (near Brest in France). Similar considerations apply for brown trout to those discussed below for Atlantic salmon.

We also note that the incidence of hybridisation between Atlantic salmon and brown trout can increase substantially in the presence of female farmed escaped salmon and that an EC project has just concluded on this aspect. We will await the publication of a summary of this report before commenting further on hybridisation.

Rainbow trout, *Oncorhynchus mykiss*, are not native to countries bordering the North Atlantic and seldom breed in the wild. Thus, the only genetic concern with escapes of this species would be that they would have indirect genetic effects (as defined below) on native salmon or brown trout populations, but by competition for resources, rather than destruction of redds.

It should also be noted that most of the effects discussed below result from the interactions between non-native reared fish and their wild conspecifics. Therefore similar problems can arise when ranched salmon enter the wrong river or when reared fish are deliberately released in stocking exercises.

Single incursions of reared salmonids into wild populations are first considered. It should, however, be noted that incursions will usually be repeated on an annual basis. This will be discussed further below.

Effects on intra population variation

Such effects can be either indirect or direct. Indirect effects (without interbreeding) could result from farmed escapes physically damaging the redds of a wild population or spreading a disease which causes high mortality among the native salmonids. The reduction in wild population size could be so severe that reduction in genetic variability would result. There appear to be no documented cases of this phenomenon but since escapes often ascend rivers to spawn later than wild populations, such effects could occur, particularly if the number of wild spawners is small (<100) and the incursion is large.

Where interbreeding occurs, direct effects result. Escapes can breed amongst themselves and with native salmon resulting in progeny of four types (wild X wild, farmed X farmed and the hybrids in both directions).

Relevant information comes from a recent field experiment in Ireland (EC AIR1.CT92.0719; Summary of Final Report), where an interaction situation was simulated in a spawning stream. In this study, wild families survived approximately twice as well as reared families during the freshwater phase. Similar results were observed with two consecutive year classes. It should be emphasised that no such survival differentials were observed with hatchery controls (a proportion of each of the individual wild, farmed and hybrid families).

Three of the groups of hybrids approximated to the mid-parent value in the field situation, though the first cohort of wild X farmed hybrids exceeded this value, perhaps implying heterosis. Relative fitness in the ocean was not investigated but results from Norway suggest that the overall performance of farmed escaped salmon compared with natives is substantially lower. Norwegian results also indicate lower reproductive fitness for farmed escaped male salmon than for females. In field experiments in northern Spain, which were part of the same AIR project, significantly better performance was observed from native salmon in freshwater than from introduced fish from Scotland.

Recommendations:
Experiments should be set up to compare the performance of native and escaped salmon during the oceanic phase and on return to the river. A similar recommendation is contained in the 1994 WGAGFM report.

In another phase of this project, the interactions between farmed escapes and native salmon were compared in two opportunist situations in the north west of Ireland. Both situations represented escapes from the same farm, from the freshwater and sea water rearing facilities respectively. Whereas genetic profiling had been used to identify individual families in the manipulated example given above, in this type of opportunist situation it was necessary to test an array of markers seeking the most discriminatory. Two were chosen, which were almost fixed for alternative types in the farmed and wild fish, at mtDNA and a minisatellite nuclear DNA locus. In both situations there was an indication of high numbers of farmed fish having escaped in one of the years studied, and in case of the sea farm there was evidence of successful spawning in nearby rivers. Insufficient discrimination was evident with the minisatellite locus to detect hybridisation. It was noteworthy that the genetic evidence of farmed fish diminished rapidly over the next two years when no further escapes seemed to have occurred.

Inferences from these experiments

Because of the fitness differentials noted above and the empirical evidence for reduction of farmed influence over time, it might be predicted that farmed progeny would decline rapidly in number. It might also be predicted that such declines would be more rapid in situations where the farmed strain is comparatively low in genetic variability or where the reared stain originates from a geographically distant area. It is not clear how much genetic variation there is in the most popular farmed strains in Europe, when compared with wild populations.

Recommendation:
The levels of variability in reared strains should be investigated using the most appropriate molecular methods. The life history characteristics and other phenotypic traits of reared strains should also be noted and compared with those of native populations into which incursions occur.

The concept that reared strains from more distant locations perform less well in introduction situations than do salmon originating from local rivers has been suggested on the basis of the results of several authors, including those involved in the project described above.

Hybrid progeny between farmed escapes and wild salmon present a situation which has been less frequently investigated, but one perhaps which should be of more concern. There is some evidence of heterosis in one example cited above, but it should be recognised that such effects might be reversed by outbreeding depression in second or subsequent generations. For example, outbreeding depression effects have been demonstrated in second generation hybrids between introduced and wild Pacific salmon in Alaska, and have been extensively discussed in the context of the western North American salmon enhancement programme. There appears to be no documentary evidence of such effects in Atlantic salmon, but this may be because no sufficiently long term studies have been conducted. Thus, it might be predicted that hybrids would decline fairly rapidly in a wild situation and that introgression would not be a major problem, unless the incursion was large and the native population very small.

All of this assumes a single introgression event. Annually repeated events, which seem much more likely, would be expected to have more serious effects and the long term productivity of affected catchments, in terms of salmon numbers could decline.

Recommendation:
If at all possible, avoid regularly repeated incursions since these will have a far more drastic effect on native populations than single events.

When using molecular methods to investigate interaction events, it is also important to identify whether one is dealing with a marker that is affected by natural selection or one that is selectively neutral, since different consequences will be predicted in each case.

Effects on inter population variation

It is generally recognised that Atlantic salmon occur in a large number of relatively isolated riverine (or sometimes tributary) populations. These populations are maintained by accurate natal homing and there is thought to be limited gene flow between them. Farmed escapes in Europe usually now consist of a very limited number of strains. This was not the case in former years, but the industry is now dominated by a few egg producers who run effective breeding programmes, for traits such as fast growth and late maturity. Thus, escapes which enter many rivers in a particular area are likely to be of a single strain, and thus massively increase artificially induced gene flow. The seriousness of such a scenario depends on the degree of local adaptation of individual populations and also on the degree of domestication of the reared strain. There have been few genetic studies directed to local adaptation, though there is abundant proof of the importance of this effect from other studies. Population differences as demonstrated by many genetic techniques are not proof of adaptation since

genetic drift is a major factor. This must particularly have been the case in the founding of populations after the Pleistocene glaciations and in the present maintenance of small populations.

Recommendation:
Genetic experiments should be designed to test for local adaptation. It is noted that the genotype by environment effect which is calculated in quantitative studies gives an estimate of the degree of local adaptation. Thus quantitative investigations should be more utilised in such experiments.

As with considerations about individual populations, repeated incursions must be regarded as much more serious than single incidents. If incursions continue on a regular basis, a situation is anticipated where the current complex population structure is replaced by a single aggregation of considerably reduced fitness. Evolutionary potential would also be considerably reduced, if such a scenario were to prevail.

Genetic means of reducing impact

Two genetic methods have been repeatedly cited in debates about alleviation of problems caused by farmed escapes. These are the use of sterile salmon or of natives or near natives, in farming.

Sterile salmon:
The present method of inducing sterility is by triploidising all-female stock. This method is neither 100% effective nor fully acceptable to the farming industry, since triploids often perform less well in certain aspects of the culture cycle (see 1996 WGAGFM report). Other methods of inducing sterility, such as transgenic techniques should soon be commercially available, but the use of transgenics in cage culture is another problematic area (see section on GMOs in the present report). The assumption underlying the use of sterilisation is that sterile salmon would not enter rivers. This aspect also requires investigation, as does how production of sterile fish might be incorporated into breeding programmes.

Recommendation:
The behaviour and ecological impact of sterile (and particularly triploid all-female) salmon should be investigated.

Use of natives or near natives in farms:
While this idea may make theoretical sense it is practically unfeasible because of the present structure of the salmon farming industry where all successful ongrowing concerns use genetically improved strains emanating from a small number of breeding companies. Since the breeders for historical reasons use predominantly Norwegian strains, the aspiration to use local strains in other farming areas such as Scotland or Ireland is impractical. Even in New Brunswick and Maine, where much of the industry uses the St. John river strain, the problem of multiple wild populations suffering incursions of a single reared strain must be considered. It should also be recognised, that even if a native strain were to be utilised in farming, it would have undergone domestication, and thus would have reduced fitness in the wild.

Thus, it must be concluded that, at present, there are no reliable genetic methods for reducing the impact of farmed escapes on wild populations and that the principle of preventing escapes from culture facilities must be followed.

Recommendation:
Genetic methods of reducing the impact of farmed escapes on wild populations should be furtherinvestigated, but meanwhile, escapes from farms must be minimised or completely eliminated.

Summary of

WGAGFM recommendations concerning genetic impact cultured --> farmed populations:

1. Experiments should be set up to compare the performance of native and escaped salmon during the oceanic phase and on return to the river. A similar recommendation is contained in the 1994 WGAGFM report.

2. The levels of variability in reared strains should be investigated using the most appropriate molecular methods. The life history characteristics and other phenotypic traits of reared strains should also be noted and compared with those of native populations into which incursions occur.

3. If at all possible, avoid regularly repeated incursions since these will have a far more drastic effect on native populations than single events.

4. Genetic experiments should be designed to test for local adaptation. It is noted that the genotype by environment effect which is calculated in quantitative studies gives an estimate of the degree of local adaptation. Thus quantitative investigations should be more utilised in such experiments.

5. The behaviour and ecological impact of sterile (and particularly triploid all-female) salmon be investigated.

6. Genetic methods of reducing the impact of farmed escapes on wild populations should be further investigated, but meanwhile, escapes from farms must be minimised or completely eliminated.

3 WORKING GROUP BUSINESS

3.1 Comments on Working Group function

With the establishment of pre-prepared position papers and specific responsibilities for chairing sessions and thematic colloquia, WGAGFM has found a working form which has substantially enhanced the efficiency of the annual meetings. Likewise, the possibility to communicate by E-mail with most members has greatly simplified the administration of the WG. However, some members have still not supplied the chairman with fax numbers and E-mail adresses. Also, from a 'geographic' point of view, the engagement by U.S. members in the WG activities is still on the low side.

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 has previously noted this problem, in the 1994 as well as in the 1995 and 1996 WG Reports. WGAGFM once more recommends 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 meetings in 1998

The attendance at the 1997 WG meeting in Gdynia (15 attending members from 11 different countries) was better than in the three previous years. During discussions on meeting place in 1998, the WG responded positively to an invitation from Dr. Tom Cross that the University College, Cork, Ireland hosted the 1998 meeting in week 14 (March 30 - April 2).

Concerning Terms of Reference for 1998, it was thus decided to recommend that:

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 populaton 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 te 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 disuss 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 strategiy 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.

APPENDIX 1

NATIONAL ACTIVITY REPORTS 1997

Studies reported in standard format

BELGIUM

Study 1
LABORATORY/RESEARCHER: Royal Belgian Institute of Natural Sciences (RBINSc), Vautierstraat 29, B-1000 Brussels / T. Backeljau and B. Winnepenninckx. University of Antwerp (RUCA), Groenenborgerlaan 171, B-2020 Antwerp, Belgium / H. De Wolf. Joint programme with: University of the Azores, P / A.M. Frias Martins, C. Brito, R. Medeiros and L. Serpa, University of Santiago de Compostela, ES / C. Olabarria, University of Leeds, U.K. / J. Grahame and P.J. Mill and Regional Technical College, Galway, EI / E. Gosling.
SPECIES: *Littorinidae* (periwinkles), particularly *Littorina striata* (Mollusca, Gastropoda and Prosobranchia).
PROJECT FUNDING: EU MAST-III program; PRAXIS (Portugal); Belgian graduate student grant by the IWT; 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 vs phenotypic plasticity).
DESIGN: The whole geographic range of *L. striata* (Macronesian 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) analysed 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 and morphometrics of shell features.
STATUS: Ph.D. project in progress; license degree theses; ongoing program within MAST-III.

Study 2
LABORATORY/RESEARCHER: Royal Belgian Institute of Natural Sciences, Vautierstraat 29, B-1000 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: *Tapes decussatus* and *T. philippinarum* (Mollusca, Bivalvia).
PROJECT FUNDING: Royal Belgian Institute of Natural Sciences.
OBJECTIVE: Genetic characterization of *T. decussatus* in the Azores as a highly isolated stock of the species (founder effects, genetic differentiation, conservation and exploitation issues); investigating relationships and possible interaction between *T. decussatus* and *T. philippinarum.*
DESIGN: Temporal sampling of *T. decussatus* in Faja de Santo Cristo (island of Sao Jorge in the Azores); additional sampling of the species and of *T. philippinarum* along European coasts.
METHODOLOGY: Allozyme electrophoresis, in the future possibly random amplified polymorphic DNA and mtDNA sequencing or RFLP analysis.

STATUS: Ph.D. thesis of T. Willems.
COMMENTS: This project has been interrupted until the position of T. Willems at the University of Vigo is secured by a grant which has been submitted at the EU TMR program. Pending on the outcome of this application the project will continue.

Study 3
LABORATORY/RESEARCHER: Katholieke Universiteit Leuven, Zoological Institute, Naamsestraat 59, B-3000 Leuven / F. Volckaert and E. Daemen in collaboration with EIFAC (glass eel bank).
SPECIES: European eel (*Anguilla anguilla*).
PROJECT FUNDING: Ph.D. fellowship and University grant.
OBJECTIVE: Characterisation of the population genetics of the European eel, including genetic structure, gene flow and selection.
DESIGN: Comparative spatial analysis of 5 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 in press in Animal Genetics (1997).
COMMENTS: Project will most likely be continued. We are looking for collaboration with fish biologists. Future application at EU-FAIR is envisaged.

Study 4
LABORATORY/RESEARCHER: Katholieke Universiteit Leuven, Zoological Institute, Naamsestraat 59, B-3000 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 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 graduate theses. The project is open ended
COMMENTS: We welcome collaboration and have funds for a postdoc.

Study 5
LABORATORY/RESEARCHER: Katholieke Universiteit Leuven, Zoological Institute, Naamsestraat 59, B-3000 Leuven / F. Volckaert and E. Daemen. Univ. Würzburg, D / M. Schmid. Univ. Padova, I / M. Bortolussi. Instituto de Acuicultura de Torre de la Sal, ES / S. Zanuy. Sepia Conseil, F / A. Brunel.
SPECIES: European eel (*Anguilla anguilla*) and seabass (*Dicentrarchus labrax*).
PROJECT FUNDING: EU AIR2-CT93-1543.
OBJECTIVE: The isolation of sex-specific molecular markers in European eel and seabass.
DESIGN: Molecular markers are isolated in model species with known sex determining systems; this expertise is translated to seabass and eel.
METHODOLOGY: Various methods to isolate sex-specific DNA sequences such AFLP, microsatellite DNA fingerprinting, SOX and Smcy genes.
STATUS: Ph.D. thesis in progress; two publications in progress; contract finishes in May 1997.
COMMENTS: Project continues with national funds.

CANADA

Study 1

LABORATORY/RESEARCHER: Applied Breeding Technology, St. Andrew's, New Brunswick, E0G 2X0 / 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, market 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 2

LABORATORY/RESEARCHER: Salmon Genetics Research Program, Atlantic Salmon Federation, St. Andrews, New Brunswick, EOG 2XO / 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 3

LABORATORY/RESEARCHER: Salmon Genetics Research Program, Atlantic Salmon Federation, St. Andrews, New Brunswick, EOG 2XO / 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 4
LABORATORY/RESEARCHER: University of Saskatchewan / P. H. Krone.
SPECIES: Zebrafish (*Danio rerio*).
PROJECT FUNDING: NSERC.
OBJECTIVE: (1). Elucidate role which heat shock proteins play during normal developmental events and in the protection of embryos from environmental stress. (2). Elucidate mechanism responsible for regulation of heat shock protein synthesis during embryonic development.
DESIGN: Isolation of cDNA and genomic clones encoding zebrafish heat shock proteins and subsequent characterization of their patterns of stage- and tissue-specific expression during normal development and during exposure to heat shock and other environmental stresses. Expression data is then used as a basis for the design of microinjection experiments in which wild-type heat shock proteins and those altered through site-specific mutagenesis are expressed in embryos. Cultured cells are being used as an in vitro model to examine the role of these proteins.
METHODOLOGY: Isolation and characterization of cDNA and genomic clones encoding zebrafish heat shock proteins. Northern blot and in situ hybridisation analyses, microinjection, tissue culture and transfection.
STATUS: Ongoing.

Study 5
LABORATORY/RESEARCHER: Institute of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, B.C. V5A 1S6 / 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:
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 6
LABORATORY/RESEARCHER: Institute of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, B.C. V5A 1S6 / 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 7
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: used. These include gene cloning, promoter analysis in tissue culture cells, characterization of transcription factors, and the development of transgenic fish.
METHODOLOGY:
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 8
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 9
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:
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 10
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 11
LABORATORY/RESEARCHER: Magaguadavic Watershed Management Association, General Delivery, St. George, New Brunswick, E0G 2Y0 and Marine Gene Probe Laboratory, Dalhousie University, Halifax, Nova Scotia, B3H 4J7 / 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, N.B. Salmon Growers Association, N.B. Depart. of Fisheries and Aquaculture, Depart. of Fisheries and Oceans, N.B. 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, N.B., 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 12
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 13
LABORATORY/RESEARCHER: Memorial University of Newfoundland / T.J. Benfey and W.S. Davison.
SPECIES: Arctic charr and brook 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 Arctic charr and brook trout.
STATUS: On hold.
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 14
LABORATORY/RESEARCHER: Dept. of Biochemistry, Memorial University, St. John´s, Newfoundland / C. M^{c}Gowan and W. Davison.
SPECIES: Brown trout and Atlantic salmon.
PROJECT FUNDING: NSERC/DFO.
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: Four linkage groups have been identified to date for brown trout. This is an on-going project.

Study 15
LABORATORY/RESEARCHER: Dept. Fisheries and Oceans, Canada, West Vancouver Laboratory, 4160 Marine drive, West Vancouver, British Columbia, V7V 1N6 / 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 16
LABORATORY/RESEARCHER: Dept. of Biology, Memorial University / S. Carr. Dept. of Biochemistry, Memorial University / W. Davison. 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 January 1996. No evidence for genetic substructuring of turbot in the North Atlantic from as far apart as Norway and the Gulf of St. Lawrence.

Study 17
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: On-going project.

Study 18
LABORATORY/RESEARCHER: Simon Fraser University, Dept. of Biological Sciences, Burnaby, B.C., V5A 1S6 / B. McKeown and S. Tang.
SPECIES: Rainbow trout.
PROJECT FUNDING: NSERC funded.
OBJECTIVE: To characterised 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: Ongoing.
COMMENTS: This genes have been identified and sequenced. Expression in various tissues and conditions have been found.

Study 19
LABORATORY/RESEARCHER: Simon Fraser University, Dept. of Biological Sciences, Burnaby, B.C., V5A 1S6 / B. McKeown and K. Poon.
SPECIES: Rainbow trout.

PROJECT FUNDING: NSERC funded.
OBJECTIVE: To characterise the ras oncogene.
DESIGN: Gene cloning and controls of expression.
METHODOLOGY: Gene cloning.
STATUS: Ongoing.
COMMENTS: This gene has been isolated and sequenced. Work is now continuing on controls of expression.

Study 20
LABORATORY/RESEARCHER: Simon Fraser University, Dept. of Biological Sciences, Burnaby, B.C., V5A 1S6 / B. McKeown and K. Poon.
SPECIES: Rainbow trout.
PROJECT FUNDING: NSERC funded.
OBJECTIVE: To identify the growth hormone receptor gene.
DESIGN: Gene cloning.
METHODOLOGY: Gene cloning.
STATUS: Ongoing.
COMMENTS: We are presently in the process of trying to clone this gene.

Study 21
LABORATORY/RESEARCHER: R. M^{c}Gowan.
SPECIES: *Danio rerio.*
PROJECT FUNDING: NSERC.
OBJECTIVE: (1) To identify whether genome imprinting exists in the zebrafish and to then exploit the unique attributes of these fish to investigate the molecular details of that process.
(2) To investigate the role of methylation and the DNA methyltransferase gene in zebrafish development.
DESIGN: Breeding of transgenetic zebrafish to non-transgenic mates and track the methylation and expression of the transgene after inheritance of the locus from either males or females. Look at methylation 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: 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 22
LABORATORY/RESEARCHER: Animal and Poultry Science, University of Guelph / M^{c}Millan and M^{c}Kay.
SPECIES: Salmonids.
FUNDING:
OBJECTIVE: Genetic improvement of commercial stocks of salmonids in the province. (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: Ongoing.

Study 23
LABORATORY/RESEARCHER: Stocks Assessment and Genetics Unit, Ontario Ministry of Natural Resources, P.O. Box 5000, Maple, ON L6A 1S9 / 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 24
LABORATORY/RESEARCHER: Stocks Assessment and Genetics Unit, Ontario Ministry of Natural Resources, P.O. Box 5000, Maple, ON L6A 1S9 and M^{c}Master University / W. Stott, P.E. Ihssen and B. White.
SPECIES: Lake trout (Salvelinus namaycush).
PROJECT FUNDING: OMNR and NSERC grants and Internship program through MBS.
OBJECTIVE: Stock differentiation in Lake trout and its application to fisheries management.
DESIGN: Lake trout from six lakes with different stocking histories are sampled and analysed with different DNA markers and estimates of their effective population size compared.
METHODOLOGY: RFLP's of mtDNA, RAPD and microsatellite DNA.
STATUS: Ongoing to end in December 1996.

Study 25
LABORATORY/RESEARCHER: NRC Institute for Marine Biosciences, 1411 Oxford Street, Halifax, NS, B3H 3Z1 / Dr. S. Douglas (project leader), in collaboration with Marine Gene Probe Laboratory, Dalhousie University / Dr. D. Cook.
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 26
LABORATORY/RESEARCHER: NRC Institute for Marine Biosciences, 1411 Oxford Street, Halifax, NS, B3H 3Z1 / Dr. S. Douglas (project leader).
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 genome mapping in this organism.
STATUS: In progress.

Study 27
LABORATORY/RESEARCHER: NRC Institute for Marine Biosciences, 1411 Oxford Street, Halifax, N.S., B3H 3Z1 and Department of Fisheries and Oceans. (DFO), PO Box 550, Halifax, N.S. B3J 2S7 / C. Bird (NRC) and E. Kenchington (DFO) project leaders.
SPECIES: *Placopecten magellanicus* (sea scallop), other scallops (*Chlamys*, *Pecten*, *Argopecten* and *Crassodoma*), oysters (*Ostrea edulis* (European Oyster) and *Crassostrea*), mussels (*Mytilus* sp.) and 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: Nucleotide sequences of ribosomal RNA genes (including their internal transcribed spacers) are being evaluated as discriminants of taxa and populations, over 24 18S rRNA gene sequences registered with Genebank. DNA nucleotide sequence is less sensitive intraspecifically but provides a measure of species relatedness and identification.
STATUS: In progress.

Study 28
LABORATORY/RESEARCHER: NRC Institute for Marine Biosciences, 1411 Oxford Street, Halifax, N.S., B3H 3Z1 / 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: In progress.

Study 29
LABORATORY/RESEARCHER: NRC Institute for Marine Biosciences, 1411 Oxford Street, Halifax, N.S., B3H 3Z1 / Dr. M. Reith (project leader).
SPECIES: *Pleuronectes americanus*, winter flounder and 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: Dept. Biology, Dalhousie Univ., Halifax, N.S. / Dr. E. Zouros, DFO, PO Box 550, Halifax, N.S. / Dr. E. Kenchington. NRC Institute for Marine Biosciences, 1411 Oxford Street, Halifax, N.S., B3H 3Z1 / C.J. Bird.
SPECIES: *Placopecten magellanicus* (sea scallop).
PROJECT FUNDING: Natural Sciences and Engineering Research Council (NSERC) of ; DFO core funds, NRC core funds.
OBJECTIVE: 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 labelling 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 analysed from one bed to determine cohort effect.
STATUS: Ongoing.

Study 31
LABORATORY/RESEARCHER: Dept. Biology, Dalhousie Univ., Halifax, N.S. / Dr. E. Zouros. DFO, Halifax, N.S. / Dr. E. Kenchington and Dr. D. Stewart.
SPECIES: Blue mussel (*Mytilus*).
PROJECT FUNDING: Natural Sciences and Engineering Research Council (NSERC) of Canada; DFO.
OBJECTIVE: The biparental transmission of mtDNA in mussels and its association with extreme sex-ratios; implications for mussel aquaculture and for geographical differentiation and speciation. All female producing lines of *M. edulis* have been produced for two generations.
METHODOLOGY: Gene sequencing; PCR identification of mitotypes; controlled breeding experiments.
STATUS: Ongoing.

Study 32
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 and U. Kuhnlein.
SPECIES: Snow crab (*Chionoecetes opilio*).
PROJECT FUNDING: Department of Fisheries and Oceans.
OBJECTIVE: (1) Description of the population structure in the Northwest Atlantic; (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) Mating system is being studied under laboratory and field conditions. Experiments describing the behaviour 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 1997. 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 is ongoing.

Study 33
LABORATORY/RESEARCHER: Institut Maurice-Lamontagne, Ministère des Pêches et des Océans, Mont-Joli / J.-M. Sévigny. Département de Biologie, Université Laval, Québec / L. Bernatchez, M. Black and S. Roques.
SPECIES: *Sebastes* sp.
PROJECT FUNDING: Department of Fisheries and Oceans.
OBJECTIVE: 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 34
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.
OBJECTIVE: 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 lagunes of the Magdalen Islands and compared with the variability detected in mussel populations cultivated under various regimes of density.
METHODOLOGY: Allozyme.
STATUS: Ongoing.

Study 35
LABORATORY/RESEARCHER: Department of Fisheries and Oceans (DFO), PO Box 550, Halifax, N.S. B3J 2S7 / E. Kenchington. NRC Institute for Marine Biosciences, 1411 Oxford Street, Halifax, N.S., B3H 3Z1 / G. Harding, D. Pezzack and C.J. Bird.
SPECIES: Homarus americanus (American Lobster).
PROJECT FUNDING: DFO core budget, NRC core budget, NSERC.
OBJECTIVE: Genetic variation in selected populations of lobster to determine stock structure (preliminary investigation).
DESIGN: The lobsters used in population comparisons were collected from three areas: the Gulf of St. Lawrence (purchased as live canners from the wharf at Arasaig); inshore Southwest Nova Scotia (Lobster Bay, Yarmouth County); offshore Georges Bank (Corsair Canyon). Sample sizes were 24, 36, and 48 respectively. Immature lobsters (60-80 mm carapace length) were used in this study to ensure that the lobsters sampled are resident to the sampling area and not immigrants or seasonally migrating animals.
METHODOLOGY: RAPD markers.
STATUS: Publication accepted in the Can. J. Fish. Aquat. Sci. Chi squared analyses of the RAPD bands in lobster samples showed no difference between populations.. The genetic distance is greatest between the southern Gulf of St. Lawrence and both inshore and offshore lobsters from Lobster Bay, N.S., and Georges Bank, respectively. Project completed.

Study 36
LABORATORY/RESEARCHER: Dept. Fisheries and Oceans, Aquaculture Division, Gulf Fisheries Centre, Moncton, N.B / Mr. T. Landry (project leader) and T. Sephton.
SPECIES: *Mercenaria mercenaria* (Bay quahaug), local wild species and aquaculture "notata" variety.
PROJECT FUNDING: Can/NB/NS Cooperation Agreement for Alternative Species Research.

OBJECTIVE: Evaluate the growth, survival and production (performance) of seedstock from 3 sources of broodstock: 1) common quahaug (F1), 2) F3 broodstock from PEI, and 3) "notata" variety
DESIGN: 3 sources of hatchery produced seed are being compared in side-by-side replicated field trials (off-bottom cages and individually tagged, randomised block design) at 3 locations in the southern Gulf of St. Lawrence for a 2 year growth experiment.
STATUS: Ongoing.

Study 37
LABORATORY/RESEARCHER: Dept. Fisheries and Oceans,, Northwest Atlantic Fisheries Centre, P.O. Box 5667, St. John's, Newfoundland, A1C 5X1 / R. Penney (project leader).
SPECIES: Blue mussel (*Mytilus edulis*) and *M. trossulus.*
PROJECT FUNDING: DFO core funding.
OBJECTIVE: 1) Delineate existing edulis - trossulus proportions. on commercial mussel culture sites 2) Survey populations of edulis and trossulus for allelic variation.
DESIGN: Twenty-two populations have been sampled since 1994, including all major commercial shellfish sites.
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 *M. trossulus* mussels are widespread in occurrence throughout Newfoundland. Typically, most sites are a mix of *M. edulis* and *M. trossulus* types. Proportionally, *M. M. edulis* usually is the dominant species at most sites. There does not appear to be any geographic separation of species nor are any other distributional patterns apparent. Analysis of the allelic variation in the 4 isozymes is continuing.

Study 38
LABORATORY/RESEARCHER: Dept. Fisheries and Oceans, Northwest Atlantic Fisheries Centre, P.O. Box 5667, St. John's, Newfoundland, A1C 5X1 / R. Penney (project leader).
SPECIES: *Modiolus modiolus.*
PROJECT FUNDING: DFO core funding.
OBJECTIVE: To determine the allozyme patterns of the horse mussel for comparison with *Mytilus edulis* and *M. trossulus.*
DESIGN: 4 sites were sampled where *M. modiolus* grows either mixed with *Mytilus* species or immediately adjacent to *Mytilus* beds.
METHODOLOGY: Four isozymes will be used, MPI, GPI, PGM and LAP.
STATUS: New project.

Study 39
LABORATORY/RESEARCHER: Dept. Fisheries and Oceans, West Vancouver Laboratory, 4160 Marine drive, West Vancouver, British Columbia, V7V 1N6 / I. I. Solar (project leader) and E. M. Donaldson.
SPECIES: Salmonids.
PROJECT FUNDING: NRC-IRAP, DFO core funding.
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. 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 40
LABORATORY/RESEARCHER: University of Guelph, Ontario Agricultural College, Guelph, Ontario, N1G 2W1 / I. McMillan (project leader).
SPECIES: Salmonids.
OBJECTIVE: Genetic improvement of commercial stocks of salmonids in the province. Development of a spring-spawning commercial rainbow trout strain for industry.
STATUS: New project.

Study 41
LABORATORY/RESEARCHER: Department of Fisheries and Oceans, Suite 400-555 West Hastings Street, Vancouver, B.C. V6B 5G3 / T. Beacham, K. Miller and R. Withler (Project Contact).
SPECIES: Pacific Salmonids.
OBJECTIVE: 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 correlations with MHC genotype. A BKD challenge of chinnook salmon is underway.

Study 42
LABORATORY/RESEARCHER: Marine Gene Probe Lab., Dalhousie University, Halifax, N.S. B3H 4J1 / Dr. C. Taggart, Dr. D. Ruzzante, D. Cook and S. Lang.
SPECIES: *Gadus morhua* (Atlantic cod).
PROJECT FUNDING: Canadian Dept. of Fisheries and Oceans.
OBJECTIVE: 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 characterised 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, N.S. B3H 4J1 / D. Cook, S. Lang, and Dr. C. Taggart.

SPECIES: *Gadus morhua* (Atlantic cod), *Gadus ogac* (Greenland cod) and *Boreogadus saida* (Arctic cod).
PROJECT FUNDING: Canadian Dept. Fisheries and Oceans.
OBJECTIVE: 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.
METHODLOGY: 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: Biology Dept., Dalhousie University, Halifax, N.S. B3H 4J1 / Dr. J. Wright (project leader).
SPECIES: Pacific herring (*Clupea harengus*).
PROJECT FUNDING: Alaska Dept. Fish and Game.
OBJECTIVE: To examine population differentiation of this species and temporal stability of allele frequencies in Prince William Sound, Alaska.
METHODOLOGY: Microsatellite markers.
STATUS: Ongoing.

Study 45
LABORATORY/RESEARCHER: Biology Dept., Dalhousie University, Halifax, N.S. B3H 4J1 / Dr. J. Wright (project leader).
SPECIES: Atlantic salmon.
PROJECT FUNDING: Department of Fisheries and Oceans.
OBJECTIVE: To evaluate stocking and enhancement of Atlantic salmon in N.S..
DESIGN: We have examined stocking practices with endogenous fish and fish from other rivers on the Grand R. and LaHave R., N.S..
METHODOLOGY: Microsatellite markers have been developed for use on scale samples as old as 50 years.
STATUS: Ongoing. We have had great success with reading scale samples.

Study 46
LABORATORY/RESEARCHER: Biology Dept., Dalhousie University, Halifax, N.S. B3H 4J1. Dr. J. Wright (project leader).
SPECIES: Atlantic salmon.
PROJECT FUNDING: Private.
OBJECTIVE: Use of microsatellite markers for pedigree analysis and breeding programmes for a Scottish aquaculture company.
METHODOLOGY: Microsatellite markers.
STATUS: Ongoing.

Study 47
LABORATORY/RESEARCHER: Biology Dept., Dalhousie University, Halifax, N.S. B3H 4J1 / Dr. J. Wright (project leader).
SPECIES: *Tilapia.*
PROJECT FUNDING: NSERC.

OBJECTIVE: 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 48
LABORATORY/RESEARCHER: Biology Dept., Dalhousie University, Halifax, N.S. B3H 4J1 / Dr. J. Wright. NRC Institute for Marine Biosciences, 1411 Oxford Street, Halifax, N.S., B3H 3Z1 / Dr. J. Wright.
SPECIES: Various toxin producing strains of dinoflagellates.
PROJECT FUNDING: NRC core budget, pending.
OBJECTIVE: 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 49
LABORATORY/RESEARCHER: Science Branch, Department of Fisheries and Oceans, PO Box 5667 St. John's, NF A1C 5X1 / V. Pepper (project leader) and 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.
OBJECTIVE: 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 John 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 50
LABORATORY/RESEARCHER: Science Branch, Department of Fisheries and Oceans, PO Box 5667 St. John's, NF A1C 5X1 / V. Pepper (project leader) and T. Nicholls.
SPECIES: Atlantic salmon (*Salmo salar*).
PROJECT FUNDING: Project implemented in 1991. Present funding: SCB Fisheries Limited, 1995: Atlantic Fisheries Adjustment Program, 1994: Department of Fisheries and Oceans, 1991: Atlantic Fisheries Adjustment Program.
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 John 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 51
LABORATORY/RESERARCHER: Biology Department and Ocean Science Centre, Memorial University of Newfoundland, St. John's, Nfld., A1C 5S7 / Dr. D. Innes (project leader), Dr. R. J. Thompson and J.E. Toro (Ph.D. Student).
SPECIES: *Mytilus edulis* and *M. trossulus* (mussels).
OBJECTIVE: Physiology, ecology and genetics of the hybridising 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 marker: 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.

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: Dept. of Ecology and Genetics, University of Aarhus / E.E. Nielsen.
SPECIES: Atlantic salmon, brown trout.
PROJECT FUNDING: In house/ Danish Institute for Fisheries Research, Dept. of Inland Fisheries.
OBJECTIVE: Estimation of effective population sizes in natural salmon and trout populations from observed temporal changes in allele/haplotype frequencies. Studies of long-term temporal changes in allele frequencies in Atlantic salmon populations and estimation of genetic relationships among Danish salmon populations which are now extinct.
DESIGN: Selected populations are sampled at certain time intervals and screened using relevant techniques. Data from populations in the 1930's -1950's have been obtained by amplifying microsatellites from old scale samples.
METHODOLOGY: Microsatellites.
STATUS: Ongoing.

STUDY 3
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 genetic variability and differentiation in and among Danish brown trout populations and hatchery strains.
DESIGN: Sampling of trout from various localities.

METHODOLOGY: Microsatellites and mtDNA.
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.
SPECIES: *Coregonus lavaretus, C. oxyrhynchus* and *C. albula.*
PROJECT FUNDING: In house.
OBJECTIVE: Estimation of genetic variability, differentiation, and gene flow among populations.
DESIGN: Screening of samples from both geographically distinct populations and from populations spawning in different rivers with outlets in the same fiord.
METHODOLOGY: Microsatellites and mtDNA.
STATUS: Ongoing.

STUDY 6
LABORATORY/RESEARCHER: University of Aarhus / E.E. Nielsen, P. Grønkjær and V. Loeschcke. Danish Institute for Fisheries Research, Dept. of Inland Fisheries, Silkeborg / M.M. Hansen. Several collaborators from the Danish Institute for Fisheries Research, Dept. of Marine Fisheries, Copenhagen.
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.
STATUS: Ongoing.

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*, in press.

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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*, in press.

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ESTONIA

Study 1

LABORATORY/RESEARCHER: Dept. of Fish Farming, Institute of Animal Husbandry, Estonian Agricultural University, Tartu / T. Paaver.
SPECIES: Sea trout and Atlantic salmon.
PROJECT FUNDING: In house.
OBJECTIVE: To follow the genetic stability of the wild and stocked populations of salmonids in Estonian coastal rivers and estimate the genetic differences between them.
DESIGN: The relatively occasional samples from test fishing and hatcheries are monitored for genetic variability of proteins.
METHODOLOGY: Allozymes, (6 polymorphic enzymes for brown trout, 3 polymorphic enzymes for Atlantic salmon), egg yolk proteins.
STATUS: Ongoing.

Study 2

LABORATORY/RESEARCHER: Dept. of Fish Farming, Inst. of Animal Husbandry, Estonian Agricultural University, 1 Kreutzwaldi St., EE2400 Tartu / R. Gross and T. Paaver. Co-operation with Dept. of Aquaculture, Swedish University of Agricultural Sciences / J. Nilsson.
SPECIES: Atlantic salmon.
PROJECT FUNDING: Estonian Fisheries Foundation.
OBJECTIVE: To examine the genetic structure in native and introduced salmon populations/stocks in Estonia.
DESIGN: Samples are collected from juvenile salmon from five rivers and two hatcheries.
METHODOLOGY: Allozymes, and PCR-amplified DNA markers (microsatellites, growth hormone genes, mtDNA genes).
STATUS: Ongoing one-year project (1996).

Study 3

LABORATORY/RESEARCHER: R.Gross, T.Paaver, A.Vasemägi. Dept. of Fish Farming, Institute of Animal Husbandry, Estonian Agricultural University, 1 Kreutzwaldi St. EE2400, Tartu, Estonia
SPECIES: Atlantic(Baltic) Salmon, Sea trout
PROJECT FUNDING: Estonian Science Foundation and Estonian Fish Capital Fund
OBJECTIVE: to reveal the genetic differences and describe population structure of introduced and hatchery reared populations vs. natural self sustaining populations of salmon and trout; screening of populations for frequency of troutXsalmon hybrids
DESIGN: sampling of juvenile salmonids from the spawning rivers and fish farms
METHODOLOGY: Allozymes, PCR amplified DNA markers - microsattelites, growth hormone genes, mtDNA genes.

STATUS: ongoing three year project (1997-1999)
COMMENTS: Cooperation and exchange of information and samples with Swedish and Finnish scientists is essential part of the study.

FINLAND

Study 1
LABORATORY/RESEARCHER: Finnish Game and Fisheries Research Institute, Helsinki / M.-L. Koljonen.
SPECIES: Atlantic salmon.
FUNDING: In house.
OBJECTIVE: Estimate stock composition of salmon catches and proportion of wild stocks in the catches.
METHODOLOGY: Genetic stock identification (GSI), allozymes.
STATUS: Ongoing.

Study 2
LABORATORY/RESEARCHER:, Acricultural Recearch Center, Institute of Animal Production, Section of Animal Breeding, Jokioinen / L. Siitonen.
SPECIES: Rainbow trout.
FUNDING: In house.
OBJECTIVE: Develop rainbow trout stocks with better growth rate.
METHODOLOGY: Selective breeding.
STATUS: Ongoing.

Study 3
LABORATORY/RESEARCHER: Agricultural Recearch Centre, Institute of Animal Production, Section of Animal Breeding, Jokioinen / K. Elo.
SPECIES: *Coregonids.*
FUNDING: In house.
OBJECTIVE: Species identification, phylogenetic analysis and genomic variation in Coregonids.
METHODOLOGY: RAPD.
STATUS: Laboratory analysis are completed. End 1997.

Study 4
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.
STATUS: Ongoing.

Study 5
LABORATORY/RESEARCHER: University of Kuopio, Department of Applied Zoology and Veterinary Medicine / H. Mölsä, T. Pitkänen, M. Reinisalo and A. Krasnov.
SPECIES: Rainbow trout.
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: Microinjections and integration assays, gene expression.
METHODOLOGY: Microinjections, mRNA, RT-PCR.

STATUS: Ongoing.

Study 6
LABORATORY/RESEARCHER: University of Oulu, Department of Biology / J. Lumme.
SPECIES: Atlantic salmon.
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 7
LABORATORY/RESEARCHER: University of Joensuu, Department of Biology / L. Kuusipalo.
SPECIES: Ciclids, clupeids and nile perch in lakes Malawi and Tanganyika.
FUNDING: FAO, Culture Fund of Finland, Women Science Foundation and in house.
OBJECTIVE: Speciation, stock identification.
METHODOLOGY: Allozymes and RAPD.
STATUS: Ongoing.

GERMANY

Study 1
LABORATORY / REASEARCHER: Dept. Physiological Chemistry I, University of Whrzburg, Biocenter, Am Hubland, 97074 Whrzburg / M. Schartl and Y. Hong.
SPECIES: *Oryzias latipes*, *Xiphophorus maculatus* and other species of *Poeciliids*.
PROJECT FUNDING: Deutsche Forschungsgemeinschaft, SFB 465, EC Biotech.
OBJECTIVE: Establishment of embryonal stem cells for homologous recombination on conservation of biodiversity, gene expression in vivo, transgenic technology.
DESIGN: Development of methods for gene-expression; promoter analysis, transgene expression; cloning of genes for growth factors, their receptors and intracellular signal transduction molecules; establishment of cell lines from blastulae of O. latipes; cell transplantation into recipient embryos; injection of DNA in oocytes and early embryos; analysis of chimeras and transgenic fish.
METHODOLOGY: Gene cloning, reporter gene assays, genetic and phenotypic analyses.
STATUS: Ongoing project.

Study 2
LABORATORY / REASEARCHER: 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*.
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 Northsea. DNA analyses are performed.
METHODOLOGY: RFLP-, RAPD- and AFLP -analyses.
STATUS: Ongoing project.

Study 3
LABORATORY / REASEARCHER: Institute of Freshwater Ecology and Inland Fisheries (IGB), Depart. of Fish Culture and Fish Pathology / K. Kohlmann.
SPECIES: *Salmo salar*.
PROJECT FUNDING: IGB.

OBJECTIVE: Genetic identification of wild Atlantic salmon stocked into R. Elbe within the framework of the AElblachs 2000@ program.
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 VNTR analyses of DNA.
STATUS: On going project.

Study 4
LABORATORY / REASEARCHER: Institute of Freshwater Ecology and Inland Fisheries (IGB), Depart. of Biology and Ecology of Fishes / Ch. Wolter.
FUNDING: IGB.
SPECIES: *Abramis brama, Blicca bjoerkna, Rutilus rutilus* and *Scardinius erythrophthalmus*.
OBJECTIVE: Estimation of individual migration between different waters and parts of waters by enzyme analyses.
DESIGN: Individuals of the 4 species were sampled from different parts of r. Spree. For comparison some samples were taken from r. Oder and its tributaries. Enzyme electrophoresis was performed.
METHODOLOGY: Enzyme electrophoresis.
STATUS: Completed project.

Study 5
LABORATORY / REASEARCHER: Institute of Freshwater Ecology and Inland Fisheries (IGB), Depart. of Fish Culture and Fish Pathology / K. Kohlmann. Inland Fisheries Institute Olsztyn, Salmonid Research Laboratory Rutki (Poland) / S. Dobosz and K. Goryczko.
PROJECT FUNDING: IGB (German part) and State Commitee 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: On going project.

ICELAND

Study 1
LABORATORY/RESEARCHER: Holar Agricultural College, IS-550 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, IS-112 Reykjavik / A. K. Danielsdottir and O.D. Jonsdottir. An EU-FAIR project in collaboration with J. Mork (University of Trondheim, Norway), T. Cross (University College Cork, Ireland), Godfrey M. Hewitt and Ciro Rico (University of East Anglia, U.K.) and R. S. Millner and M. Nicholson (Directorate of Fisheries Research, MAFF, U.K.).
SPECIES: Cod (*Gadus Morhua*), hake (*Merluccius merluccius)*, blue whiting (*Micromesistius poutassou*) and poor cod (*Trisopterus minututs*).
PROJECT FUNDING: MRI and EU FAIR.
OBJECTIVE: Eluciating 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: Heamoglobins, allozymes and anonymous cDNA RFLP.
STATUS: Four year project. Cod sampling has started, analysis of samples started in October 1996.

Study 3
LABORATORY/RESEARCHER: Marine Research Institute (MRI), c/o Biotechnology House, IS-112 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, heamoglobins and anonymous cDNA RFLP.
STATUS: Three to four 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, IS-112 Reykjavik / A.K. Danielsdottir and S. Gudjonsson.
SPECIES: Brown trout (*Salmo trutta*).
PROJECT FUNDING: In house and the Icelandic Science fund.
OBJECTIVE: Genetic variation in wild populations of landlocked and anandromous brown trout in Iceland.
DESIGN: Mapping of gene frequencies.
METHODOLOGY: Allozymes.
STATUS: Samples from 13 locations have been analysed and the study is ongoing.

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 and mtDNA cytochrome b sequencing.
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, IS-550 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: Multi-disiplinary 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.

IRELAND

Study 1

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: Samples from three sources are used: a) fish farm, b) isolated river above impassable waterfall, i.e. no upstream movement of fish to this system.
METHODOLOGY: MtDNA, RAPDs and genetic fingerprinting.
STATUS: One year project which started in August 1996.

Study 2

LABORATORY/RESEARCHERS: Department of Genetics, Trinity College, Dublin / E.P. Cunningham, D. Bradley and A. Norris. Industrial partner: Hydro Seafood Fanad.
SPECIES: Atlantic Salmon, *Salmo salar.*
PROJECT FUNDING: Forbairt Scientific Research Programme and 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 for 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 and 1 Norwegian river.
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 3

LABORATORY/RESEARCHER: Recombinant DNA Group, Department of Microbiology, University College, Galway / R. Powell.
SPECIES: Pacific oyster, *Crassostrea gigas.*
PROJECT FUNDING: EC FAIR.
OBJECTIVE: Development of a molecular karyotype system for the Pacific oyster.
DESIGN: Aim is to define clones that mark specific chromosome pairs and develop a chromosomal karyotype system based on such molecular markers.
METHODOLOGY: Large insert genomic libraries are constructed using *E. coli* cosmid and P1 vectors. Clones with the insert ranging from 10 to 100 kb will be chosen and used in *in situ* hybridisations with oyster chromosomes.

Study 4

LABORATORY/RESEARCHER: Recombinant DNA Group, Department of Microbiology, University College, Galway / R. Powell.
SPECIES: *Aeromonas salmonicida* (atypical strain).
PROJECT FUNDING: EC AIR.

OBJECTIVE: Improved identification and taxonomic analysis of atypical isolates of this fish pathogen to (i) develop definitive diagnostic procedures for identification and (ii) quantify the detrimental effects of this group on native fish.
METHODOLOGY: 16S rRNA sequence analysis, ribotyping, RAPD and PFGE analysis.

Study 5
LABORATORY/RESEARCHER: Recombinant DNA Group, Department of Microbiology, University College Galway / R. Powell.
SPECIES: Atlantic salmon, brown trout and rainbow trout.
PROJECT FUNDING: EC FAIR.
OBJECTIVE: Microsatellite-based genetic maps of salmonid species.
STATUS: Three year project to describe a low resolution genetic map of the selected salmonid species using PCR assays for microsatellite loci. Collaborative project with Norwegian, French, Danish, Scottish and Canadian research groups.

Study 6
LABORATORY/RESEARCHER: Recombinant DNA Group, Department of Microbiology, University College Galway / R. Powell. Salmon Research Agency of Ireland / D. Cotter and T. Gallagher.
SPECIES: *Aeromonas salmonicida*, Atlantic salmon, brown trout and eel.
PROJECT FUNDING: Irish Marine Institute - Operational Programme for Fisheries.
OBJECTIVE: Molecular ecology of fish pathogen *Aeromonas salmonicida.*
STATUS: Two year project to map the presence of *Aeromonas salmonicida* in an Irish river catchment. The pathogen detection techniques utilised, and compared, are PCR/DNA probe, ELISA and bacteriology. Resident and migratory fish stocks in the catchment are collected and monitored each month.

Study 7
LABORATORY/RESEARCHER: National Diagnostics Centre, University College, Galway / T. Smith and S. Hanley.
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.
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: 2 years from Dec. 1994.

Study 8
LABORATORY/RESEARCHER: National Diagnostics Centre, University College, Galway / T. Smith, S. Martin and P. O'Dea.
SPECIES: Salmon (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 San attempt to isolate novel genes. Duration: 3 years (Jan. 96-Dec 98).

Study 9
LABORATORY/RESEARCHER: National Diagnostics Centre, University College, Galway (NDC) / F. Gannon, T. Smith, C. Stenson and A. McNair.
SPECIES: Salmon (Galway) and Mammalian (others).
PROJECT FUNDING: EU Biotech programme (210,000ECU).
OBJECTIVE: Isolation and characterisation of the genes encoding liver-enriched transcription factors HNF 1 and HNF 3 from salmon. Analysis of regulatory regions and control of expression during development. TITLE: The HNF3 / forkhead and HNF 1 transcription factor families: structural/functional analysis and developmental role.
DESIGN: cDNA library screening to isolate cDNA sequences and genomic library screening to isolate genomic clones and promoter regions.
METHODOLOGY: The Galway group is involved in the isolation and characterisation of genes and promoters including sequence analysis, DNA / protein binding studies.
STATUS: Ongoing studies on HNF 3 gene as well as Salmon Apo AI and HNF 1 promoters being carried out during 1997. Duration: 3 years (Sept. 94 - Sept. 96).

Study 10
LABORATORY/RESEARCHER: School of Science, Regional Technical College, Galway / E. Gosling and I. Wilson. Also one UK, one Belgian and one Portuguese partner.
SPECIES: Periwinkle species, *Littorina* (*L. saxatilis* group, *L. littorea* and *L. striata*).
PROJECT FUNDING: EC MAST III CT95-0042.
OBJECTIVE: 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.
METHODOLOGY: Morphometrics, allozymes, DNA microsatellites, RAPDs, SSCPs, thermal tolerance studies.
STATUS: Three year project finishing in January 1999.

Study 11
LABORATORY/RESEARCHER: Biochemistry Department, University College Galway / L. Byrnes and K. Gately.
SPECIES: Atlantic salmon, *Salmo salar.*
PROJECT FUNDING: Forbairt.
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 and transgenic trout.

STATUS: Final year of project.

Study 12
LABORATORY/RESEARCHER: Biochemistry Department, University College Galway / L. Byrnes and M. Schmitz. 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-disiplinary 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 on 1st December, 1996 to continue for 3 years.

Study 13
LABORATORY/RESEARCHER: Salmon Research Agency of Ireland / P. McGinnity. Also one Irish, two UK and two Spanish partners.
SPECIES: Atlantic salmon.
PROJECT FUNDING: EU AIR 1 3003 92 719.
OBJECTIVE: To study the genetic impact of introduced non native Atlantic salmon on natural populations.
DESIGN: (a) Simulation of a farm escape to a natural stream contained by high specification fine screened trap, individual fish identified to family using DNA minisatellites, (b) a study of temporal changes, a consequence of farm escapes, in the genetic composition of juvenile salmon populations from selected rivers in North-West Ireland.
METHODOLOGY: Establishment of experimental population, hatchery control, trap and field monitoring, sampling, minisatellite DNA and Mitochondrial DNA (QUB), Allozymes (UCC).
STATUS: Three year project completed January 1996.

Study 14
LABORATORY/RESEARCHER: Salmon Research Agency of Ireland / P. McGinnity.
SPECIES: Atlantic salmon.
PROJECT FUNDING: Atlantic Salmon Trust, Salmon Research Agency of Ireland.
OBJECTIVE: To study the adaptive significance of genotypic variation at the Malic enzyme locus *MEP-2**.
DESIGN: Establishment of hatchery population, observation population study 1, monitoring of a number of West of Ireland populations, relate *MEP-2** genotype and performance.
METHODOLOGY: Allozyme analysis.
STATUS: Four year part-time project completed June 1996.

Study 15
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 16
LABORATORY/RESEARCHER: Salmon Research Agency of Ireland / R. Poole. Also 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 to be finished in March 1997.

Study 17
LABORATORY/RESEARCHER: Salmon Research Agency of Ireland / D. Cotter. Also one Irish, two Scottish and one Norwegian partner.
SPECIES: Atlantic salmon.
PROJECT FUNDING: 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 18
LABORATORY/RESEARCHER: Aquaculture Development Centre, University College, Cork / T. Cross, L. Bourke and J. Coughlan. Also one Irish (Salmon Research Agency), two UK and two Spanish partners.
SPECIES: Atlantic salmon, *Salmo salar.*
PROJECT FUNDING: EC AIR1.CT92.0719.
OBJECTIVE: To quantify genetic variability in wild salmon populations throughout the European range.
DESIGN: Parr samples from 14 European rivers from Russia to Spain, with one additional Canadian sample, were screened for the loci listed below.
METHODOLOGY: Allozymes, minisatellite DNA SLPs, mtDNA, MHC genes.
STATUS: Three year project which finished in January 1996.

Study 19
LABORATORY/RESEARCHERS: Aquaculture Development Centre, University College, Cork / T. Cross, R. FitzGerald, J. Coughlan and P. Galvin. Also 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.
METHODOLOGY: Microsatellite loci.
STATUS: Two year project finishing in October 1997.

Study 20
LABORATORY/RESEARCHER: Aquaculture Development Centre, University College, Cork / T. Cross and E. Daeme. Katholike University of Leuven, Belgium / F. Volkaert.
SPECIES: Eel, *Anguilla anguilla.*

PROJECT FUNDING: EC HCM.
OBJECTIVE: To investigate population structure of European eels.
DESIGN: Samples of elvers from Italy, Ireland and Sweden were screened for four microsatellite loci.
METHODOLOGY: Microsatellite loci and mtDNA.
STATUS: One year study which finished in March 1996.

Study 21
LABORATORY/RESEARCHER: Aquaculture Development Centre, University College, Cork / T. Cross and P. Galvin. Also two UK partners.
SPECIES: Whiting, *Merlangius merlangus*.
PROJECT FUNDING: EC FAR MA.3.781.
OBJECTIVE: To develop molecular markers for use in stock discrimination.
DESIGN: Samples were taken from throughout the range including the Black Sea.
METHODOLOGY: PCRable minisatellite DNA loci.
STATUS: Three and a half year project which finished in January 1996. Substantial differences were evident only between eastern Atlantic and Black Sea samples.

Study 22
LABORATORY/RESEARCHER: Aquaculture Development Centre, University College, Cork / T. Cross, P. Galvin, J. Coughlan. Also 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 a macro and micro-geographic scale.
DESIGN: Samples are being collected for macrogeographic studies and additional loci are being characterised.
METHODOLOGY: Minisatellite DNA SLPs, MHC and transferrin genes.
STATUS: Four year project from April 1996.

Study 23
LABORATORY/RESEARCHER: Aquaculture Development Centre, University College, 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 SLPs.
STATUS: One year project from March 1996.

Study 24
LABORATORY/RESEARCHER: Aquaculture Development Centre, University College, Cork / T. Cross, P. Galvin 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: Parr samples have been collected from the hatchery and five rivers.
METHODOLOGY: Minisatellite and microsatellite DNA SLPs.
STATUS: Four year project from November 1996.

NORWAY

Study 1

LABORATORY/RESEARCHER: Department of Fisheries and Marine Biology, University of Bergen (DFMB) / Gunnar 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 abundant 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.
COMMENTS: Cooperation has been established with several fisheries research institutes around the North Sea and Iceland.

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 Marineforskning, Clesund.
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 distribution 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).
STATUS: Studies on haemoglobins and allozymes by electrophoresis and isoelectric focusing have been going on since 1987; the last years with main emphasise on Icelandic and Greenland waters. From 1995 DNA-analyses have been included with the main emphasise 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, and very little is known about the structure west of the Davis Strait.

Study 3

LABORATORY/RESEARCHER: Department of Fisheries and Marine Biology, University of Bergen (DFMB) / G. Nævdal.
SPECIES: Herring, *Clupea harengus.*
PROJECT FUNDING: The Norwegian Research Council.
OBJECTIVE: To describe the esterase and haemoglobin variation in herring and to reveal the genetic and ontogenetic background control mechanisms.
DESIGN: A series of samples of herring distributed on potential population and different life stages are being analysed.
METHODOLOGY: Isoelectric focusing.
STATUS: The project is started in winter 1996, and will last for one year.
COMMENTS: The project is terminated by the end of 1996. The results so far indicate that esterases may be used , while haemoglobins show both ontogenetic and in vitro variations which make them of little use in for population studies.

Study 4

LABORATORY/RESEARCHER: Department of Fisheries and Marine Biology, University of Bergen (DFMB) / G. Nævdal.
SPECIES: Northern shrimps, *Pendulous borealis.*
PROJECT FUNDING: No special funding (student thesis).
OBJECTIVE: To reveal, describe and utilise genetic variation in northern shrimps.
DESIGN: An extensive material have been collected in coastal and offshore waters Northwest of Iceland and in the Denmark Strait. The samples have been analysed by standard starch gel electrophoresis.
STATUS: Frequency distributions of three polymorphic systems are being used to study the structure of shrimps in the mentioned areas. Very clear differences were found between shrimps from different areas in Icelandic waters and the Denmark Strait. The project is now terminated.
COMMENTS: Studies on shrimps were intended to be continued, but could not be funded.

Study 5
LABORATORY/RESEARCHER: Department of Fisheries and Marine Biology, University of Bergen (DFMB) / G. Nævdal.
SPECIES: Roughead grenadier, *Macrourus berglax.*
PROJECT FUNDING: No special funding (student thesis).
OBJECTIVE: To reveal, describe and utilise genetic variation in the grenadier.
DESIGN: Material has been collected in areas between Iceland and Greenland and in the Norwegian Sea. The analyses so far have revealed several polymorphic systems.
STATUS: The results are encouraging so far, and the results are now being written as a thesis.
COMMENTS: The analyses are carried out by a foreign student which also have to take several courses in different disciplines to be accepted as a graduate student at the University of Bergen.

Study 6
LABORATORY/RESEARCHER: Institute of Marine Research (IMR), Bergen / È' 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: Genetically marked smolt produced and released in 1994. Allozyme and DNA minisatellites baseline data on wild and released stocks done. Fry will be sampled and screened in 1996 and 1997.
COMMENTS: Collaborative work on minisatellites with John B.Taggart, Univ. of Stirling. The study does not include transgenic fish, but employ multigeneration farmed salmon as a model to investigate impacts from transgenic fish potentially used in fish farming in the future. Return rates of released smolt has been very low, which gives a weak gene pulse and difficulties in detecting gene flow. Coincide with low returns in wild salmon stock.

Study 7
LABORATORY/RESEARCHER: Institute of Marine Research (IMR), Bergen / È. 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 allele 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, altogether some 800 individuals from at least 8 year classes. Gradual elevation in frequency of *125 allele from 0.49 to 0.65 which corresponds to frequency the allele in «escaped» spawners in the river.
COMMENTS: Collaborative work with Dr. Kjetil Hindar at NINA.

Study 8
LABORATORY/RESEARCHER: Institute of Marine Research (IMR), Bergen / O. Skaala.
SPECIES: Brown trout (*Salmo trutta*).
PROJECT FUNDING: Norwegian electricity industry.
OBJECTIVE: Quantify the contribution of wild and stocked populations to trout fisheries in L. Tinnsjøen.
DESIGN: Genetic screening of wild and released stocks.
METHODOLOGY: Starch gel electrophoresis, statistics by Pella.
STATUS: Major difference between donor stock over 50 years and local samples. Small differences among local samples (inlets, outlet and lake). Heterozygote excess in four loci in one inlet. Additional sample of pelagic trout was collected and about to be genotyped before final testing.
COMMENTS: Collaborative work with Prof. Jan Heggenes, Institute of Biology and Nature conservation, Ås Agricultural University.

Study 9
LABORATORY/RESEARCHER: Institute of Marine Research (IMR), Bergen / È' Skaala.
SPECIES: Atlantic salmon and Brown trout.
PROJECT FUNDING: The Norwegian Sea ranching programme (PUSH).
OBJECTIVE: A) Investigate gene flow from sea ranched salmon to wild stock, and species hybridisation. B) Genetic comparison of three salmon stocks employed under the ranching programme.
DESIGN: A) Genetically marked smolt released from a small stream with salmon and sea trout. Genetic screening of various year classes done. B) Genotyping by polymorphic isozyme loci, extend on microsatellites if funding makes it possible.
METHODOLOGY: Allozymes.
STATUS: A) Several year classes screened. Fry was also collected and screened i 1996, but abundance of salmon fry was low, probably due to low water levels and ice during winter. B) All three stocks genotyped and compared.
COMMENTS: Very low return rates of released groups gives weak gene pulse and difficulties in detecting gene flow. Coincide with low returns of wild salmon in the area.

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.
COMMENTS: A DNA library has been established, and several sibling groups have been produced during 1996.

Study 11
LABORATORY/RESEARCHER: Department of Aquaculture, Institute of Marine Research (IMR), Bergen / G. Dahle.
SPECIES: Cod (*Gadus morhua*).
PROJECT FUNDING: Norwegian Research Council.
OBJECTIVE: Study modern mini- and microsatellite loci variation in historic material.
DESIGN: Otolithes have been sampled and stored for many decades at IMR. These old otolithes are being used to analyse the genetic composition of historic samples of cod with mini- and microsatellite primers.
METHODOLOGY: Mini- and microsatellite analysis.
STATUS: Two year project ending in 1996.

Study 12
LABORATORY/RESEARCHER: Institute of Marine Research (IMR), Bergen / K.E. Jørstad.
SPECIES: European lobster (*Hommarus gammarus*).
PROJECT FUNDING: Norwegian Sea Ranching Programme.
OBJECTIVE: Genetic comparison of cultured and wild lobsters; genetic monitoring of artificial production.
DESIGN: Sampling of wild broodstock, released and recaptured cultured lobsters. Comparison with samples of wild stock at Kvitsøy and nearby regions.
METHODOLOGY: Starch gel electrophoresis, polymorphic enzymes.
STATUS: Reporting 1997.
COMMENTS: The work is part of Norwegian lobster stock enhancement programme.

Study 13
LABORATORY/RESEARCHER: Institute of Marine Research (IMR), Bergen / K.E. Jørstad.
SPECIES: Mainly herring (*Clupea harengus*).
PROJECT FUNDING: IMR.
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 14
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 15
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 16
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 starting in 1996.

Study 17
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 1996.

Study 18
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 to be 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: University of Oslo / I.B. Mjølnerød. Norwegian Institute for Nature Research (NINA) / K. Hindar, U.H. Refseth and K.S. Jakobsen.
SPECIES: Atlantic salmon.
PROJECT FUNDING: Research Council of Norway.
OBJECTIVE: Analyse genetic variation detected by allozymes and nuclear DNA markers.
DESIGN: Compare levels of genetic variation in two wild and one farmed population.
METHODOLOGY: Allozymes; multi-locus and single-locus minisatellites.
STATUS: Three-year project to be completed 1996.

Study 20
LABORATORY/RESEARCHER: Univ. 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 21
LABORATORY/RESEARCHER: Univ. of Trondheim, Biological Station / J. Mork and M. Giæver.
SPECIES: Blue whiting (*Micromesistius poutassou*).
PROJECT FUNDING: The Norwegian Research Council, grant NF 108 093/110.
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 (N=5025) from a tight sampling grid in the relevant areas, during and outside the spawning season.
METHODOLOGY: Allozymes and minisatellites.
STATUS: Two year project to be end reported in 1997.
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 22
LABORATORY/RESEARCHER: Univ. of Trondheim, Biological Station / J. Mork.
SPECIES: Cod (*Gadus morhua*).
PROJECT FUNDING: Institutional.
OBJECTIVE: Study of the long term stability of haemoglobin and allozyme 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 1997.

Study 23
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 24
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.
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, transcribed sequences, mtDNA.
STATUS: Cod and blue whiting allozyme analyses are a jour (>3000 specimens each), and poor cod are underway. DNA minisatellite analyses are ongoing for blue whiting , cod, and poor 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: Inland Fisheries Institute; Salmonid Research Laboratory Rutki, Institute of Freshwater Ecology and Inland Fisheries Berlin and Warsaw University of Agriculture / K. Goryczko, S. Dobosz, K. Kohlmann and A. Zynczynski.
SPECIES: Rainbow trout.
PROJECT FUNDING: Committee of Scientific Research (CSR) and Institutional.
OBJECTIVE: To improve the breeding value of rainbow trout.
DESIGN: Family selection from outbred broodstock the 100 F_1 families were started in 1991. In 1994 from the 10 selected families the 100 F_2 families were produced and reared during 1995. Growth and mortality were monitored.
METHODOLOGY: Each family reared separately until the end of the first season, then the fish is tagged (PIT tags), amount of families culled (60), fish reared in one pond until sexual maturity.

Study 2

LABORATORY/RESEARCHER: IFI; Salmonid Research Laboratory Rutki / K. Goryczko, S. Dobosz and H. Kuzminski.
SPECIES: Sea trout.
PROJECT FUNDING: Institutional.
OBJECTIVE: Vistula sea trout gene bank.
DESIGN: Freshwater broodstock produced from representative group of river ascending sea trout.
METHODOLOGY: A sample of 50 g of fertilised eggs from each wild female spawned were taken, incubated and reared at SRL. A 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.
STATUS: Fourth and second year life. Elder fish second spawn.
COMMENTS: Project aimed at protection of genetic diversity in a valuable strain maintained by stocking.

Study 3

LABORATORY/RESEARCHER: IFI; Salmonid Research Laboratory Rutki / K. Goryczko, S. Dobosz and H. Kuzminski. University of Agriculture and Technology, Olsztyn, Dept. of Basic Fishery Sciences / M. Luczynski.
SPECIES: Whitefish.
PROJECT FUNDING: CSR grant no ZO22/S3/94/01.
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 fish were produced. The biochemical genetic study of farmed whitefish were realised.
STATUS: Second spawning season of farmed fish; 4 million eggs were obtained, 100 000 fry and 80 000 summer fingerlings (from the first spawn) were stocked.
COMMENTS: Farmed broodstock enabled enhancement and reproduction projects realisation without curtailing of wild broodstock.

Study 4

LABORATORY/RESEARCHER: Inland Fisheries Institute / Olsztyn. M. Zolkiewicz.
SPECIES: Rainbow trout.

PROJECT FUNDING: Institutional.
OBJECTIVE: Genetic monitoring of rainbow trout hatchery stocks to detect changes and take measures preventing unwanted inbred.
DESIGN: Farmed stocks trough Poland were sampled and analysed for genetic variation.
METHODOLOGY: Allozymes.
STATUS: To be repeated periodically.

Study 5
LABORATORY/RESEARCHER: Olsztyn University of Agriculture and Technology, Department of Basic Fishery Sciences / M. Luczynski and collaborators.
SPECIES: Whitefish (*Coregonus lavaretus*), bream (*Abramis brama*), pikeperch (*Stizostedion lucioperca*), river lamprey (*Lamperta fluviatilis*) 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 location, storing tissue samples at -25° C and analysing them using allozymes and various other techniques, or transporting live fish to the laboratory for cytogenetic studies. Sample collection each year during the spawning season and on the spawning site/migration route of each species.
METHODOLOGY: Allozymes, cytogenetics, RFLP PCR amplified mtDNA, morphology.
STATUS: Some jobs completed and published, some other ongoing, some scheduled for coming years.
COMMENTS: Tissue samples can be made available for interested researches.

Study 6
LABORATORY/RESEARCHER: Olsztyn University of Agriculture and Technology, Department of Basic Fishery Sciences / M. Luczynski and collaborators. Collaboration with Sea Fisheries Institute in Gdynia / R. Bartel.
SPECIES: Sea trout (*Salmo trutta*).
PROJECT FUNDING: Institutional.
OBJECTIVE: To assess genetic differentiation between the sea trout ascending Vistula River and Pomeranian rivers; to restore a unique population of the sea trout ascending Vistula River.
DESIGN: Collection of specimens during the spawning migration, storing tissue samples at -25°C and analysing them using allozymes and various other techniques. Sampling smolts reared in the Salmonid Research Laboratory Rutki, transporting live fish to the laboratory for cytogenetic studies.
METHODOLOGY: Allozymes, cytogenetics, RFLP PCR amplified mtDNA, mini - and micro-satellite analysis of nuclear DNA.
STATUS: Allozymes completed, cytogenetics currently underway, mtDNA currently underway, nuclear DNA. Scheduled for coming years.
COMMENTS: Tissue samples can be made available for interested researchers; guidance and assistance in developing nuclear DNA studies needed.

Study 7
LABORATORY/RESEARCHER: Sea Fisheries Institute in Gdynia /R. Bartel. Collaboration with the Fish Farm "Aquamar", Miastko / A. Marczynski and with Olsztyn University of Agriculture and Technology, Department of Basic Fishery Sciences /M. Luczynski.
SPECIES: Baltic salmon (*Salmo salar*).
PROJECT FUNDING: Committee for Scientific Research.
OBJECTIVE: To restore populations of the Baltic salmon ascending Polish rivers.
DESIGN: Development of the farmed breeding stock based on the breeding program designed to minimise inbred and to maximise effective population size, rearing and releasing of smolts, periodical evaluation of the amount of genetic variation in the gene pool of the breeding stock.

METHODOLOGY: Breeding, hatchery operations including smolt rearing, allozymes.
STATUS: Currently underway.
COMMENTS: Will be carried out in coming years, if necessary cytogenetics, RFLP PCR amplified mtDNA, mini- and micro-satellite analysis of nuclear DNA will be included.

Study 8
LABORATORY/RESEARCHER: Olsztyn University of Agriculture and Technology, Department of Basic Fishery Sciences / M. Luczynski and collaborators. Ohio State University, school of Natural Resources, Columbus, USA / K. Dabrowski (Principal Investigator).
SPECIES: Northern pike (*Esox lucius*).
PROJECT FUNDING: U.S. - Poland Maria Sklodowska-Curie Joint Fund II.
OBJECTIVE: To obtain gynogenetic diploid, triploid and sex-reversed (neo-males) individuals and all-female stocks.
DESIGN: Triploid fish are obtained due to the thermal shock applied towards fertilised eggs, diploid gynogenetic fish are obtained due to egg activation with UV-irradiated sperm followed by a thermal shock, sex-reversal is carried out by feeding the gynogenetic larvae (genetically females) with food stuffs supplemented with respective hormones.
METHODOLOGY: Chromosome engineering, hormonal sex reversal, sperm cryoconservation, allozymes, cytogenetics, rearing.
STATUS: Currently underway since 1994 and due for completion in 1997.
COMMENTS: This represents an experimental study.

Study 9
LABORATORY/RESEARCHER: Sea Fisheries Institute, Gdynia / R. Wenne and E. Wlodarczyk
SPECIES: Sea trout (*Salmo trutta*).
PROJECT FUNDING: Institutional.
OBJECTIVE: To study population genetic structure of the sea trout in Poland.
DESIGN: Collection of 6 samples (40 specimens each) from Polish rivers. Fin clippings are stored in ethanol.
METHODOLOGY: RFLP analysis of PCR amplified mtDNA segment.
STATUS: Ongoing.

PORTUGAL

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. Univ. of Porto / Dr. C. Thiriot. Observatoire Oceanologique de Villefarnce-sur-Mer, Univ. 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 rpecense 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). Univ. 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

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: Salmon Research Institute / H. Jansson.
SPECIES: Atlantic salmon and brown trout.
PROJECT FUNDING: National funds.
OBJECTIVE: Genetic monitoring 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 3
LABORATORY/RESEARCHER: Salmon Research Institute / H. Jansson. Department of Genetics, Uppsala University / K. Fredga and H. Tegelström.
SPECIES: Atlantic salmon × 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, the National Board of Fisheries.
METHODOLOGY: Allozymes and mitochondrial DNA.
STATUS: Three year study 1997-1999.

Study 4
LABORATORY/RESEARCHER: Department of Zoology, Uppsala University / J. Dannewitz.
SPECIES: Atlantic salmon.
PROJECT FUNDING: Institutional and regional funds.
OBJECTIVE: To study the influence of egg-size on survival, growth and subsequent choices of life-histories in Atlantic salmon juveniles.
DESIGN: Fish from different sized eggs are followed from hatching until the last individuals leave the water system as smolts. At certain stages, samples are taken and survival- and growth-rates of fish from different sized eggs are examined.
METHODOLOGY: Variation at microsatellite loci.
STATUS: Two year study started in 1997.

Study 5
LABORATORY/RESEARCHER: The National Board of Fisheries / T. Öst and T. Järvi.
SPECIES: Brown trout.
OBJECTIVE: To study the performance of genetic difference between wild and sea-ranched anadromous brown trout from a common origin.
DESIGN: Hatchery reared fish from wild and stocked parents are analysed to detect possible genetic changes.
METHODOLOGY: Mitochondrial DNA.

Study 6
LABORATORY/RESEARCHER: Department of Aquaculture, Swedish University of Agricultural Sciences / J. Nilsson.
SPECIES: Atlantic salmon.
PROJECT FUNDING: Swedish Council for Forestry and Agricultural Research.
OBJECTIVE: To study genetic variation within and among populations in the Baltic, to compare wild and cultivated populations in R. Vindelälven and to estimate possible loss of genetic variation resulting from the M74 syndrome.
DESIGN: Comparisons of samples from Baltic Sea rivers and from subpopulations within rivers.
METHODOLOGY: DNA microsatellites and mtDNA AFLP.
STATUS: Started in 1995. New funding will be applied for in 1997.

UNITED KINGDOM

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: On-going - 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 & 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: On-going.

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: Wellcome 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 phagocytic capacity in the face of challenge. Allozyme electrophoresis at enzyme loci.
STATUS: On-going.

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 & 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: On-going.

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 + 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 on-going 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 Labortaory, 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 chainreaction (PCR) based analysis of mitochondrial and nuclear.
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: SOAFD Marine and Freshwater Fisheries Laboratories / E. Verspoor and collaborators.
SPECIES: Atlantic salmon.
PROJECT FUNDING: SOAFD and EC AIR1-CT92-0719.
OBJECTIVE: To assess whether the documented interbreeding of farm Atlantic salmon, which ascended the River Polla in 1989 and 1990, with the wild stock has resulted in genetic changes to the juvenile populations in the river.
DESIGN: Samples of juvenile Atlantic salmon from two year classes were collected from the lower, middle and upper reaches of the river pre-spawning of farm fish in 1989. The genetic composition of these fish will be compared with post spawning juvenile samples from the same locations taken in 1991 and differences related to the genetic make-up of adult farm Atlantic salmon ascending the river.
METHODOLOGY: Allozymes, RFL analysis of PCR amplified mtDNA, mini- and micro-satellite analysis of nuclear DNA, PCR amplification of structural gene nDNA.
STATUS: Currently underway and due for completion in 1995.

COMMENTS: This represents an opportunistic study.

Study 9
LABORATORY/RESEARCHER: SOAFD Marine and Freshwater Fisheries Laboratories / E. Verspoor and collaborators.
SPECIES: Atlantic salmon.
PROJECT FUNDING: SOAFD and EC AIR1-CT92-0719.
OBJECTIVE: To assess whether genetic differences among stocks are relevant to their biological performance during the juvenile freshwater phase in the wild in ways that relevant to their fitness.
DESIGN: Simultaneously spawned eggs of different regional stocks and their hybrids have been planted out within 48 hrs of fertilisation in artificial redds using a random stratified planting strategy in a small experimental river where natural spawning is precluded. Performance parameters such as egg mortality, hatching and emergence timing, developmental state, maturation, growth and smoltification will be compared among groups.
METHODOLOGY: Stock groups will be genetically marked using RFLP's PCR amplified mtDNA and single locus minisatellite fingerprinting.
STATUS: Currently underway and due for completion in 1996.
COMMENTS: This represents an experimental study.

Study 10
LABORATORY/RESEARCHER: SOAFD Marine and Freshwater Laboratories / A. Youngson and J. Webb.
SPECIES: Atlantic salmon.
PROJECT FUNDING: SOAFD and Atlantic Salmon Trust.
OBJECTIVE: To determine the frequency of farm escapes among Atlantic salmon in the coastal Atlantic salmon fisheries in Scotland.
DESIGN: Regular sampling of Atlantic salmon taken by four representative coastal net fisheries off Scotland.
METHODOLOGY: Identification of farm fish on basis of body morphology and scale analysis.
STATUS: Started in 1992 and ongoing.
COMMENTS: This represents an opportunistic study.

Study 11
LABORATORY/RESEARCHER: SOAFD Marine and Freshwater Laboratories / E. Verspoor.
SPECIES: Atlantic salmon.
PROJECT FUNDING: SOAFD.
OBJECTIVE: To determine the nature and extent of population structuring of Atlantic salmon in Scotland within and among rivers so as to assess the impact of farm escapes on natural structure.
DESIGN: Geographic sampling of within and among river genetic variation and statistical analysis of differentiation.
METHODOLOGY: Allozymes, mtDNA, mathematical modelling.
STATUS: Started in 1989 and ongoing.

Study 12
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: On-going.

Study 13
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: On-going.

Study 14
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: On-going.

Studies reported in non-standard format

Canada
Fisheries and Oceans Canada
4160 Marine Drive, West Vancouver, B.C., Canada
(Reported by R. Devlin)

1) Production of transgenic salmon with enhanced growth and altered reproductive capability using "all-salmon" gene constructs.

2) Characterization of Y-chromosomal DNA probes from salmon for use in monosex all-female culture

3) Development of DNA-based diagnostics for several Microsporean and Myxosporean parasites to assist with management of infection in sea-farm facilities.

4) 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.

5) Development of a RAPD linkage map for Chinook salmon.

6) Development of a sensitive PCR-based assay for CYPIA1 gene expression to evaluate the biological effects of xenobiotic exposure.

APPENDIX 2

TERMS OF REFERENCE 1997 (C. Res. 1996, 2:31).

The **Working Group on the Application of Genetics in Fisheries and Mariculture** (Chairman: Prof. J. Mork, Norway) will meet in Gdynia, Poland, from 17-21 February 1997 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) review the new molecular techniques recently developed and implemented in population genetic studies, with a view to evaluate their merits so far in studies of marine finfish and shellfish;

c) review and evaluate the variety of computer software packages now available for population genetic analysis, in order to come up with recommendations for tools that are suitable for different types of problems;

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

e) provide quantitative information on the escape of fish from mariculture operations in the context of genetic composition in relation to wild stocks, and advice on the means by which this impact could be controlled and report to ACME before its June 1997 meeting [OSPAR 1997/4.1].

The Working group will report to the Mariculture Committee at the 1997 Annual Science Conferance.

APPENDIX 3

WGAGFM MEETING FEB. 17–21, 1997 IN GDYNIA, POLAND.

PARTICIPANTS

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APPENDIX 4

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