Mariculture Committee

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REPORT OF THE

WORKING GROUP ON THE APPLICATION OF GENETICS IN FISHERIES AND MARICULTURE

Reykjavik, Iceland
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International Council for the Exploration of the Sea

Conseil International pour l'Exploration de la Mer

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1 INTRODUCTION

As decided in ICES C.Res.1998/2:41, adopted at the 1998 Annual Science Conference in Cascais, Portugal, the Working Group on the Application of Genetics in Fisheries and Mariculture [WGAGFM] (Chair: J. Mork, Norway) met at the Marine Research Institute in Reykjavik, Iceland, 12–15 April 1999, to deal with its Terms of Reference for 1999 (Annex 1).

1.1 Attendance and Meeting Place

There are currently 50 appointed members and observers in the WGAGFM (Annex 2). Of these, 22 members from 16 ICES Member Countries attended the 1999 WGAGFM meeting in Reykjavik (Annex 3). Countries represented (number of persons in parenthesis) were Belgium (1), Canada (2), Denmark (1), Estonia (1), Finland (1), Germany (1), France (1), Iceland (2), Ireland (3), Norway (3), Poland (1), Portugal (1), Sweden (1), UK (2), USA (1). As in the five previous years, the representation on the quantitative genetics was lower than on the qualitative genetics side.

The Icelandic Marine Research Insitute (Director Jóhann Sigurjónsson), offered excellent rooms and facilities for the WGAGFM meeting in Skulagata 4, and all arrangements in connection with the meeting were extremely well organised by our hostess Anna Kristin Danielsdottir. WGAGFM is very pleased with all the efforts by her, MRI, and the Ministry of Fisheries to make our meeting effective and enjoyable.

1.2 Working Format

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 a final plenary consensus discussion and editing for the WGAGFM report.

- J. Mork and T. Cross chaired business and open scientific sessions (ToR (a))
- E. Verspoor chaired 'Molecular markers in breeding' (ToR (b))
- B. Chatain chaired 'Triploidy in aquaculture species ' (ToR (c))
- E. Kenchington chaired 'Protecting marine biodiversity (ToR (d))
- A. Ferguson chaired 'Genetic tags in study & management of wild stocks' (ToR (e))
- B. Chatain chaired 'The gender of fish' (ToR (f))
- W. Davidson chaired 'Patenting of technology' (ToR (g))
- W. Davidson chaired 'Genetic tissue authentication in forensics' (ToR (h))
- A. Danielsdottir collected and compiled the National Activity Reports (ToR (j))
- M.-L. Koljonen chaired 'The HELCOM request' (ToR (k))
- A. Danielsdottir chaired '*Sebastes mentella* genetics and management' (ToR (l))

ToR (i) 'Experimental design and statistical frames' was not addressed (see Section 2.i.).

The session Chairs were responsible for leading the respective colloquia, the subsequent plenary sessions, and for preparing the final report text from their sessions. All members were asked beforehand to collect national activity reports from their respective countries and bring with them (on diskette) to Reykjavik for inclusion in the Report. The Working Group decided that, as in the four previous years, the preparation of the WG Report should mainly be done by the members present at the meeting. A preliminary version of the Report were made available on the (external) WGAGFM homepage for final comments by members before finalisation and submission to the ICES Secretariat.

2 TERMS OF REFERENCE FOR 1999

2.a. General population genetic topics related to fisheries and mariculture

This session was scattered throughout the meeting, and served mainly to identify topics for the Terms of Reference for the year 2000.

2.b. Molecular markers in breeding

Based on a position paper by Eric Verspoor, 'The Potential for using Molecular Markers in the selective Breeding of Fish Species in Mariculture'; adopted by WGAGFM in Reykjavik 1999.

Introduction

At present less than 1 % of world-wide aquaculture production is based on genetically improved strains (Gjdrem, 1997). In contrast, the use of improved breeds, at least for cattle, poultry, sheep, turkeys and pigs, has become a competitive necessity in the modern market place and provides the most cost-effective basis for food production.

Production potential is determined by the genetic character of a cultivated stock. To neglect genetic improvement is to ignore one half of the production equation and precludes realization of the full potential of aquaculture species. In light of the importance of genotype-environment interactions, genetic and non-genetic components of the equation need to be optimized in concert.

The biological potential for the genetic improvement of fish and shell fish stocks is considerable. Most, if not all, fish species contain heritable variation for traits important to production such as growth rate, disease resistence, etc. (e.g., Tave, 1993). Furthermore, they can be cultivated in large numbers at high densities and have a high fecundity providing the basis for higher selection intensities and more rapid genetic improvement of production traits than is possible for many terrestrial species.

Obstacles to Genetic Improvement

A number of factors lie behind the current low level of utilization of genetically improved fish stocks in aquaculture. Most obvious is the fact that most farmed fish species have only come to be extensively cultivated relatively recently. However, even then in many cases selective development is seldom included as part of stock management in a rigorous manner. This is probably due, in part at least, to a general lack of formal training in genetics among most fish culturists (Tave, 1993) and has led to a focus in aquaculture operations on the more immediate and obvious production gains to be made from improving diets, health management, and other rearing conditions such as water quality (Tave, 1993).

Genetic improvement has been undertaken for a few species such as the common carp, rainbow trout, Atlantic salmon, and some species of tropical fish (Tave, 1993). However, even for these exceptions, the genetic improvement programmes are still in their infancy. The fact that reared stocks are generally just a few generations removed from the wild is a major reason for this but it also arises because, even though the importance of genetic management is recognized in these species, major technical obstacles to the development of effective selective breeding programmes still remain.

Traditional approaches to selection exploit sophisticated statistical procedures to partition performance variation into genetic and environmental components based on observed patterns of inheritance of variation among relatives. By so doing, individuals which are most likely to be genetically superior with regard to the trait of interest can be identified. However, the traditional approach is often difficult to implement for fish species due to problems in tracking pedigrees and the relatedness of individuals.

External fertilization allows matings to be controlled but newborn offspring of aquatic fish species are generally indistinguishable, and too small and numerous for physical tagging to be a viable option. At the same time, for many species, the alternative of isolating individuals in tagged enclosures is also not an option for logistic or biological reasons. Even where individuals can be isolated, as for example may be the case for shellfish or family groups of finfish, having to the costs of individual enclosures may limit the numbers which can be so reared. This can severely limit selection intensities and rates of genetic improvement.

Even where isolated rearing of individuals or families is possible, it poses another problem. Specialized facilities are needed where the environment can be controlled to ensure uniform rearing conditions to facilitate the disentanglement

of the genetic and environmental components of performance variation and the selection of genetically superior individuals or family groups. However, construction of such facilities is often beyond the reach of many aquaculture operations.

A further problem in applying traditional selection approaches arises in species such as salmon as a consequence of their semelparity, i.e., most fish spawn once before dying. This precludes retrospective selection of breeders based on offspring performance. This approach has very successfully been applied in selection programmes for terrestrial species such as cattle and poultry.

A general problem posed in all traditional selection programmes, be they for terrestrial or aquatic species, is having to selection for superior genotypes on the basis of phenotype. Phenotypically superior individuals or families are not necessarily genetically superior for the performance traits of interest. As the environmental component in the variation in performance among individuals increases, there is an increased probability that the phenotypic performance of individuals or families will deviate from that defined by their genotype. The consequence is that there will be an increasing proportion of selected individuals which will only be phenotypically and not genetically superior, reducing the effective selection intensity and the realized rate of genetic improvement. This drawback is compounded by problems in statistically portioning phenotypic variation into environmental and genetic components (Falconer and Mackay, 1996; Lynch and Walsh, 1998).

Applying Molecular Markers

In the ideal population for selective breeding, each individual would be born with a unique, heritable and visible mark that could be instantly deciphered, and linked each individual unambiguously to both its parents. Furthermore, variants possessed by for the genes affecting performance would be similarly visibly decipherable to allow direct selection of genetic types disposed to the desired level of performance. With molecular markers it is possible, in principal at least, to approach this ideal. The extent to which it can be approached is considered here.

Most fish species are likely to have in the order of 50,000 to 100,000 genes. Even if less than 1 % of these are variable, and these have only two alleles, each individual can be expected to be genetically unique. As this uniqueness results from a combination of genetic variation inherited equally from both parents, this uniqueness will unambiguously link offspring to their parents. This molecular variation thus serves as a marker of an individuals pedigree.

Molecular variation at the level of the DNA is also ultimately responsible for heritable performance variation. Thus by resolving molecular variation at loci responsible for quantitative trait variation (i.e., quantitative trait loci or QTLs), or loci physically linked to them, selection can be directed on the genes themselves. If so, by using such molecular performance markers, all selected individuals would be certain to be genetically, and not just phenotypically, superior.

Molecular Pedigree Analysis

Molecular pedigreeing is concerned with establishing the relationship between parents and offspring, from which all other relationships can be determined. The more parent/offspring combinations distinguishable, the better, given that the objective of most selective breeding programmes is to maximize selective intensity (i.e., minimize the % of families selected) while minimizing inbreeding (maximizing the absolute numbers of family groups used to produce the next generation).

In practice, to be useful, a molecular system of pedigreeing should be able to discriminate a minimum of several hundred families. Furthermore, in an ideal world it would also allow discrimination of individuals within families. The latter is important if both among- and within-family selection are to be carried out, as it allows individual performance to be tracked and their performance over time to be evaluated.

Assignment power, i.e., the ability to discriminate individuals and families, and establish relatedness, increases with the number of variable loci used and with the number of alleles at each locus. This has been discussed previously by Ferguson *et al.* (1995), Verspoor (1995) and others. Simulations (Villaneauva, unpublished) show that five loci with 10 alleles at each locus would be sufficient to discriminate 400 different family groups. The requirements for individual discrimination within families will depend on the numbers of sibs to be discriminated but is likely to require an order of magnitude more loci.

Development of molecular pedigreeing methods in most species has over the last decade focused on satellite DNA loci, a class of nuclear genes first identified in humans, but now known to be common in most species. These loci, of which there are two basic types—minisatellites and microsatellites—generally display high levels of allelic diversity,

frequently having 10 or more allelic variants. Estimates place the number of potential microsatellite loci present in the fish genome at in the order of 100,000, and for minisatellite loci at 10,000 (Park and Moran, 1994).

Initial work focused on minisatellites loci, stretches of nDNA composed of variable numbers of tandemly repeated sequences 9 to 100 bases long. In fishes, this class of loci was first identified in relation to Atlantic salmon (Taggart and Ferguson, 1990a,b) and brown trout (Prodöhl *et al.*, 1994), though the methodology could be applied to a wide range of species (Prodöhl *et al.*, 1995). Ferguson *et al.* (1995) have used minisatellite-based pedigree analysis to analyse performance variation in growth and seaward migration of different families of wild, farm and farm/wild hybrid Atlantic salmon families planted out in an Irish stream. However, their use has now been largely eclipsed by microsatellites, composed of tandemly repeated units made up of 2–6 bases.

Microsatellite loci, and particularly those loci involving 3 and 4 base repeat motifs for which allelic variation is more easily resolved, offer a number of advantages over minisatellites as molecular markers (O'Connell and Wright, 1997). Being able to be resolved by polymerase chain reaction (PCR) technology means that only crude DNA extracts from small non-destructively sampled quantities of tissue such as fish scales, mucus and fin clips are required for typing, and semi-automated DNA screening systems can be used. Furthermore, in contrast to minisatellite loci, microsatellite loci can be selected which have unambiguously resolved discrete non-overlapping allelic classes, minimizing the amount of typing required to resolve relationships. With minisatellites, two people took one year to screen 2000 fish for 6 loci, which gave a correct assignment of ~ 90 % of fish to family in a group of 100 families. Now with microsatellite technology, 2000 fish can be screened for 10 loci in less than a month allowing assignment to family > 99 % of fish from mixtures of 500+ families. Overall, the estimated saving in time and costs is estimated to be approximately 10-fold, with the cost of typing an individual now in the order of the £5 mark per 7 loci.

Microsatellite loci have already been used by Herbinger *et al.* (1995) to assess paternal and maternal effects on juvenile growth and survival in a farm population of rainbow trout. (Doyle *et al.*, 1995) have used them to discriminate family groups of cod (*Gadus morhua*) to assess the intensity of interfamily selection in the laboratory in the early post-hatch period. In these cases, offspring assignment was to known parental types. However, with sufficient levels of variability, family discrimination may also be achievable in the absence of parental information (Blouin *et al.*, 1996), in relation to family discrimination in wild mice populations. A number of research programmes are already under way to develop microsatellite markers for a range of other fish species including Atlantic salmon, rainbow trout, brown trout, Atlantic cod (*Gadus morhua*), Pacific herring (*Clupea harengus pallasi*), sea bass (*Dicentrarchus labrax*), tilapia (*Oreochromis* spp.) and brook char (*Salvelinus fontinalis*) (O'Connell and Wright, 1997) as well as many other species of interest in aquaculture.

The full potential of microsatellite-based pedigree analysis has yet to be exploited in selective breeding programmes, largely due to cost. Cost is determined by both the unit cost of typing, which in turn is determined by the resolution required (a greater number of loci need to be screened to resolve individuals as opposed to families) and to the number of times typing must be carried out (number of individuals and the number of times each individual is typed). As molecular pedigree information is detached from the individual, use of molecular approaches alone requires that a fish be retyped each time its performance is evaluated or individuals are selected. As a fish may need to be evaluated many times during its development and selection carried out at a number of stages, a solely molecular approach would involve a large amount of typing which would still likely become uneconomic with current technology. This problem may be resolved in the future using microchip-based genotyping. This not only has the potential to bring down costs but also makes it conceivable that genotyping may be able to be carried out on site within minutes, based simply on the analysis of DNA from mucus or scales taken from the surface of a fish.

Until typing costs are brought down such that individual resolution becomes possible, exploitation of microsatellite-based pedigreeing technology will require innovatively designed selection programmes such as the walk-back selection model proposed by Doyle and Herbinger (1994). In general, these will involve the physical tagging and biopsy of individuals when they are large enough to be marked, with microsatellite analysis based on the biopsy used to assign individuals to family. Exactly how the combined physical tagging and molecular pedigreeing are combined is likely to depend on the particular biology of a species, the available resources, and the particular selective objectives set out for a given programme. This will in turn dictate the selective gain that can be achieved and will need to be carefully evaluated in each individual case.

Molecular Markers for QTLs

The detection of QTL markers in fish and their use in selective breeding programmes has recently been reviewed by Poompuang and Hallerman (1997). For the detection of markers for major QTLs affecting performance, it is necessary to have a large number of molecular markers which are distributed across the majority of a species' genome. The more markers utilized and the more polymorphic the marker loci, the greater the likelihood of detecting an association with a

QTL. Poompuang and Hallerman (1997) suggest that based on the size of the zebrafish genome, and considerations that marker loci selected are randomly rather than evenly spaced through out the genome, 150 markers would be needed to ensure that 90 % of the genome was within 20 centimorgans of a marker. This is the distance at which a segregating QTL can be expected to be reliably mapped to a marker locus (Soller *et al.*, 1976). This number can be reduced where markers have been mapped and ones that are more evenly spaced across the genome can be selected. Alternatively, numbers of markers can be reduced by focusing on only one part of the genome, though this risks not detecting some or all of the main QTLs which might exist.

To be useful in QTL detection, marker loci must be variable in at least one parent. The probability that this will occur in a given mating being analysed increases as the allelic diversity and heterozygosity at a locus increases. A number of different technologies are available (Park and Moran, 199) and could be applied (Poompuang and Hallerman, 1997) but the most promising source of molecular markers is likely to be hypervariable microsatellite loci. These appear to be numerous in most fish species and to be widely dispersed in fish genomes based on available mapping studies. However, any polymorphic locus can be used including those for allozymes, and in some species recent efforts have been directed at the development of expressed sequence tags (ESTs), e.g., LahbibMansais *et al.* (1999), which can be used to resolve variable loci for regions of the DNA whose sequences are transcribed (i.e., from which mRNA transcripts are produced). The use of these regions in the search for QTLs and QTL markers may be particularly productive given that, unlike microsatellites, they represent transcribed loci likely to be functionally important in cellular processes important to organismal performance.

The detection of markers for QTLs, and the identification of QTLs themselves, is facilitated by the development of a genetic map which identifies the physical linkage relationships among the markers used. These can be established by segregation analysis of informative crosses where marker loci are polymorphic in at least one parent. Development of a basic genetic map for a species can be facilitated by carrying out segregation analysis for all markers on a common set of crosses. This avoids the need for duplicating crosses and costly repetition of linkage assessments and available resources can be focused on extending the numbers of mapped markers. Additionally, it ensures that all research groups are working from the same baseline. Once a map is developed, it can be used to identify sets of markers best suited for general surveys of QTL linkage and, once these have been found, to identify local groups of markers among which that showing the strongest linkage can be selected. By allowing pre-selection of optimal marker combinations, a marker map provides the information needed to design the most cost-effective and sensitive experimental design for detecting the optimal marker from those available. Maps are essential where a researcher wishes to ascertain the chromosomal position of a QTL.

The analysis of marker linkage with performance variation can be carried out within crosses made either between species, inbred lines (lines where individuals differ with regard to a particular performance trait and can be expected to be fixed for alternative variants), or between different individuals within an outcrossing population. In general, the most informative crosses can be expected to be those where the parental types used show the greatest divergence both with regard to performance and allelic variation at marker loci. In any case, only those loci which are polymorphic within the cross and for which parents possessed different allelic variants will be informative. Each mating design has different merits depending on the available biological material and should be carefully considered. Their relative merits are discussed in detail by Poompuang and Hallerman (1997) as well as Lynch and Walsh (1998) and Falconer and Mackay (1996). Depending on the approach taken, a variety of statistical procedures can be used to test for linkage between performance and marker variation and establish whether a QTL marker is present or not. Discussion of these methods can be found in the same references.

The methodology for the detection of QTLs, or of variation at physically linked genes, which are associated with and mark QTL variation is currently still being developed (Falconer and Mackay, 1996; Poompuang and Hallerman, 1997; Lynch and Walsh, 1998) and its application to selective breeding programmes to date is limited. Only one case of the identification of molecular markers for QTLs in fish has been reported in the literature; Jackson *et al.* (1998) who reported finding microsatellite variation marking two independent QTLs for upper temperature tolerance in rainbow trout (*Onchorynchus mykiss*). However, many QTLs have been identified in plants and mammalian species, and a number of research programmes aimed at identifying molecular markers for QTLs, or the QTLs themselves, in fish are currently under way (see Activities Reports).

In addition to the major challenge of detecting even the major QTLs (those genes which account for 5+% of observed genetically based performance variation), considerable development work needs to be done on integrating QTL-based selection procedures into traditional selection programmes. Given the need for addressing multiple traits simultaneously in breeding programmes using selection indices, optimal approaches to integrating molecular marker based genetic information will need to be developed and tested. However, progress in the field is likely to be rapid, largely as a result of work on mammalian species, and the coming years are likely to see an increasing number of markers for QTLs identified in fishes and their exploitation in selective breeding programmes for fish stock improvement.

Conclusions

Molecular markers offer considerable potential for aiding in the genetic improvement of cultivated fish stocks. Using microsatellite-based technology, resolving power can now easily be developed and applied cost-effectively for the simultaneous discrimination of several hundred families in mixed family groups. In contrast, while individual resolution is possible to develop, the costs of screening the numbers of microsatellites required is unlikely to be cost-effective.

The use of QTL markers in programmes aimed at the selective improvement of fish stocks is at present precluded. Only a few QTL markers are likely to have been identified in a limited number of species and approaches for their effective use in selection programmes need to be worked out.

At present, the use of molecular markers is far from a panacea for the genetic improvement of fish stocks. However, at least with regard to obtaining pedigree information, molecular markers are likely to be able to be used to increase selection intensities and thus rates of genetic improvement in a cost-effective manner. With the methodologies and technologies advancing rapidly, this is likely to become increasingly true.

RECOMMENDATIONS

Support research and development work to identify suites of microsatellite loci in mariculture species:

- *to facilitate pedigreeing within selective breeding programmes, particularly for newly cultivated species where specialized rearing facilities required for traditional approaches are not cost-effective or feasible;*
- *to monitor levels of genetic diversity among aquatic organisms used to found mariculture stocks, and during the course of breeding programmes as a result of selection and inbreeding;*
- *to provide molecular markers for quantitative trait loci (QTLs).*

Support the development of new technology that makes microsatellite-based pedigree analysis more rapid and cost effective such that greater selection intensities can be economically applied and rates of realizable genetic improvement increased.

Support research to develop analytical frameworks for the most cost-effective utilization of molecular pedigreeing within traditional phenotype-based selective breeding programmes.

Support research programmes to develop molecular marker based genome maps to facilitate the identification of QTLs and QTL markers.

Support research to identify QTLs for important mariculture traits, such as growth and disease resistance, and to identify molecular markers for tracking QTLs and allowing their direct selection in genetic stock improvement programmes.

Support research to develop generalized frameworks for the optimal utilization of QTL markers within traditional phenotype-based selection procedures in selective breeding programmes for cultivated aquatic organisms.

Support research to develop genetic improvement programmes based on marker-assisted selection (MAS) techniques.

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2.c. Triploidy in aquaculture

Based on a position paper, 'Tripoidy in Mariculture: Status and Perspectives', by Pierre Boudry and Beatrice Chatain; adopted by WGAGFM in Reykjavik 1999.

Introduction

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

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

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

2) As sexually matured animals can be of lower quality for consumption than non-matured ones (e.g., in salmon and oyster), sterility (or reduced gametogenesis) improves the marketing value of the products.

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

The present paper aims to review the present status of triploidy in mariculture in order to establish specific recommendations.

Methodology

Fish

The natural occurrence of triploids among vertebrates is rare (Bogart, 1980; Shultz, 1980; Cormier and Neiheisel, 1993). Spontaneous triploid individuals in normally diploid species have been reported among amphibians, reptiles, and birds (Ohno *et al.*, 1963; Witten, 1978; Tiersch and Figiel, 1991). Among fish, spontaneous triploidy has been reported in both wild and hatchery populations of rainbow trout (Cuellar and Uyeno, 1972; Gold and Avise, 1976; Gold, 1986). Dawley *et al.*(1985) reported triploids resulting from hybridization in natural populations of the sunfish genus *Lepomis*. Flajshans *et al.* (1993) also reported spontaneous triploidy in tench (*Tinca tinca* L.). A more detailed review may be find in Benfey (1989).

The artificial induction of triploidy has been reported in many aquaculture species. In fish, triploidy is generally induced by the retention of the second polar body by pressure or thermal shocks. An alternative is based on mating of tetraploids and diploids to obtain all-triploid stocks. In fish numerous reports have described the techniques to induce polyploidy (triploidy and tetraploidy), as reviewed by Thorgaard and Allen (1987), Ihssen *et al.* (1990) and Purdom (1993). Artificial triploidy, in particular, has been induced with variable success in several freshwater species for which artificial fertilization techniques have been developed. To date, more than a hundred different species have been investigated for triploidy or tetraploidy (see Table 1). However, if we exclude the pioneering work on flatfishes by Purdom (1972), results on chromosome set manipulations in marine fish remain confined to the last decade only. In Europe, most of the work was done on the sea bass, *Dicentrarchus labrax* L. and on the sea bream *Sparus auratus*, which are highly valued marine teleosts of major economic importance in the Mediterranean and European Atlantic areas. In sea bass, for example, different reports have been published concerning the induction of triploidy (Carrillo *et al.*, 1993; Zanuy *et al.*, 1994; Colombo *et al.*, 1995; Gorshkova *et al.*, 1995; Curatolo *et al.*, 1996; Felip *et al.*, 1997), and tetraploidy (Curatolo *et al.*, 1996). All these authors report more or less comparable methods for chromosome set manipulation in this species using either thermal or hydrostatic shocks.

As a general rule, the range of triploidy yielded varied considerably for different induction protocols applied to a wide variety of fish species. Nevertheless, as techniques are refined, very high percentages of triploid induction are often achieved, specially with high pressure treatment. This technique seems to be the most reliable, allowing 80 % to 100 % triploidy (Benfey and Sutterlin, 1984; Chourrout, 1984, Lou and Purdom, 1984; Peruzzi and Chatain, in press). Still, the survival resulting from such a treatment is highly variable and dependent on a strong maternal effect (Moffett and Crozier, 1995; Peruzzi *et al.*, in press).

Shellfish

In shellfish, ploidy manipulation has been reviewed by Baumont and Fairbrother (1991). The induction of triploidy has been reported in many species, such as mussels *Mytilus galloprovincialis* (Kiyomoto *et al.*, 1996), *M. edulis* (Yamamoto and Sugawara, 1988), *M. chilensis* (Toro and Sastre, 1995), oysters *Ostrea edulis* (Gendreau and Grizel, 1990; Hawkins *et al.*, 1994), *Saccostrea commercialis* (Nell *et al.*, 1994), *Crassostrea gigas* (Allen *et al.*, 1986; Desrosiers *et al.*, 1993), *C. virginica* (Allen and Bushek, 1992; Barber *et al.*, 1992), pearl oysters *Pinctada sp.* (Durand *et al.*, 1990; Jiang *et al.*, 1993), scallops *Pecten maximus* (Beaumont, 1986) clams *Tapes dorsatus* (Nell *et al.*, 1995), *Tapes philippinarum* (Utting and Child, 1994), *Mercenaria mercenaria* (Buzzi and Manzi, 1988) and abalone.

Triploidy is induced in fertilised eggs at meiosis I or meiosis II by the suppression of polar body I or polar body II formation. In contrast with fish ploidy manipulation, the most frequently used method is based on treatment of fertilised eggs using cytochalasin B. Other protocols are based on pressure shock, heat shock, and chemical treatment using 6-dimethylaminopurine (6-DMAP) (Desrosiers *et al.*, 1993).

An alternative method is based on the mating of tetraploids and diploids to obtain all-triploid stocks (Guo *et al.*, 1996). The production of tetraploids by chemical treatment of gametes of diploid individuals has been attempted in several species: *C. gigas*: (Cooper and Guo, 1989, Guo *et al.*, 1994); *O. edulis* (Gendreau and Grizel, 1990), *M. galloprovincialis* (Komaru *et al.*, 1995; Scarpa *et al.*, 1993). In most cases, tetraploid embryos were obtained but they showed very low or no survival. More recently, tetraploids have been obtained in *C. gigas* by inhibiting polar body I in eggs from triploids (Guo and Allen, 1994b). These tetraploids are fully fertile and can be easily crossed with diploids to produce all-triploid progenies (Guo *et al.*, 1996).

Growth

The main rationales for the use of the triploidy techniques in fish and shellfish culture are the production of sterile populations. Indeed, triploidy generally induces sterility (or strongly reduces gametogenesis). From a general point of view, better performance of triploids over diploids is expected, primarily for growth. In the early years, it was expected that in vertebrates, triploids may develop larger bodies because they have more chromosomes per cell than observed in plants. In plants, cell size is correlated with ploidy level and the body size itself is correlated with the cell size. However, studies of polyploid amphibians showed that although polyploids do have larger cells, their ultimate body size is not significantly larger than that of diploids (Fischberg, 1944; Fankhauser, 1945). In fish as well, it has been demonstrated that triploids have larger cells (Purdom, 1972; Thorgaard and Gall, 1979; Benfey *et al.*, 1984) but they do not necessarily reach larger ultimate size than diploids, nor do they generally grow faster, at least up to sexual maturity. The increase in cell size is compensated for by a decrease in the number of cells (Beatty and Fisher, 1951). In shellfish however, Guo and Allen (1994a, 1994b) have re-introduced the idea that triploids are bigger due to larger cells.

Triploids do not have normal gonadal development, they have much lower somatic indices, and are often functionally sterile because they produce aneuploid gametes (Allen *et al.*, 1986; Benfey *et al.*, 1986; Wang *et al.*, 1999). Hence, they might grow faster as the energy normally allocated to reproduction in diploids can be reallocated to growth in triploids. Consequently, triploidy should be of greater interest in species with high allocation to reproduction. In fish, results are equivocal and some studies report a slight growth advantage of triploids, whereas others found similar (case of sea bream, Sugama *et al.*, 1992; sea bass when triploids are reared separately from diploids, Zanuy, pers. comm.) or even reduced growth of triploids compared to diploids (case of sea bass when triploids are reared together with diploids, Chatain, unpublished data). Nevertheless, it can be said as a general rule that during the immature stage, performance differences between diploid and triploid fish appear to be species specific (see Krasznai and Marai, 1966; Taniguchi *et al.*, 1986; Thorgaard, 1986 for reviews) and that triploids have a better growth in later stages after maturation (case of the rainbow trout, Ihssen *et al.*, 1991; red sea bream, Sugama *et al.*, 1992; Atlantic salmon, Boeuf *et al.*, 1994). Triploids issued from the crossing of tetraploid and diploid fish show a similar pattern (Blanc *et al.*, 1987).

In shellfish, the higher performance of triploids over diploids is more frequently observed. The energy normally allocated to reproduction can then be reallocated to growth. Consequently, reserves are accumulated as glycogen and are not transformed into unpalatable lipids (e.g., in *Saccostrea commercialis*: Cox *et al.*, 1996, in *Mytilus galloprovincialis*: Kiyomoto *et al.*, 1996, in *Mercenaria mercenaria*: Eversole *et al.*, 1996, in *Crassostrea gigas*: Goulletquer *et al.*, 1996). In *C. gigas*, a multi-site comparative study of the performance of triploids and diploids was performed in France by IFREMER (Boudry *et al.*, 1998). The mean individual total weight was significantly higher in triploids compared with diploids in 4 different sites. There was a clear north-to-south cline for the difference in total weight. This result could be due to a positive correlation between summer temperature and reproductive effort in this species. Recently, Wang *et al.* (1999) showed that triploids issued from crosses between tetraploids and diploids were significantly bigger than triploids issued from cytochalasin treatments.

Survival

In addition to effects on growth, triploid fish have somewhat lower early survival than their diploid controls, while survival of yearlings to the age of sexual maturity seems not significantly different between triploid and diploid (case of rainbow trout, Quillet *et al.*, 1988; Atlantic salmon, Boeuf *et al.*, 1994; Pacific salmon, Pifferer *et al.*, 1994; red sea bream, Sugama *et al.*, 1992; sea bass, Chatain unpublished data; Pacific oyster, Gérard unpublished data). Sugama *et al.* (1992) noticed differences among families and emphasized an important quantitative genetic aspect of triploids—unequal inheritance. Guo *et al.* (1990) also reported that the growth of rainbow trout triploids was significantly affected by maternal strain effects, suggesting that identification and use of specific strains and crosses within that species might lead to improved growth of triploid offspring.

A similar consideration with respect to parental species contributions arises when triploid hybrids are produced. As a result of often lackluster results from simple hybridization, triploidy and hybridization have often been studied simultaneously and performances (mostly survival) of triploid groups were often found to exceed that of their diploid counterparts (Chevassus *et al.*, 1989; Oshiro *et al.*, 1991; Goryczko *et al.*, 1992; Gallbreath and Thorgaard, 1992; Blanc *et al.*, 1992; McKay *et al.*, 1992; Gray *et al.*, 1993). Particular interest lies in hybrids with specific resistance to disease in their triploid form (Dorson and Chevassus, 1985; Parson *et al.*, 1986).

Heterosis

Better performances of triploids are also expected because triploidy potentially induces higher mean heterozygosity. In fish, this has been clearly demonstrated by Allendorf and Leary, 1984. In sea bass, such correlations with survival in rearing conditions were also demonstrated (Bierne, unpublished data). In many shellfish species, high heterozygosity has been shown to be positively correlated with growth (for a review, see Zouros, 1987, but also Britten, 1996). Indeed, in *Ostrea edulis*, Hawkins *et al.* (1994) report faster growth in meiosis I triploids than in meiosis II triploids or in their diploid siblings. Relative heterozygosity of meiosis I triploids and meiosis II triploids depends on recombination (Beaumont and Fairbrother, 1991). Heterozygosity at a given locus depends not only on which meiotic division is suppressed, but also on recombination between the locus and the centromere. More knowledge about recombination and establishment of linkage maps would be of great interest on this question.

Quality

Sterility (or reduced gametogenesis) may improve the marketing value of the products as sexually matured animals can be of a lower quality for consumption than non-matured. Indeed, sterility in some species (e.g., salmonids, oysters) suppresses some of the undesirable phenomena associated with reproduction such as the deterioration in flesh quality, reduced appetite, reduced feed conversion efficiency, and post-spawning mortality. For example, in male triploid trout, the fat content in viscera and in muscle is particularly low and similar to that of mature diploids while the contrary is observed in triploid females (Chevassus *et al.*, 1988). High water content in muscle was also noticed as a typical sign of a maturation stage, it particularly decreases flesh quality, and that is absent in triploid females. This offers to producers the possibility of standardizing their products and just for this reason, in France, triploid trout are used for fish devoted to processing, the triploidy being obtained by thermal or pressure shocks. In oysters, The limited gametogenesis in triploids versus diploids leads to a higher glycogen content and lower lipid content during the summer period (Goulletquer *et al.*, 1996; Boudry *et al.*, 1998). These characteristics give triploid oysters a much better quality of flavour in summer (Allen and Downing, 1991).

Environment

The sterility of triploids can also be of interest itself, quite apart from other physiological or morphological considerations. Indeed, triploidy is often consider as a possible tool for the protection of genetic ressources against aquaculture escapees or to ensure genetic confinement of transgenic organisms. Nevertheless, as was shown previously, if the triploidization methods are not lacking, unfortunately their reproducibility still has to be improved before triploidy could be practically used. In species where all-triploid stocks can be obtained by mating tetraploids and diploids, the management of tetraploids should be carefully considered.

Moreover, even after overcoming such a technical problem, other problems may still remain, for example, the fact that triploid males in some species develop secondary sexual characteristics and even testosterone levels like those of diploid males as is the case with plaice (Lincoln, 1981). If their spawning behavior sufficiently mimics that of diploid males, they could mate with diploid females though such mating would produce no viable progeny because triploids either produce no sperm or aneuploid sperm. Consequently, if large numbers of triploids were introduced into a wild population, they could suppress the natural reproduction of the wild stock if the triploid males competed successfully with the diploid males for females. Cases where males were able to produce sperm are not rare as shown in rainbow

trout (Thorgaard and Gall, 1979). In contrast, no external sexual differentiation was observed in triploid brook trout, and gonado-somatic indices for males were even lower than those for triploid male rainbow trout (Ihssen *et al.*, 1990). In sea bass, males were not fluent but testis size was about half the size of diploid ones and sperm was non-functional (Colombo *et al.*, 1995; Peruzzi *et al.*, in press). In shellfish, triploids are often not fully sterile as shown in oysters, where gametes from triploids have been used to produce tetraploids (Guo and Allen, 1994b). More information on the relative fecundity of triploids compared to diploids and tetraploids is needed.

Considering more confined environments, triploids may also be used for population control. For example, uncontrolled reproduction is undesirable in tilapia culture (Penman *et al.*, 1987) because it can lead to overcrowded, stunted, poor-quality fish. Sterile fish permit more precise control of the number of fish per tank or pond until harvest and hence better rearing conditions. Exotic species that possess desirable characteristics not found in native fish may be also useful in fisheries if concerns about their uncontrolled reproduction could be overcome. For example, grass carp are desirable for the control of aquatic vegetation, but the introduction has been prohibited in many waters because they might reproduce and invade habitats used by native fish. Triploid grass carp, however, are sterile (Allen *et al.*, 1986) yet retain the feeding habits of diploids; these fish are permitted for vegetation control and now available commercially (Wiley and Wike, 1986).

Apart from these typical aquacultural considerations, triploidy sometimes finds other applications such as in the case when it is used to reduce the aggressive behavior of aquarium species such as the fighting fish, *Betta splendens* (Kavumpurath and Pandian, 1992). The aggressive behavior of the fish was related to its reproductive behavior.

RECOMMENDATIONS

1) Triploidy should be considered as one of the most effective and quick ways to improve productivity in aquaculture, mostly in species showing high allocation to reproduction, associated with decreased quality of matured animals. In that perspective, research on triploidy should be supported.

2) Triploidy should be especially favoured when the gene flow between aquaculture stocks and wild populations is to be reduced to preserve wild genetic resources, or to ensure the protection of selective breeding progress.

3) Total sterility of male and female triploids should be carefully examined before any further use is made of this technique for genetic confinement.

4) In the case of tetraploid production, its use to produce all-triploid progenies (by the way of mating them with diploids) should be considered. In that case, the management of these tetraploids should be carefully examined.

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Table 1. Examples of triploidy induction by retention of the second polar globule in fish species.

Genius and species	n	TAF (min)	TT	TD (min)	SP (°C/atm)	FT (°C)	ΔT	Energy (ΔT.DT)	Efficiency (%)	Survival (%)	References
Anabantidae											
Betta splendens	3	2.5	h	3	42				100	67 % at feeding	Varadaraj Pandian 1990
Bagridae											
Ictularus	2	5.0	co	60	5						Wolters *et al.* 1981
Centrarchidae											
Micropterus salmoides	3	5.0	p	1	500	22			100	60 % before hatching	Garret *et al.* 1992
Pomoxis	2	5.0	h	1–5	36–40	22*	16*	48*			Baldwin *et al.* 1990
Pomoxis	2	5.0	co	45–60	5	22*	−17*	−892*			Baldwin *et al.* 1990
Pomoxis annularis	3	5.0	h	1–5	36–40	21–23	13–19	48	10	60	Baldwin *et al.* 1990
Pomoxis annularis	3	5.0	co	45	5	21–23	−16–18	−765	24	40	Baldwin *et al.* 1990
Pomoxis annularis	3	5.0	co	60	5	21–23	−16–18	−1020	92	30	Baldwin 1990
Cichlidae											
Oreochromis	2	15	co	60	4–11	25*	−17.5*	−1050*			Valenti 1975
Oreochromis	2	2.5	h	3	42	25*	17*	51*			Varadaraj 1990
Oreochromis aureus	2–3	5.0	h	3.5	40.8–41.3	25*	16*	56			Penman *et al.* 1987
Oreochromis mossambicus	2–3	5.0	h	3.5	40.8–41.3	25*	16*	56*			Penman *et al.* 1987
Oreochromis mossambicus	2–3	2.5	h	3	42	25*	17*	51*			Varadaraj Pandian 1988
Oreochromis mossambicus	3	2.5	h	3	42	25*	17*	51*	100	67 % at feeding	Varadaraj Pandian 1990
Oreochromis niloticus	2–3	2.5	h	3	42	25*	17*	51*			Varadaraj Pandian 1990
Oreochromis niloticus	2–3	4.0	h	2–7	40.5–41	25*	15.75*	71*			Chourrou Itshovich 1984
Cyprinidae											
Brachydanio	2	3.0	p	5	542	25*					Streisinger *et al.* 1981
Brachydanio rerio	3	2.5	h	4	41	25	16	64	100	69 % of control	Kawumpurath Pandian
Brachydanto rerio	3	5–16	p	6–7	629–677	25*					Onazanto 1981
Catla catla	4	19–22	cc[a]	2	40				65		Reddy *et al.* 1990
Carassius	2	2.0	co	20	3						Jiang 1982
Ctenopharyngodon	2	1.0	h	5–15	2						Stanley Sneed 1974

Table 1. Continued.

Genius and species	n	TAF (min)	TT	TD (min)	SP (°C/atm)	FT (°C)	ΔT	Energy (ΔT.DT)	Efficiency (%)	Survival (%)	References
Ctenopharyngodon idella	3	fast	p		250–562						Cassani Cates 1986
Cyprinus carpio	2	5–15	co	60	4	20	–16	–960	100	56 % of incubated eggs	Nagy *et al.* 1978
Cyprinus carpio	3	1–9	co	45	0	20	–20	–900		50 % of incubated eggs	Gervais *et al.* 1980
Cyprinus carpio	3	5.0	co	30	0	23	–3	–690		10	Ueno 1984
Cyprinus carpio	3–2	3–5	co	30	–4–0	21*	–23*	–690*		36 % of incubated eggs	Wu *et al.* 1986
Cyprinus carpio	3	15	co	60	0	23	–23	–1380		5	Muiswinkel *et al.* 1986
Cyprinus carpio	3	15			0–4	22*	–20*			2.86	Linhart *et al.* 1986
Cyprinus carpio		3–5	h	2	39	20	19	38		50	Hollebecq *et al.* 1986
Cyprinus carpio		1–2 or 1–9	co	45	0	24	–24	–1080		54	Komen *et al.*
Cyprinus carpio	2[b]	40	h	2	40	22	18	36	100	10.5	Nagy 1986
Cyprinus carpio	2[b]	30	h	2	40	24	16	32	100	18	Komen *et al.*
Cyprinus carpio	2	1–2	co	45–60	0–2	20	–18–20	–990	100	31 % 1–2d after hatching	Cherfas *et al.* 1990
Cyprinus carpio	3	1.5–2.5	co	45–60	0–2	22*	21*	1092*	50	85	Cherfas *et al.* 1990
Cyprinus carpio	2[b]	28–30	h	2	40	24	16	32	100	15 % at 96h	Komen *et al.* 1990
Cyprinus carpio	2	1.0	co	45	0	24	–24	–1080	100	50 % viables	Komen *et al.* 1987
Cyprinus carpio	3		h	2–2.5	39 at 39.5	20 at 25	14 at 19.5	37.7			Cherfas Hulata 1992
Cyprinus carpio	4		h	2–2.5	39.5 at 40	20 at 25	14.5 at 20	38.8			Cherfas Hulata 1992
Cyprinus carpio	2	27–29.2							100	50 % at 24h	Rothbard 1991
Cyprinus carpio	3	6.0	h	1.5	41	20	21	31.5	100	70 % of control	Recoubrastky *et al.* 1992
Hypophtalmichtys nobilis	3	4.0	p	1.5	500	26			100 % at 20 h		Aldridge *et al.* 1990
Hypophtalmichtys nobilis	4	36	p	1.5	500	26					Aldridge *et al.* 1990
Labeo rohita	3	7	h	1–2	42	29	13	19.5	12		Reddy *et al.* 1990
Labeo rohita	4	19–22	h	2	39	29	10	20	70		Reddy *et al.* 1990
Labeo rohita	4	19–22	co	10	10–15	29	–14–19	–165	55		Reddy *et al.* 1990
Misgurnus	2	5.0	co	30	–2						Oshiro 1987
Misgurnus	2	5.0	p	6-8	484						Vasetskii *et al.* 1984
Rhodeus ocellatus ocellatus	2–3	5.0	co	60	0.2						Uneo Arimoto 1982

Table 1. Continued.

Genius and species	n	TAF (min)	TT	TD (min)	SP (°C/atm)	FT (°C)	ΔT	Energy (ΔT.DT)	Efficiency (%)	Survival (%)	References
Cyprinodontidae											
Orysias	2	3.0	h	2	41						Naruse *et al.* 1985
Percidae											
Perca flavescens	3	5.0	h	25	28–30	11	18	450		50	Malison *et al.* 1992
Perca flavescens	3	5.0	p	12	560	11				60	Malison *et al.* 1992
Perca flavescens	4	192	p	24	560	11					Malison *et al.* 1992
Osmeridae											
Plecoglossus	2	6.0	co	60	0						Taniguchi *et al.* 1987
Plecoglossus	2	6.0	p	6	580						Taniguchi *et al.* 1988
Pleuronectidae											
Pseudopleuronectes americanus	2		co	180	0	5	–5	–900	100	18days	Hornbeck Burke
Pseudopleuronectes americanus	3		co	190	0	5	–5	–950		34days	Hornbeck Burke
Pleuronectes	2	5–25	co	120–240	0	7*	–7*	1260*			Purdom 1969
Pleuronectes platessax	2–3	15	co	60	–1–0	7*	–6.5*	–390			Lincoln 1980
Platichtys flessus											
Pleuronectes platessa	2	20	co	240	-0.5	7	–7.5	–1800	97	56.7 % at 20d	Purdom Lincoln
Pleuronectes platessa	2	20	co	240	-0.5	7	–7.5	–1800	93 5	64.4 % at 20d	Purdom Lincoln
Salmonidae											
Truites	2–3	25	h	20	26.5	10	16 5	330	98	75–80 % at 100d	Chevassus Quillet Chourrout
Oncorhynchus keta	2–3	10	h	10	29						Seeb *et al.* 1988
Oncorhynchus kisutch	2–3	20	h	10	27						Parsons 1986
O.kisutch	2–3	10	h	10	29						Seeb *et al.* 1988
Oncorhynchus	2	10–50	h	10	28						Chourrout 1980
Oncorhynchus	2	10–50	p	10	560						Chourrout 1980
O. mykiss	2	5.0	p	6	670						Onozato
O. mykiss	2–3	40	h	10–15	27–28	10*	17.5*	218*			Lincoln Scott 1983
O. mykiss	2–3	20	h[c]	10	27	10*	17*	170*			Parsons *et al.* 1986
O. mykiss	2–3	10	h	10	28–29	10*	18.5*	185*			Thorgard 1983
O. mykiss	2–3	10	h	10	28	10*	18*	180*			Scheerer Thorgard 1983
O. mykiss	2–3	40	h	10	28	10*	18*	180*			Thompson Scott 1984
O. mykiss		25	h	20	26	10*	16*	320*			Guyomard 1984
O. mykiss	2–3	20–45	h	10	27–30	10*	18.5*	185*			Chourrout 1980
O. mykiss	2–3	25	h	20	26	10*	16*	160*			Chourrout 1980
O. mykiss *O. kisutch*	2–3	10	h	20	27	10*	17*	340*			Parsons *et al.* 1986
O. mykiss	3	20–25	h	20	26.5	10*	16.5*	330*			Vauchez 1991
O. mykiss	3	25	h	20	26	10*	16*	320*			Chourrout Quillet. 1982
O. mykiss	3	0–45	h	10–15	27–28	10*	17.5*	218*			Lincoln Scott. 1983
O. mykiss	3	10	cc[c]	10	27	10	17	170	80	68 % at hatching	Anders 1980

Table 1. Continued.

Genius and species	n	TAF (min)	TT	TD (min)	SP (°C/atm)	FT (°C)	ΔT	Energy (ΔT.DT)	Efficiency (%)	Survival (%)	References
O. mykiss	3	30	h	17	27	8.7	18.3	311	93 % at 35d 86 % at 160j		Guo *et al.* 1990
O. mykiss	3	10	ch		pH=10, 100 ml water, 750 mg NaCl, 28 mg KCl, 1.11 mg $CaCl_2$				33	6.3	Ueda *et al.* 1988
O. mykiss	3	10	ch	20	pH=10, 100 ml water, 780 mg NaCl, 28 mg KCl, 1.11 mg $CaCl_2$				80	14.7	Ueda *et al.* 1988
O. mykiss	2[b]	210	h	5	31.5	10	21.5	107	16 % larval stage		
O. mykiss	2[b]	20 at 25	h	5	26.5	10*	16.5*	82*	63 % feeding		
O. mykiss	2	220	h	5	31.5	10*	21.5*	107*	100	50 % of control at eyed stage	
O. mykiss	2	5–15	p	6–7	630–726				100		Yamazaki 1983
O. tshawytscha	2	8, 16 or 24[d]	h	20	25	10	15	300	100	50 % of control at 55d	Levandusky *et al.* 1990
Brown trout	2	25–45	h	20	26	10	16	200	100	29 % at 90d	Quillet *et al.* 1990
Salmonidés	3	20	h		26	10	16	416		75 % of control at 95-105d	Quillet Chevassus Chourrout 1987
Salmonidés hybrides	2–3		h	10	28						Scheerer Thorgaard 1983
Salmonidés hybrides	2–3	25	h	20	26	10*	16*	416*			Chevassus Guyomard Chourrout 1983
Salvelinus fontinalis	3	15	h	10	28	10	18	504	62 % of control at 7-8 month		Dubé *et al.* 1990
Salmo	2	10	h	28							Johnstone 1985
Salmo salar	2–3	20	h	12	30	10*	20*	240*			Johnstone 1985
Salmo salar	2	1–5	h	20	26	10	16	320	10	70 % of control at feeding	Quillet Gaignon 1990
Salmo salar	3	1, 5, or 10	h	10 or 20	26–29	10	16–19	175,350		89 % of control at feeding	Quillet Gaignon 1990
Salmo trutta	2–3	25	h	20	26	10*	16*	320*			Guyomard 1986
Salmo trutta	2–3	5–45	h	10	29	10*	19*	190*			Araï Wilkins 1987
Salmo trutta	3	.05	h	10–15	28	10	18	225	100		Quillet *et al.* 1990
Salmo trutta fontinalis	2–3	10	h	10	28						Scheerer Thorgaard 1983
Salmo trutta salvelinus	2–3	10	h	10	28						Scheerer Thorgaard 1983
Sparidae											
Acanthopagrus schlegeli	3	1.5	co	5–25					90		Arakawa *et al.* 1987

Table 1. Continued.

Genius and species	n	TAF (min)	TT	TD (min)	SP (°C/atm)	FT (°C)	ΔT	Energy (ΔT.DT)	Efficiency (%)	Survival (%)	References
Pagrus major	3	3.0	co	5–15		21–23			100	70 % at hatching 40 % at swimming stage	Arakawa *et al.* 1987
Pagrus major	2	3.0	h	2.5	35	18	17	42.5	100	74 % 3d after hatching	Sugama *et al.* 1990
Pagrus major	3	3.0	co	12	0	18	–18	–216	100	64 %[e] of control at feeding	Sugama *et al.* 1990
Sparus aurata	3		h	10	28	18	10	100		35 % 5d after fertili-sation	Glamuzina Jug-Dujakovic[f]

n: degree of ploidy (2: gynogenesis, 3: triploidy, 4 tetraploidy)

TAF: Timing After Fertilisation for the shock application

TT: Type of Treatment (h: hot shock, co: cold shock, p: pressure shock, ch: chemical)

TD: Treatment Duration

SP: shock power in Celcius degrees or in atmospheres

FT: fertilisation temperature;

ΔT: difference between TF and D: difference between the shock temperature and the fertilisation temperature

TE: Thermal Treatment Energy expressed by the product of ΔT and DT

a: cold shock is inefficient;

b: endomitotic individual;

c: autumn and spring lines have different treatment optimum;

d: similar survival for 3 TAF;

e: no difference in survival at 10 months between 2n and 3n;

f: freshwater fish have different optima compared to marine fish;

*: data issued from another publication.

The efficiency is the maximal percentage of success in the ploidy modification. The survival, evaluated at different times according to the experiments, is also the maximal percentages cited by authors. The calculation of the intensity (ΔT. DT) allows the comparison between thermal shocks done at different temperatures and takes into account the treatment duration.

2.d. Measures for protecting marine genetic diversity

Based on a position paper by Ellen Kenchington, 'Protecting Marine Genetic Diversity'; adopted by WGAGFM in Reykjavik 1999.

Introduction

The preservation of marine genetic resources is now viewed as one of the major aims of conservation. Today, the integrity of entire coastal ecosystems is threatened as a result of human activities (cf., Beatley, 1991), and public concern has prompted governments to adopt new policies to protect natural resources for humanity. In January 1992, at the Earth Summit in Rio de Janeiro, over 145 countries signed the legally binding agreement referred to as the Convention on Biological Diversity (CBD). Those Signing Parties agreed on a programme for implementing the CBD with respect to marine and coastal biodiversity at their second Conference of Parties (COP) held in Jakarta, Indonesia in 1995. De Fountaubert *et al.* (1996) provide an overview of the CBD and related international agreements in *Biodiversity in the Seas* and present an analysis intended to help the Parties implement the Jakarta Mandate.

The CBD defines diversity as 'the variability among living organisms from all sources, including, inter alia, terrestrial, marine and other aquatic ecosystems and the ecological complexes of which they are part: this includes diversity within species, between species and of ecosystems.' The genetic value of resources was one of the many components acknowledged, both for its role in maintaining traditional fishery and mariculture practices, and for the potential benefits of new products derived from those resources.

The economic value of genetic resources has recently been focused on the tremendous potential associated with new products for medical, pharmaceutical and biotechnological applications. Bioprospecting, that is, actively collecting marine organisms and researching potential applications for marine resources, is currently an active pursuit in many countries. The economic success associated with relatively recent discoveries such as *Taq* polymerase, an enzyme discovered from hyperthermophilic bacteria living in deep-sea vents (estimated annual sales of US$100 million), and new cancer-fighting drugs derived from molluscs and bryozoans that are currently in pre-clinical and clinical trials, have been invaluable in directing public attention to the importance of protecting marine diversity for the future (de Fontaubert *et al.*, 1996). Recognizing the potential for the more developed nations to exploit the natural resources of those less technically advanced, the CBD established a new international regime for the transfer of 'genetic resources' (defined as 'genetic material of actual or potential value'). The CBD affirms each Party's sovereign right to control access to its genetic resources, while requiring that the Party make efforts to facilitate access for other Parties. It also requires the users of genetic resources to take measures to promote equitable sharing of the benefits, including technologies, with the providers of those resources (de Fontaubert *et al.*, 1996).

Five priorities toward implementation of the CBD were identified in the Jakarta Mandate, and eight action items were recommended to support those priorities. Those action items are:

1) institute integrated coastal area management (ICAM), including community-based coastal resource management, and prevention and reduction of pollution from land-based sources;

2) establish and maintain marine protected areas for conservation and sustainable use;

3) use fisheries and other marine living resources sustainably;

4) ensure that mariculture operations are sustainable;

5) prevent introduction of and control or eradicate, harmful alien species;

6) identify priority components of biodiversity and monitor their status and threats to them;

7) build capacity to use and share the benefits from marine genetic resources; and

8) take responsibility for transboundary harm and global threats to marine biodiversity.

Many ICES Member Countries have moved toward implementation of these actions, however, the process has been slow as it requires major changes in policies and programmes.

This paper will deal with Item 2, the establishment of protected areas, specifically with respect to protecting marine genetic diversity. Marine protected areas (MPAs) are coastal or oceanic management areas designed to conserve biodiversity which includes diversity within species, between species and of ecosystems; there are currently over 1,000 MPAs ranging in size from 3 km^2 to 40,0000 km^2 (de Fontaubert *et al.*, 1996). Included in the objectives of many MPAs are the protection of endangered species and the maintenance or restoration of viable populations of native species and of genetic diversity. Extinction, extirpation, hybridization and loss of genetic variation within populations represent the major categories of threat to genetic diversity within species (cf., Ryman *et al.*, 1995). The genetic resource management plan for a MPA will therefore depend upon the nature of the threat to genetic diversity. When endangered species are implicated, the immediate concern will be to avoid extinction, requiring the maintenance of viable populations in the short-term (cf., Polunin, 1983; Meffe, 1986). This short-term preservation of endangered species presents a special challenge as the extant representatives are in most cases the remnants of a formally larger and more diverse gene pool. Longer-term goals require the maintenance of genetic diversity in the species to allow for adaptation to environmental change and for continued speciation in order to maintain evolutionary flexibility for the future (e.g., Fisher, 1930; Soule, 1980; Polunin, 1983).

Total genetic variation within a species can be partitioned into variation within and among populations. Natural selection acts within populations, while the genetic potential of the species to adapt to environmental changes depends on the total genetic diversity represented among populations. In most marine species, where the parents produce large numbers of offspring, there is large scope for local selection. It is necessary to maximize both types of variation to maintain full potential for evolutionary change within a species.

The above considerations apply equally to marine plant and animal species, although there is a tendency for marine algae to be overlooked in discussions of marine biodiversity (Van Oppen *et al.*, 1996).

Within-Population Variance

Population size is the single most important factor in sustaining a high level of genetic variation within a population of a species. Population size here refers to the genetically effective population size (*Ne*), and not the number of individuals in a population (*N*). Population geneticists define *Ne* as 'the size of an idealized population that would have the same amount of inbreeding or of random gene frequency drift as the population under consideration (Kimura and Crow, 1963)'. *Ne* is considered to be the most appropriate variable for assessing population viability (Barton and Whitlock, 1997). Maintaining large *Ne* increases the likelihood that favourable mutations will become widespread and deleterious ones will be eliminated. *Ne* is nearly always less than *N* because generally not all individuals in a population are reproductive at spawning time. Estimates of *Ne* are under the influence of sex ratio, variation in the number of offspring per family, and fluctuating population numbers. The influence of these variables on *Ne* is presented by Meffe (1986) and summarized here.

Sex ratio is an important determinant of genetic variation. If the sex ratio of breeders departs from 1:1, *Ne* and genetic variation will be reduced. The effective population size with respect to sex ratio is determined as:

$$Ne = 4(Nm \times Nf)/(Nm + Nf),$$

where *Nm* and *Nf* are the numbers of male and female breeders, respectively (Frankel and Soule, 1981). It can be seen that an effective population of 50 males and 50 females is nearly 2.8 times larger, genetically, than one of 10 males and 90 females.

Variation in the number of offspring per family is expected to follow a Poisson distribution (where variance = mean). If certain matings produce disproportionately more offspring, biasing the representation of contributed gametes in the next generation, *Ne* will be lower. A biased progeny distribution will affect *Ne* approximately by:

$$4N/(2 + \sigma^2),$$

where σ^2 is variance in progeny distribution (Franklin, 1980). The effective population size can be drastically reduced by disproportionate offspring production. For example, consider two theoretical populations in a 1:1 sex ratio, one with 1000 females reproducing following a Poisson distribution with a mean of 2 offspring and a σ^2 of 2, and the other consisting of 1 female producing 1001 offspring and the remaining 999 females producing 1 each (mean of 2 offspring, a σ^2 of 31.6). In the first instance, *Ne* will be 1000 while in the second, *Ne* is reduced to 119.

Whenever a population declines, the genetic variance for all future generations is contained in the few survivors. Since those individuals represent only a sample of genetic variance contained in the original population, *Ne* is reduced by

fluctuations to low levels. This effect is known as FPS, fluctuation in population size. *Ne* is affected by the harmonic mean of population sizes in each generation, or:

$$1/Ne = 1/t\ (1/N_1 + 1/N_2 + \ldots + 1/N_t),$$

where t = time in generations (Franklin, 1980). Population fluctuations can be seen to be very important to *Ne* by applying this equation to two theoretical populations, one of 100 fish for each of 5 consecutive generations for an arithmetic mean of 100, and one with a similar mean but with a population fluctuating each generation as 100, 10, 300, 10, and 80. In the first scenario, $Ne = 100$ while in the second, $Ne = 22$, a 78 % reduction effected through population crashes. Estimates of FPS are dependent on time-scale. As more census records are incorporated, estimates of FPS tend to increase (Vucetich and Waite, 1998). Estimates of *Ne* based on only a few annual counts (i.e., 10) are statistically quite unreliable and long-term population monitoring is necessary to provide a framework for interpreting *Ne* estimates calculated from census data (Vucetich and Waite, 1998).

The above aspects are all highly relevant to three closely related problems associated with small populations: genetic bottlenecks, genetic drift and inbreeding. Genetic bottlenecks arise as a result of a sudden and dramatic decline in numbers resulting in a remnant population with less overall variation than the original population. The degree of change in genetic diversity will depend upon the range present in the original population, the relative magnitude of the decline in numbers and the degree of randomness applied to the selection of survivors. Genetic bottlenecks generally result in both the reduction in variance of quantitative traits and in the loss of rare genetic variants. Because a small number of individuals contain most of the genetic variation in the original population, a bottleneck must be very severe and prolonged in order to drastically reduce the amount of quantitative variation. However, the loss of rare genetic variants (e.g., alleles occurring at frequencies of 5 % or less) may be important in the long-term ability of the species to adapt to changing environmental conditions.

Genetic drift, the random change in gene frequencies, can ultimately produce fixed loci (no genetic variance) through a repeated loss of variance in a small population. In the short-term, drift can significantly reduce the proportion of polymorphic loci, the average number of alleles per locus, and average heterozygosity per individual. Genetic drift can be an important factor in both hatchery breeding programmes and natural stocks.

Inbreeding depression results from the mating of individuals which are related by common ancestry. Closely related individuals will have more genes in common due to descent compared to individuals randomly selected from the population. The result of inbreeding is an increase in homozygosity which differentially affects different fitness and other characters of low heritability (Falconer, 1981). While inbreeding occurs in nature, it poses a special problem to the mariculture of aquatic organisms which have practical constraints on *Ne*. In such situations, the effects of inbreeding can be mitigated through selective mating.

There is no absolute measure of inbreeding, as the degree to which it occurs is measured against the base population. The increase in inbreeding per generation is the usual means of evaluating this occurrence and is related to the effective population size:

$$\Delta F = 1/(2Ne).$$

Such traits as fecundity, fertility, age-at-maturity, clutch size, growth, and survivorship can be greatly depressed by inbreeding. Frankel and Soule (1981) document that a ΔF of 10 % may result in a 25 % decrease in population reproductive performance and suggest that even a ΔF of 1 % ($Ne = 50$) is only tolerable in the short term. Recent theoretical work suggests that population sizes of 1000 to 5000 are required for long-term viability of the population, to avoid reduction in fitness traits (Lynch, 1996). Previous values of 500 or more brood stock (Frankel and Soule, 1981) appear to have been underestimated.

Among-Population Variance

While many species have free-drifting larvae, gametes or spores (approximately 70 % of marine invertebrates species have pelagic larvae; Mileikovsky, 1971) and are ultimately distributed over a wide area, local populations can often be discerned. Populations which are reproductively isolated, with little or no gene flow between them, will tend to genetically diverge either through different selective forces or through genetic drift. Ultimately this leads to speciation. Salmonids represent a group with high levels of among-population variance resulting from their homing behaviour at spawning time (e.g., Gharrett and Smoker, 1993). In such species, it is important that variance among naturally isolated populations be preserved and that their relative isolation be maintained wherever possible. The diversity contained among-populations may be important in the long-term ability of the species to adapt to environmental change.

Managers may also exploit this variability in matching gene pools to environmental conditions in re-stocking new or environmentally altered sites. Conversely, when formerly widespread populations with large *Ne* are artificially separated by human or other means (e.g., causeways blocking migration routes), supplemental gene flow through human intervention may be necessary to prevent the genetic problems faced by small populations detailed above.

When populations are isolated for long periods of time, closely-linked genes may become co-adapted, that is, they have evolved to work well with one another. Subsequent hybridization of such populations may result in co-adapted gene complexes breaking down, resulting in a loss of fitness over subsequent generations (Hindar *et al.*, 1991). This phenomenon is known as out-breeding depression and has been documented in Atlantic salmon (Stahl, 1981; Emlen, 1991).

Protection of Marine Genetic Diversity

In contrast to some ecological indices of community health, the effective population, *Ne*, is the significant unit in the management of genetic diversity. However, the point between individuals where the transfer of genetic material no longer occurs (generally at the species level) represents a unique evolutionary lineage that cannot be replaced once lost. The management of populations serves only to protect and preserve the variation represented at the species level. Unfortunately, for *most* marine species, data on population structure and the range and geographic partitioning of genetic and morphological variation are lacking (although many species have been studied). The development of management plans to protect marine genetic diversity may have to proceed without the full complement of genetic information needed to develop an optimal design for a given species.

These uncertainties and popular misconceptions have often become the focus of debate, diverting attention from the need to establish important safeguards for the protection of genetic diversity (cf., Currens and Busack, 1995). A pervasive popular misconception, that because sea water is a continuous medium there is a great deal of genetic interchange among populations of marine species, and therefore little need to protect populations but rather only representative individuals (cf., Thorne-Miller and Catena, 1991), is in direct opposition to the requirements for management of genetic diversity detailed above. This misconception is further endorsed by the fact that genetic divergence of populations or even species is not necessarily reflected in detectable morphological difference, incorrectly inferring genetic homogeneity to the layperson. In fact, there is considerable discussion as to whether oceanic species can become endangered (cf., Beverton, 1990; Culotta, 1994; Hunstman, 1994; Malakoff, 1997).

The concept of genetic risk assessment for fishery scientists and managers is still relatively new (e.g., Currens and Busack, 1995; Allendorf *et al.*, 1997) but may provide a framework for decision making in light of uncertainty. Genetic risk assessment is ecological risk assessment (Lackey, 1994) that emphasizes the systematic identification and characterization of vulnerability to losses of genetic diversity categorized as extinction, loss of within-population genetic variability, loss of among-population variability and domestication or loss of fitness in a wild population held in captivity. Loss(es) is evaluated at biological endpoints (e.g., individuals, populations, species, or communities) and the vulnerability of the system is assessed through evaluation of proposed safeguards and their associated risks (Currens and Busack, 1995; Allendorf *et al.*, 1997). The incorporation of genetic risk assessment into the planning of MPAs is likely to increase given the knowledge gaps discussed above. Allendorf *et al.* (1997) have provided a set of criteria for assigning priorities to salmonid stocks where the large number of threatened breeding populations (300+ in the Pacific Northwest USA) poses problems for implementing protection. In this approach, multispecies considerations can be evaluated as co-ocurring species with similar profiles can be grouped together to secondarily rank the benefit of proposed conservation measures. Allendorf *et al.*'s (1997) method has been advocated for the conservation of brown trout genetic diversity in Britain and Ireland (http://www.qub.ac.uk/bb/prodohl/TroutConcert/TroutConcert.htm). While there are inherent problems with this approach (Given and Norton, 1993; Allendorf *et al.*, 1997), including the acceptance of possible loss of genetic diversity and breeding populations, it may be useful in situations where a large number of stocks warrant protection.

With the difficulties associated in establishing MPAs, it is likely that in cases where the immediate objective is genetically based, it will be to preserve endangered species. Few attempts have thus far been made to involve local populations in the collection of threatened biological resources or in the management of protected areas (but see NINA, 1990; Allendorf *et al.*, 1997). The preservation of endangered species may involve enhancement of the surviving population(s) through captive breeding (Fleming, 1994). Hatcheries producing seed for enhancement purposes will have different strategies from those producing mariculture stock (Smith, 1998). In the former, the largest feasible *Ne* representative of the base stock should be initiated and maintained, and selective mating should be performed to minimize inbreeding effects (Meffe, 1986). *Ne* can be further maximized by maintaining equal sex ratios of breeding adults, insuring even progeny distribution by selective mating and culling of excess offspring, and avoiding population crashes. Periodic introduction of wild individuals from the same locality as the original brood stock into the captive population, or release of one sex for interbreeding with the resident population, will mitigate against the further erosion

of quantitative genetic variation. Maintenance of captive stocks for enhancement purposes should be of short duration to avoid extensive artificial selection for hatchery conditions (Meffe, 1986) and only used when other means of re-establishing the threatened populations (e.g., habitat restoration) have been exhausted (Tringali and Bert, 1998).

The Nature of Marine Protected Areas

Marine protected areas are physical units and so physical boundaries must be defined such that they meet the objectives of genetic conservation. The size and location of these boundaries will vary according to many factors, but will relate to the area occupied by the effective population in both time and space. In species that are territorial, sessile or relatively small it may be possible to protect entire breeding populations in relatively small geographic units. For highly motile organisms (e.g., pelagic fish), it may only be possible to preserve spawning aggregations unless expansive areas (or chains of contiguous smaller areas) are preserved throughout the distribution of the species. Where the proportion of genetic variation among-populations is high, MPAs should be established which will protect each threatened population. Selection of MPAs on the basis of rare or pristine habitats or landscapes (Urban *et al.*, 1987) may indirectly protect associated endemic species in advance of discovery (e.g., sea-mounts, Koslow and Exon, 1995; deep-sea vents, Grassle, 1991).

In addition to determining physical boundaries surrounding MPAs, decisions must be made on what activities are permitted within those boundaries; marine protected areas are not necessarily no-fish zones. At this point, the establishment of MPAs may conflict with historical commercial and recreational use of the area. In determining what activities are permissible, managers must also consider enforcement issues. Currently, there is strong evidence that selection generated by fishing is directly affecting fitness traits such as size-at-age and age-at-maturation without necessarily reducing the populations to near extinction levels (see reviews by Smith, 1994; Law, 1999). In some cases, there has been a loss of genetic diversity associated with overfishing (e.g., Smith *et al.*, 1991; Merkouris *et al.*, 1998). In addition, there may be indirect genetic effects caused by the stress-disturbance of fishing gear. From the perspective of preserving genetic diversity and also establishing base populations against which unprotected populations can be compared, fishing should be completely banned in MPAs established for the conservation of genetic resources.

RECOMMENDATIONS

1. *Genetic resource management plans for MPAs should consider both the short-term preservation of endangered species as well as longer-term goals for the maintenance of genetic diversity within species in order to allow for adaptation to environmental change and for continued speciation that are needed to maintain evolutionary flexibility for the future.*

2. *Genetic risk assessment for fishery scientists and managers should be incorporated into MPA decision-making processes in light of uncertainty.*

3. *Captive rearing to speed the process of recolonization should only be used as a last resort when other means of rehabilitation (such as habitat restoration, fishery protection, elimination of pollution) have been exhausted. If this option is used widely, published genetic principles should be utilized.*

4. *Where genetic diversity among populations is high, MPAs should be established to protect as much of this diversity as possible.*

5. *When the objective of an MPA is to protect genetic diversity, the protected boundaries should encompass all or part of the breeding distribution of prioritized species or populations, as well as taking other considerations into account. This information should then be incorporated into modeling approaches based upon numbers that are routinely used to establish MPAs.*

6. *Rare or pristine habitats or landscapes, such as sea-mounts or deep-sea vents which support previously undescribed species, should be considered for MPA designation as an indirect approach to protecting genetic diversity.*

7. *Fishing and other human activities should be restricted in MPAs established for the conservation of genetic resources in a way that is consistent with the genetic objective. In most cases this is likely to mean no fishing. In this way base populations will be established against which unprotected populations can be compared.*

8. *Genetic expertise should be included in the planning groups to establish MPAs.*

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2.e. Genetic tags in management of wild stocks

Based on a position paper by Andy Ferguson, 'VNTR Genetic Tags in the Study and Management of Wild Stocks'; adopted by WGAGFM in Reykjavik 1999.

Background

The outline brief in this ToR was to consider new areas of investigation resulting from the development of VNTR markers over the past five years. So the basic question that I want to consider is, what advances are possible through the use of VNTRs as markers for the study and management of wild stocks of exploited marine organisms? VNTRs (Variable Number Tandem Repeats) are generally separated, on the basis of the length of the repeat unit, into microsatellites (repeat unit of 1–6 bp) and minisatellites (repeat unit 9–64 bp). As only a few groups are still working with minisatellites, I will concentrate on microsatellites although many conclusions in respect of one type are applicable to the other. However, mutation processes, and thus assumptions in data analyses, may be different for the two types.

Since 1993 the application of microsatellite tags to the study of fisheries stocks has increased exponentially and although only a limited number of studies has been published to date, many studies are in progress. The relative ease with which a large number of highly variable markers can be obtained for most species has encouraged many workers to adopt microsatellites as their marker of choice. While early studies were frustrated somewhat by problems in the use of dinucleotide repeats, recent methods (e.g., Kijas *et al.*, 1994) for selectively enriching libraries for tri- and tetra-nucleotide repeats has overcome these problems to a great degree. Using these methods, and with automated sequencing, it is now possible for an experienced worker to obtain 50 microsatellite primer sets, primarily tri- and tetra-repeats, for a species in about three months. Automated multiplexed screening now makes it possible for large numbers of individuals/loci to be processed relatively rapidly, although considerable effort may be required to optimise conditions in the first place. There is also considerable capital cost for the automated equipment required. Certainly screening of several hundred individuals for 8 loci in a day by one worker is now a realistic proposition. The potential number of microsatellites available is enormous, possibly in excess of 100,000 for many species. Allelic variability is high, with 5–50 alleles being commonly found at many loci in most species. Thus the potential markers available far exceed our technical capacity to deal with them at present. Future advances involving nanotechnology (e.g., 'DNA chips') seems likely to overcome this within the next decade.

Properties of microsatellites

Application of any type of molecular variant as a genetic tag requires knowledge of the mechanisms by which the variant is generated and influenced by various evolutionary processes. Thus, estimation of various population parameters of interest in a fisheries context is dependent on the mutation model, and to some extent the rate of mutation, assumed for microsatellites. At least four different mutation models have been suggested to apply to microsatellites (Estoup and Angers, 1998). It would also appear that mutation processes vary greatly among loci, and possibly among alleles at a single locus, and this makes the construction of generalized models very difficult, if not impossible. Selection of a suite of loci with similar and easily assessed mutation parameters may be crucial for accurate estimates of population parameters (Estoup and Angers, 1998). There would appear to be a selective constraint on the upper size limit of microsatellite alleles. For example, Dermitzakis *et al.*, (1998) found that for two closely linked microsatellite loci in the fish *Sparus aurata*, there was a negative covariance in repeat number between the two loci. This suggests a mechanism that maintains the combined length of alleles at the two loci below a constrained size. Selection appears also to occur on the difference between alleles at a single locus, i.e., there are size constraints on how different two alleles can be. Given the importance of spatial organisation in gene regulation such size constraints are not unexpected. From the point of view of their use as markers, purifying selection for microsatellites is, however, no different than purifying selection that acts against particular alleles in coding regions. More important is selection on linked alleles. As microsatellites sometimes occur in intron regions, it is to be expected that they are almost certainly influenced by such 'hitchhiking' selection although the extent of this will depend on the microsatellite mutation rate (Wiehe, 1998). The idea of microsatellites as truly neutral markers is almost certainly invalid in spite of the many statements to the contrary in the literature. As with any other class of marker, basic information on the inheritance, sex linkage and co-linkage is required.

Data analysis

Basically three types of data are available from nuclear molecular markers: individual genotype data at one or more loci, derived allele frequencies, and evolutionary relationships among alleles. The different evolution of microsatellites from other markers and their higher allelic variability has necessitated the development of new methods of data analysis, even for conventional analyses. Until recently, most analyses were based on allele frequencies in a sample of 50 – 100 individuals assumed to represent the population from which they were drawn, although this assumption has

only rarely been tested. More recently, methods of analysing individual genotype data and also of making use of phylogenetic information among alleles have been developed.

The power and conceptual simplicity of individual-based genotype analyses, as opposed to population-based ones, become possible because of the enormous amount of information present in large numbers of highly variable loci. The large number of alleles detectable at most microsatellite loci means that when only a moderate number (5+) of loci are used, most individuals will have unique multi-locus genotypes. With individual-based analyses it is generally better to examine more loci than to have larger sample sizes. This is an important consideration given the large sample sizes necessary to estimate accurately population-based statistics (e.g., O'Connell and Wright, 1997; Ruzzante, 1998).

One of the potential advantages of microsatellites over other makers such as allozymes is that the evolutionary relationships of the alleles are potentially available. However, obtaining phylogenetic information for microsatellite alleles is very dependent on the mutation process involved. Thus, if a stepwise mutation model (SMM) holds, then the fewer the number of repeat differences, the closer the alleles are in a genealogical sense. A major problem in the use of microsatellites is homoplasy. While this is likely to be increasingly a problem the more distantly related the populations, it may also be problematic for closely related ones. Thus, alleles with the same number of repeats may not be homologous and may be different in terms of sequence. Ideally all microsatellite alleles and flanking regions should be sequenced to reduce this problem. However, as with any markers, congruence among many unlinked markers will increase confidence in the conclusion.

Population structure and gene flow

Determination of the genetic population structure and the extent of inter-population gene flow for exploited species has been a major goal of fisheries geneticists for many decades. This involves both testing for significant sample differentiation and quantifying differentiation among samples. One of the simplest ways of identifying population admixture is as a result of deficit of heterozygotes in a mixed sample—the Wahlund effect (Wahlund, 1928). Wright (1931, 1943) introduced his F-statistics as a means of using allele frequency data to quantify population subdivision and estimate gene flow. These statistics have formed the backbone of much of the analysis of molecular data over the past 35 years. Thus these analyses have generally involved heterogeneity testing of genotype or allele frequencies together with F statistics and genetic distance measures. The higher mutation rate and thus the higher level of allelic variability of microsatellites potentially increase the power of tests for population differentiation. As expected, studies with microsatellites on marine fishes have, in some cases, shown much higher levels of genetic differentiation than found with previous genetic markers. They have also identified population structure within geographical areas previously thought to be genetically homogenous (e.g., Rico *et al.*, 1997; Ruzzante *et al.*, 1997; O'Connell *et al.*, 1998; Shaw *et al.*, 1999).

However, even highly variable microsatellites will not necessarily detect differences among significantly differentiated populations. For population-based analyses, a low level of gene flow between populations will result in genetic uniformity. From a management point of view, however, stocks with 10 % exchange per generation are largely self-recruiting stocks and still need to be treated as separate management units. Such levels of gene flow will result in genetic uniformity as far as conventional population-based statistics are concerned. Theoretically, even with a 10 % level of migration individuals within a stock should still on average be more closely related than individuals belonging to different stocks. Determining relatedness beyond the first order would however require a very large number of loci. Use of 40 loci, with a mean heterozygosity of 0.75, would result in a misclassification of 8 % of individuals when comparing unrelated to half sibs (Blouin *et al.*, 1996). A phylogenetic tree, based on allele sharing at a large number of loci, should be largely monophyletic with respect to each sample if population structure exists. One of the challenges for fisheries population geneticists is to come up with empirically validated methods for examining genetic structure in species with moderate levels of gene flow.

Many studies have attempted to estimate gene flow by converting *Fst*, or an analogous measure (*Gst, Rst, ρst, Φst, θ,* etc.), into N_em, generally with little or no consideration of the basic assumptions involved (Ferguson, 1994). This approach requires that populations are at equilibrium with respect to mutation, migration and genetic drift, an assumption unlikely to hold for most fisheries stocks. It also assumes similarity of population sizes, migration rates and symmetrical migration, again almost certainly untenable for most fisheries organisms. Also the results for N_em can be very different for the different '*Fst*' measures (Charlesworth, 1998). For example, Allen *et al.*, (1995) reported an almost 8-fold discrepancy between the migration values obtained between two colonies of grey seals estimated using *Fst*, *Rst* and Slatkin's private allele methods. Newer methods based on coalescence theory avoid the need to assume symmetry of migration rates or equal population sizes (Beerli, 1998) and in general these approaches appear to be superior to allele frequency based methods. Coalesence is based on allele genealogies, i.e., allele diversity is traced back through mutations to ancestral alleles with two genealogies coalescing when they have a common ancestor. Individual based analyses potentially allow the analysis of contemporary levels of gene flow as opposed to historical gene flow

estimates derived from *Fst* and other indirect approaches (Estoup and Angers, 1998). Another approach making use of genealogical information is Templeton's (1998) nested clade analyses of haplotype trees which allows an evolutionary nested analysis of the spatial distribution of genetic variation in a much more precise way than traditional *F* statistics. This approach also allows a separation of population structure from population history. Other potential methods of gene flow estimation will be dealt with below.

Temporal genetic changes

Fragmentary DNA can be successfully isolated from scales. There is generally sufficient fragments of several hundred base pairs to allow successful amplification of microsatellites (Nielsen *et al.*, 1997). As scale collections exist for many exploited fish species since the end of last century, there is the potential to look at genetic changes over the past 100 years or more. This opens up a number of exciting prospects and various studies are under way.

Estimation of effective population size and demographic fluctuations

A number of methods have been suggested for estimating both current and long-term effective population sizes from genetic data. These include use of temporal variation in allele frequencies that can be obtained, for example, from scale collections. As effective population size is a key element in fisheries management, it is perhaps rather surprising that more effort has not been put into the development and validation of methods in this respect.

Stock of origin/mixed stock fisheries

A key question for managing fisheries according to quotas is to know the stock of origin of a particular catch or the proportion attributable to each stock for a mixed stock fishery. The Genetic Stock Identification (GSI) approach uses a maximum likelihood approach to estimate the combination of potential source populations that best explain the genotype frequencies observed in the catch. It cannot be used to assign individual fish to a particular source but estimates the most likely fraction of each source to yield the allele frequencies observed in a catch. Although microsatellite data can be used to enhance standard GSI estimations, new possibilities are opened up using such data. One alternative is to produce a phylogeny of individuals based on sharing of alleles across microsatellite loci, the large number of alleles at these loci making this a feasible proposition. Another is the use of assignment tests. Assignment tests are based on multilocus genetic data and make use of both individual genotypes and overall population allele frequencies. This test calculates the likelihood of a single multilocus genotype coming from several populations based on the allele frequencies at each locus in each population. The higher the allelic variability, and the more loci used, the greater the power of this method. Assignment tests also provide a means of identifying migrants and thus of directly estimating current levels of migration. Other possible approaches include 'artificial intelligence' neural networks.

Breeding behaviour and fitness

Possibly the major advance made possible by the use of microsatellites is in the identification of parent-offspring combinations. This can be carried out readily for a closed population where all potential parents are available but is more problematic in an open population. Various programs have been produced which perform maximum likelihood estimations of parentage. The approach has been widely used to work out mating strategies in fish and organisms, e.g., Atlantic salmon (Thompson *et al.*, 1998). The ability to identify parentage means that individual reproductive success, i.e., fitness, can be directly estimated in field situations.

Genetic tagging in ranching/supplementation

Microsatellite genetic tagging opens up many new possibilities especially as it allows tagging of early life history stages such as eggs and larvae. Often attempts at ranching or supplementation of natural stocks have been not adequately monitored due to the cost of tagging individuals and of rearing them to a sufficient size for physical tagging. Thus if the parentage of the individuals being used for ranching/supplementation is known then these individuals can be identified at harvest and thus differentiated from native individuals.

Genetic tags have been used to trace movements of individuals in species with relatively low numbers e.g., turtles (Bowen, 1995) and humpback whales (Palsboll *et al.*, 1997). In the latter case, the same individuals were identified in samples taken in the summer and winter feeding grounds in the North Atlantic, allowing details of migrations to be determined in the same manner as for physical tags. Analysis was carried out using six microsatellite loci in DNA obtained from skin biopsy or sloughed skin. The ability to identify individuals also enabled estimation of numbers based on the mark-recapture technique. This study demonstrates that genetic tagging can be effective even in large

populations of wide-ranging and inaccessible mammals such as cetaceans. The advantages and disadvantages of genetic tagging relative to physical tagging is a point for discussion.

Experimental studies on fitness/impact of deliberate and inadvertent releases

One of the main problems in carrying out field-scale experiments on aquatic organisms is that such experiments need to be started at the egg stage and this precludes the use of traditional physical tagging. The use of microsatellite profiling overcomes this problem as has been demonstrated in an experiment looking at the relative performance of native, farmed and hybrid salmon under natural river conditions (McGinnity *et al.*, 1997). Similar experiments could be carried out in the marine environment using natural or artificially enclosed bays.

Conclusions

There is considerable potential in the use of microsatellite markers for the study and management of wild stocks. However, the technical ability to acquire data has now outstripped our understanding of the basic molecular processes underlying microsatellite variation and also the ability to analyse the data in a statistically rigorous manner. Good empirical test situations are required to validate the various possibilities outlined above. As with all genetic markers, the maximum value is obtained when the genetic information is integrated with detailed demographic and ecological data.

RECOMMENDATIONS

1. *Microsatellites should be more fully exploited as markers for the study and management of fisheries populations.*

2. *Microsatellites should be used alongside other marker types and genetic information integrated with detailed demographic and ecological data. Novel insights are often possible as a result of non-congruence of different marker types and/or genetic and other data.*

3. *Further research is urgently required, involving both geneticists and modellers, to test empirically the newer methods of analyses which have been proposed and to develop and test further methods. Attention should be given to methods for: determining population structure and estimating gene flow; estimation of effective population size; population(s) of origin of a catch; determining relatedness in open populations. Of particular importance is the need to develop new methods which allow examination of population structure where there is moderate gene flow but where the populations are still largely self-sustaining.*

4. *The use of microsatellites should be subject to the following recommendations if optimum and valid results are to be obtained:*

    a) *information, where possible, should be obtained on inheritance, sex linkage and co-linkage before microsatellites are used as tags for any species;*

    b) *where methods of analysis are used that involve the use of genealogical relationships among alleles, the mutation mechanism assumed should be validated for the loci used;*

    c) *where Hardy-Weinberg deficits are found in a sample, additional primer sets should be designed as one possible way of correcting for non-amplification due to mutation in the primer region ('null alleles'.); where deficits still persist with new primers, caution should be exercised in the interpretation of the data;*

    d) *information on temporal variability of genotype frequencies should be available and taken into consideration.;*

    e) *adequate sampling strategies should be used as appropriate to the species under study. (See WGAGFM 1998 Report for further details.) This should also involve proper a priori planning of sampling and ongoing refinement of sampling based on initial results;*

    f) *for population-based analyses, replicate sampling should be carried out to check that samples reflect the population from which they were derived;*

    g) *individual-based analyses, which are potentially much more powerful, should be used alongside 'traditional' sample-based statistics;*

h) in all statistical analyses, consideration should be given to the assumptions involved in underlying models as these assumptions are often clearly violated.

5. *Workers involved with a particular species should:*

a) use a consensus set of microsatellites, at least as part of a suite of loci, to allow inter-calibration of studies;

b) establish a database of primer sequences and PCR conditions for the species, preferably on the WWW;

c) establish specific study groups to deal with the above recommendations.

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2.f. The gender of fish

Based on a position paper by Filip Volckaert, 'On the Gender of Marine Teleosts'; adopted by WGAGFM in Reykjavik 1999.

Abstract

Gender in fish shows a high degree of plasticity under the influence of genetic and environmental factors. Sex-ratio selection is the driving force in its evolution. As most commercially important marine fish have incompletely described sex-determining systems, an integrated approach involving field observations, breeding, genome mapping and molecular studies is necessary for sex-specific life history traits to become better understood.

Introduction

Sex-ratio selection is the driving force responsible for the evolution of sex-determining mechanisms (Bull, 1983). Each time at reproduction the reshuffling and combination of male and female genetic factors shapes a new set of genotypes with their accompanying phenotypes. During the life cycle, selection will act on these traits. Thus sexual selection will determine the chances sperm and eggs will carry genetic information to the next generation.

The evolution of sex-determining systems is influenced by parental sex ratio genes, parental-effect sex determiners and zygotic sex determiners (Werren and Beukenboom, 1998). It is not only the factors in the embryo (sex determination sensu stricto) which play a role, but also factors issuing from the parents. Parental sex ratio genes are known to influence the sex ratio in a large number of taxa, including fishes. The parental choice of the spawning environment in the case of environmental sex determination (ESD) may be a crucial factor influencing sex. Several fish species are known to be influenced by temperature (*Oreochromis mossambicus*, *Menidia menidia*, and *Pomatoschistus minutus*) (Baroiller *et al.*, 1995; Conover and Heins, 1987; Kvarnemo, 1995).

Parental-effect sex determiners act within the developing zygote and may be induced by either the maternal or paternal parent. Messenger RNA (mRNA) or proteins (including gametic imprinting) are the common agents. Imprinting affects

mammals and invertebrates such as *Drosophila* through differential methylation of CpG residues in the male and female genome (John *et al.*, 1996) and has been reported in the zebrafish *Danio rerio* (McGowan and Martin, 1997).

Finally zygotic sex determiners group those factors which initiate from the zygote itself and act on its sex. Nuclear sex-determining genes are the most commonly reported factors in fish, but also mitochondria and heritable microbes may play a role.

Various reviews have been published on sex determination in fish and lower vertebrates (Bull, 1981, 1983; Chourrout, 1988; Crews, 1993; Kallman, 1984; Kirpichnikov, 1987; Mittwoch, 1996; Morescalchi, 1992; Price, 1984; Strüssmann and Patino, 1995) and even more cover sex differentiation (Francis, 1992; Schreibman *et al.*, 1994; Shapiro, 1989; Blazquez *et al.*, 1998). The subject draws a lot interest, largely fuelled by progress in mammalian and invertebrate (*Caenorhabditis* and *Drosophila*) systems (see Science, 1998, 281: 1979–2010).

Evolution of sex chromosomes

Sex-determining systems are amazingly diverse; hermaphrodites, gonochorists, paternal genome loss, paternal heterogamety, maternal heterogamety, dilocus or polygenic sex determination, and environmental sex determination are some of the systems encountered in fish. Charlesworth (1991) identified four characteristics of sex chromosomes, regardless of the taxon involved: (1) the failure of the X and Y chromosome to recombine over most of their length; (2) the genetic inertness of much of the Y chromosome; (3) dosage compensation of the activity of the X chromosomal loci; and (4) the accumulation of repeated DNA sequences on the Y chromosome. Also, mutations should occur more commonly in males because of the larger number of cell divisions in spermatogenesis compared to oogenesis (Haldane, 1947). Conceptually close to these thoughts, Kirpichnikov (1987) presented a model of cytogenetic evolution where sex chromosome patterns in teleosts evolved to (1) clearly differentiated sex chromosomes (gonosomes) with male or female heterogametes or multiple sex chromosomes, from (2) the absence of karyological sex-related differences but the presence of sex-specific genomic DNA sequences as detected by genetic analysis, and from (3) the absence of DNA specific sequences and gonosomes but the presence of a conspicuous sexual dimorphism, although possibly in variable ratios.

Heterogamety, that is, males or females with heteromorphic sex chromosomes (heterosomes), has been observed in only an estimated 10 % of all fish species studied (Morescalchi, 1992). Single chromosome systems have been identified amongst others in *Bathylagus*, *Lampanyctus* and *Gobiodon* (Chourrout, 1988). Examples of XY sex determination (male heterogamety) are found in the family of the Salmonidae (Thorgaard, 1977; Prodöhl *et al.*, 1994; Devlin *et al.*, 1991), channel catfish *Ictalurus punctatus* (Liu *et al.*, 1996), common carp *Cyprinus carpio* which has an XY major system with in selected inbred populations autosomally linked minor genes (Komen *et al.*, 1996), guppy *Poecilia reticulata* (Nanda *et al.*, 1992), medaka *Oryzias latipes* (Aida, 1936) and African catfish *Clarias gariepinus* (Galbusera *et al.*, 1999; Liu *et al.*, 1995). The ZZ/ZW system (female heterogamety) is less common but has been documented amongst others in *Gambusia*, plaice, Dover sole and *Leporinus*. A well-studied triallelic system is found in natural populations of *Xiphophorus maculatus*. Three types of chromosomal constitution are known in females, WY, WX, XX, and two types in males, XY and YY (Kallman, 1984). However, the genetic sex of numerous species has been identified solely from karyotypes assuming that heteromorphic chromosomes represent sex chromosomes. This assumption has proven to be dubious.

The molecular basis of sex determination

If the cytogenetic range of sex determination is diverse and known in only a small group of fish species, its molecular basis remains unknown. Molecules interact within a cell and among cells in response to various stimuli. Sex determination could be viewed just as a signal which initiates a cascade of reactions responsible for the phenotypic sex. The most likely moment such gene cascade is initiated, is during development when initially a single cell and later on a growing number of cells differentiate. Two phases, namely the initiation of cell fate, i.e., the single trigger which starts the process, and its consecutive stabilisation, i.e., the process during which the phenotypic sex is more or less irreversibly established, may be identified (Place, 1997). Otherwise formulated, what makes the initially female 'Anlage' to remain female or to be shifted towards a male phenotype in early development, knowing that the mammalian genetic sex and possibly the genetic sex of fishes (Beullens *et al.*, 1997) is initially female. In mammals this involves the regulation of the gene cascade by the regulatory elements such as *Sry*, *Sox9* and *Dax1* (Marin and Baker, 1998). In teleosts, the *Sry* gene is not conserved (Tiersch *et al.*, 1992), but the mammalian genes *Sox9* and *DMTR1* have potential for evolutionary conservation in lower vertebrates. Both have been observed in birds and *DMTR1* has homologues in *Drosophila* and *Caenorhabditis* (Raymond *et al.*, 1998).

If the molecular basis of the sex-determining system in fish is thus poorly known, there is general agreement on the pivotal role of the enzymes aromatase and reductase in steroidogenesis. Unlike mammals where ovarian development is

independent of estrogens, in lower vertebrates estrogens play a crucial role in the development of the ovary. In all vertebrates, the formation of estrogens from androgens is catalysed by aromatase (Piferrer *et al.*, 1994). Progesterone is transformed in androstenedion which is either transformed directly to testosterone, or through estrone to estradiol, or through 11 keto-androstenodion to 11 keto-testosteron. Temperature dependence of sex ratios may be linked to temperature dependence of aromatase, say even of modification of the intracellular pH (Jeyasuria and Place, 1997). The question remains whether either the balance between estrogens and androgens, or the regulation of aromatase is the driving force of gonadal development.

Anyway, the hypothalolo-pituitary-gonadal axis in lower vertebrates is the principal determinant of sex, where sensory perception of the external environment may determine the functionally active phenotypic sex (Merchant-Larios, 1997). The role of the brain as initial site of phenotypic sex determination differentiates fish from mammals, where the gonad seems to be the driving force in sex determination (Francis, 1992; Crews, 1993). This explains why Environmental Sex Determination (ESD) is so common in lower vertebrates. ESD does not necessarily exclude the possibility of a heritable genetic system. It is perfectly possible to have initially a genetically determined system which is rather unstable and which switches under specific conditions (population density and physical environmental factors) to an ESD system.

The list of fish with ESD is rather extensive, which indicates that the phenomenon is evolutionarily significant. Temperature affects species such as Atlantic silverside *Menidia menidia* (Conover and Heins, 1987; Lagomarsino and Conover, 1993), Nile tilapia *Oreochromis niloticus* (Baroiller *et al.*, 1995), *Oreochromis aureus* (Desprez *et al.*, 1997), sand goby *Pomatoschistus minutus* (Kvarnemo, 1995) and sockeye salmon *Oncorhynchus nerka* (Craig *et al.*, 1995). Among-family variation for temperature-dependent sex determination in reptiles has been observed by Rhen and Lang (1998). Social factors affect the sex ratio amongst others in saddleback wrasse *Thalassoma duperrey* (Larson, 1997) and the Midas cichlid *Cichlasoma citrinellum* (Francis and Barlow, 1993). Breeding experiments indicate that there is some genetic basis since it is possible to select for sex ratio in Atlantic silverside (Conover *et al.*, 1992) and hirame (Yamamoto, 1999).

Tools to elucidate the genetic sex

Given the poor understanding of sex determination in fish, various approaches can be envisaged to clarify the role of sexual selection and the functioning of sex determination. Four principle fields of action (with some level of overlap) may contribute: field observations, controlled breeding, genome mapping and molecular characterisation. In progressing from the field to molecular experiments, increasing levels of understanding will be achieved.

Field observations

Natural populations have reached an equilibrium in their genetic and phenotypic sex ratio in response to local selective pressures. Within-population density may modulate the operational sex ratio (Bull, 1983), but high quality information on the proportion of genetic and phenotypic sex ratios as well as operational sex ratios is missing. The former is observed on the basis of sex-specific genetic markers (genetic sex) and of the proportion of females, males and intersexes (phenotypic sex), the latter on the proportion of males and females effectively participating in spawning (a value which may be several times smaller than the observed phenotypic sex ratio). Generally marine fish are batch spawners where the genetic contribution of the parents at the moment of spawning itself and in the survival rate of the larvae may differ considerably. For example, the sex ratio of European eel is suspected to be different in northern and southern populations. Also, the proportion of male and female sea bass on the spawning grounds is thought to be variable although not quantified in great detail. In both cases, ESD is suspected to modify the sex ratio.

Standard fisheries data give limited attention to phenotypic sex ratios, although the consequences are major as this may represent sex-specific growth and thus production. No attention is paid to the genetic sex or the operational sex ratios. Judging from Table 2, which compiles gender information relevant to fish either captured or cultured in the North Atlantic Ocean, the poor quality of our knowledge is obvious. This is a cause for concern, since the fishery is size-selective and thus sex-specific to a certain degree, while aquaculture struggles with biased sex ratios, often skewed towards the slower growing sex (Beullens *et al.*, 1997).

Controlled breeding

The most obvious experimental method to test for the heritability of gender is the observation of the sex ratio by controlled breeding. If the sex ratio of the progeny is under a variety of experimental conditions systematically proportional to 1:1, a diallelic single-locus system is operational. If a further test on the basis of ploidy manipulated parents (gynogenesis or androgenesis) or of sex-reversed parents (neo-males or neo-females) gives unisexual females or males, a heterogametic male (XX/XY) (Liu *et al.*, 1995; Galbusera *et al.*, 1999) or heterogametic female (ZW/ZZ) system may be envisaged (Howell *et al.*, 1995). However, if the sex ratio is under a variety of environmental conditions

systematically skewed and different from 1:1, a multiallelic (as in *Xiphophorus maculatus*) (Schartl, 1988) or multilocus system may be functional. The action of minor and major genes may further complicate genetic sex determination (Komen *et al.*, 1992).

If the sex ratio of controlled crosses under a number of different environmental conditions seems extremely variable, environmental sex determination may be part of the sex-determining system (Yamamoto, 1999). In such cases, more experiments under controlled conditions (average and range of temperature, salinity, feeding regime and population density) should be made (Conover *et al.*, 1992).

Genome mapping

An alternative to locate the sex-determining system is the use of sex-linked markers based on its detection in a second generation (F_2) backcross. In this case a phenotypic, protein or DNA sequence (either coding or non-coding) is tracked closely linked to the sex-determining system. Due to the limited cross-over between marker and sex-determining region, it represents a faithful indicator of the genetic sex. Several sex-linked colour markers have been identified in the medaka (Aida, 1936), swordtail (Schartl, 1988) and guppy.

The methodology to isolate molecular markers can be divided in coding or non-coding sex-linked markers. Non-coding sequences are either unique or repetitive (microsatellite and minisatellite DNA) and located close to the sex-determining system; they show thus limited levels of recombination (genetic hitchhiking). Chromosome-specific non-coding DNA sequences have been identified by subtractive hybridisation in species with heterologous sex chromosomes (e.g., *Oncorhynchus tshawytscha* (Devlin *et al.*, 1991) and *Leporinus* (Nakayama *et al.*, 1994)) but failed in rainbow trout (Nakayama *et al.*, 1994) and several other unreported cases. Since most gDNA of the sex chromosome is highly repetitive, such strategy results most likely in probes of non-coding gDNA. Restriction fragment length polymorphisms (RFLP) linked to the sex chromosome have been identified in *Xiphophorus* (Schartl, 1988). Fingerprinting with certain motifs of simple tandem repeated DNA sequences are sex-specific in, for example, the guppy *Poecilia reticulata* (Nanda *et al.*, 1992).

A promising approach to isolate sex-specific sequences in a species with heterosomes is represented by the Randomly Amplified Polymorphic DNA (RAPD) or a modified form Amplified Fragment Length Polymorphisms (AFLP) (Vos *et al.*, 1995). This technique, in which a single random primer is used to amplify fragments of genomic DNA by PCR, has already been used successfully to isolate sex-specific probes in birds (Griffiths and Tiwari, 1993). Heterologous sequences such as Bkm, ZFY and Sry isolated in other vertebrates do not hybridise sex-specifically in rainbow trout (Lloyd *et al.*, 1989; Ferreiro *et al.*, 1989), the coral reef fish *Anthias squamipinnis* (Watchel *et al.*, 1991), or channel catfish (Tiersch *et al.*, 1992). In general, the above-mentioned probes usually seem to be specific to the species they were developed for; cross-hybridisation with other teleosts is restricted.

Coding sex-linked markers, i.e., genes or their products, are less common but some have been studied in greater detail. Allozyme markers have been identified, e.g., in brown trout (Prodöhl *et al.*, 1994) and American catfish (Liu *et al.*, 1996). Because there is a product involved, detection at the protein level either by electrophoresis or blotting is easy.

The *Smcy* gene coding for the HYK^k epitope (Scott *et al.*, 1995; Wang *et al.*, 1995) has been identified in many organisms and is sex-linked, although not involved in the regulation of genetic sex as initially thought. The H-Y antibody has been attributed to the heterogametic sex in many vertebrates and invertebrates (reviewed by Kallman, 1984). The heterogametic genetic sex of fish has been identified in this way in amongst others *Xiphophorus maculatus*, medaka *(Oryzias latipes)* and *Haplochromis burtoni* (Pechan *et al.*, 1979), the coral reef fish *Anthias squamipennis* (Pechan *et al.*, 1986), guppy *Lebistes reticulatus* (Shalev and Huebner, 1980), rainbow wrasse *Coris julis* (Reinboth *et al.*, 1987) and rudd *Scardinius erythrophthalmus* (Köhler *et al.*, 1995). Cyprinidae (*Rutilus rutilus*, *Carassius auratus* and *Barbus tetrazona*) and primitive fish such as Salmonidae (*Oncorhynchus mykiss* and *Salvelinus alpinus*) show equal absorption of H-Y in males and females (Müller and Wolf, 1979). Although no sex difference at the level of H-Y was found in *Salvelinus*, *Salmo*, *Rutilus*, *Carassius* and *Barbus* (Müller and Wolf, 1979) and although it has been hard to obtain reproducible results (most likely linked to the quality of the antigen), there is consensus that the H-Y antibody binds to the somatic tissues of the heterogametic sex.

After the recombination rates of a large number of phenotypic and genetic markers have been identified on a panel of F_2 backcrosses and organised on a linear map, syntenic groups of markers become apparent. In case of a high density map, the number of linkage groups should correspond to the number of chromosomes. Such full scale genomic maps are available for a growing number of fishes such as tilapia *Oreochromis niloticus* (Kocher *et al.*, 1998), salmonids (Lundin *et al.*, 1998), swordtail *Xiphophorus maculatus* (Morizot *et al.*, 1998) and American catfish (Liu *et al.*, 1999). The most dense map (705 markers for 25 chromosomes) is available on the zebrafish *Danio rerio* (Knapik *et al.*, 1998), an established model in developmental biology with unfortunately an unidentified sex determining system.

Finally, large sex-specific probes are hybridised with fluorescence *in situ* hybridisation (FISH) to the karyotype such that physical positioning of the linkage groups is achieved (Nanda *et al.*, 1999).

Molecular characterisation

A reductionist approach to the identification of the genetic sex involves the identification of the gene cascade. It is assumed that by detailing the interactions between genes and their response to external stimuli, the observed sex ratios can be explained. This approach is paying off in mammals, the fruit fly and the nematode worm *Caenorhabditis*. In this perspective, the functional comparison of the *SOX9* and *DMRT1* across taxa looks promising (see above).

Sex differentiation

Processes which relate to the phenotypic expression of gender in males and females are known as sex differentiation (Blazquez *et al.*, 1998). Sex in fish has typically a higher level of plasticity than in higher vertebrates. All types of sexuality such as gonochorists, unisexuals, sequential and consecutive hermaphrodites are found across taxa. Temperate water fishes of the North Atlantic are no exception (Table 2). Those species which have been studied show sex ratios varying between rather stable 1:1 ratios to highly variable ratios often implying environmental plasticity (e.g., European eel). Gonadal differentiation takes place after hatching at a moment when environmental factors may act on the undifferentiated gonad (the so-called critical period) or after the gonad has fully developed. In the former case, temperature and pH play a role in the sex differentiation of Atlantic silverside (Conover *et al.*, 1992) and *Xiphophorus* (Rubin, 1985), respectively. In reptiles temperature regulates the production of aromatase/reductase and estrogen/androgen receptors in females and males, respectively (Crews, 1996). In the latter case, social factors (including hierarchical social structure or density regulation) influence sex differentiation (Shapiro, 1992). Under laboratory conditions only low doses of exogenous sex steroids are required to switch gender (Buellens *et al.*, 1997a, 1997b; Blazquez *et al.*, 1998). These experiments showed that the earliest stages in the development of the undifferentiated gonad are critical since they are the most sensitive. Generalisations remain speculative as the wide evolutionary divergence in fishes may be the cause for interspecific differences.

RECOMMENDATIONS

Some general suggestions may be made on the gender of fish as related to fisheries and aquaculture:

1) *The genetic sex of fish merits more research, given the important phenotypic implications of gender in fisheries and aquaculture.*

2) *Field data on the genetic, phenotypic and operational sex ratio should be collected systematically across the natural range.*

   Rationale: A wide diversity of sex-determining systems may be expected because of the broad evolutionary divergence within the phylum. Therefore, a common pattern explaining all aspects of gender in fish may not necessarily exist.

3) *Research into sex determination mechanisms is required, especially the aromatase/reductase systems that may be evolutionarily conserved.*

4) *Sex determination should be studied in selected model and commercial species such as a salmoniform, a perciform and a gadiform.*

   Rationale: Concentrating efforts on a few marine species with a well-controlled reproductive cycle and an excellent breeding potential has the highest chances of success.

5) *A multidisciplinary study of sex determinism, including breeding, ploidy manipulation, genome mapping, gene regulation, developmental biology and careful field work, is recommended.*

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Table 2. Sex determination, gender and sex ratio of selected North Atlantic fishes.

Family	Species	Genetic sex	ESD	Sex ratio (M/F)	Reference
Anguillidae	*Anguilla anguilla*, European eel	?	yes	0.9:0.1 to 0.4:0.6	Degani and Kushnirov 1992
	Anguilla rostrata, American eel	?	yes		
Atherinidae	*Menidia menidia*, Atlantic silverside		yes	gonochorist	Conover and Heins 1987
Clupeidae	*Clupea harengus*, Atlantic herring	?			
Gadidae	*Gadus morhua*, Atlantic cod	?			
Gasterosteidae	*Gasterosteus aculeatus*, three-spine stickleback	?	yes	gonochorist; 1:1	Lindsey 1962
Gobiidae	*Pomatoschistus minutus*, sand goby	?	yes	gonochorist; 1:1 to 1:3	Kvarnemo 1995
Labridae	*Symphodus (Crenilabrus) melops*, corkwing	?		protogynous hermaphrodite + sex reversal	
	Coris julis, rainbow wrasse	H-Y positive		diandric protogynous	Reinboth *et al.* 1987
Moronidae	*Dicentrarchus labrax*, sea bass	multi-allelic or multi-locus	yes	2/1 to 1/2	Blazquez *et al.* 1998
	Morone saxatilis, striped bass	?			
Pleuronectidae	*Pleuronectes platessa*, plaice	ZZ/ZW			Purdom and Lincoln 1973
	Pseudopleuronectes americanus, winter flounder	?			
Salmonidae	*Salmo salar* Atlantic salmon	XY/XX		gonochorist; 1/1	
	Salmo trutta, brown trout	XY/XX		gonochorist; 1/1	Prodöhl *et al.* 1994
Scombridae	*Scomber scombru*, Atlantic mackerel	?			
Soleidae	*Solea solea*, Dover sole	ZZ/ZW?		gonochorist; 1/1	Howell *et al.* 1995
Scophthalmidae	*Scophthalmus maximus*, turbot	?			
	Hippoglossus hippoglossus, halibut	?			
Sparidae	*Sparus aurata*, sea bream	-		Protandrous hermaphrodite	
Thunnidae	*Katsuwonus pelamis*, Skipjack tuna	?			

2.g. Patenting of technology; a potential problem for research

Based on a position paper by Willie Davidson, adopted by WGAGFM in Reykjavik 1999.

Biotechnology patent law operates under the same general legal principles as other areas of patent law. However, the nature of biotechnology raises many unique patent law issues. Biotechnology law is becoming more complex in all industrialised nations and what can and cannot be patented is often determined by the jurisdiction rather than by a set of commonly agreed arguments.

In general, there are two items that can be afforded protection of intellectual property by a patent:

1) materials, and

2) processes.

For a patent to be granted, the invention must satisfy three criteria:

1) the invention must have the potential to be useful,

2) it must not be immediately obvious to a practitioner in the field, and

3) the invention's commercial use must not be contrary to public policy.

Examples of inventions related to biotechnology that may be patented include:

A. Products

1) genes, including modified genes, expression vectors, and probes,

2) proteins, including modified proteins, monoclonal antibodies, and receptors,

3) other chemical compounds such as small organic molecules,

4) cells including unicellular organisms (bacteria and fungi), animal cells, and plant cells.

B. Methods

1) a new and non-obvious procedure such as micro-injection of DNA into a pro-nucleus,

2) the isolation of a recombinant protein produced by a transgenic organism such as a fusion oleosin-fish growth hormone from the oil body of rapeseed.

Note that methods of medical treatment are not directly patentable in Canada but can be in the United States.

The most contentious issue is the patentability of complex life forms. Biotechnology is now providing the capability to produce novel genetically modified organisms that are commercially valuable and industrially useful. Patent protection has already been sought in many countries.

The United States courts have ruled that higher life forms are patentable subject matter and the United States Patent Office routinely grants claims. The only prohibition in the United States is against patenting of humans. Japan grants claims to plants and animals.

Genetically engineered plants and animals are not patentable in Canada. In Canada, plant varieties produced through traditional cross-breeding techniques may be protected under the Plant Breeders Rights Act and this was amended as recently as 1998 to allow plant variety protection to be obtained for all species of plants. However, Canadian authorities have taken a different approach to patenting life forms from that of the United States and Japan. Although Canadian and United States courts use virtually the same definition of 'invention', the conclusions reached differ substantially. The test case is the 'Harvard mouse'. The inventors claimed a technique for inserting a gene which increases sensitivity to cancer (an oncogene) into the DNA of animal cells and this enabled them to clone lab test animals (generally mice but the claim was for any non-human mammal) which are useful for testing and identifying any potential cancer-causing compounds. In 1998 the Federal Court upheld a refusal by the Canadian Patent Office to allow claims to the 'Harvard mouse'. In its decision the court recalled a decision made by the Federal Court of Appeal in the 'Pioneer' case which involved an application to patent a new soy plant made through traditional cross-breeding (something the United States Patent Office will allow). Both the 'Harvard mouse' and the 'Pioneer' cases raise the issue of whether there is patentable subject matter in a living product that results when natural processes are allowed to run their course. The Commissioner of Patents allowed claims to a plasmid and to a process of injection of the plasmid into a fertilised egg as these are produced under the full control of the inventor and are reproducible. They thus fall under the definition of a

'manufacture' or ' composition of matter' under Section 2 of the Patent Act. The Commissioner would not allow the inclusion of a non-human mammal on the grounds that the inventors did not have full control over all the characteristics of the resulting mammal. The reservations were based on the fact that after the engineered oocyte was created, it was nature that determined the growth and differentiation of the mice and the subsequent breeding of a stable population. In 'Pioneer', the Court of Appeal decided that in spite of human efforts involved in breeding the new soy plant, the plant and its seed were not patentable because they were grown by means of a natural biological process.

The situation in Europe is similar to that of Canada. Provisions of the UK and European patent laws prohibit a valid patent claim based on an essentially biological process. It is not possible to have a valid patent claim that is so narrow in scope as to cover only a group of organisms comprising a Avariety@. Patents are also not granted for inventions that are judged morally offensive. However, there is no legal definition of Amoral offensiveness@ and the decision rests with the Patent Offices and Courts in each country and ultimately with public opinion.

In Europe two laws could potentially bar the patentability of transgenics. The first is the provision of the European Patent Convention (EPC), a treaty that predates the EU and was signed by the nations that are now members of the EU. Under this convention, a single European Patent Office (EPO) was established as a central location for examining all European patent applications. Under EPC Article 53(a) inventions whose commercial use would be contrary to public policy are not patentable. Under EPC Article 53(b) the law excludes 'plant and animal varieties' from patentability. The problem with the EPC is that interpretations of its provisions vary from country to country and, as a result, the EPO and EU member states have selectively decided to issue or reject transgenic patents. Two contradictory cases illustrate this confusion.

The first is the 'Harvard mouse'. In the first round of patent examinations, the EPO rejected Harvard's application, excluding it under Article 53(b) as a 'variety'. It could not come to a conclusion if the 'Harvard mouse' violated public policy under Article 53(a). The EPO's Board of Appeals sent the case back stating that the claims embraced 'animals' not just a 'variety' and this laid the groundwork for circumventing exclusion under Article 53(b). The issue of morality (public policy) was handled by weighing the environmental risks and the potential for cruelty to animals against potential benefits to mankind. The Appeals Board found no danger to the environment because the mouse would only be handled in controlled circumstances and no release into the wild was intended. They also concluded that animal suffering would also decrease in the long term as fewer animals would be required for experimentation. It was made very clear that this decision was strictly for the 'Harvard mouse' case alone. The EPO therefore, granted the patent to the 'Harvard mouse'.

The second case involved Plant Genetic Systems (PGS), a company based in Belgium, which sought a patent for a transgenic plant. The EPO granted the patent and this was challenged by Greenpeace. In this instance, the Appeals Board re-examined and revoked the patent. In reaching its decision, the EPO found nothing immoral in the patenting of the plant under Article 53(a), but rejected the application under Article 53(b) because it interpreted the PGS plant as an unpatentable 'plant variety'. Despite this rejection, the Board went on to grant a patent on the transgenic plant cells and seeds under a section of Article 53(b) that allows the patenting of the product of a microbiological process. The Board's explanation for this ruling was that plant cells and seeds are classified as 'microorganisms'. Under the EPO's definition, a single cell capable of reproduction falls into this category no matter what the biology of the parent organism. So, claims to genetically modified plant cells and to a process producing genetically modified plants were held to be patentable whereas claims to the plants themselves were held unpatentable.

Applicants for European patents for transgenics are confronted with a dilemma. The EPO has granted a patent for a transgenic in the case of the 'Harvard mouse' but rejected a patent for a transgenic in the case of the PGS plant. What is and is not patentable when it comes to fish has still to be determined. However, the following gives some illustrations of what can be anticipated:

1) Patents have been granted for fish expression vectors including growth hormone genes in all of these countries.

2) Patents have been issued that cover the process of producing transgenic salmon.

3) Patents have been granted for probes able to detect the sex of a specific species of salmon.

4) A patent has been issued by the EPO for a transgenic *Bacillus* species that can produce fish antifreeze proteins (note that this comes under the microorganism category).

RECOMMENDATION

It is recommended that the WGAGFM monitor patent decisions that have implications for fisheries and mariculture and report them annually.

2.h. Genetic tissue authentication in forensics

Based on a position paper by Willie Davidson and Geir Dahle, adopted by WGAGFM in Reykjavik 1999.

Introduction

Authentication of fish and seafood products is becoming more necessary because of increased international trade and the introduction of laws requiring that products are labelled with official names. Enforcement officers must be able to identify the flesh of samples to determine if an animal has been hunted illegally (i.e., without a permit, or out of season) and to ensure accurate labelling to prevent the substitution of cheaper species. Customs officers and conservation groups require methods for identifying restricted and endangered species or products that are derived from them. In addition, with the introduction of new strains or breeds of animals through genetic engineering (i.e., transgenic animals) producers want a technique that can verify their own particular variety. There is therefore, a need for rapid, accurate and reliable methods for determining the species origin of a biological sample.

This working paper will review some of the procedures currently available to authenticate the species origin of raw and processed seafood products and then describe in depth recently developed procedures that take advantage of the resiliency of DNA, the genetic material, in the sample. Recent reviews, one specifically on fish and seafood (Bossier, 1999) and others of a more general nature (Davidson, 1998; Ashurst and Dennis, 1998), are recommended reading.

Protein-Based Methods

Electrophoretic techniques are probably the most commonly used methods for analysing the protein components of meat and meat products (AOAC, 1984). They are particularly useful for fresh or freshly frozen meat. Water-soluble proteins are extracted and separated according to their charge, size or isoelectric point on gels made from polyacrylamide, starch or agarose or on cellulose acetate strips. The proteins are visualised using a non-specific protein stain such as Coomassie blue or a more sensitive silver staining procedure. Isoelectric focusing (IEF) gels of sarcoplasmic proteins from fish muscle revealed that this is a reliable method for species identification although some intra-specific variation was noted (Lundstrom, 1981). The Institut Français de la Recherche pour l'Exploitation de la Mer (IFREMER) has produced a catalogue of 34 fish families that contains the scientific names of the fish and drawings and photographs of water-soluble proteins separated by IEF (Durand *et al.*, 1985). Despite the widespread use of IEF of muscle proteins for fish species identification (Rehbein *et al.*, 1995a), it should be noted that this procedure was unable to distinguish between the closely related bluefin tuna (a regulated species with tight quota restrictions) and yellowfin tuna (an unregulated species with no quota restrictions) (Bartlett and Davidson, 1991).

Proteins are denatured by heating during the cooking or processing of meat. For most practical purposes, this limits protein electrophoresis for species identification to fresh or freshly frozen meat samples although heat-stable proteins (e.g., parvalbumins) can be extracted and analysed by IEF in the presence of a denaturant such as urea (Hsieh *et al.*, 1997). The major drawback to protein electrophoresis is the need for standards with which to compare the unknown. A comparison with diagrams of standard electropherograms (see http://vm.cfsan.fda.gov//frf/rfe2fgs.html) can provide an initial indication of what species might be candidates as the species of origin, but confirmation of the identity of the actual species by electrophoretic analysis requires reference samples to be run beside the unknown on the same gel.

Need for Alternative Approach

The disadvantages of the methods outlined above are a consequence of their dependence on the ability to characterise proteins. Many proteins are heat-labile, lose their biological activity soon after death, are subject to modification in different cell types, and their presence in a sample is a function of the cell type being examined. Moreover, electrophoretic, immunological and chromatographic methods do not detect all the differences that may occur between two proteins. For example, electrophoresis will only detect changes in the net charge of two proteins, and immunological techniques require different antigenic determinants to be present on the proteins under investigation. The difference in the structure of a homologous protein from one species to another resides in the organism's genetic material, namely its DNA. It is therefore preferable to examine the DNA in a biological sample if one wishes to know its species origin.

Advantages of DNA

DNA is the same in every cell type of an individual. Thus, it does not matter if one isolates DNA from a blood stain, a muscle extract or a liver sample. The information content is also greater in DNA than in proteins. DNA can be isolated from seafood products that have undergone the most intense processing (e.g., extensive cooking and then autoclaving after canning in the case of tuna). Heat is the scourge of the protein-based procedures because it denatures the molecule under investigation. In contrast, the heat denaturation of DNA into its two constituent strands is a prerequisite step in all processes that involve DNA analysis.

The question then becomes what segment of the genome (DNA) will best serve this purpose. Genomes have regions that reflect their evolutionary history in that they are common to a broad range of related species. Other regions are more variable and taken together combine to give an individual-specific 'genetic fingerprint' (Jeffreys *et al.*, 1985). The trick is to select a segment that is sufficiently variable that it differs from species to species but not so variable that every individual is different at this genetic locus. The segment of choice should also be present in multiple copies in the cell so that small quantities of extractable DNA are more likely to contain it. To date, the majority of work that has used DNA to identify the species origin of food or other biological specimens has employed repetitive genomic DNA (e.g., ribosomal DNA) or else a region of the mitochondrial genome that is present in several thousand copies per cell.

Polymerase Chain Reaction (PCR)

Methods of analysis that use DNA are primarily based on the polymerase chain reaction (PCR). The fundamental part of PCR relies on the specific hybridization of oligonucleotides of defined sequence to denatured, single-stranded template or target DNA. In order to carry out PCR one has to have some *a priori* knowledge of the nucleotide sequence of the DNA that is to be amplified. In particular, the sequences flanking the target must be known. This allows two oligonucleotide primers, approximately 20 to 30 nucleotides in length, that are complementary to the flanking regions of the target DNA to be chemically synthesised. After the DNA sample has been isolated, it is heated to approximately 95 °C to denature it and make it single-stranded. It is then allowed to cool to 45 °C–60 °C in the presence of excess primers and these oligonucleotides hybridise to their complementary sequences such that they face each other on the two opposing strands. The reaction mixture also contains all four of the deoxyribonucleotide triphosphates, which are the building blocks of DNA, and a heat-stable DNA polymerase that catalyses the synthesis of new DNA by extending the primer and incorporating the appropriate base under the direction of the DNA template. The temperature of the reaction is raised to approximately 70 °C, which is the optimum temperature of the heat-stable polymerase, and the elongation step takes place. This process effectively doubles the segment of DNA that is targeted by the primers. At this point the cycle is repeated and the newly formed DNA, as well as the original sample, act as targets for the primers and templates for the synthesis of more DNA. As each cycle effectively doubles the amount of DNA specifically between the primers, ten cycles produce 2^{10} or a 1000-fold increase in the target DNA and twenty cycles will yield 10^6 copies.

Forensically Informative Nucleotide Sequencing (FINS)

A comparison of known sequences of mitochondrial DNA from a variety of different animals revealed several highly conserved regions. This information enabled the construction of primers that could be used pairwise in combination with PCR to amplify homologous regions of the mitochondrial genome from representatives of all vertebrate classes (Kocher *et al.*, 1989). This combination of PCR/direct sequence analysis permitted an examination of the dynamics of mitochondrial DNA evolution in animals and paved the way for the development of FINS (Forensically Informative Nucleotide Sequencing), a procedure for the identification of the species origin of biological samples (Bartlett and Davidson, 1992a, 1992b).

The initial driving force behind the development of the FINS technique was the difficulty of enforcing the quotas and regulations governing the bluefin tuna fishery off the east coast of Canada (Bartlett and Davidson, 1991). Bartlett and Davidson (1991), therefore, turned to PCR/direct sequencing analysis of a segment of the mitochondrially encoded cytochrome b gene as a means of distinguishing these tuna species in the genus *Thunnus*. Although variation was observed within each of the four tuna species, distinct differences which characterise each of the species were found. These species-specific markers in the nucleotide sequence of the cytochrome b gene make it possible to use this procedure and a phylogenetic analysis to determine unambiguously the species identity of muscle, or other tissue, from an individual tuna. This method was adopted by Canada's Department of Fisheries and Oceans and, after a few test cases, fishermen were made aware of the procedure and the illegal fishing of bluefin tuna essentially stopped.

FINS is composed of four steps. First, DNA is extracted from the sample. Second, a specific segment of the isolated DNA is amplified using PCR and the universal cytochrome b primers of Kocher *et al.* (1989) or another pair designed specifically for the species of interest. The third step is the determination of the nucleotide sequence of the amplified DNA, and the fourth is a comparison of this sequence with standard sequences in a database. The process has been used

successfully to identify the species origin of a wide variety of seafoods including smoked salmon and mackerel, pickled herring, salted cod, partially cooked battered cod, canned salmon, and canned tuna (Bartlett and Davidson, 1992a).

Several factors have to be considered before a specific segment of DNA is selected for its ability to be used to identify the species origin of a sample. As described above, this region of the genome has to accept mutations quickly enough so that closely related organisms have different sequences but not so rapidly that the amount of variation within a species is substantial. The length of sequence must be long enough to permit the detection of sequence differences between congeneric species, but bearing in mind that the DNA in the sample will be degraded. A protein coding region of a gene is a good choice as amplification or sequencing errors are more likely to be detected by translating the nucleotide sequence and comparing the inferred amino acid sequence with known amino acid sequences of the gene. Highly conserved, invariant amino acids should be maintained. The creation of a database of sequences is a lengthy process for one lab and, therefore, it is desirable to take advantage of a gene whose sequence has been determined in many diverse organisms by many groups. The mitochondrially encoded cytochrome b satisfies these criteria admirably. A 307 base pair segment of the cytochrome b gene corresponding to amino acids 33 to 134 can be amplified in all vertebrates tested to date using the primers described by Kocher *et al.* (1989). This latter point is very important as it allows one to amplify the DNA without any prior knowledge of what the biological origin of the sample might be. Part of the nucleotide sequence of this gene has been determined for hundreds of vertebrates. (These sequences can be found by searching GenBank at http://www.ncbi.nlm.nih.gov). The cytochrome b gene is very popular with population geneticists and molecular systematists and for this reason the amount of sequence information on this gene continues to expand.

FINS is a rapid, accurate and reproducible procedure that is based on established techniques. It is not subject to operator bias and can be performed independently in any lab equipped to carry out simple standard molecular biology. This means that there is no need for standards to be run along side the sample. The method has been accepted by the court system in Canada and is the method of choice for Canada's Department of Fisheries and Oceans and the Canadian Wildlife Service if there is any question concerning the species identification of a biological sample.

The FINS process has also been used to determine the molecular genetic identification of whale meat and dolphin products from commercial markets in Korea and Japan (Baker and Palumbi, 1994; Baker *et al.*, 1996). Instead of the cytochrome b gene, these researchers decided to use a 550 base pair fragment of the mitochondrial DNA control region. This is the most variable segment of the mitochondrial genome and the rationale for choosing this region rather than the cytochrome b gene was that it could potentially reveal the populations from which the samples were derived in addition to the species. This proved to be the case in some instances. A total of 34 sequences were obtained from 31 products and at least eight species were represented. Some of the species identified in the products from the Japanese and Korean commercial markets were not consistent with recent catch records made available to the International Whaling Commission. The results of this survey raised questions concerning the legality of some of the products tested and point to the need for a standardized form of commercial labelling of whale products. It also suggested that the international and domestic management of whale products was not being practised as it should be. The FINS process is regarded as an important tool for monitoring the regulations covering the international trade in whale products and the domestic sale from unregulated local whaling or fisheries by-catch. As with the bluefin tuna problem that existed in Canada, the realisation that there is now a reliable method for detecting cheating in the whaling industry might prove useful in conserving these mighty mammals of the sea.

The identification of the species in canned tuna presents a special problem. Unlike canned salmon which is usually smoked and then canned without much in the way of heat treatment, tuna is first cooked thoroughly and then autoclaved in the can. This leads to extensive degradation of the DNA in the sample and DNA isolated from commercial cans of tuna has an average size of 100 base pairs (Unseld *et al.*, 1995). This precludes the use of the cytochrome b primers that flank the 307 base pair region of the cytochrome b gene for use in the FINS process with these samples. A comparison of cytochrome b sequences from a wide variety of tuna and tuna-like species (Block *et al.*, 1993) provided the information for the design of primers that amplified a 59 base pair fragment (123 base pairs including the primers) (Unseld *et al.*, 1995). This allowed the FINS procedure to be carried out on 30 commercially distributed cans of tuna and it was possible to determine a reliable sequence in each case. However, although this region of the cytochrome b gene provides unambiguous molecular markers for the identification of nine of the eleven tuna species commonly used by the canning industry, it was not able to distinguish between *Thunnus thynnus* (bluefin) and *Thunnus albacares* (yellowfin) tuna.

These modifications of the original FINS process illustrate the widespread utility of this procedure. However, having to sequence the amplified product as part of the FINS process to identify the species from which a sample is derived is both time-consuming and expensive. It is, however, necessary if one wishes to obtain an unambiguous identification. In other cases it is desirable to have methods that can be used as a rapid screen for detecting possible violations of regulations. The following sections illustrate techniques that have been developed to satisfy these demands.

Species-specific primers

As has been demonstrated in the FINS process, the specificity afforded by the hybridization of two oligonucleotide primers to total extractable DNA allows PCR to amplify a particular region of a genome. By a judicious choice of primers, it should therefore be possible to design primers that are specific for one species and only that species. This approach has been taken to determine the species designations of commercially available lots of caviar (DeSalle and Birstein, 1996). The increasing demand for caviar has had a serious impact on the population sizes of commercial sturgeon species. The traditional method for identifying the species of a particular shipment of caviar has relied upon such factors as egg size, appearance, smell, texture, and colour. De Salle and Birstein (1996) compared the sequences of portions of the cytochrome b genes and 12S and 16S ribosomal RNA genes from all extant sturgeons and paddlefishes and designed pairs of primers specific for diagnostic nucleotide substitutions for each of the three main commercial Russian sturgeon species: beluga sturgeon (*Huso huso*); Russian sturgeon (*Acipenser guelenstaedt*); and sevruga (*Acipenser stellatus*). They then surveyed 23 commercially available lots of caviar purchased in New York and found that five of them were misrepresentations. Three of these samples came from species that are threatened with extinction. The development of this procedure provides wholesalers with an alternative to the crude identification methods presently in use and should assist international conservation efforts to preserve what is left of commercial sturgeon populations. In addition, it should help protect the other species that are likely to be substituted as the commercial species disappear.

Amplification of Restriction Site Polymorphisms (Amp-FLP)

The cost of sequencing amplified fragments of DNA or designing species-specific primers has led several investigators to investigate the possibility of using the patterns generated when amplified DNA is subjected to restriction enzymes to distinguish the species origin of fish and seafood products. This method has been used successfully to differentiate between canned tuna and bonito (Ram *et al.*, 1996) and to identify flatfish species (Cespedes *et al.*, 1998). The Amp-FLP technique has a far lower resolving power in relation to sequence analysis, but it may prove useful for constructing databases for local applications.

PCR-Single Strand Conformational Polymorphisms (PCR-SSCP)

PCR-single strand conformational polymorphism (PCR-SSCP) is a variation on the theme of IEF that uses PCR products instead of proteins for rapid species identification. It is based on the observation of Orita *et al.* (1989) that single-stranded DNAs that differ by one or more nucleotide substitutions have different mobilities when separated by neutral polyacrylamide gel electrophoresis. Hara *et al.* (1994) developed this assay for many species of fish. In their protocol, DNA is extracted from the sample and a fragment of approximately 110 base pairs is amplified using a pair of primers that correspond to highly conserved regions of the 28S ribosomal RNA gene. The region between the primers was known to be highly variable between species, both in sequence and in length (Naito *et al.*, 1992), and this provides the resolving power of the system to discriminate between species. The amplified fragment is denatured by heating in a formamide solution or using strong base and then applied to a 12 % native polyacrylamide gel. After electrophoresis, the gel is silver-stained and the resulting banding patterns are diagnostic of different species. Twelve taxa of fish were examined by this technique and the indications were that this method could be used for species typing using small samples such as eggs or larvae.

PCR-SSCP has also been used for fish species identification of canned tuna (Rehbein *et al.*, 1995b). Two different regions of the cytochrome b gene were selected for amplification, the 123 base pair region described by Unseld *et al.* (1995) and another region that gave a product of 148 base pairs. Separation of the denatured 123 base pair fragment on 7.5 % or 10 % polyacrylamide gels revealed species-specific patterns for six different tunas and bonitos which were different from the pattern for bluefin and yellowfin tuna which, as expected, were identical. The universal application of PCR-SSCP to species identification of fishery products has been described by Rehbein *et al.* (1997) and demonstrated by Pharmacia Biotech (Application Note 18-1108-90). In this latter report it was shown that the 307 base pair segment of the cytochrome b gene can be used to resolve fish species. PCR-SSCP provides a rapid screening method for the analysis of the species origin of a biological sample. The main disadvantage of this technique is that, like IEF, it is necessary to run reference standards and samples side by side on the same gel. The information content of a PCR-SSCP result is obviously less than that of the FINS procedure. However, it is possible to cut out bands from the PCR-SSCP gel and determine the nucleotide sequence of them after reamplification. By this means the two methods complement one another. PCR-SSCP can also indicate that a sample contains mixtures from different organisms. Here again, species identification would be possible by examining the DNA sequences of the multiple bands.

Arbitrary primers (AP-PCR, RAPD)

In 1990 two groups independently discovered that PCR would work if only one primer were added to the amplification reaction. The rationale for this result is that the single primer is binding to identical sequences that happen to be facing one another on opposite strands of the DNA. The primers used in this procedure tend to be smaller (often only ten nucleotides) than those usually used in primer pair PCR reactions. This procedure was termed arbitrarily primed PCR (AP-PCR) by Welsh and McClelland (1990) and randomly amplified polymorphic DNA (RAPD) by Williams *et al.* (1990). It is the latter terminology that has generally been adopted and 'RAPDs' are now accepted as genetic markers. RAPD analysis requires no *a priori* knowledge of the sequence of DNA that is amplified. In fact, it is by chance alone that a segment of DNA is amplifiable in a RAPD reaction. This means that many primers can be synthesized and tested to see if they produce any products. This provides a very quick and inexpensive means of screening organisms for genetic variation. The technique is expected to sample evenly throughout the genome and variant alleles are identified by the presence or absence of a PCR product, or a change in its size, as determined by agarose gel electrophoresis followed by staining the gel with ethidium bromide. The absence of a band on a gel where one is expected is explained by a change in the sequence of one of the priming sites such that the primer no longer binds to it. Allelic bands of different size reflect insertions or deletions between the priming sites. RAPD markers are dominant and this leads to one of the drawbacks of this technique. The presence of a band on a gel is observed in samples from either a homozygote (present/present) or a heterozygote (present/absent) individual in diploid organisms. Therefore, this procedure is particularly useful for distinguishing species and strains of haploid microorganisms or for discriminating between highly inbred varieties of plants. The combination of arbitrary primers and PCR has become one of the most common approaches for searching for novel genetic markers but its application to species identification in fish has been limited. RAPD analysis has been used to differentiate between penaeid prawns (Meruane *et al.*, 1997), distinguish Atlantic salmon and brown trout (Elo *et al.*, 1997), and to analyse the amount of genetic variation among species of tilapia (Dinesh *et al.*, 1996).

Microsatellites

No description of PCR methods and applications, especially their use for authentication purposes, would be complete without mention of microsatellites. Microsatellites are tandemly repeated, short (two to five base pairs) simple sequences that are widely dispersed throughout the genomes of all eukaryotes (Weber and May, 1989). As genetic markers, microsatellites are both efficient and informative. Mutations in repeat number can be detected as size differences in the PCR products of loci which are amplified using oligonucleotide primers specific to the non-repetitive flanking regions of the microsatellite. Microsatellites have also become the markers of choice for forensic work for individual identification of humans (Fregeau and Fourney, 1993). As the PCR products of microsatellites are not large, DNA that has been degraded by exposure to the elements can be examined by microsatellite analysis whereas 'DNA fingerprinting' using minisatellite markers requires high molecular weight DNA and many orders of magnitude more material (Jeffreys *et al.*, 1985). The resolving power of microsatellites for forensic work is not restricted to humans. Poaching cases often revolve around the ability to match samples taken from a suspect with those from a 'kill site' in the field. Microsatellites are available for a growing number of marine organisms and databases are being developed in many labs. Microsatellites are particularly useful in this respect as the DNA isolated from tissue at a 'kill site' is often degraded and samples from a suspect may consist of a few flakes of blood on the blade of a knife or fish scales.

Summary

It is evident that there is a wide variety of techniques available for carrying out fish and seafood authentication. These range from quick screening methods to distinguish between two species through more sophisticated methods that can be used to identify the biological source of samples when there is no immediate indication of the species of origin. Choosing the most appropriate method comes down to cost and the state of the product being analysed. There are several companies (including Bio-ID Corporation Ltd., in St. John's, Canada and Atlangene, in Nantes, France) and government labs that routinely offer the services necessary for seafood authentication.

RECOMMENDATIONS

Enforcement agencies should be made aware of the techniques currently available for species identification and other forensic applications and should be encouraged to use them whenever appropriate.

It might seem desirable to produce databases for diagnostic DNA sequences similar to those available for muscle protein electropherograms. However, these databases are currently available on a commercial basis in North America and Europe from the companies listed above. There is little incentive, therefore, for independent groups to replicate what these commercial enterprises have already done.

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2.i. Experimental design and statistical framework

This ToR could not be handled because the Workshop from which the disucssion was supposed to have its input had to be cancelled due to lack of funding.

2.j. National Acitivity Reports

The National Activity Reports are compiled in Annex 4.

2.k. HELCOM request concerning genetic effects of released cultured fish

Based on a position paper by Marja-Liisa Koljonen, adopted by WGAGFM in Reykjavik 1999.

Conclusions

- Enough evidence exists to conclude that some changes have occurred in both the diversity levels of the marker genes and the inherited life-history traits.
- Some changes are inevitable in future, too, because artificial reproduction cannot be completely similar to natural reproduction.
- There is no return to the original state of the Baltic salmon populations.

RECOMMENDATIONS

If HELCOM wants the information necessary to manage genetic change and Baltic stock characteristics in the future then it is recommended that:

1. *Present existing data from Finland about genetic composition of populations before human impact, estimated by means of DNA-analyses from old scales and crossing experiments, should be used.*
2. *Additional data from the other Baltic Sea countries should be collected on diversity levels in historical populations and compared to that of present populations.*
3. *Changes in life-history traits should be assessed from historical information of age and size structure of the spawning stocks. However, to which extent these changes are genetic cannot be exactly assessed, because they mainly result from selective fishery and possibly also from changes in sea environment.*
4. *Future monitoring of genetic changes (at least of diversity levels of the marker genes) is recommended.*
5. *Studies of the changes in the viability (fitness) of the reared populations in the wild are recommended.*

2.1. *Sebastes mentella* in the Irminger Sea

Based on two documents presented by Anna Danielsdottir, 'Studies of the genetic relationships between deep-seas and oceanic *Sebastes mentella* in the Irminger Sea' by T. Johansen, A.K. Danielsdottir, K. Meland & G. Nævdal, and 'Genetic Differentiation of Deep-Sea and Oceanic *Sebastes mentella* in the Irminger Sea based on microsatellite Markers' by A.K. Danielsdottir & S.L. Jonsdottir. Below are the conclusions and recommendations on this matter made by the WGAGFM after its discussions in Reykjavik 1999.

Summary

Significant differences in allele frequencies based on preliminary studies on three polymorphic isozyme loci (IDHP, MEP & PGM) and three polymorphic microsatellite loci (SEB45, SEB25 and SEB31) of the deep-sea and oceanic types *S. mentella* in the Irminger Sea indicate that the two types represent separate genetic stocks.

Differences between Icelandic and Irminger Sea deep-sea *S. mentella* are smaller but significant, indicating also probably distinct genetic stocks.

Heterogeneity among samples of deep-sea and oceanic types *S. mentella* in the Irminger Sea, respectively, could indicate substructuring within each group and awaits further study. These are preliminary results from a bigger study of 30 isozyme loci and eight microsatellite loci and sample size of 2000 individuals. Results and discussion on the annual and seasonal heterogeneity within the deep-sea and oceanic *S. mentella* types will wait until the sample analysis is completed.

RECOMMENDATIONS

Based on the preliminary genetic studies to date, the deep-sea and oceanic types S. mentella in the Irminger Sea are unlikely to belong to the same breeding stock and should be managed as separate stock units.

Further research is needed to verify the preliminary genetic results on the two types and to determine if there is additional substructuring within each type.

3 WORKING GROUP BUSINESS

3.1 Comments on Working Group Function

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

It is noted with satisfaction that the U.S. as well as Estonia in 1999 joined the group of active members in the Working Group. Scientifically, the representation of scientists with a quantitative genetics background is too low, and ways to remedy this situation should be explored.

The attendance at the WGAGFM meetings, however, has been steadily increasing each year since 1994. Currently, 48 persons are appointed as members and two as observers of WGAGFM. At this year's meeting in Reykjavik, 22 members from 16 different ICES Member Countries attended.

3.2 Suggestions for WG ToRs and Meeting Place in 2000

During discussions on meeting place in the year 2000, the WG responded positively to a generous invitation from Dr Filip Volckaert, the Catholic University in Leuven, Belgium, to host the WGAGFM meeting in week 14 (3–6April) in 2000. Concerning Terms of Reference and venue, WGAGFM agreed in plenary to recommend that:

The Working Group on the Application of Genetics in Fisheries and Mariculture [WGAGFM] (New Chair: Dr Michael Møller Hansen, Denmark) will meet in Leuven, Belgium, 3–6 April 2000, to:

a) continue the review of general population genetic topics in fisheries and mariculture, with emphasis on the utilisation of possibilities lying in the combination of qualitative and quantitative genetics;

b) review principles for prioritasion of marine finfish and shellfish populations for conservation;

c) review the status of Artificial Intelligence and Neural Networks as tools in population studies based on input requested from the SIMWG;

d) compile an updated list of patents in molecular biology which potentially may interfere with population genetic research;

e) review potential genetic implications of recent research on endocrine disruptors;

f) review the possibility and feasibility of developing coordinated genetic databases for enhancing understanding of genetic diversity in fish species;

g) review genetic implications of commercial fisheries on deep-water fish stocks;

h) explore the question of trade-offs between genetic gain and loss of genetic variability in breeding programmes (how to minimize inbreeding in intense breeding);

i) prepare updated protocols of fishery and mariculture genetic research in ICES Member Countries, and identify the scope for enhanced international cooperation.

3.3 Justifications for Proposed 2000 ToRs

a) WGAGFM is a relatively informal forum where members should feel free to discuss and update each other on practical and theoretical problems related to genetics of marine species. Experience has shown that there is a need for an open scientific session at the annual meetings, where topics that are not necessarily listed in the Terms of Reference can be enlightened by the competence and experience existing in the WG. Not least have those topics which need competent input from both qualitative and quantitative genetics benefitted from these discussions.

b) With limited resources available for conservation of marine finfish and shellfish populations, it is important to provide biologically based prioritisation guidelines for managers in order to save maximum biodiversity. This has been done for many other species (see Given and Norton, 1993), including freshwater fish (Pacific salmon, brown trout). The general principles of the procedure are to rank populations within a species according to: 1) current viability status, and 2) biological consequences of extinction (genetic, evolutionary, and ecological). Together, these rankings can serve as a tool for prioritisation of possible action. So far, a formalised list of criteria for prioritisation of marine finfish and shellfish populations has not been produced. Since the marine ecological paradigm is so different from that of freshwater (or terrestrial), the criteria applied there may not all be valid for marine populations or can be made more specific.

c) WGAGFM has earlier communicated with the Stock Identification Methods Working Group (SIMWG) concerning methodology, and wants through a request to SIMWG to be updated about the current status of the use of Artificial Intelligence and Neural Networks in stock identification so that WGAGFM can have an updated discussion on these methodologies at its next meeting in Leuven, Belgium in April 2000.

d) Further to the discussion on ToR (g) Patenting of gene technology at its 1999 meeting, WGAGFM feels a need for an updated list of relevant patents.

e) Endocrine disruptors are chemical substances interacting with hormone regulatory systems or acting as hormones themselves. An enormous amount of chemicals are produced and widely used for a broad range of human purposes. Most of these substances are released to the environment and a growing number is suspected to act as endocrine disruptors. There is already evidence from laboratory experiments that some endocrine disruptors lead to a shift in sex ratio to one or the other sex in fish and there is also reason to believe that some may as well cause sterility. A disproportion in sexes or sterility may lead to genetic effects such as reduction of effective population sizes and thereby loss of genetic diversity. WGAGFM wants to summarise the scientific literature and formulate recommendations for additional research required to estimate the effects of endocrine disruptors on the genetics of fish/shellfish and recommendations on how to protect genetic diversity.

f) Molecular technology is being increasingly used by various laboratories in different countries to characterise genetic diversity in fish species, including the accumulation of DNA sequence data, to understand population

structuring and local adaptation. To fully assess broader geographical patterns and structuring across regions, and gain the maximum insight, the results of different research programmes need to be coordinated to allow integrated analysis of combined data sets. The best way for this to be pursued needs to be considered, including the establishment of common 'core' loci for screening and interlaboratory calibration of genotype assignment.

g) Landings of deep-water fish species from trawl fisheries on the continental slope have increased and fishing pressure on deep-water fish species is likely to continue to increase. The lack of reliable catch and effort data and of biological information on the stocks of deep-water species makes it difficult to establish the stocks sustainability. There is evidence that deep-water species tend to be slow growers, to mature late and to be long-lived. Such life-history traits make these stocks particularly vulnerable to exploitation. Moreover, the geographical distribution of deep-water species may extend into international waters and the implications of uncontrolled fisheries in these areas must be considered. WGAGFM feels it is appropriate to review this topic with a view to presenting recommendations for research and management, if possible.

h) Increased rates of inbreeding as the result of selection decisions will have a negative effect on future genetic response through reduction in genetic variance and a negative impact on future performance if inbreeding affects the selected trait. This can be particularly important in small highly selected nucleus populations and when selection is based on breeding values based on best linear unbiased prediction (BLUP) which is likely to result in higher levels of inbreeding than when mass selection is practiced. A number of methods have been proposed to attain high rates of genetic response with moderate to low inbreeding. These methods vary from very simplistic approaches such as minimum coancestry matings to methods that require quite sophisticated programming techniques. The best method to be used will depend on the population structure, selection intensity and the heritability of the traits under selection. WGAGFM proposes a review of the best methods to restrict inbreeding as they apply to an aquaculture population under selection.

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

ANNEX 1: TERMS OF REFERENCE

ICES C.Res.1998/2:41

The Working group on the Application of Genetics in Fisheries and Mariculture [WGAGFM] (Chair: Prof. J. Mork, Norway) will meet in Reykjavik, Iceland, from 12–15 April 1999, to:

a) continue the review of general population genetic topics in fisheries and mariculture, with emphasis on the utilisation of possibilities lying in the combination of qualitative and quantitative genetics;

b) review the potential of molecular markers as tools in breeding programmes;

c) review and discuss the status and future development of triploidy in aquaculture species;

d) review and evaluate measures used for protecting marine genetic diversity;

e) review the use of genetic tags in the study and management of wild stocks;

f) review problems and potential remedies concerning determining the gender of fish;

g) review patenting of technology as a potential problem in genetic research on marine species;

h) review genetic tissue authentication for forensic purposes;

i) review basic experimental design and statistical framework when using highly variable genetic markers in various species;

j) prepare updated protocols of fishery and mariculture genetic research in ICES Member Countries, and identify scopes for enhanced international cooperation;

k) define the information required to be able to evaluate the genetic effects of releases of cultured fish for use by WGBAST, and prepare for how the resulting material can be handled in early 2000 as a contribution to a chapter on 'Baltic fish stocks, diseases and ecosystem effects' for the HELCOM Fourth Periodic Assessment of the State of the Marine Environment of the Baltic Sea, 1994-1998 [HELCOM 1999/3];

l) review all available genetic results to make conclusions about how *Sebastes mentella* in the Irminger Sea and adjacent waters should be structured into stocks or populations in order to obtain an optimal biological management;

Some of the above Terms of Reference are set up to provide ACME with the information required to respond to requests for advice/information from the Helsinki Commission

WGAGFM will report to ACME before its May/June 1999 meeting, and to the Mariculture Committee at the 1999 Annual Science Conference.

Justifications for the1999 ToRs

a) WGAGFM currently acts as a relatively informal forum where members can feel free to discuss and update each other on practical and theoretical problems related to genetics of marine species. Experience has shown that there is a need for an open scientific session at the annual meetings, where topics that are not necessarily listed in the Terms of Reference can be enlightened by the competence and experience existing in the WG. Not least have those topics which need competent input from both qualitative and quantitative genetics benefitted from these discussions.

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

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

c) The induction of triploidy has been reported in many aquaculture species (fish: salmonids, seabass, seabream, turbot, etc. shellfish: mussels, oysters, scallops, clams, etc.). In fish, triploidy is generally induced by pressure or thermal shocks, while in shellfish it is induced by the suppression of polar body formation in fertilised eggs. An alternative method is based on the mating of tetraploids and diploids to obtain all-triploid stocks. Tetraploids have been obtained in several species (rainbow trouts, carps, oysters, mussels). As triploidy induces sterility (or strongly reduces gametogenesis), better performance of triploids over diploids can be explained by two main factors: 1) the energy normally allocated to reproduction in diploids can be reallocated to growth in triploids, consequently, triploidy will be of greater interest in species with high allocation to reproduction. Additionally, triploidy potentially induces higher mean heterozygosity, which has been shown to be positively correlated with growth in many shellfish species. 2) As sexually matured animal can be of lower quality for consumption than non-matured (e.g., oysters), sterility (or reduced gametogenesis) improves the marketing value of the products. Additionally, the recent need of sterily to ensure a genetic confinement of transgenic organisms led to new interest in triploidy. The present status of triploidy and its potential for future development in aquaculture species will be reviewed by the WGAGFM to establish specific recommendations.

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

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

f) Unlike mammals, the genotypic and phenotypic sex of bony fish varies among species. Halibut, seabass, European eel and turbot among others show variable sex ratios in natural and cultured populations, presumably in response to environmental and population dynamical factors. So far, no common sex determining system has been observed but the aromatase gene seems to play a pivotal role in sex differentiation. Only 10 % of all fish species have been reported to carry sex chromosomes, but few have been verified by breeding. The few sex probes developed (Rainbow trout, Chinook salmon, guppy and medaka amongst others) have been proven to be species-specific. Consequently the determination of the genetic sex remains problematic and causes major economic losses in aquaculture. WGAGFM wants to review current knowledge and discuss future ways to go to solve these problems, with a view to give recommendations to ICES as to the kind of fundamental and applied research (e.g., genetics, molecular biology, and endocrinology) needed.

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

h) Several of the laboratories represented in the Working Group have been engaged in work with the identification of animal tissues for forensic purposes, and there is a growing need to coordinate research and methodology in this

field. WGAGFM feels it is time to establish a status of this field, and to look into the possibilities for a better international network/coordination.

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

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

k) This work is required in relation to a request from the Helsinki Commission (HELCOM).

l) The North-Western Working Group (NWWG) recommends that all available genetic results related to the stock structure of *S. mentella* in the Irminger Sea be evaluated to permit better biological management of the stock.

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ANNEX 4: 1999 NATIONAL ACTIVITY REPORTS

BELGIUM

Study 1
LABORATORY/RESEARCHER: Royal Belgian Institute of Natural Sciences (RBINSc), Brussels / T. Backeljau, B. Winnepenninckx and H. De Wolf. Joint program with University of the Azores, Portugal / A.M. Frias Martins, C. Brito and R. Medeiros. The Natural History Museum London / D. Reid. University of Leeds, U.K. / J. Grahame and P.J. Mill. Regional Technical College, Galway, Ireland / E. Gosling.
SPECIES: *Littorinidae* (periwinkles), particularly *Littorina striata* (Mollusca, Gastropoda and Prosobranchia).
PROJECT FUNDING: EU MAST-III program; PRAXIS (Portugal); graduate student grant by the IWT (Belgium); Joint Basic Research Project grant by the Fund for Scientific Research (Flanders).
OBJECTIVE: Integrating population genetic and morphological variation for evolutionary research. Phylogeny of Littorinidae.
METHODOLOGY: Electrophoresis of allozymes and radular myoglobins; random amplified polymorphic DNA; development of microsatellite DNA markers and Single Strand Conformation Polymorphisms (SSCP); DNA sequencing; morphometrics of shell features.
STATUS: Undergraduate theses; ongoing program within MAST-III.

Study 2
LABORATORY/RESEARCHER: Royal Belgian Institute of Natural Sciences, Brussels / T. Backeljau. In collaboration with the University of Vigo, ES / T. Willems, J. Troncoso and A. Sanjuan. University of the Azores, P / A.M. Frias Martins and C. Brito.
SPECIES: *Artemia salina*, Rotifera.
PROJECT FUNDING: Own funding.
OBJECTIVE: Genetic characterisation of strains of *Artemia salina* and Rotifera.
METHODOLOGY: DNA fingerprinting (RAPD and AFLP).
STATUS: Under development.
COMMENTS: Funding requested.

Study 3
LABORATORY/RESEARCHER: Katholieke Universiteit Leuven, Zoological Institute, Leuven / F. Volckaert.
SPECIES: European eel (*Anguilla anguilla*).
PROJECT FUNDING: Own funding.
OBJECTIVE: Population structure, including gene flow and selection.
METHODOLOGY: DNA microsatellites and mitochondrial DNA sequence variation, allozymes.
STATUS: several publications in progress. DNA microsatellite primers published in Animal Genetics (1997).

Study 4
LABORATORY/RESEARCHER: Katholieke Universiteit Leuven, Zoological Institute, Leuven / F. Volckaert and E. Gysels.
SPECIES: Gobies (*Pomatoschistus minutus, P. microps* 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 structure of three sympatric populations of gobies along the European continental shelf, including gene flow and selection.
METHODOLOGY: Allozyme electrophoresis and mitochondrial DNA sequence variation and polymorphisms.
STATUS: Ph.D. project and graduate thesis in progress; project is open-ended; we welcome collaboration.
COMMENTS: collaboration welcome

Study 5
LABORATORY/RESEARCH: Katholieke Universiteit Leuven, Zoological Institute, Leuven / F. Volckaert and T. Huyse.
SPECIES: Monogenea: *Gyrodactylus* sp. and their goby (*Pomatoschistus*) and stickleback (*Gasterosteus*) hosts.
PROJECT FUNDING: IWT (Flemish research fund for applied research) and Belgian Ministry of Scientific Affairs (project "Sustainable development of the North Sea").
OBJECTIVE: Co-evolution between parasites of genus *Gyrodactylus* and *Pomatoschistus* hosts.
METHODOLOGY: Phylogenetic characterisation of ITS nuclear region and phylogeographic characterisation with sequence variation at COI mitochondrial locus.
STATUS: Ph.D. project in progress; project is open-ended; we welcome collaboration.

Study 6
LABORATORY/RESEARCH: Katholieke Universiteit Leuven, Zoological Institute, Leuven / F. Volckaert and 11 other teams (Coordinator: B. Chatain, IFREMER, Palavas-les-Flots, France).
SPECIES: Sea bass (*Dicentrarchus labrax*).
PROJECT FUNDING: EU -Concerted Action (DGXIV).
OBJECTIVE: To establish a concerted programme for strain testing of sea bass.
METHODOLOGY: Informal and formal meetings, reports.
STATUS: In progress since 01.01.98.

Study 7
LABORATORY/RESEARCH: Katholieke Universiteit Leuven, Zoological Institute, Leuven / F. Volckaert.
SPECIES: Dover Sole (*Solea solea*).
PROJECT FUNDING: Belgian Ministry of Scientific Affairs (project "Sustainable development of the North Sea).
OBJECTIVE: To detail gene flow throughout the natural range.
METHODOLOGY: Characterisation of the mitochondrial DNA genome by means of RFLP analysis and sequencing.
STATUS: In progress.
COMMENTS: Biopsies of 30 mature fish from spawning grounds are welcome.

Study 8
LABORATORY/RESEARCH: Agricultural Research Centre-Ghent, Department of Sea Fisheries, Oostende.
SPECIES: Flatfishes initially, later on all seafood or seafood products.
PROJECT FUNDING: Own funding.
OBJECTIVE: To develop DNA -based methods for authentication of commercially important species (unprocessed and processed).
METHODOLOGY: RAPD, SSCP and AFLP.
STATUS: Project started in September 1997.
COMMENTS: The project aims at the development of reliable, reproducible, cheap and easy DNA techniques suitable to construct a database.

Study 9
LABORATORY/RESEARCH: Department Sea Fisheries, Oostende / D. Delbare and R. De Clerck. Joint project with: CEFAS Fisheries Laboratory, Lowestoft, United Kingdom / R. Millner, and the Danish Institute for Fisheries Research (DIFMAR), Charlottenlund, Denmark / M. Winter.
SPECIES: *Scophthalmus rhombus* (brill) (Pisces, Pleuronectiformes).
OBJECTIVE: To describe the stock structure of the brill in relation to fisheries, by comparison of biological parameters (growth, sex ratio, age of maturity, stage of maturity, and gonadosomatic index) and genetic variation.
METHODLOGY: Mitochondrial DNA sequencing of the control region.
STATUS: Project in progress.

Study 10
LABORATORY/RESEARCH: RIVO-DLO, IJmuiden, The Netherlands / H. Heesen. Department of Sea Fisheries, Oostende / E. Ongenae, D. Delbare and R. De Clerck.
SPECIES: *Psetta maxima* (turbot) and *Scophthalmus rhombus* (brill) (Pisces, Pleuronectiformes).
PROJECT FUNDING: European Commission, Directorate General XIV Fisheries.
OBJECTIVE: Preliminary assessments of two important by-catch species to provide data on mortality and recruitment combined with the precise identification of unity stocks.
METHODOLOGY: Mitochondrial DNA sequencing of the control region, SSCP and AFLP.
STATUS: Project started on 01.01.98.

CANADA

Study 1
LABORATORY/RESEARCHER: NRC Institute for Marine Biosciences, Halifax / S. Douglas, project leader. Marine Gene Probe Laboratory, Dalhousie University / 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: Research largely completed. Data being prepared for publication.

Study 2
LABORATORY/RESEARCHER: NRC Institute for Marine Biosciences, Halifax / S. Douglas and M. Reith, project leaders.
SPECIES: *Pleuronectes americanus*, winter flounder.
PROJECT FUNDING: NRC core budget.
OBJECTIVE: Establishment of an EST database from winter flounder.
DESIGN: DNA libraries established from a number of tissues of a winter flounder.
METHODOLOGY: Random cDNA clones from several winter flounder libraries (pyloric caeca, intestine and spleen) are being sequenced and identified by comparing against the genetic databases. This identifies clones containing sequences of highly expressed genes and gives valuable information regarding codon usage (necessary for design of PCR primers). The EST data provides a starting point for isolating genes and for genome mapping in this organism.
STATUS: Currently approximately 1200 ESTs in the database from the following libraries: stomach, pyloric caeca, spleen, intestine and pancreas. Project is suspended at this point due to lack of funding to proceed further.

Study 3
LABORATORY/RESEARCHER: NRC Institute for Marine Biosciences, Halifax / S. Douglas project leader.
SPECIES: *Pleuronectes americanus*, winter flounder.
PROJECT FUNDING: NRC core budget.
OBJECTIVE: Ontogeny of digestive enzyme activity in teleost fish.
DESIGN: cDNA clones are isolated from gut tissues of winter flounder and used to probe expression patterns.
METHODOLOGY: Portions of genes are amplified by PCR using primers based on conserved motifs. Genes are sequenced and used to develop assays for gene expression (molecular and microscopic).
STATUS: Probes for several genes related to the digestion process have been prepared and conditions for probing tissue slices and examining these by microscopy have been established. The work is ongoing.

Study 4
LABORATORY/RESEARCHER: NRC Institute for Marine Biosciences, Halifax / S. Douglas project leader.
SPECIES: *Pleuronectes americanus*, winter flounder.
PROJECT FUNDING: NRC core budget.
OBJECTIVE: Investigation of malpigmentation in flatfish using molecular biological techniques.
DESIGN: The gene for a critical enzyme in the biochemical pathway leading to melanin is being cloned.
METHODOLOGY: Portions of the gene are being amplified by PCR using primers based on conserved motifs. The gene will be sequenced and used to assay expression at the molecular level.
STATUS: In progress. Little progress was made on this project during the past year, partly due to lack of time. It is being continued in the current year, pending review of IMB research priorities.

Study 5
LABORATORY/RESEARCHER: NRC Institute for Marine Biosciences, Halifax (NRC). Department of Fisheries and Oceans (DFO). C.J. Bird (NRC) and E. Kenchington (DFO) project leaders.
SPECIES: *Placopecten magellanicus* (sea scallop), other scallops (*Chlamys*, *Pecten*, *Argopecten*, *Crassodoma*), oysters (*Ostrea edulis* (European oyster), *Crassostrea* (mussels *Mytilus* sp.), clams (*Mactromeris*).
PROJECT FUNDING: NRC and DFO core budget.
OBJECTIVE: Genetic discriminants and markers for bivalves.
DESIGN: DNA samples are being examined for animals from a number of locations.
METHODOLOGY: Microsatellites and nucleotide sequences of ribosomal RNA genes (including their internal transcribed spacers) are being evaluated as discriminants of taxa and populations. Microsatellites in particular are valuable for labelling pedigreed broodstock and checking the provenance of aquacultured stocks. DNA nucleotide sequence is less sensitive intraspecifically but provides a measure of species relatedness.

STATUS: Research ended. Papers are being drafted..

Study 6
LABORATORY/RESEARCHER: NRC Institute for Marine Biosciences, Halifax / M. Ragan, project leader. Atlantic Veterinary College, Charlottetown / R. Cawthorn). St Mary's University, Halifax / T. Rand. 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: Characterisation 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: Characterisation 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) characterisation of protistan parasites of economic importance in aquaculture.
STATUS: Most of this work has been published and new targets have been identified for further work.

Study 7
LABORATORY/RESEARCHER: NRC Institute for Marine Biosciences, Halifax / M. Reith, project leader.
SPECIES: *Pleuronectes americanus*, winter flounder, other marine fish species.
PROJECT FUNDING: NRC core budget.
OBJECTIVE: To undertake a search for sex-linked DNA markers in flatfish.
DESIGN: Various cDNA markers isolated from reproductive and other tissues of male and female flounder are being isolated and compared for expression and for segregation in male and female fish.
METHODOLOGY: Molecular biology tools are being used to obtain probes that reveal polymorphic loci and to examine segregation of these alleles in male and female fish (test for linkage with sex-determining locus).
STATUS: This project has been set aside for the time being. Several approaches aimed at identifying sex-specific markers were tried but none successfully.

Study 8
LABORATORY/RESEARCHER: NRC Institute for Marine Biosciences, Halifax / M. Ragan, project leader. DFO Nanaimo / M. Kent.
SPECIES: Selected microsporidian protists parasitic in salmon and other marine fish.
PROJECT FUNDING: NRC and DFO core budgets.
OBJECTIVE: Characterisation of protistan parasites of fish and shellfish using sequence data from random genomic fragments; funding for more extensive genomic sequencing of a selected parasite is being sought.
DESIGN: DNA samples are being obtained for target parasites.
METHODOLOGY: Partial characterisation of protistan parasites of fish using DNA sequence data. Design and application of oligonucleotide probes for detection of protistan parasites and search for potential targets for therapeutants.
STATUS: All attempts to get clean parasite DNA were unsuccessful. All salmon tested were multiply infected with more than one of the target parasite species. This study will be revisited once a method for getting "clean" parasite material has been obtained.

Study 9
LABORATORY/RESEARCHER: NRC Institute for Marine Biosciences, Halifax / M. Ragan, project leader. Natural History Museum, London, UK / M. Embley.
SPECIES: Selected microsporidian protist parasitic in marine fish.
PROJECT FUNDING: NRC core budgets and European grant money (for Embley).
OBJECTIVE: Characterisation of the parasite using sequence data from ESTs; funding for more extensive genomic sequencing of a selected parasite is being sought.
DESIGN: cDNA samples are being sent to Halifax for sequencing.
METHODOLOGY: Partial characterisation of protistan parasites of fish using DNA sequence data. Design and application of oligonucleotide probes for detection of protistan parasites and search for potential targets for therapeutants.
STATUS: Moving ahead very well. Several hundred EST's were obtained and the project is moving towards random sequencing of some genomic clones as the cDNA library is now yielding mostly repeats of previously obtained ESTs.

Study 10
LABORATORY/RESEARCHER: NRC Institute for Marine Biosciences, Halifax / M. Reith project leader. University of New Brunswick / C. McGowan and T. Benfy.
SPECIES: Atlantic halibut.
PROJECT FUNDING: NRC core budget and strategic NSERC grant.

OBJECTIVE: Microsatellite DNA markers are being obtained to assist in estimating genetic divergence of wild stocks and to aid in future broodstock selection in aquaculture.
DESIGN: Initial fish were taken from broodstock at DFO St. Andrews; others to be added.
METHODOLOGY: Microsatellites are being cloned and sequenced from genomic DNA and primers are designed to amplify specific microsatellites by PCR. The products are resolved on an automated DNA sequencer.
STATUS: Several useful markers have been characterised and used to make an initial assessment of an F1 population derived from matings of the broodstock. An initial manuscript describing this work has been submitted for publication.

Study 11
LABORATORY/RESEARCHER: NRC Institute for Marine Biosciences, Halifax / S. Johnson (project leader), V. Ewart and N. Ross.
SPECIES: *Lepeophtheirus salmonis* (sea louse on salmon host).
PROJECT FUNDING: NRC core budget and NRC- NSERC grant.
OBJECTIVE: Identification of proteins that are potential therapeutic targets or vaccine antogen candidates.
METHODOLOGY: Random cDNA clones from a sea louse library are being sequenced and identified by comparing against the genetic databases. This identifies clones containing sequences of highly expressed genes. The library is also being probed for specific target genes such as proteases that might play a role in sea lice modification of salmon mucous.
STATUS: Several interesting EST's have been identified for further study.

Study 12
LABORATORY/RESEARCHER: NRC Institute for Marine Biosciences, Halifax / L. Brown (project leader) and C. Sensen.
SPECIES: ISAV (ISA virus on salmon host).
PROJECT FUNDING: NRC core budget.
OBJECTIVE: Genomic sequencing of a local strain of this virus with a view to identifying targets for therapeutants or vaccines.
METHODOLOGY: The virus genome will be sequenced using standard genome sequencing techniques.
STATUS: In progress.

Study 13
LABORATORY/RESEARCHER: NRC Institute for Marine Biosciences, Halifax / M. Ragan, project leader. DFO, Moncton / S. McGladdery.
SPECIES: QPX.
PROJECT FUNDING: NRC and DFO core budgets.
OBJECTIVE: Identification of the QPX organism and development of a diagnostic probe for the presence of the organism.
METHODOLOGY: Comparison of protistan parasites of fish and shellfish using sequence data from nuclear ssu-rRNA genes. Design and application of oligonucleotide probes for detection of the Quahog parasite.
STATUS: Beginning.

Study 14
LABORATORY/RESEARCHER: Marine Gene Probe Lab., Dalhousie University, Halifax / C. Taggart, 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 characterized spawning groups.
STATUS: First phase of project complete, all samples have been collected and lab work completed. At this stage several stocks have been defined. Further collections of spawning stocks and mixed stock have been made and laboratory work on new samples is complete, analysis of results for temporal stability and also for mixed stock analysis is now underway.

Study 15
LABORATORY/RESEARCHER: Marine Gene Probe Lab., Dalhousie University, Halifax / D. Cook, D. Ruzzante, S. Lang and C. Taggart.
SPECIES: *Gadus morhua* (Atlantic cod); *Gadus ogac* (Greenland cod): *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.
METHODOLOGY: Blood of fin clip samples as available collected from three species, DNA extracted and run on all microsatellites available.
STATUS: Data has been collected from approximately 5,000 adult Atlantic cod (for other purposes) data collection complete for Arctic cod and is presently being collected for Greenland cod. To date results have shown identification of Arctic cod will be 100 % accurate, results for Greenland cod show that approximately 30 % of known Greenland cod cannot be distinguished beyond a 80 % probable level, differences do exist but there is low probability of northern cod showing these profiles so identity is not unique.

Study 16
LABORATORY/RESEARCHER: Marine Gene Probe Lab., Dalhousie University, Halifax / D. Ruzzante, D. Cook and C. Taggart.
SPECIES: *Gadus morhua* (Atlantic cod).
PROJECT FUNDING: Canadian Dept. Fisheries and Oceans.
OBJECTIVE: To determine whether or not there is evidence of genetic differentiation between Spring and Fall spawning cod populations on the Scotian Shelf.
STATUS: In progress.

Study 17
LABORATORY/RESEARCHER: Marine Gene Probe Lab., Dalhousie University, Halifax / D. Ruzzante, D. Cook and C. Taggart.
SPECIES: *Gadus morhua* (Atlantic cod).
PROJECT FUNDING: Canadian Dept. Fisheries and Oceans.
OBJECTIVE: Larval cod aggregations on the Scotian Shelf and off Newfoundland: source-sink populations.
STATUS: In progress.

Study 18
LABORATORY/RESEARCHER: Marine Gene Probe Lab., Dalhousie University, Halifax / D. Ruzzante, D. Cook and C. Taggart. US / D. Townsend and I. Kornfield.
SPECIES: *Gadus morhua* (Atlantic cod).
PROJECT FUNDING: NSF.
METHODOLOGY: Comparative analysis of larvae known to originate on Browns or Georges Bank, and of larvae which may have been carried from Browns to Georges with microsatellites and anonymous nuclear markers.
OBJECTIVE: Larval exchange between Georges Bank and Browns Bank.
STATUS: Samples of spawning adults from Georges and Browns Banks have been collected to confirm temporal stability of observed differences. Larvae and juveniles have been obtained from Browns Bank to establish identity of potential donor stock during the last episodic flux.

Study 19
LABORATORY/RESEARCHER: Marine Gene Probe Lab., Dalhousie University, Halifax / D. Ruzzante, D. Cook and C. Taggart.
SPECIES: *Gadus morhua* (Atlantic cod).
PROJECT FUNDING: Canadian Dept. Fisheries and Oceans.
OBJECTIVE: Assessment of historical DNA from cod populations in the NW Atlantic.
METHODOLOGY: DNA is extracted from archived otolith collections.
STATUS: Laboratory work complete analysis in progress.

Study 20
LABORATORY/RESEARCHER: Marine Gene Probe Lab., Dalhousie University, Halifax / D. Cook, A. Macpherson and C. Taggart.
SPECIES: Capelin.
PROJECT FUNDING: Canadian Dept. Fisheries and Oceans.
OBJECTIVE: Development of dinucleotide and tetranucleotide probes for capelin.

STATUS: New project.

Study 21
LABORATORY/RESEARCHER: Marine Gene Probe Lab., Dalhousie University, Halifax / D. Cook and C. Taggart.
SPECIES: Shark species.
PROJECT FUNDING: FAO.
OBJECTIVE: Development of species identification markers for shark species.
STATUS: Anonymous markers have been developed which can distinguish all species of sharks tested to date, now awaiting the collection of more species to expand the range. Methods have been developed to extract DNA from dried shark fins and speciation has been confirmed on these samples.

Study 22
LABORATORY/RESEARCHER: Marine Gene Probe Lab., Dalhousie University, Halifax / D. Ruzzante, D. Cook and C. Taggart. Memorial University of Newfoundland / S. Goddard.
SPECIES: *Gadus morhua* (Atlantic cod).
PROJECT FUNDING: Canadian Dept. Fisheries and Oceans.
OBJECTIVE: Genetic differences between inshore and offshore Atlantic cod (*Gadus morhua*) off Newfoundland.
METHODOLOGY: Microsatellite markers, blood antifreeze level (to assign overwintering location as inshore or offshore).
STATUS: There are two papers published on this subject: The first describes evidence of genetic structure between inshore and offshore cod off Newfoundland (Ruzzante *et al.* 1996 CJFAS 53:634-645). The second provides evidence of temporal stability of the genetic structure at the scale of 2 to 4 years (Ruzzante *et al.* 1997 CJFAS 54:2700-2708).

Study 23
LABORATORY/RESEARCHER: Marine Gene Probe Lab., Dalhousie University, Halifax / D. Ruzzante, D. Cook and C. Taggart.
SPECIES: *Gadus morhua* (Atlantic cod).
PROJECT FUNDING: Canadian Dept. Fisheries and Oceans.
OBJECTIVE: The genetics of a larval cod aggregation and genetic identification of a larval cohort in relation to some oceanographic features (Gyre-like eddies) are determined.
METHODOLOGY: Six microsatellite DNA loci were assessed for polymorphism.
DESIGN: Cod larvae were sampled repeatedly over a 3-week period from an aggregation on Western Bank.
STATUS: There is one paper published on this subject: Ruzzante *et al.* 1996 CJFAS 53:2695-2705.

Study 24
LABORATORY/RESEARCHER: Marine Gene Probe Lab., Dalhousie University, Halifax / D. Ruzzante, D. Cook and C. Taggart.
SPECIES: *Gadus morhua* (Atlantic cod).
PROJECT FUNDING: Canadian Dept. Fisheries and Oceans.
OBJECTIVE: To describe broad and fine-scale genetic structure among cod populations in the NW Atlantic.
METHODOLOGY: Microsatellite markers.
STATUS: There are two papers on this topic: the first (Bentzen *et al.* 1996 CJFAS 53:2706-2721) describes evidence of genetic structure at ocean basin and continental shelf scales. Another manuscript has been provisionally accepted in Molecular Ecology (Ruzzante *et al.*) describing emerging evidence of genetic structure among cod populations from throughout the species range in the NW Atlantic in relation to oceanographic features (gyre-like circulations or eddies that might act as retention mechanisms for eggs and/or larvae) and spatio-temporal differences in peak spawning time.

Study 25
LABORATORY/RESEARCHER: Marine Gene Probe Lab., Dalhousie University, Halifax / D. Ruzzante.
SPECIES: *Gadus morhua* (Atlantic cod).
PROJECT FUNDING: Canadian Dept. Fisheries and Oceans.
OBJECTIVE: A comparison of several measures of genetic distance and population structure with microsatellites.
METHODOLOGY: Microsatellite markers.
STATUS: This work is now published D. Ruzzante (1998, CJFAS 55(1)).

Study 26
LABORATORY/RESEARCHER: Marine Gene Probe Lab., Dalhousie University, Halifax / D. Ruzzante, D. Cook, T. McParland, and C. Taggart.
SPECIES: *Gadus morhua* (Atlantic cod).
PROJECT FUNDING: Canadian Dept. Fisheries and Oceans.
OBJECTIVE: Genetic identification of inshore/offshore cod stock components in the Newfoundland region.

METHODOLOGY: Microsatellite markers.
STATUS: Samples have been collected from Bay populations and from offshore populations in the same areas, laboratory processing of samples is complete and analysis is underway.

Study 27
LABORATORY/RESEARCHER: Marine Gene Probe Lab., Dalhousie University, Halifax / D. Cook, A. Macpherson and C. Taggart.
SPECIES: *Clupea harengus harengus* (Atlantic herring).
PROJECT FUNDING: Pelagic Research Council.
OBJECTIVE: To define the population, genetic, structure of the Atlantic herring in the Scotia Fundy region, and to relate the structure or lack there of to the known oceanographic patterns in the area.

Study 28
LABORATORY/RESEARCHER: Marine Gene Probe Lab., Dalhousie University, Halifax / D. Cook, S. Lang and C. Herbinger. NRC Institute for Marine Biosciences, Halifax / S. Douglas, project leader.
SPECIES: *Pleuronectes americanus*, winter flounder.
PROJECT FUNDING: MGPL 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: Samples have been collected from along the Scotian Shelf, Saint Mary's Bay and Georges Bank
METHODOLOGY: Dinucleotide microsatellites have been cloned and sequenced from genomic DNA and primers designed to amplify specific microsatellites by PCR. The products are resolved on an automated DNA sequencer.
STATUS: Microsatellites have been developed and laboratory work is complete, analysis is underway.

Study 29
LABORATORY/RESEARCHER: Institut Maurice-Lamontagne, Ministère des Pêches et des Océans, Mont-Joli / J.-M. Sévigny and B. Sainte-Marie.
SPECIES: Snow crab (*Chionoecetes opilio*).
PROJECT FUNDING: Department of Fisheries and Oceans.
OBJECTIVE: (1) Description of the population structure in the Northwest Atlantic; (2) Description of inter-cohort genetic variability.
DESIGN: (1) Mature males of different size classes were sampled at several sites in the Gulf of St. Lawrence and the Atlantic. (2) Several cohorts collected at the same sampling site are being analysed.
METHODOLOGY: Morphometry, allozyme, mtDNA, microsatellite DNA.
STATUS: Description of the population structure and inter-cohort variability is ongoing.

Study 30
LABORATORY/RESEARCHER: Institut Maurice-Lamontagne, Ministère des Pêches et des Océans, Mont-Joli / B. Sainte-Marie, A. Rondeau and J.-M. Sévigny. Département des Sciences Animales, Université McGill, Sainte-Anne-de-Bellevue / N. Urbani and U. Kuhnlein. Département de pathologie et biologie cellulaire, Université de Montréal / G. Sainte-Marie.
SPECIES: Snow crab (*Chionoecetes opilio*).
PROJECT FUNDING: Department of Fisheries and Oceans.
OBJECTIVE: Study of the snow crab mating system.
DESIGN: 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: Microsatellite DNA, histology.
STATUS: Both wild and laboratory primiparous females commonly mated with several males whose ejaculates are stored and stratified in the spermathecae. Genetic typing of the offspring revealed single paternity and indicated that the last mate to inseminate the female before oviposition gained paternity of the clutch. The field study and laboratory experiments on mating behaviour are ongoing.

Study 31
LABORATORY/RESEARCHER: Institut Maurice-Lamontagne, Ministère des Pêches et des Océans, Mont-Joli. J.-M. Sévigny, M. Black and A. Valentin. Département de Biologie, Université Laval, Québec / L. Bernatchez 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. Morphometric analyses is also used for species identification in the Gulf of St. Lawrence. The project is carried out in collaboration with Dr. G. Naevdal and T. Johansen of Bergen University.
METHODOLOGY: Allozyme, mtDNA, rDNA and microsatellite DNA.
STATUS: Ongoing.

Study 32
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 of different lagunes of the Magdalen Islands is described and compared with the variability detected in mussel cultivated under various regimes of density. Bioenergetic and genetic parameters are also examined in relation to susceptibility of blue mussels to summer mortality.
METHODOLOGY: Allozyme, metabolic parameters.
STATUS: Allozyme analyses have shown important difference in heterozygosities among the stocks of the Magdalen Islands. The stock showing the highest degree of multiple-locus heterozygosity is also the most resistant to summer mass mortality. The results support the hypothesis that high levels of heterozygosity are related with lower cost of maintenance. Ongoing.

Study 33
LABORATORY/RESEARCHER: Dept. Fisheries and Oceans, Canada, Northwest Atlantic Fisheries Centre, St. John's, Newfoundland / R. Penney (project leader).
SPECIES: Blue mussel (*Mytilus edulis*), *M. trossulus.*
PROJECT FUNDING: DFO core funding.
OBJECTIVE: 1) Delineate existing *edulis* - *trossulus* proportions at commercial mussel farms; Survey wild and cultured populations of edulis & trossulus for multilocus allelic variation; explore relationships among multilocus genotype and phenotypic expression of commercially important characters.
DESIGN: Multilocus genotype and shell morphometry data from twenty-five wild and fifteen cultured populations have been sampled since 1994. A rearing experiment is ongoing at 3 farms.
METHODOLOGY: Laboratory analysis using electrophoretic techniques is underway. Five isozymes are being used, MPI, GPI, PGM, ODH, and LAP. Samples are being classified to species using the MPI.
STATUS: The initial survey is now complete. Results indicate *trossulus* mussels are widespread in occurrence throughout Newfoundland. Typically, most sites are a mix of *edulis* and *trossulus* types. Proportionally, *edulis* usually is the dominant species at most sites. There does not appear to be any geographic separation of species nor are any other distribution patterns apparent. Sites in close (< 5 km.) proximity have similar scales of genetic variation as was found over the entire study area, a coastline of over 9600 km. Culture sites generally have proportionally more *M. trossulus* compared to wild sites. Significant inter- and intraspecific relationships have been found among multilocus genotype, shell shape, and shell weight. The rearing experiment got underway in August, 1998. Its purpose is to determine whether significant inter- and intraspecific relationships exist among multilocus genotype, growth, and survival.

Study 34
LABORATORY/RESEARCHER: Dept. Fisheries and Oceans, Canada, Northwest Atlantic Fisheries Centre, St. John's, Newfoundland / R. Penney (project leader).
SPECIES: *Modiolus modiolus.*
PROJECT FUNDING: DFO core funding.
OBJECTIVE: To determine the allozyme patterns of the horse mussel for comparison with *Mytilus edulis* and *M. trossulus.*
DESIGN: In 1997-98, 4 sites were sampled where *M. modiolus* grows either mixed with *Mytilus* species or immediately adjacent to *Mytilus* beds.
METHODOLOGY: Allelic variation was studied at 7 loci: MPI, GPI, PGM, LAP, EST, AAP and AP. Electromorphs in *M. modiolus* were compared to known electromorphs of *Mytilus* spp.
STATUS: This study was completed in 1998. We were unable to adequately resolve MPI for *M. modiolus.* Electromorph patterns at the GPI locus are virtually 100 % diagnostic for discriminating *M. modiolus* from *Mytilus* spp. Other loci were partially diagnostic. This technique has potential applicability as a screening tool to identify *M. modiolus* individuals among mixed species spat cohorts.

Study 35

LABORATORY/RESEARCHER: Dept. Fisheries and Oceans, Canada, Northwest Atlantic Fisheries Centre, St. John's, Newfoundland / R. Penney (project leader), F. Corbett. Thimble Bay Farms Ltd. / T. Mills.

SPECIES: *Placopecten magellanicus.*

PROJECT FUNDING: DFO core funding.

OBJECTIVE: Bio-economic modelling of scallop farming.

DESIGN: A pilot scale scallop production system was run during 1989-91 to determine appropriate input parameters to a bio-economic model.

METHODOLOGY: The effects of stocking density, intermediate culling, net mesh size, and spat grading on growth and survival were studied in structured experiments at a commercial scallop farm. All labour, capital costs, and operating costs were recorded. These data are extrapolated to simulate the startup and operation of a commercial scale farm. Economic viability of farming scallops is forecasted using standard financial evaluation techniques of NPV and IRR.

STATUS: Analysis of the growth and survival data from the pilot scale trials has been completed. Results from these analyses are now being used as input into the simulation modelling exercise.

Study 36

LABORATORY/RESEARCHER: Science Branch, Department of Fisheries & Oceans, PO Box 5667 St. John's, NF / 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: Repeated measurement of performance and subsequent pedigree analysis of spawning history of each of the two salmon strains to establish breeding strategy for both strains.

STATUS: - Grand Codroy performance was inferior for first generation aquaculture salmon. - Grand Codroy strain outperformed industry standard strain during second generation on growing. Industry interest in the GCR strain culminated in incubation of 1.6 million eggs in 1997 and further propagation of the line in the fall of 1998. Pedigree analysis of the GCR brood stock now has illuminated potential founder effects for which genetic strategy is being developed.

Study 37

LABORATORY/RESEARCHER: Science Branch, Department of Fisheries & Oceans, PO Box 5667 St. John's, NF / V. Pepper (project leader), T. Nicholls.

SPECIES: Atlantic salmon (*Salmo salar*).

PROJECT FUNDING: Project implemented in 1991. Program complete 1998. Funding: SCB Fisheries Limited, 1995. Atlantic Fisheries Adjustment Program, 1994. Department of Fisheries & 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. mixed-sex, diploid SJR). Monthly sampling to document: growth (G), mortality (Z), biomass elaboration (G-Z) and Food Conversion Ratio.

METHODOLOGY: Pressure shock, blood sampling, flow cytometry.

STATUS: Through the first cycle of estuarine performance evaluation, all-female triploid salmon out performed all other salmon in the industry net-pens. At the time of grading for marked, HOG weight for the triploid salmon was 28 % larger than for the control group of diploids. There were very few jaw deformities in this year-class. However, the second year-class of triploid salmon performed very poorly in the estuarine cages and suffered a high incidence of jaw deformities (53 %), poor survival (37 % 3n vs. 68 % 2n) and inferior growth (i.e., final mean weight 90 % of the control group mean). Industry has reacted to this lack of cause-and-effect in husbandry practices by destroying the third cycle of triploid salmon during the hatchery phase of the life cycle. However, industry is continuing with an alternate strain of triploid salmon (Gaspé) from a commercial supplier to evaluate its performance in the Bay d'Espoir estuarine fjord environment.

Study 38

LABORATORY/RESEARCHER: Dept. Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, N.S. / E. Kenchington (contact) in collaboration with K. Freeman and A. Glass. St. Andrew's Biological Station, St. Andrew's, N.B. / S. Robinson.

SPECIES: Blue mussel (*Mytilus edulis*), *M. trossulus.*

PROJECT FUNDING: DFO core funding; Biology Department, Dalhousie University, Halifax, Nova Scotia, Canada.

OBJECTIVE: To determine whether mussel growers can optimise the proportion of *M. edulis: M. trossulus* spat on their collectors through seasonal and/or depth adjustments to their collection strategy.

DESIGN: Spat were collected on replicate collector ropes put out and removed at spaced intervals over the spawning season. Another similar set of replicate lines were deployed at the beginning of the spawning season and removed at the end. Collectors were placed at two sites in Nova Scotia, and one site in New Brunswick, where both mussel species were identified using molecular techniques. Individual spat were removed from the lines and species identifications (including hybrids) were made from three depth classes (1, 3, 5 m below surface).

METHODOLOGY: An internally transcribed spacer (ITS) region of the nuclear rRNA gene array is amplified and digested with *Hha* I to reveal diagnostic banding patterns with agarose gel electrophoresis.

STATUS: Samples from one location have been fully identified and preliminary observations of the data suggest a strong depth preference bias in the field between these two species. A difference in the proportions of spat of the two species over time was also noted. The identification of spat from the second location is underway and we plan to repeat the field collection at one site again in 1999 in order to evaluate the consistency of these results.

Study 39

LABORATORY/RESEARCHER: Biology Dept., Dalhousie University, Halifax, Nova Scotia and Dept. Fisheries and Oceans, Bedford Institute of Oceanography, Dartmouth, N.S. / E. Kenchington (project leader) in collaboration with E. Zouros, L. Cao, Y. Shi, A. Glass and M. Cox.

SPECIES: Blue mussel (*Mytilus edulis*), *M. trossulus.*

PROJECT FUNDING: Natural Sciences and Engineering Research Council of Canada.

OBJECTIVE: To determine the role of mtDNA and its unique inheritance pattern (DUI) in sex determination and sex ratio.

METHODOLOGY: The fate of sperm mitochondria in developing embryos of known pedigree (wrt sex ratio) is tracked using fluorescent markers and confocal microscopy. Various hypotheses concerning the replication advantages of different mitotypes present in these species are being addressed using immunological and molecular techniques.

STATUS: Ongoing.

Study 40

LABORATORY/RESEARCHER: Institute of Marine Biology of Crete, Crete, Greece / E. Zouros. Dept. Fisheries & Oceans, Dartmouth, N.S. / E. Kenchington. NRC Institute for Marine Biosciences, Halifax / C. Bird.

SPECIES: *Placopecten magellanicus* (sea scallop).

PROJECT FUNDING: Natural Sciences and Engineering Research Council (NSERC) of Canada; DFO core funds, NRC core funds.

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: A publication on the microsatellite markers appeared in the Journal of Shellfish Research (December 1997). Final year.

Study 41

LABORATORY/RESEARCHER: Dept. Biology, Dalhousie University, Halifax / E. Zouros (project leader), L. Cao, Y. Shi. DFO, Dartmouth, N.S. / E. Kenchington.

SPECIES: Blue mussel (*Mytilus edulis*), *Mytilus trossulus* (*Placopecten magellanicus*).

PROJECT FUNDING: Natural Sciences and Engineering Research Council (NSERC) of Canada; DFO core funding.

OBJECTIVE: Genetics of natural and contained populations (stock discrimination, population structure, hybridisation and dispersal-genetic improvement of stocks used in aquaculture).

METHODOLOGY: Molecular markers of nuclear and mitochondrial DNA, induction of triploidy, sex manipulation.

STATUS: Ongoing. A large number of publications in primary research journals detailing mtDNA inheritance and sex determination in mussels, molecular genetics of natural populations of scallops and significance of enzyme variation for growth and viability in oysters and scallops.

Study 42
LABORATORY/RESEARCHER: Dept. of Biochemistry, Memorial University, St. John's, Newfoundland / C. M^{c}Gowan and W. Davidson.
SPECIES: Brown trout and Atlantic salmon.
PROJECT FUNDING: NSERC.
OBJECTIVE: Genome mapping of Salmo species.
DESIGN: Hybrid families have been produced and segregation of alleles at different loci is being examined.
METHODOLOGY: Genetic markers being examined include: RAPD, microsatellites and expressed sequence tags (cDNA's).
STATUS: Six linkage groups have been identified to date for brown trout and five for Atlantic salmon This is an ongoing project.

Study 43
LABORATORY/RESEARCHER: Dept. of Biology, Memorial University / S. Carr. Dept. of Biochemistry, Memorial University / W. Davidson. Department of Fisheries and Oceans, St. John's, Newfoundland / R. Bowering.
SPECIES: Greenland halibut (turbot).
PROJECT FUNDING: CCFI.
OBJECTIVE: Population structure of Greenland halibut in the North Atlantic.
DESIGN: 40 turbot from 7 sampling sites across the North Atlantic were examined for genetic variation within and between samples.
METHODOLOGY: Sequence analysis of a 400 bp region of the cytochrome b mitochondrial DNA was examined.
STATUS: Completed. No evidence for genetic substructuring of turbot in the North Atlantic from as far apart as Norway and the Gulf of St. Lawrence. (Published in *CJFAS* Vis *et al.* 1997).

Study 44
LABORATORY/RESEARCHER: Dept. of Biology, Memorial University, St. John's, Newfoundland / S. Carr.
SPECIES: Atlantic cod.
PROJECT FUNDING: NSERC.
OBJECTIVE: Population structure of Atlantic cod.
DESIGN: Many samples of cod from all over the North Atlantic have been examined for evidence of population structuring.
METHODOLOGY: Mitochondrial DNA and microsatellites.
STATUS: Ongoing project.

Study 45
LABORATORY/RESEARCHER: Stocks Assessment and Genetics Unit, Ontario Ministry of Natural Resources, Maple / P.E. Ihssen and G.Wm. Martin.
SPECIES: Atlantic salmon, aurora trout, brook trout, brown trout, Chinook salmon, trout, lake whitefish, coho salmon and rainbow trout.
PROJECT FUNDING: Ontario Ministry of Natural Resources (OMNR).
OBJECTIVE: Monitoring of OMNR hatchery stocks for maintenance of genetic variability.
DESIGN: Successive year classes of hatchery-reared fish of the above species are monitored for approximately 50 allozyme loci. In cases where the phenotypes of the original parents were determined, comparison is made with succeeding year classes to determine if there has been a loss of genetic variability. In the case of Atlantic salmon, Chinook salmon and lake whitefish, gametes are collected from wild fish. For all other species, gametes are collected from hatchery brood stock.
METHODOLOGY: Starch gel and cellulose acetate electrophoresis of cathodal and general muscle protein and 23 allozyme systems.
STATUS: Ongoing.

Study 46
LABORATORY/RESEARCHER: Dept. of Biochemistry, Memorial University, St. John's, Newfoundland / J. Johansen and W.S. Davidson.
SPECIES: Arctic charr.
PROJECT FUNDING: NSERC.
OBJECTIVE: Marker assisted selection of broodstock.
DESIGN: Families have been produced and association genetics is being carried out to relate microsatellite alleles with growth.
METHODOLOGY: Genetic markers being examined are microsatellites.
STATUS: In progress.

Study 47
LABORATORY/RESEARCHER: Biology Department and Ocean Science Centre, Memorial University of Newfoundland, St. John's, Nfld. / D. Innes (project leader), Raymond, J. Thompson and J.E. Toro (Ph.D. student).
SPECIES: *Mytilus edulis*, *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

Study 48
LABORATORY/RESEARCHER: Memorial University of Newfoundland / C. McGowan and W.S. Davidson.
SPECIES: Atlantic salmon and brown trout.
PROJECT FUNDING: Natural Sciences and Engineering Research Council of Canada.
OBJECTIVE: To develop a method to distinguish normal XY males from sex-reversed XX males.
DESIGN: Screening a library of primers for any that show differences in DNA fragment sizes after PCR amplification.
METHODOLOGY: RAPD technique - randomly amplified polymorphic DNA based on screening a library of oligonucleotide primers (each 10 base pair long) on DNA purified from male and female Atlantic salmon and brown trout.
STATUS: Ongoing.
COMMENTS: The RAPD technique has been used successfully to determine the sex of birds and plants. 300 to 400 markers were tested on Arctic charr and brook trout but no sex specific Marker was found.

Study 49
LABORATORY/RESEARCHER: Dept. Fisheries and Oceans, Aquaculture Division, Gulf Fisheries Centre, Moncton / T. Landry (project leader) and T.W. Sephton.
SPECIES: *Mercenaria mercenaria* (Bay quahaug), local wild species and aquaculture "notata" variety.
PROJECT FUNDING: Can/NB/PEI Cooperation Agreement for Alternative Species Research.
OBJECTIVE: Evaluate the growth, survival and production of seedstock from two sources of broodstock: 1) F three broodstock from PEI, and 2) "notata" variety broodstock.
DESIGN: Two source of seed are being compared in side by side replicated field trials at three locations in the southern Gulf of St. Lawrence for a two year growth experiment.
STATUS: Second and final year.

Study 50
LABORATORY/RESEARCHER: Department of Anatomy and Cell Biology, University of Saskatchewan, Health Sciences Building, Saskatoon / P. Krone.
SPECIES: Zebrafish (*Danio rerio*).
PROJECT FUNDING: NSERC.
OBJECTIVE: Regulation and role of heat shock proteins (hsps) during normal embryonic development. Regulation and role of heat shock proteins in following exposure to environmental stress.
DESIGN: Embryos at different stages of embryonic development are treated with the environmental stressors (heat shock, etc.) and the corresponding morphological and molecular changes are assessed. Overexpression of hsps and dominant negative forms hsps and examination of subsequent effects on development as above. Pharmacological inhibition of hsp function and examination of subsequent effects on development as above.
METHODOLOGY: Recombinant DNA techniques (cDNA cloning, etc; whole mount in situ hybridisation for the examination of tissue specific patterns of gene expression; Northern and Southern blot analysis; microscopy (stereo, compound and compound w/ DIC).
STATUS: Project ongoing.

Study 51
LABORATORY/RESEARCHER: Department of Anatomy and Cell Biology, University of Saskatchewan, Health Sciences Building, Saskatoon, Saskatchewan / P. Krone.
SPECIES: Zebrafish (*Danio rerio*).
PROJECT FUNDING: Canadian Network of Toxicology Centres.
OBJECTIVE: Assessment of molecular and cellular effects of endocrine-disrupting compounds on embryonic development.

DESIGN: Treatment of embryos with putative endocrine disrupting compounds and examination of subsequent (morphological and molecular) impact on development.
METHODOLOGY: Recombinant DNA techniques (cDNA cloning, etc; whole mount in situ hybridisation for the examination of tissue specific patterns of gene expression; Northern and Southern blot analysis; microscopy (stereo, compound and compound w/ DIC).
STATUS: Project ongoing.

Study 52
LABORATORY/RESEARCHER: Simon Fraser University, Dept. of Biological Sciences, Burnaby / B. McKeown and S. Tang.
SPECIES: Rainbow trout.
PROJECT FUNDING: NSERC funded.
OBJECTIVE: To characterise the SPARC (secreted protein acidic and rich in cystine) and PLP (proteolipid protein) genes.
DESIGN: Gene cloning and controls of expression.
METHODOLOGY: Gene cloning.
STATUS: Project ongoing.
COMMENTS: These genes have been identified and sequenced. Expressions in various tissues and conditions have been found.

Study 53
LABORATORY/RESEARCHER: Simon Fraser University, Dept. of Biological Sciences, Burnaby / 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: Project ongoing.
COMMENTS: This gene has been isolated and sequenced. Work is now continuing on controls of expression.

Study 54
LABORATORY/RESEARCHER: Simon Fraser University, Dept. of Biological Sciences, Burnaby / B. McKeown and K. Poon.
SPECIES: Rainbow trout.
PROJECT FUNDING: NSERC funded.
OBJECTIVE: To identify the growth hormone receptor gene.
METHODOLOGY: Gene cloning.
STATUS: Project ongoing.
COMMENTS: We are presently in the process of trying to clone this gene.

Study 55
LABORATORY/RESEARCHER: Zoology Dept., University of Guelph, Guelph, Ontario / J.S. Ballantyne (project leader) with P.D.N. Hebert, E. Boulding and P. Wright.
SPECIES: Arctic charr.
PROJECT FUNDING: NSERC Strategic Grant.
OBJECTIVE: Enhancement of Arctic charr aquaculture in Canada.
STATUS: Project ongoing.

Study 56
LABORATORY/RESEARCHER: Sciences and Technology Dept., Laval University, Quebec / J. de la Noue (project leader) with S.L. Scott.
PROJECT FUNDING: NSERC Strategic Grant.
OBJECTIVE: Enhanced oral delivery of microbial phytase.
METHODOLOGY: Using novel pH-sensitive polymers to improve fish growth performance and reduce phosphorus discharge from aquaculture production.
STATUS: Project ongoing.

Study 57
LABORATORY/RESEARCHER: Oceanography Dept., University of Quebec, Rimouski / H.I. Browman (project leader).

PROJECT FUNDING: NSERC Research Grant.
SPECIES: Cod (*Gadus morhua*).
OBJECTIVE: Effects of solar ultraviolet radiation, maternal condition, quality and temperature on survivorship, growth and feeding performance of cod larvae.
STATUS: Project ongoing.

Study 58
LABORATORY/RESEARCHER: Biology Dept., University of Ottawa, Ottawa, Ontario / F. Chapleau (project leader).
PROJECT FUNDING: NSERC Research Grant.
SPECIES: Flatfish.
OBJECTIVE: Phylogeny and the evolution of life history traits in flatfishes.
STATUS: Project ongoing.

Study 59
LABORATORY/RESEARCHER: Zoology Dept., University of Guelph, Guelph, Ontario / R.G. Danzmann (project leader).
PROJECT FUNDING: NSERC Research Grant.
SPECIES: Salmonids.
OBJECTIVE: Genetics of development, fitness and life-history variability in salmonid fishes.
STATUS: Project ongoing.

Study 60
LABORATORY/RESEARCHER: Zoology Dept., University of Toronto, Toronto, Ontario / H.H. Harvey (project leader).
PROJECT FUNDING: NSERC Research Grant.
SPECIES: White sucker (*Catostomus commersoni*).
OBJECTIVE: Phenotypic plasticity and genetic polymorphism in the white sucker.
STATUS: Project ongoing.

Study 61
LABORATORY/RESEARCHER: Zoology Dept., University of British Columbia, Vancouver, B.C. / J.D. McPhail (project leader).
PROJECT FUNDING: NSERC Research Grant.
SPECIES: Sticklebacks and charr.
OBJECTIVE: Hybridisation, natural selection and genetic divergence in sticklebacks and charr.
STATUS: Project ongoing.

Study 62
LABORATORY/RESEARCHER: Faculty of Medicine, University of Ottawa, Ottawa, Ontario / M. Ekker (project leader).
PROJECT FUNDING: NSERC Research Grant.
SPECIES: Zebrafish (*Danio rerio*).
OBJECTIVE: Functional analysis of dlx homeoproteins in transgenic zebrafish embryos.
STATUS: Project ongoing.

Study 63
LABORATORY/RESEARCHER: Department of Biological Sciences, University of Calgary, Calgary, Alberta / L. Gedamu (project leader).
PROJECT FUNDING: NSERC Research Grant.
SPECIES: Rainbow trout.
OBJECTIVE: Rainbow trout metallothionein gene regulation.
STATUS: Project ongoing.

Study 64
LABORATORY/RESEARCHER: Zoology Department, University of Manitoba, Winnipeg, Manitoba / R.A. McGowan (project leader).
PROJECT FUNDING: NSERC Research Grant.
SPECIES: Zebrafish (*Danio rerio*).
OBJECTIVE: Dominance modification and genome imprinting in zebrafish; to investigate the role of methylation and the DNA methyltrasferase gene in zebrafish development.

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

Study 65
LABORATORY/RESEARCHER: Department of Biology, University of New Brunswick / T. J. Benfey (project leader).
PROJECT FUNDING: NSERC Strategic Grant, Department of Fisheries & Oceans, NRC Institute of Marine Biosciences, Marine Mariculture Inc., R&R Finfish Developments Ltd., Stolt SeaFarm Inc.
SPECIES: Halibut.
OBJECTIVE: Development of all-female halibut stocks for aquaculture.
METHODOLOGY: Genetic and hormonal manipulations used successfully to develop all-female stocks of salmon and trout for aquaculture will be tested for halibut.
STATUS: Project ongoing.

Study 66
LABORATORY/RESEARCHER: Dept. Fisheries and Oceans, Aquaculture Division, Gulf Fisheries Centre, Moncton, N.B / Mr. T. Landry (project leader), R. Tremblay and B. Gillis.
SPECIES: *Mytilus edulis* (Mussel), PEI wild and cultured stocks.
PROJECT FUNDING: DFO and PEI AFRI.
OBJECTIVE: Evaluate the qualitative contribution of the wild stock versus the cultured stocks and the interaction of mussel mariculture and wild mussel fisheries.
DESIGN: Four bays in PEI (sites) are being investigated. Enzyme polymorphisms are analysed with electrophoretic techniques to describe the genetic variability of wild and cultured mussels.
STATUS: Ongoing.

Study 67
LABORATORY/RESEARCHER: University of Guelph, Ontario Agricultural College, Guelph, Ontario / 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. (1) Comparison of growth, mortality, maturation rates and reproductive traits among four strains of spring-spawned rainbow trout and two management groups (1991 year class). (2) Comparison of early growth, maturation and mortality in crosses of three of four strains from (1) (1994 year class). (3) Initiation of additional crosses of three of the four strains in (1) (95/96 year class). (4) Development of computer models to compare inbreeding and rates of genetic progress under different genetic improvement strategies.
DESIGN: Characterisation of four pure strains and crosses between 95/96 year classes.
METHODOLOGY: Measurements of growth, mortality, maturation rates and reproductive traits. Development of computer models.
STATUS: On going.

Study 68
LABORATORY/RESEARCHER: Department of Fisheries and Oceans, Canada, Vancouver / T. Beacham, K. Miller, 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 correlation with MHC genotype. A BKD challenge of Chinook salmon is underway.

Study 69
LABORATORY/RESEARCHER: Dept. of Fisheries & Oceans, West Vancouver, B.C / R. Devlin.
SPECIES: Salmon.
OBJECTIVE: Production of transgenic salmon with enhanced growth and altered reproductive capability using "all-salmon" gene constructs.
STATUS: Ongoing.

Study 70
LABORATORY/RESEARCHER: Dept. of Fisheries & Oceans, West Vancouver, B.C / R. Devlin.
SPECIES: Salmon.
OBJECTIVE: Characterisation of Y-chromosomal DNA probes from salmon for use in monosex all-female culture.
STATUS: Ongoing.

Study 71
LABORATORY/RESEARCHER: Dept. of Fisheries & Oceans, West Vancouver, B.C / R. Devlin.
SPECIES: Salmon.
OBJECTIVE: Development of DNA based diagnostics for several Microsporean and Myxosporean parasites to assist with management of infection in sea-farm facilities.
STATUS: Ongoing.

Study 72
LABORATORY/RESEARCHER: Dept. of Fisheries & Oceans, West Vancouver, B.C / R. Devlin.
SPECIES: Salmon.
OBJECTIVE: Examination of the potential for hybridisation between Atlantic and Pacific salmon with regard to the possible reproductive interaction between escaped farmed Atlantic salmon and wild Pacific salmon stocks.
STATUS: Ongoing.

Study 73
LABORATORY/RESEARCHER: Dept. of Fisheries & Oceans, West Vancouver, B.C / R. Devlin.
SPECIES: Chinook Salmon.
OBJECTIVE: Development of a RAPD linkage map for Chinook salmon.
STATUS: Ongoing.

Study 74
LABORATORY/RESEARCHER: Dept. of Fisheries & Oceans, West Vancouver, B.C / R. Devlin.
SPECIES: Salmon.
OBJECTIVE: Development of a sensitive PCR-based assay for CYPIA 1 gene expression to evaluate the biological effects of xenobiotic exposure.
STATUS: Ongoing.

Study 75
LABORATORY/RESEARCHER: Applied Breeding Technology, St. Andrew's, New Brunswick / Dr. J. Bailey (project leader).
SPECIES: Atlantic salmon (*Salmo salar*).
FUNDING: Atlantic Canada Opportunities Agency, Canadian Institute of Biotechnology, Department of Fisheries and Oceans, National Research Council, New Brunswick Department of Fisheries and Aquaculture, New Brunswick Salmon Growers Association.
OBJECTIVE: To establish four Atlantic salmon strains for aquaculture.
DESIGN: Growth and developmental traits are monitored in both fresh and sea water for each year class of each strain. Selection is carried out when the fish have spent 18 months in sea water and the broodstock population is reduced from approximately 5000 to 800. Spawning takes place the following year with a population of approximately 400 fish.
METHODOLOGY: Selection is based on an index to increase percent 1+ smolts, percent non-grilse, M.et length and resistance to bacterial kidney disease. In one of the strains, selection was based on truncated mass selection for market length.
STATUS: Ongoing.
COMMENTS: Substantial genetic gains of significant economic value to salmon farmers have been made.

Study 76
LABORATORY/RESEARCHER: Salmon Genetics Research Program, Atlantic Salmon Federation, St. Andrews, New Brunswick / G. Friars, J. Bailey and F. O'Flynn. University of New Brunswick / T. Benfey and A. McGeachy.
SPECIES: Atlantic salmon (*Salmo salar*).

PROJECT FUNDING: Atlantic Canada Opportunities Agency, Canadian Institute of Biotechnology, Department of Fisheries and Oceans, National Research Council, New Brunswick Department of Fisheries and Aquaculture, New Brunswick Salmon Growers Association.
OBJECTIVE: To compare the aquacultural performance of diploid and triploid Atlantic salmon.
DESIGN: Mixed-sex triploid groups of Atlantic salmon were made in all SGRP aquaculture strains. All-female triploid groups were made in two SGRP strains and have all-female diploid contemporaries. Growth and survival is being monitored in both fresh and sea water.
METHODOLOGY: A 2.7 litre pressure vessel was used to produce triploid salmon. Fertilisation with mono-milt produced all-female groups. At the parr stage blood samples were taken to test the ploidy level of the fish by flow cytometry.
STATUS: Completed.

Study 77
LABORATORY/RESEARCHER: Salmon Genetics Research Program, Atlantic Salmon Federation, St. Andrews, New Brunswick / G. Friars, J. Bailey and F. O'Flynn. Research and Productivity Council / S. Griffiths.
SPECIES: Atlantic salmon (*Salmo salar*).
PROJECT FUNDING: Atlantic Canada Opportunities Agency, Canadian Institute of Biotechnology, Department of Fisheries and Oceans, National Research Council, New Brunswick Department of Fisheries and Aquaculture, New Brunswick Salmon Growers Association.
OBJECTIVE: To investigate genetic variation in resistance to Bacterial Kidney Disease (BKD).
DESIGN: Samples of parr and smolt from three SGRP strains were challenged with *Renibacterium salmonimum.*
METHODOLOGY: Heritability values were estimated, based on full-sib families, for survival and time to death.
STATUS: Project Completed.
COMMENTS: The information obtained from this study was used to include resistance to BKD as an index trait in the selection of broodstock.

Study 78
LABORATORY/RESEARCHER: Institute of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby / B. P. Brandhorst, G. Corley-Smith and J. Chinten Lim.
SPECIES: *Danio rerio* (zebrafish).
PROJECT FUNDING: NSERC.
OBJECTIVE: The production of diploid androgenetic fish and their use as a genetic tool.
DESIGN: The female genome is eliminated by X-ray irradiation, and the first cleavage is inhibited by heat shock. Polymorphic DNA markers are used to assess transmission from the female and male parents.
METHODOLOGY: DNA markers.
STATUS: Numerous diploid androgenotes have been produced with a success rate of 1-2 %. These have a normal appearance and have been bred. A manuscript has been submitted. Haploid androgenotes have been produced with an efficiency of up 30-50 %. This should allow their use in haploid genetic mutational screens, and production of a male meiotic cross-over map in collaboration with J. Postlethwait (U. Oregon) is near completion. Currently, the focus is on improving the efficiency of production of androgenotes and assessing the sex of androgenotes and their progeny, which may be informative about sex determination, another interest of the laboratory.
COMMENTS: The extensive DNA Marker data provides compelling evidence for the production of androgenotes with little or no leakage of maternal genes. The methods may be adaptable to other fish. A paper on this work was published in *Genetics* **142** (1996):1265-1276.

Study 79
LABORATORY/RESEARCHER: Institute of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby / B. P. Brandhorst, G. Corley-Smith and J. Chinten Lim.
SPECIES: *Oncorhynchus nerka* (sockeye salmon).
PROJECT FUNDING: None at present.
OBJECTIVE: Development of a method for the rapid identification of stock specific DNA markers.
DESIGN: Random amplified polymorphic DNA (RAPD) analysis using fluorescent primers is being applied to bulked DNA samples of spawning sockeye salmon from adjacent and distant geographic regions, in an effort to establish the efficacy of a method for quickly identifying stock specific markers. Sequence analysis of distinctive amplification products, if any, should result in the production of highly specific PCR primers allowing for rapid DNA typing on small amounts of material.
METHODOLOGY: RAPD analysis using fluorescent primers and an ABI automated DNA sequencer, running GeneScan software.
STATUS: The sensitivity and reliability of RAPD analysis is considerably improved with the use of fluorescent primers and high resolution polyacrylamide gel electrophoresis. Application to identification of stock specific DNA markers is just beginning.
COMMENTS: This is a proof of concept project, not part of a planned long term program.

Study 80
LABORATORY/RESEARCHER: Departments of Clinical Biochemistry and Biochemistry, University of Toronto / C.L. Hew.
SPECIES: Winter flounder (*Pleuronectes americanus*), ocean pout (*Macrozoarces americanus*).
PROJECT FUNDING: Medical Research Council of Canada.
OBJECTIVE: To investigate the molecular mechanisms controlling the seasonal and hormonal regulated synthesis of fish antifreeze proteins, and to explore the use of antifreeze protein genes in conferring freeze resistance to other fish species.
DESIGN: These include gene cloning, promoter analysis in tissue culture cells, characterisation of transcription factors, and the development of transgenic fish.
METHODOLOGY: Gene cloning, transcripted factors and transgenics.
STATUS: We have demonstrated that the winter flounder contains both extracellular and intracellular AFPs. These have raised further questions on the structure and function, regulation and evolution of AFPs (Gong *et al.*, 1996. Skin antifreeze protein genes of the winter flounder, *Pleuronectes americanus*, encode distinct and active polypeptides without the secretory signal sequences. *J. Biol. Chem.* In Press).

Study 81
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, characterisation 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 82
LABORATORY/RESEARCHER: Departments of Clinical Biochemistry and Biochemistry, University of Toronto / C.L. Hew.
SPECIES: Atlantic salmon (*Salmo salar*).
PROJECT FUNDING: Natural Sciences and Engineering Research Council of Canada.
OBJECTIVE: The objective is the development of transgenic salmon beneficial to aquaculture. these include: (i) the transfer of antifreeze protein gene (AFP) for freeze resistance; (ii) the transfer of growth hormone gene (GH) for growth enhancement; and (iii) the transfer of lysozyme gene (LYZ) for disease resistance.
DESIGN: These genes (AFP, GH, LYZ) were injected separately into salmon eggs by gene transfer. The inheritance and expression of the transgene is being studied.
METHODOLOGY: Transgenetics.
STATUS: Positive transgenic fish have been accomplished for AFP and GH gene transfer. GH transgenic fish grow 5 to 10 times faster than the control and the inheritance of transgenes to F2 generation is established (See Gong and Hew 1995), Transgenic fish in aquaculture and developmental biology. Current Topics in *Developmental Biology* **30**:177-214.

Study 83
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 84
LABORATORY/RESEARCHER: Stocks Assessment and Genetics Unit, Ontario Ministry of Natural Resources, Maple / P.E. Ihssen and G.Wm. Martin.
SPECIES: Atlantic salmon, aurora trout, brook trout, brown trout, Chinook salmon, trout, lake whitefish, coho salmon and rainbow trout.
PROJECT FUNDING: Ontario Ministry of Natural Resources (OMNR).
OBJECTIVE: Monitoring of OMNR hatchery stocks for maintenance of genetic variability.
DESIGN: Successive year classes of hatchery-reared fish of the above species are monitored for approximately 50 allozyme loci. In cases where the phenotypes of the original parents were determined, comparison is made with succeeding year classes to determine if there has been a loss of genetic variability. In the case of Atlantic salmon, Chinook salmon and lake whitefish, gametes are collected from wild fish. For all other species, gametes are collected from hatchery brood stock.
METHODOLOGY: Starch gel and cellulose acetate electrophoresis of cathodal and general muscle protein and 23 allozyme systems.
STATUS: Ongoing.

DENMARK

Study 1
LABORATORY/RESEARCHER: Danish Institute of Agricultural Sciences / L.-E. Holm. Collaborators: B. Høyheim, Norway (Coordinator); J. Taggart, Scotland; R. Powell, Ireland; R. Guyomard, France; R. Danzmann, Canada.
SPECIES: Rainbow trout.
PROJECT FUNDING: In house / EU (SALMAP).
OBJECTIVE: Construction of genetic maps in rainbow trout, brown trout and Atlantic salmon. Generation of comparative genetic maps. Maps will be primarily based on microsatellites.
DESIGN: Resource families shared between laboratories. Generation of genetic markers, primarily microsatellites, and typing of these in the families.
METHODOLOGY: Microsatellites.
STATUS: Ongoing (1997-1999).

Study 2
LABORATORY/RESEARCHER: Danish Institute of Agricultural Sciences / C. Bendixen and L.-E. Holm Collaborators: R. Powell, Ireland (Coordinator); J. Taggart, Scotland; B. Høyheim, Norway.
SPECIES: Atlantic salmon.
PROJECT FUNDING: In house / EU (SALGENE).
OBJECTIVE: Generation of expressed sequence tags (ESTs) from Atlantic salmon. Analysis of tissue specific expression of selected genes.
DESIGN: Sequencing of clones from tissue specific cDNA libraries.
METHODOLOGY: Construction of cDNA libraries from different tissues from Atlantic salmon. Sequencing of these and comparison with known sequences.
STATUS: Ongoing (1999-2001).

Study 3
LABORATORY/RESEARCHER: Danish Institute for Fisheries Research, Dept. of Inland Fisheries, Silkeborg / E.E. Nielsen.
SPECIES: Atlantic salmon.
PROJECT FUNDING: In house.
OBJECTIVE: Studies of long-term temporal changes in allele frequencies at loci that are possibly affected by selection.
DESIGN: Geographically distinct populations are analysed. Variation is analysed on a temporal scale by amplifying DNA from old scale samples.
METHODOLOGY: ScnDNA.
STATUS: Ongoing. Started 1998, due to end by 2000.

Study 4
LABORATORY/RESEARCHER: Danish Institute for Fisheries Research, Dept. of Inland Fisheries, Silkeborg / M.M. Hansen. University of Aarhus / S. Østergaard and V. Loeschcke.
SPECIES: Brown trout.
PROJECT FUNDING: In house.
OBJECTIVE: Estimation of genetic variability and differentiation in and among Danish brown trout populations and

hatchery strains. Analysis of metapopulation dynamics (extinction-recolonisation).
DESIGN: Sampling of trout from various localities.
METHODOLOGY: Microsatellites (from tissue and old scale samples), mtDNA.
STATUS: Ongoing.

Study 5
LABORATORY/RESEARCHER: Danish Institute for Fisheries Research, Dept. of Inland Fisheries, Silkeborg / M.M. Hansen, D.E. Ruzzante and E.E. Nielsen.
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 6
LABORATORY/RESEARCHER: Danish Institute for Fisheries Research, Dept. of Inland Fisheries, Silkeborg / M.M. Hansen. Pike microsatellites developed by J. Taggart, University of Stirling, U.K.
SPECIES: *Coregonus lavaretus, C. "oxyrhynchus", Esox lucius.*
PROJECT FUNDING: In house.
OBJECTIVE: Estimation of phylogeographic patterns and genetic differentiation.
DESIGN: Screening of samples from geographically distinct and morphologically divergent populations.
METHODOLOGY: Microsatellites and mtDNA. New tetranucleotide microsatellites have been developed for pike.
STATUS: Ongoing.

Study 7
LABORATORY/RESEARCHER: Danish Institute for Fisheries Research, Dept. of Inland Fisheries, Silkeborg / E.E. Nielsen and M.M. Hansen. Several collaborators from the Danish Institute for Fisheries Research, Dept. of Marine Fisheries, Copenhagen. University of Aarhus / P. Grønkjær and V. Loeschcke.
SPECIES: Cod.
PROJECT FUNDING: The Danish Ministry of Agriculture and Fisheries.
OBJECTIVE: Studies of the genetic population structure of cod in the South-eastern part of Kattegat, the Danish Belt Sea and in the Baltic Sea area. Estimation of the possible drift of juvenile cod into the Baltic Sea and the contribution of Belt Sea cod to the fishery in the Baltic Sea area.
DESIGN: Sampling of cod larvae and adult spawners from various localities.
METHODOLOGY: Microsatellites and other molecular markers.
STATUS: Started 1998, due to end by 2000.

Study 8
LABORATORY/RESEARCHER: Danish Institute for Fisheries Research, Dept. of Inland Fisheries, Silkeborg / M.M. Hansen (coordinator) + 22 other participants from laboratories in Europe and Canada.
SPECIES: Brown trout.
PROJECT FUNDING: EU FAIR.
OBJECTIVE: Concerted Action on brown trout population genetics (TROUTCONCERT). The objectives are to promote collaboration among laboratories that are active in research on population genetics of brown trout, to harmonise the use of genetic markers, to give recommendations for a European strategy for management and conservation of the species, and to establish databases on relevant literature, available genetic markers and data from published and unpublished studies. The databases are made publicly accessible on the World Wide Web (www.qub.ac.uk/bb/prodohl/TroutConcert/TroutConcert.htm).
DESIGN: Concerted action, i.e. network among laboratories.
METHODOLOGY: Workshops, exchange visits among laboratories, common databases and WWW facilities.
STATUS: Two-year project (1998-1999).

Study 9
LABORATORY/RESEARCHER: Danish Institute for Fisheries Research, Dept. of Inland Fisheries, Silkeborg / D. Ruzzante,
SPECIES: Brown trout.
PROJECT FUNDING: The Danish Ministry of Agriculture and Fisheries.
OBJECTIVE: Mixed-Stock analysis of anadromous brown trout in the Limfjord, Denmark.

DESIGN: Collection of reference samples from tributaries and from mixed aggregations in the Limfjord.
METHODOLOGY: Microsatellites
STATUS: Started 1998, due to end by 2000.
Recent Danish publications:
Hansen, M.M. & Mensberg, K.-L.D. (1998). Genetic differentiation and relationship between genetic and geographical distance in Danish sea trout (*Salmo trutta* L.) populations. *Heredity,* 81, 493-504.
Hansen, M.M., Mensberg, K.-L.D. & Berg, S. (1999). Postglacial recolonisation patterns and genetic relationships among whitefish (*Coregonus sp.*) populations in Denmark, inferred from mitochondrial DNA and microsatellite markers. *Molecular Ecology,* 8, 239-252.
Holm, L-E (1998). Omy0002DIAS: A highly polymorphic dinucleotide microsatellite in rainbow trout (Oncorhynchus mykiss). *Animal Genetics,* 29, 465.
Holm, L-E & Brusgaard, K (1999). Two polymorphic dinucleotide repeats in rainbow trout (*Oncorhynchus mykiss*). *Animal Genetics,* 30, in press.
Nielsen, E.E., Hansen, M.M. & Mensberg, K.-L.D. (1998). Improved primer sequences for the mitochondrial ND1, ND3/4 and ND5/6 segments in salmonid fishes. Application to RFLP analysis of Atlantic salmon. *Journal of Fish Biology,* 53, 216-220.
Nielsen, E.E., Hansen, M.M. & Loeschcke, V. (1999). Genetic variation in time and space: Microsatellite analysis of extinct and extant populations of Atlantic salmon. *Evolution,* 53, 261-268.
Nielsen, E.E., Hansen, M.M. & Loeschcke, V. (1999). Analysis of DNA from old scale samples. Technical aspects, applications and perspectives for conservation. *Hereditas in press.*

ESTONIA

Study 1
LABORATORY/RESEARCHERS: Dept. of Fish Farming, Institute of Animal Science, Estonian Agricultural University, Tartu / R. Gross, T. Paaver and A. Vasemägi.
SPECIES: Atlantic (Baltic) salmon and sea trout.
PROJECT FUNDING: Estonian Science Foundation, Estonian Fisheries Foundation.
OBJECTIVE: To reveal genetic differentiation and structure of natural and hatchery salmon and trout populations, estimate the influence of population size and stocking of hatchery fish on gene pools of natural populations, reveal frequency of salmon x trout hybrids in salmon rivers.
DESIGN: Samples are taken from parr, caught by electrofishing in Eastern Baltic salmon rivers or from juveniles, reared in hatcheries of Estonia, Latvia and Russia.
METHODOLOGY: PCR-amplified DNA markers (microsatellites, growth hormone genes, mtDNA genes), allozymes.
STATUS: Ongoing (1997-1999).

FINLAND

Study 1
LABORATORY/RESEARCHER: Finnish Game and Fisheries Research Institute, Helsinki./ M.-L. Koljonen, University of Helsinki. Department of Animal Science / J. Tähtinen and M. Säisä.
SPECIES: Atlantic salmon, brown trout.
PROJECT FUNDING: In house, Academy of Finland.
OBJECTIVE: Phylogeography of Atlantic salmon in the Baltic Sea. Estimate stock composition of salmon catches and proportion of wild stocks in the catches.
METHODOLOGY: Genetic stock identification (GSI), allozymes, microsatellites.
STATUS: Ongoing.

Study 2
LABORATORY/RESEARCHER: Agricultural Research Centre, Department of Animal Breeding, Jokioinen / L. Siitonen.
SPECIES: Rainbow trout.
PROJECT FUNDING: In house.
OBJECTIVE: Develop rainbow trout stocks with better growth rate.
METHODOLOGY: Selective breeding.
STATUS: Ongoing.

Study 3
LABORATORY/RESEARCHER: University of Joensuu, Department of Biology / J. Vuorinen.
SPECIES: Coregonids.
PROJECT FUNDING: In house.
OBJECTIVE: Evolution and taxonomy of Holarctic Coregonids.
DESIGN: Mapping of gene frequencies.
METHODOLOGY: Enzyme electrophoresis, mtDNA, sequencing (collaboration), SINEs (collaboration).
STATUS: Ongoing.

Study 4
LABORATORY/RESEARCHER: Department of Ecology and Systematics, University of Helsinki / C.R. Primmer and T. Aho. Finnish Game and Fisheries Research Institute / J. Piironen.
SPECIES: Arctic charr.
PROJECT FUNDING: In house, Academy of Finland, Helsinki University, FGFRI.
OBJECTIVE: Analysis of genetic variability in Finnish hatchery stocks. Comparison with wild caught fish. Stock identification. Phylogeography of Nordic populations.
METHODOLOGY: Microsatellites.
STATUS: Complete (1998-1999). There is an article in press in *Hereditas* on these topics.

Study 5
LABORATORY/RESEARCHER: Department of Ecology and Systematics, University of Helsinki / T. Aho, C.R. Primmer. Finnish Game and Fisheries Research Institute / J. Piironen.
SPECIES: Land-locked salmon.
PROJECT FUNDING: In house, Academy of Finland, Helsinki University, FGFRI.
OBJECTIVE: Analysis of genetic variability in Finnish hatchery stocks. Comparison with wild caught fish. Analysis of the effects of broodstock composition on genetic variability.
METHODOLOGY: Microsatellites.
STATUS: In progress (1998-1999).

Study 6
LABORATORY/RESEARCHER: Department of Ecology and Systematics, University of Helsinki / T. Aho, C.R. Primmer and K. Lahti. Finnish Game and Fisheries Research Institute / J. Piironen.
SPECIES: Brown trout.
PROJECT FUNDING: In house, Academy of Finland, Helsinki University, FGFRI.
OBJECTIVE: Analysis of genetic variability in Finnish hatchery stocks. Phylogeography of Nordic populations. Comparison of genetic variation in lake, river and sea forms.
METHODOLOGY: Microsatellites.
STATUS: In progress (1999-2000).

Study 7
LABORATORY/RESEARCHER: Department of Ecology and Systematics, University of Helsinki / C.R. Primmer and T. Aho. Finnish Game and Fisheries Research Institute / J. Piironen.
SPECIES: European grayling.
PROJECT FUNDING: In house, Academy of Finland, Helsinki University, FGFRI.
OBJECTIVE: Analysis of genetic variability in Finnish hatchery stocks. Phylogeography of Nordic populations.
METHODOLOGY: Microsatellites, mtDNA.
STATUS: In progress (1999-2000).

Study 8
LABORATORY/RESEARCHER: University of Oulu, Department of Biology / J. Lumme.
SPECIES: Atlantic salmon, *Gyrodactylus salaris*, thymalli, lavareti etc.
PROJECT FUNDING: Academy of Finland.
OBJECTIVE: Postglacial history of Atlantic & Baltic salmon and their parasites.
METHODOLOGY: Mitochondrial DNA, PCR, RFLP, sequencing.
STATUS: Ongoing.

Study 9
LABORATORY/RESEARCHER: University of Kuopio, Department of Applied Zoology and Veterinary Medicine / H. Mölsä, T. Pitkänen, M. Reinisalo and A. Krasnov.
SPECIES: Rainbow trout.
PROJECT FUNDING: Ministry of Agriculture and Forestry, Ministry of Interior Affairs, Academy of Finland, In house.
OBJECTIVE: Enhanced growth and metabolism of rainbow trout via gene transfer technology.
DESIGN: Microinjections and integration assays, gene expression.
METHODOLOGY: Microinjections, mRNA, RT-PCR.
STATUS: Ongoing.

Study 10
LABORATORY/RESEARCHER: University of Joensuu, Department of Biology / L. Kuusipalo.
SPECIES: *Salmo trutta*, Ciclids, clupeids and Nile perch in lakes Malawi and Tanganyika.
PROJECT FUNDING: FAO, Culture Fund of Finland, Women's Science Foundation, The Foundation of Research of Natural Resources in Finland.
OBJECTIVE: Speciation, stock identification.
METHODOLOGY: Allozymes and RAPD, microsatellites.
STATUS: Ongoing.

FRANCE

Study 1
LABORATORY/RESEARCHERS: Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER) Palavas les Flots, France / E. Saillant, B. Chatain and B. Menu. Syndicat des Sélectionneurs Avicoles et Aquacoles Français (SYSAAF), Rennes / P. Haffray. Laboratoire de Physiologie des Poissons (INRA) Rennes / A. Fostier. Laboratoire de Biologie Animale de l'Université de Montpellier II, Montpellier / R. Connes. CNRS URA 1493, Laboratoire génome et populations, Montpellier / F. Bonhomme. CNRS GDR 1005, Laboratoire d'Ichtyologie, Paris / C. Ozouf-Costas.
SPECIES: *Dicentrarchus labrax*.
PROJECT FUNDING: EC FAIR.CT97.3886.
OBJECTIVE: To produce monosex female populations.
METHODOLOGY: To study environmental factors influence.
STATUS: Three year project started in 1998.

Study 2
LABORATORY/RESEARCHERS: Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER) Palavas les Flots / B. Chatain, H. Chavanne. Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER) La Tremblade, France / P. Boudry. Centre National pour le Recherche Scientifique (CNRS), UPR 6090, Lab. Génome et Populations, Montpellier / F. Bonhomme and C. Lemaire. Institut National de Recherche en Agriculture (INRA), Jouy en Josas, France / M. Vandeputte, M. Dupont-Nivet and R. Guyomard. University of Leuven, Leuven, Belgique / F. Volckaert. Institute of Marine Biology of Crete (IMBC), Heraklio, Grèce / G. Kotoulas, C. Bartagias and P. Divanach. University of Padova, Padova, Italie / L. Colombo, A. Libertini and T. Paternello. Institute of Oceanography and Limnology (IOLR), Eilat, Israel / W. Knibb, G. Gorshkova and S. Gorshkov. Universidade de Algarve, Faro, Portugal / R. Castilho and M.-Th. Dinis. Institute of Murcia (IEO), Murcia, Espagne / A. Garcia-Alcazar. University of Malaga,

Malaga, Espagne / M.-C. Alvarez. Institute de Aquacultura de Torre de la Sal (CSIC), Torre de la Sal, Espagne / S. Zanuy, M. Carillo, G. Martinez and A. Gomez. University of Stirling, Stirling, United Kingdom / Brendan Mac Andrew and D. Penman.
SPECIES: *Dicentrarchus labrax.*
PROJECT FUNDING: EC FAIR.CT97.3886.
OBJECTIVE: To gather the main European laboratories involved in aquaculture in a network in order to harmonise genetic strategies and research procedures to be able to propose to the E.U., after two years, a common efficient international programme devoted to comparative strain testing of a commercially important fish, the seabass, *Dicentrarchus labrax.*
METHODOLOGY: Concerted Action.
STATUS: Three year project started in 1998.

Study 3
LABORATORY/RESEARCHERS: Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER) Palavas les Flots / C. Fauvel. Laboratoire de Physiologie des Poissons (INRA) Rennes / A. Fostier. Laboratoire de Biologie de la reproduction des Poissons, Université de Bordeaux / F. Lemaine.
SPECIES: *Dicentrarchus labrax.*
PROJECT FUNDING: IFREMER.
OBJECTIVE: Mastering of controlled reproduction.
METHODOLOGY: Fine morpho-functional description of gametes and application to gamete management (hormonal stimulation, sperm preservation).
STATUS: 4 year project started in 1995.

Study 4
LABORATORY/RESEARCHERS: Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER) Palavas les Flots / J.C. Falguière, B. Chatain and S. Peruzzi.
SPECIES: *Dicentrarchus labrax.*
PROJECT FUNDING: IFREMER.
OBJECTIVE: To produce sterile fish and to evaluate their rearing performances.
METHODOLOGY: Triploidy.
STATUS: Project started in 1995.

Study 5
LABORATORY/RESEARCHERS: Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER) Palavas les Flots / B. Menu. Institut de Génétique Humaine (IGH), Montpellier / P. Berta.
SPECIES: *Dicentrarchus labrax.*
PROJECT FUNDING: IFREMER.
OBJECTIVE: Research of the Sox 9 gene.
METHODOLOGY: Triploidy.
STATUS: Project started in 1998.

Study 6
LABORATORY/RESEARCHERS: Centre Océanographique du Pacifique - IFREMER-Tahiti, Laboratoire d'Aquaculture Tropicale, Tahiti / E. Goyard and AQUACOP.
SPECIES: *Penaeus stylirostris.*
TITLE: Importation of genetic variability among inbred domesticated strains of *P. stylirostris* through refrigerated spermatophores of wild males.
PROJECT FUNDING: IFREMER.
OBJECTIVE: To increase genetic variability available for developing new genetic improvement programs.
METHODOLOGY: Refrigeration of spermatophores of wild males, rearing in quarantine, physical and molecular tagging
STATUS: Two years project started 1998.

Study 7
LABORATORY/RESEARCHERS: Centre Océanographique du Pacifique - IFREMER-Tahiti, Laboratoire d'Aquaculture Tropicale, Tahiti / E. Goyard and AQUACOP.
SPECIES: *Penaeus stylirostris.*
TITLE: Comparison between 2 genetic improvement strategies: exploitation of heterosis between inbred lines versus population improvement and control of inbreeding.
PROJECT FUNDING: IFREMER.
OBJECTIVE: To compare the efficiency of improvement schemes.

METHODOLOGY: Production of inbred lines, hybrids and selection among a population with a large genetic basis. Testing in farming conditions; control of homozygocity through microsatellite markers.
STATUS: 4 years project starting 2000.

Study 8
LABORATORY/RESEARCHERS: Centre Océanographique du Pacifique - IFREMER-Tahiti, Laboratoire d'Aquaculture Tropicale, Tahiti / E. Goyard and AQUACOP.
SPECIES: *Penaeus stylirostris.*
TITLE: Optimisation of mass selection for better growth.
PROJECT FUNDING: IFREMER.
OBJECTIVE: To produce improved lines with the best selection strategy.
METHODOLOGY: Assessment of early selection criteria, production of a control line and of a improved line.
STATUS: Project started 1994 (5th generation produced), transfer of genetic improvement to the producers starting 1999.

Study 9
LABORATORY/RESEARCHERS: Centre Océanographique du Pacifique - IFREMER-Tahiti, Laboratoire d'Aquaculture Tropicale, Tahiti / E. Goyard and AQUACOP.
SPECIES: *Penaeus stylirostris.*
TITLE: Selection of a "100 % green tail" line.
PROJECT FUNDING: IFREMER.
OBJECTIVE: To produce a line genetically marked to provide a inter-tank control.
METHODOLOGY: Family selection.
STATUS: Project started 1996.

Study 10
LABORATORY/RESEARCHERS: CNRS URA 1493, IRD, Laboratoire Génome et Populations, Sète France / C. Daguin, F. Bonhomme, S.R. Caetano, M. Ohresser, V. Rolland, E. Desmarais and Ph. Borsa.
SPECIES: *Mytilus edulis, Mytilus galloprovoncialis.*
PROJECT FUNDING: URM 16 IFREMER / LGP.
OBJECTIVE: To study *Mytilus* phylogeography, to study the M. edulis / M. galloprovincialis hybrid zone along the French coast.
METHODOLOGY: Nuclear DNA markers, DNA sequencing.
STATUS: Started in 1997.

Study 11
LABORATORY/RESEARCHERS: CNRS URA 1493, IRD, Laboratoire Génome et Populations, Sète France / G. Hoarau, R. Grandperrin and Ph. Borsa.
SPECIES: *Beryx splendens.*
PROJECT FUNDING: LGP, IRD.
OBJECTIVE: To study population genetics of *Beryx splendens* in the south-western Pacific Ocean.
METHODOLOGY: Nuclear DNA markers, DALP, PCR-SSCP.
STATUS: Started in 1988.

Study 12
LABORATORY/RESEARCHERS: CNRS URA 1493, IRD, Laboratoire Génome et Populations, Sète France / Ph. Borsa and F. Bonhomme.
SPECIES: Multi-species project.
PROJECT FUNDING: LGP, IRD, PNDBE.
OBJECTIVE: To study intra-specific zoogeography of the Mediterranean sea and the near Atlantic Ocean.
METHODOLOGY: Allozymes, ADNmt.
STATUS: Started in 1996.

Study 13
LABORATORY/RESEARCHERS: CNRS URA 1493, IRD, Laboratoire Génome et Populations, Sète France / F. Bonhomme, M. Potier, C. Perrin, S. Arnaud, V. Castric, C. Poux and Ph. Borsa
SPECIES: *Decapterus russelli, D. macrosoma.*
PROJECT FUNDING: LGP, IRD, IFREMER, PNDBE.
OBJECTIVE: To study population genetics of fished stocks of *Decapterus russelli* in the Indo-Pacific region.
METHODOLOGY: Mitochondrial DNA markers (D-loop, cytochrome *b*) PCR-SSCP, DNA sequencing.
STATUS: Started in 1997.

Study 14
LABORATORY/RESEARCHERS: CNRS URA 1493, Laboratoire Génome et Populations, Sète France / F. Bonhomme and C. Lemaire.
SPECIES: *Dicentrarchus labrax.*
PROJECT FUNDING: LGP, IFREMER.
OBJECTIVE: To study population genetics of seabass, to study genetic differentiation between open sea and costal populations.
METHODOLOGY: Microsatellite markers, DNA sequencing, gene markers.

Study 15
LABORATORY/RESEARCHERS: Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER) La Tremblade, France / A. Gérard, P. Boudry, S. Lapègue, S. Heurtebise; J.P. Baud, J.L. Martin, M. Héral, S. Bougrier, P. Garen, F. Cornette, D. Buestel, J.F. Sammain and J. Moal. Natural Environment Research Council (NERC) Plymouth Marine Laboratory / A.J.S. Hawkins and A.J. Day. Centre National de la Recherche Scientifique (CNRS) Observatoire Océanologique de Villefranche sur Mer / C. Thiriot and A. Leitao. University College Galway (UCG) Department of Zoology / N.P. Wilkins, R. Powell, J.A. Houghton, S. Hubert, G. Rafferty; 5-Institute of Marine Biology of Crete (IMBC) Genetics department / G. Kotoulas, A. Magoulas, A. Ekonomaki, S. Darivianakis and E. Zouros. Centre National de la Recherche Scientifique (CNRS) Laboraoire Génome et Populations / F. Bonhomme, J.J. Versini, F. Cornette and V. Hervouet.
SPECIES: *Crassostrea gigas.*
PROJECT FUNDING: EC FAIR PL-95-421.
OBJECTIVE: To study relationships between genetics, physiology and growth in the Pacific oyster *C. gigas.*
METHODOLOGY: Genetic markers (allozymes, microsatellites), physiology (oxygen consumption, feeding processes, protein turn-over, proteolitic activities), cytogenetics (aneuploidy, FISH).
STATUS: 5 year project started in 1996.

Study 16
LABORATORY/RESEARCHERS: Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER) La Tremblade France / E. Bédier, A. Gérard, P. Boudry, S. Lapègue, F. Blouin and N. Cochennec.
SPECIES: *Ostrea edulis.*
PROJECT FUNDING: IFREMER.
OBJECTIVE: Selective breeding for resistance to bonamiosis.
METHODOLOGY: Selective breeding, pair mating, microsatellites.
STATUS: Project started in 1985.

Study 17
LABORATORY/RESEARCHERS: Institut Français de Recherche pour l'Exploitation de la Mer (IFREMER) La Tremblade France / A. Gérard, P. Boudry and S. Lapègue. Centre National de la Recherche Scientifique (CNRS) Observatoire Océanologique de Villefranche sur Mer / C. Thiriot and A. Leitao.
SPECIES: *Crassostrea gigas.*
PROJECT FUNDING: Région Poitou-Charentes.
OBJECTIVE: To evaluate aneuploidy in *C. gigas* populations in France, to study the causes of aneuploidy.
METHODOLOGY: Cytogenetics.
STATUS: Project started in 1999.

GERMANY

Study 1
LABORATORY/RESEARCHER: Institute of Animal Husbandry and Genetics (IAG), University of Göttingen, Göttingen / G. Hörstgen-Schwark and A. Müller-Belecke.
SPECIES: *Oreochromis niloticus.*
PROJECT FUNDING: Deutsche Forschungsgemeinschaft (DFG).
OBJECTIVE: Development and performance testing of homozygous tilapia lines and their crosses.
DESIGN: Mitotic and meiotic gynogenesis, test cross diallels including separate and communal testing of genetic groups.
METHOLOGY: UV treatment of sperm, heat treatment of activated tilapia eggs.
STATUS: Ongoing project.

Study 2
LABORATORY/RESEARCHER: Institute of Animal Husbandry and Genetics (IAG), University of Göttingen, Göttingen / G. Hörstgen-Schwark and S. Huang.

SPECIES: *Brachydanio rerio.*
PROJECT FUNDING: IAG and Friedrich Naumann Stiftung.
OBJECTIVE: Development of test fish populations of defined genetic variability for ecotoxicological studies.
DESIGN: Mitotic and meiotic gynogenesis, test cross diallels between homozygous lines, performance testing under unpolluted water conditions and reference tests according to the German Chemical Control Act.
METHOLOGY: UV treatment of sperm, heat-, cold- and pressure treatments of activated zebra fish eggs for suppression of first mitosis.
STATUS: Ongoing project.

Study 3
LABORATORY/RESEARCHER: Institute of Animal Husbandry and Genetics (IAG), University of Göttingen, Göttingen / G. Hörstgen-Schwark, J.-N. Meyer. Institute of Inland Fisheries (inc.) (IIF), Groß Glienicke / H. Wedekind. Research Center for Animal Production and Technology of the Faculty of Agriculture (RCAPT), University of Göttingen / H.-J. Langholz and K. Eder.
SPECIES: *Oncorhynchus mykiss.*
PROJECT FUNDING: IAG, IFF, RCAPT.
OBJECTIVE: Comparison of growth, carcass- and meat quality of heat-shocked and tetraploid-derived triploid and diploid rainbow trout.
DESIGN: Paternal half sib families, consisting of heat-shocked and tetraploid derived triploid rainbow trout and diploid controls have been raised under the same environmental conditions till fish were slaughtered at 2.5 years (at the beginning and the end of spawning season).
METHOLOGY: Measurements and classifications of the outer product quality (growth, body proportions, carcass composition) and the inner product quality (physical-technological-, chemical- and sensorial criteria).
STATUS: Ongoing project.

Study 4
LABORATORY/RESEARCHER: Institute of Animal Husbandry and Genetics (IAG), University of Göttingen, Göttingen / J.-N. Meyer, G. Hörstgen-Schwark, I. Jenneckens and A. Müller-Belecke. Institute of Inland Fisheries (inc.) (IIF), 14476 Groß Glienicke / H. Wedekind.
SPECIES: *Acipenser* spp., *Oreochromis* spp.
PROJECT FUNDING: DFG.
OBJECTIVE: Identification of species, populations (lines) within species and hybrids between species by the use of gene markers.
DESIGN: Collection of adequate samples from different origins
METHOLOGY: Enzyme electrophoresis and DNA analyses (multilocus DNA fingerprinting, RAPD, SSRa-PCR, AFLP).
STATUS: Ongoing project.

STUDY 5
LABORATORY/RESEARCHER: Institute of Fishbiology and Institute of animal breeding and genetics, Technical University of Munich –Weihenstephan / M Baars and O. Rottmann.
SPECIES: *Thymallus thymallus.*
PROJECT FUNDING: Landesfischereiverb and Bayerne.V.
OBJECTIVE: In an ecological work on grayling differences in growth rate and maximal growth were found in Bavarian grayling populations. These differences are to be correlated to DNA polymorphism.
DESIGN: Populations from three Bavarian river-systems will be sample and analysed.
METHODOLOGY: DNA analyses.
STATUS: Ongoing project.

Study 6
LABORATORY/RESEARCHER: Bundesforschungsanstalt fhr Fischerei, Institut for Fisheries Ecology / J. Trautner. University of Hamburg, Institut for Hydrobiology and Fisheries Research / W. Nellen.
SPECIES: *Oncorhynchus mykiss, Zoarces viviparus* and *Limanda limanda.*
PROJECT FUNDING: Ministry of Agriculture.
OBJECTIVE: Population structure of wild populations and hatchery strains of *O. mykiss,* wild populations of *Z. viviparus* and *Limanda limanda.* Estimation of intraspecific biodiversity.
DESIGN: *O. mykiss* species have been sampled from hatcheries and Canadian lakes and Rivers and *Z. viviparus and L. limanda* from the North and Baltic Sea. DNA analyses are performed.
METHODOLOGY: RFLP-, RAPD- AFLP- and mtDNA -analyses.
STATUS: Ongoing project.

Study 7
LABORATORY/RESEARCHER: Northrhine-Westfalian Agency for Ecology, Land and Forestry / Northrhine-Westfalian Office for Agriculture Development in Recklinghausen (LÖBF NRW) / J. Lehmann and F.-J. Stürenberg.
SPECIES: *Salmo salar* and *Salmo trutta trutta.*
PROJECT FUNDING: Land Northrhine-Westfalia/NRW.
OJECTIVE: Genetic identification and characterisation of wild Atlantic salmon and *Salmo trutta trutta* in the Rhenanian drainage and Weser system of NRW. Eyed eggs and fingerlings from eight wild populations used for reintroduction for the Rhine were reared up to analyses at LÖBF.
METHODOLOGY: Enzyme electrophoresis (allozyme genotyping) and flow-catofluorometic determination of relative DNA contents of cell nuclei (relative genome sizes).
STATUS: Ongoing project.

Study 8
LABORATORY/RESEARCHER: Institute of Freshwater Ecology and Inland Fisheries (IGB), Depart. of Fish Culture and Fish Pathology, Berlin / K. Kohlmann. University of South Bohemia, Research Institute of Fish Culture and Hydrobiology, Vodnany, Czech Republic / M. Flajšhans. Academy of Sciences of Czech Republic, Institute of Animal Physiology and Genetics, Department of Genetics, Libechov, Czech Republic / V. Šlechtova, V. Šlechta.
PROJECT FUNDING: IGB (German part) and Ministry of Agriculture (Czech part).
SPECIES: *Tinca tinca.*
OBJECTIVE: Genetic characterisation of wild and cultured populations; genetic improvement of cultured strains.
DESIGN: Tench from wild and cultured populations were collected in Germany and Czech Republic. The examination of growth rate, food conversion efficiency, survival and product quality in the different populations will be accompanied by investigations on their genetic structure.
METHODOLOGY: Enzyme electrophoresis is performed, DNA analysis will be introduced. Performance tests are carried out under warm water conditions in a closed recirculating system.
STATUS: Ongoing project.

Study 9
LABORATORY/RESEARCHER: Institute of Freshwater Ecology and Inland Fisheries (IGB), Depart. of Fish Culture and Fish Pathology, Berlin / K. Kohlmann. Inland Fisheries Institute Olsztyn, Department of Salmonid Research Rutki, Zukowo, Poland / S. Dobosz and K. Goryczko.
PROJECT FUNDING: IGB (German part) and State Committee for Scientific Research Poland (Polish part).
SPECIES: *Oncorhynchus mykiss.*
OBJECTIVE: Genetic improvement of rainbow trout performance.
DESIGN: Family selection based on mixed half and full sib families. Heritability estimates for body weight and length have been calculated. Influence of parental body weight on progeny performance has been studied. Effectiveness of family selection will be evaluated by comparing the actual selected generation with the unselected control population.
METHODOLOGY: Performance tests (separate incubation of eggs and rearing of fry until tagging as one summer old fingerlings, communal rearing later on) with control measurements of growth (at half year intervals) and sexual maturation.
STATUS: Ongoing project.

Study 10
LABORATORY/RESEARCHER: Institute of Freshwater Ecology and Inland Fisheries (IGB), Depart. of Biology and Ecology of Fishes, Berlin / A. Ludwig and C. Wolter.
SPECIES: Different species of Cyprinids.
PROJECT FUNDING: Bundesministerium für Forschung und Technologie.
OBJECTIVE: Investigation of population as well as sub-population structure. Estimation of intraspecific and interspecific biodiversity.
DESIGN: Development of marker systems for gene flow within different sampling points.
METHODOLOGY: Sequence and microsatellites analyses.
STATUS: Ongoing project.

Study 11
LABORATORY / REASEARCHER: Institute of Freshwater Ecology and Inland Fisheries (IGB), Depart. of Biology and Ecology of Fishes, Berlin / A. Ludwig and F. Kirschbaum.
SPECIES: *Acipenser sturio.*
PROJECT FUNDING: Deutsche Forschungsgemeinschaft, KI 189/11-1.
OBJECTIVE: Genetic characterisation of historical stocks of *Acipenser sturio* originated in German waterways. Comparison with samples from other sturgeon catches of European waters, especially the Gironde population with respect to the reestablishment of *Acipenser sturio* in German waterways.

DESIGN: Museum samples of *Acipenser sturio* were collected originating from different River systems and from the North Sea. DNA analyses are performed.
METHODOLOGY: Sequence analyses.
STATUS: Ongoing project.

Study 12
LABORATORY/RESEARCHER: Zoologisches Institut, LMU of Munich / Tautz, U. Schliewen, K. Rassmann and C. Englbrecht.
SPECIES: *Salmo trutta* and *Salvelinus alpinus*.
PROJECT FUNDING: Federal Ministry of environment (UBA).
OBJECTIVE: 1) to study changes in genetic variability (connected with changes in behaviour, morphology, etc.) of autochthonous species resulting from crossing with non-autochthonous species (e.g. stocking). 2) To establish a model to estimate the risk of releasing genetically manipulated organisms.
DESIGN: *Salmo trutta* species have been sampled in many brooks within Bavaria. *Salvelinus alpinus* samples were obtained from major prealpine lakes and small alpine lakes of Germany. Morphological and genetic analyses are performed.
METHODOLOGY: Sequencing of mtDNA, Microsatellite-analyses.
STATUS: Ongoing project.

Study 13
LABORATORY/RESEARCHER: Zoological Institute I, University of Heidelberg, Heidelberg / A. Schreiber.
SPECIES: *Salmo trutta, Thymallus, Cottus, Gobio, Chondrostoma* and *Lampetra*.
PROJECT FUNDING: Fisheries authorities.
OBJECTIVE: Genetic population structure as basis for conservation management.
DESIGN: Population samples from wild stocks in different river basins are investigated.
METHODOLOGY: Enzyme electrophoresis, RAPDs, morphometry.
STATUS: Ongoing project.

Study 14
LABORATORY/RESEARCHER: UFZ-Centre for Environmental Research, Dept. Community Ecology / B. Lauchstaedt.
SPECIES: *Rutilus rutilus*.
PROJECT FUNDING: UFZ-Centre for Environmental Research.
OBJECTIVE: Habitat fragmentation in riverside forest waters of the "Biosphaerenreservat Mittlere Elbe".
DESIGN: Sampling of 24 sites from the Elbe river and backwaters at different stage of isolation.
METHODOLOGY: Allozyme analyses.
STATUS: Ongoing project.

Study 15
LABORATORY/RESEARCHER: UFZ-Centre for Environmental Research, Dept. Community Ecology / B. Lauchstaedt and B. Haenfling
SPECIES: 24 Central European *Cyprinid* species.
PROJECT FUNDING: UFZ-Centre for Environmental Research.
OBJECTIVE: Phylogenetic relationship among and within *cyprinid* subfamilies.
DESIGN: Sampling of 24 cyprinid species of German waters, including all genera occurring in Central Europe
METHODOLOGY: Allozyme analyses.
STATUS: Ongoing project.

ICELAND

Study 1
LABORATORY/RESEARCHER: Holar Agricultural College, Saudarkrokur / E. Svavarsson.
SPECIES: Arctic charr.
PROJECT FUNDING: The National Research Council and the Agricultural Productivity Fund in Iceland.
OBJECTIVE: To determine genetic parameters, i.e. heritability and genetic correlation of economically important traits of Arctic charr in Aquaculture. The results will be utilised in a national breeding program of Arctic charr.
DESIGN AND METHODOLOGY: Data are collected from charr in the first 2 or 3 year classes of the Arctic charr breeding program. Each year class is made up of 100 - 120 full sib families, with two or three families pr. sire. The families are reared for two and a half year from hatching. Data are collected on growth, sexual maturity at different life stages, flesh coloration and possibly fat content of fish. Data are analysed after standard methods in animal breeding.

STATUS: The project started in 1993 and is planned for four years. Preliminary results for the first year class have been published in Iceland. A revised project plan has been sent to the Research Council for the years 1996 - 1998. Continued work according to the revised plan will depend on funding.
COMMENTS: The project is in co-operation between the Agricultural school at Hólar in North Iceland, that is in charge of the actual breeding program for Arctic charr, the Institute of Freshwater Fisheries and the Agricultural Research Institute. The breeding program is funded by the Agricultural Productivity Fund in Iceland.

Study 2
LABORATORY/RESEARCHER: Marine Research Institute (MRI), c/o Biotechnology House, Reykjavik / A.K. Danielsdottir, O.D.B Jonsdottir and O.Y. Atladottir. University of Trondheim, Norway / J. Mork; University College Cork, Ireland / T. Cross and P. Galvin; University of East Anglia, U.K. / G. M. Hewitt and C. Rico; Directorate of Fisheries Research, MAFF, U.K. / R. S. Millner and M. Nicholson.
SPECIES: Cod (*Gadus Morhua*), hake (*Merluccius merluccius*), blue whiting (*Micromesistius poutassou*) and poor cod (*Trisopterus minututs*).
PROJECT FUNDING: MRI and EU FAIR.
OBJECTIVE: Cod stock structure in Icelandic waters and calibration of different molecular Markers for use in discrimination and management of cod, blue whiting, hake and poor cod.
METHODOLOGY: Haemoglobin's, allozymes and anonymous cDNA RFLP.
STATUS: Four year project. Cod sampling has started, analysis of samples started in October 1996. (1996-2000).

Study 3
LABORATORY/RESEARCHER: Marine Research Institute (MRI), c/o Biotechnology House, Reykjavik / A.K. Danielsdottir (project leader), I. Hansen, H.B. Jonsdottir and S.L. Jonsdottir.
SPECIES: Redfish (*Sebastes mentella*).
PROJECT FUNDING: MRI, The National Research Council of Iceland and various trawlers.
OBJECTIVE: Study the genetic population structure of oceanic and deep-sea *S. mentella* in Irminger sea and Icelandic waters.
DESIGN: Redfish samples from different locations Southwest of Iceland and the Irminger Sea.
METHODOLOGY: Allozymes, haemoglobin's and anonymous cDNA RFLP.
STATUS: Three to five year project. Redfish sampling and analyses started summer 1995.
COMMENTS: The project is in collaboration with University of Bergen, Norway / T. Johansen and G. Naevdal. Département de Biologie, Université Laval, Québec / L. Bernatchez and S. Roques.

Study 4
LABORATORY/RESEARCHER: Institute of Freshwater Fisheries, c/o Biotechnology House, Reykjavik / A.K. Danielsdottir and S. Gudjonsson. Danish Institute for Fisheries Research, Dept. of Inland Fisheries, Silkeborg / M.M. Hansen (coordinator) + 21 other participants from laboratories in Europe and Canada.
SPECIES: Brown trout (*Salmo trutta*).
PROJECT FUNDING: In house, the Icelandic Science fund and EU FAIR
OBJECTIVE: Genetic variation in wild populations of landlocked and anandromous brown trout in Iceland. Concerted Action on brown trout population genetics (TROUTCONCERT). The objectives are to promote collaboration among laboratories that are active in research on population genetics of brown trout, to harmonise the use of genetic markers, to give recommendations for a European strategy for management and conservation of the species, and to establish databases on relevant literature, available genetic markers and data from published and unpublished studies. The databases will be made publicly accessible on the World Wide Web (WWW).
DESIGN: Mapping of gene frequencies. Concerted action, i.e. network among laboratories.
METHODOLOGY: Allozymes. Workshops, exchange visits among laboratories, common databases and WWW facilities.
STATUS: Samples from 13 locations have been analysed and the study is ongoing. Two-year project (1998-1999).

Study 5
LABORATORY/RESEARCHER: University of Iceland, Department of Biology, Reykjavik / E. Arnason.
SPECIES: Cod, salmon, brown trout and Arctic charr.
PROJECT FUNDING: In house and the Icelandic Science fund.
OBJECTIVE: Genetic population structure and species variation.
DESIGN: Mapping of gene frequencies and sequence variation.
METHODOLOGY: RFLP of mtDNA, mtDNA cytochrome b sequencing and microsatellite markers.
STATUS: Ongoing.

Study 6
LABORATORY/RESEARCHER: Stofnfiskur ltd., private fishfarmers / J. Jonasson.
SPECIES: Atlantic salmon.

PROJECT FUNDING: Icelandic Government, private.
OBJECTIVE: Use selective breeding to improve economically important traits in rearing of salmon in landbased units and net pens.
DESIGN: Produce 100-200 families a year for selection.
STATUS: Started in 1991, ongoing.

Study 7
LABORATORY/RESEARCHER: Stofnfiskur Ltd., private fishfarmers / J. Jonasson.
SPECIES: Atlantic salmon.
PROJECT FUNDING: Icelandic Research Council.
OBJECTIVE: Establish rearing methods by using geothermal heat and light regimes to accelerate growth and age at maturity to shorten the generation interval to increase response to selection.
DESIGN: Produce 100-150 families a year.
STATUS: Started in 1993-1997.

Study 8
LABORATORY/RESEARCHER: Stofnfiskur Ltd., private fishfarmers / J. Jonasson.
SPECIES: Atlantic salmon.
PROJECT FUNDING: Icelandic Research Council.
OBJECTIVE: Salmon quality. Estimate heritabilities for fat content and genetic correlation between fat content and other life history traits in salmon farming.
METHODOLOGY: Use Tory-fish fat meter to measure fat content.
DESIGN: Produce 100-150 families a year.
STATUS: Started in 1995-1997.

Study 9
LABORATORY/RESEARCHER: Stofnfiskur Ltd. / J. Jonasson. Saebyli ltd. / S. E. Stefansson. Institute of Freshwater Fisheries / A. Gudnason. The Marine Research Institute,, A. Steinarsson.
SPECIES: Red Abalone.
PROJECT FUNDING: Icelandic Research Council.
OBJECTIVE: Estimate genetic parameters for body weight, survival and shell and meat proportion, in the attempt to plan a breeding program for red abalone culture in Iceland to reduce production cost for coming years.
METHODOLOGY: Classic selective breeding programs.
DESIGN: Produce 100-150 full- and half-sib families a year.
STATUS: Started in 1996-1999.

Study 10
LABORATORY/RESEARCHER: University of Iceland, Holar Agricultural College, Saudarkrokur / S. Skulason and D. Gislason. Joint population genetic laboratory of the Marine Research Institute and Institute of Freshwater Fisheries, c/o Biotechnology House, Reykjavik / A.K. Danielsdottir. Also one partner in Ireland, one in Sweden and one in Scotland. In collaboration with the University of Guelph / M. Ferguson.
SPECIES: Arctic charr, *Salvelinus alpinus.*
PROJECT FUNDING: EU FAIR-CT-96-1981.
OBJECTIVE: Development of sustainable aquaculture of Arctic charr.
DESIGN: Multidisciplinary approach involving fish-farmers, ecologists, brood stock managers, fish husbandry experts and molecular biologists. Holistic approach encompassing a variety of techniques to provide a sound scientific basis for the development of this species for aquaculture.
METHODOLOGY: Genetic variation in wild populations and domesticated stains from Iceland, Scotland Ireland and Sweden by the use of microsatellites.
STATUS: Project started in September 1997, to continue for 2 years.

IRELAND

Study 1
LABORATORY/RESEARCHER: Department of Zoology, University College Dublin / L. Sullivan, B. Danilowicz and E. Duke.
SPECIES: Atlantic herring.
FUNDING: Fisheries Research Centre, Enterprise Ireland, University College Dublin.
OBJECTIVE: To establish the extent of genetic (microsatellite) variation among Irish herring populations and determine if there are distinct spawning populations on individual gravel beds.

DESIGN: Collect egg stage herring on specific gravel beds; collect adult herring from each major spawning location around Ireland; perform genetic analyses on collections taken over two years.
METHODOLOGY: Microsatellite analysis.
STATUS: Three year project started November 1998.

Study 2
LABORATORY/RESEARCHER: National Diagnostics Centre, NUI, Galway / T. Smith, S. Martin, O. McMeel and M. Cairns and EU partner laboratories.
SPECIES: Rainbow trout, tilapia (Galway).
FUNDING: EU FAIR programme (CT 97-3796) (Coordinator –Jean-Francois Baroiller, Rennes).
OBJECTIVE: Identification of genes involved in sex determination in salmonids.
METHODOLOGY: PCR-based cloning of differentially expressed genes during sex determination using SSH and DD RT-PCR. Molecular analysis of genes isolated by differential cloning.
STATUS: 3 year project funded until December 2000.

Study 3
LABORATORY/RESEARCHERS: National Diagnostics Centre, NUI, Galway, T. Smith, O. McMeel, B. Cleary and M. Cairns and EU partner laboratories.
SPECIES: Atlantic salmon, Rainbow trout, (Galway).
FUNDING: EU Biotech programme (CT 97-0554) (Coordinator: Dr Terry Smith, Galway).
OBJECTIVE: Assessment of biological containment and gene flow in transgenic sterile fish.
METHODOLOGY: Inhibition of GnRH protein production in trout, tilapia and zebrafish by antisense mRNA expression. Molecular approaches to induce controllable and reversible sterility in fish.
STATUS: 3 year project funded until December 2000.

Study 4
LABORATORY/RESEARCHERS: National Diagnostics Centre, NUI, Galway / T. Smith, S. Martin and P. O'Dea and EU partner laboratories.
SPECIES: Rainbow trout (Galway).
FUNDING: EU FAIR programme (CT 95-0666) (Coordinator: Daniel Chourrout, Bergen).
OBJECTIVE: Molecular basis of fish immunity for disease resistance.
METHODOLOGY: PCR-based library construction and cloning of differentially expressed genes using SSH. Molecular analysis of genes isolated by differential cloning.
STATUS: 3 year project funded until April 1999.

Study 5
LABORATORY/RESEARCHERS: National Diagnostics Centre, NUI, Galway / T. Smith, S. Martin and A. Sangrador.
SPECIES: Rainbow trout (Galway).
FUNDING: EU FAIR programme TMR (GT 97-3020).
OBJECTIVE: Identification of genes involved in the non-specific immune response of rainbow trout.
METHODOLOGY: Immuno-stimulation of trout head-kidney primary leukocyte cells. PCR-based library construction and cloning of differentially expressed genes using SSH. Molecular analysis of genes activated by immuno-stimulation isolated by differential cloning.
STATUS: 2 year TMR project funded until October 2001.

Study 6
LABORATORY/RESEARCHERS: National Diagnostics Centre, NUI, Galway / S. Martin, T. Smith, O. McMeel, A. O'Keefe and M. Cairns.
SPECIES: Atlantic salmon.
FUNDING: Enterprise Ireland Basic Research Programme.
OBJECTIVE: Identification of the role of thyroid stimulating hormone in smoltification of Atlantic salmon.
METHODOLOGY: Molecular analysis of thyroid stimulating hormone gene expression during development and smoltification. Isolation and characterisation of TSH subunits, Northern blot analysis and study of TSH expression in primary pituitary cells.
STATUS: 3year project funded until October 2001.

Study 7
LABORATORY/RESEARCHERS: Department of Microbiology and National Diagnostics Centre, NUI, Galway / R. Powell, T. Smith, S. Martin and K. Glennon.
SPECIES: *Lepeophtheirus salmonis.*

FUNDING: Marine Institute Operational Programme for Fisheries (1994-1999) 97.IR.MR.011.
OBJECTIVE: Cloning and characterisation of *Lepeophtheirus salmonis* microsatellite genetic elements as useful tools for the study of sea lice ecology.
METHODOLOGY: Construction and characterisation of short-insert genomic plasmid libraries of *L.salmonis*. Screening of libraries and isolation of microsatellite sequences. Characterisation of microsatellites for polymorphism information content and evaluation of distinct sea-lice populations for heterogeneity.
STATUS: 2 year project funded until December 1999.

Study 8
LABORATORY/RESEARCHER: Salmon Research Agency of Ireland / P. McGinnity. Queens University Belfast. National University of Ireland, Cork. Marine Institutes Fisheries Research Centre.
SPECIES: Atlantic salmon.
FUNDING: Salmon Research Agency of Ireland, Marine Institute.
OBJECTIVE: Field and hatchery experiments, utilising molecular markers, to determine the genetic effects of farmed escaped salmon on natural populations; relative marine survival and comparative performance of F2 hybrids and back crosses in freshwater.
DESIGN: Simulation of a farm escape to a natural stream contained by high specification fine screened trap, individual fish identified to family using DNA microsatellites, release of hatchery reared control population smolts to sea, capture and DNA profiling of returning adults.
METHODOLOGY: Establishment of experimental population, hatchery control, trap and field monitoring, microtagging, cold branding, sampling, microsatellite DNA analysis.
STATUS: Project started 1998, ongoing.

Study 9
LABORATORY/RESEARCHER: Salmon Research Agency of Ireland / D. Cotter with one Irish, two Scottish and one Norwegian partners.
SPECIES: Atlantic salmon.
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, and simulation of escapes from sea cages.
STATUS: Main programme completed October 1998, ocean ranching aspects of project still ongoing.

Study 10
LABORATORY/RESEARCHER: Trinity College Dublin & Hydro / A. Norris. Seafood Fanad.
SPECIES: Atlantic salmon.
FUNDING: Hydro Seafood Fanad.
OBJECTIVE: To implement new family selection program for a number of economically important traits. DNA markers will be used to estimate relatedness and parentage and to monitor any losses in genetic variation as the program advances.
DESIGN: Establish new family population each year for four years. Parentage identification and selective breeding to begin in three years.
METHODOLOGY: Quantitative genetics analysis and microsatellite analysis.
STATUS: Ongoing.

Study 11
LABORATORY/RESEARCHER: Biochemistry Department, National University of Ireland, Galway / L. Byrnes and K. Gately.
SPECIES: Atlantic salmon, *Salmo salar*.
PROJECT FUNDING: BioResearch Ireland.
OBJECTIVE: To examine the regulation of salmon transferrin gene expression, particularly during smoltification.
DESIGN: Promoter of salmon transferrin gene has been isolated.
METHODOLOGY: DNA sequence analysis, electrophoretic mobility shift assays, DNase footprinting, functional assays of promoter activity in cell lines.
STATUS: Final year of project.

Study 12
LABORATORY/RESEARCHER: Biochemistry Department, National University of Ireland, Galway / L. Byrnes, J. Hill and A. Kelly. Also two partners in Iceland, and one each in Sweden and Scotland.

SPECIES: Arctic charr, *Salvelinus alpinus*.
PROJECT FUNDING: EU FAIR-CT-96-1981.
OBJECTIVE: Development of sustainable aquaculture of Arctic charr.
DESIGN: Multi-disciplinary approach involving fish-farmers, ecologists, brood stock managers, fish husbandry experts and molecular biologists
METHODOLOGY: Holistic approach encompassing a variety of techniques to provide a sound scientific basis for the development of this species for aquaculture.
STATUS: Project started in December, 1996, to continue for three years.

Study 13
LABORATORY/RESEARCHER: Recombinant DNA Group, Department of Microbiology, National University of Ireland, Galway / R. Powell.
SPECIES: Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*), brown trout (*Salmo trutta*).
FUNDING: EU FAIR Programme 1996-1999.
OBJECTIVE: Generation of highly informative DNA markers and genetic marker maps of salmonid fishes (SALMAP). Research involves the cloning, isolation and design of PCR assays targeting repetitive microsatellite DNA sequences in the genome of the selected salmonids. The objectives are to define low density genetic maps for the three selected salmonid species.
STATUS: 3 year project due for completion in January 2000.

Study 14
LABORATORY/RESEARCHER: Department of Microbiology, National University of Ireland, Galway / R. Powell.
SPECIES: Pacific oyster (*Crassostrea gigas*).
PROJECT FUNDING: EU FAIR Programme 1995-1999.
OBJECTIVE: Development of a molecular karyotype system for Pacific oyster. Research involves the construction of large-insert genomic DNA libraries of Pacific oyster using *E. coli* cosmid vectors. The aim is to define clones that mark specific chromosome pairs and develop a chromosomal karyotype system based on such molecular markers.
STATUS: 5 year project due for completion in November 2001.

Study 15
LABORATORY/RESEARCHER: Department of Microbiology, National University of Ireland, Galway / R. Powell.
SPECIES: Atlantic salmon (*Salmo salar*).
PROJECT FUNDING: Enterprise Ireland Basic Science Programme 1997-2000 and EU FAIR Programme 1998-2001.
OBJECTIVE: Generation of a genetic body map for Atlantic salmon. Research involves the construction of cDNA libraries from four tissues of Atlantic salmon. Partial DNA sequencing will be used to generate ESTs and define the major abundant messenger RNA transcripts in the selected tissues.
STATUS: 3 year projects due for completion in 2002.

Study 16
LABORATORY/RESEARCHER: Department of Microbiology, National University of Ireland, Galway / R. Powell.
SPECIES: *Aeromonas salmonicida* in salmonid and non-salmonid species.
PROJECT FUNDING: No current dedicated funding.
OBJECTIVE: Improved identification and taxonomy of atypical isolates of the fish pathogen *Aeromonas salmonicida*. Research is underway on a genetic, biochemical and immunological analysis of new isolates of 'atypical' *Aeromonas salmoncida* presently being isolated from a large range of diseased sea- and fresh-water fish species. The objectives are (i) to develop definitive diagnostic procedures for the identification of this bacterial group, and (ii) to quantify the detrimental effect of this group on native fish resources.
STATUS: Ongoing project

Study 17
LABORATORY/RESEARCHERS: Aquaculture Development Centre, National University of Ireland, Cork / T. Cross, P. Galvin and J. Coughlan plus 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: Three novel minisatellite loci have been developed for each species and are currently being screened. A 242bp MHC fragment has been sequenced in disparate members of all species, directed RFLP designed and screening is in progress.
METHODOLOGY: PCRable minisatellites, MHC genes.
STATUS: Four year project from April 1996.

Study 18
LABORATORY/RESEARCHERS: Aquaculture Development Centre, National University of Ireland, Cork / P. Galvin, T. Cross and Y. Kelly.
SPECIES: Atlantic salmon, *Salmo salar.*
PROJECT FUNDING: Irish Electricity Supply Board.
OBJECTIVE: To assist the breeding programme on the river Shannon by carrying out molecular studies. To study wild population structure using GSI.
DESIGN: Many hatchery and wild samples are being screened.
METHODOLOGY: Microsatellites.
STATUS: One year project from January 1999.

Study 19
LABORATORY/RESEARCHERS: Aquaculture Development Centre, National University of Ireland, Cork / T. Cross, M. Cross and P. Galvin..
SPECIES: Atlantic salmon, *Salmo salar.*
PROJECT FUNDING: EU Interreg.
OBJECTIVE: To study genetic variability in the hatchery strain in the river Erne and wild populations from nearby rivers and those throughout the country, to advise on ranching programme.
DESIGN: In addition to the geographic element, parr samples of two cohorts are being screened to test for temporal variability, as are archival scale collections.
METHODOLOGY: Microsatellites.
STATUS: Four year project from November 1996.

Study 20
LABORATORY/RESEARCHERS: Aquaculture Development Centre, National University of Ireland, Cork / T. Cross, P. Galvin and E. Dillane, plus three UK and two Spanish, three French, one Portuguese and one Greek partner or associate.
SPECIES: Ommastrepid squid, *Illex coindetii* and *Todaropsis eblanae* (Cork).
PROJECT FUNDING: EC FAIR.CT96.
OBJECTIVE: To search for population structure throughout the north east Atlantic and Mediterranean range, with later concentration on aspects of fishery interest.
DESIGN: In addition to work at Cork, four other cephalopod species are being studied (*Octopus vulgaris, Loligo vulgaris* and *forbesi, Sepia officinalis*), and GIS, life history aspects and automated ageing are also being investigated.
METHODOLOGY: Microsatellites.
STATUS: Three year project which finished in March 2000.

Study 21
LABORATORY/RESEARCHERS: Aquaculture Development Centre, National University of Ireland, Cork / T. Cross, R. FitzGerald, J. Coughlan, M. Stefansson, P. Galvin, M. Mulcahy and R. Hoare, plus Norwegian and Dutch groups.
SPECIES: Turbot, *Scophthalmus maximus,* halibut *Hippoglossus hippoglossus.*
PROJECT FUNDING: EU FAIR Programme.
OBJECTIVE: To compare genetic variability in experimental strains of turbot and halibut, and also to study wild population structure in these species.
DESIGN: Three strains of each species are held at varying temperatures (and salinities in the case of turbot) at the Bergen Large Scale Facility, and growth, immunology, stress physiology and genetics investigated. Six or more wild samples are also taken from throughout the ranges for genetic characterisation.
METHODOLOGY: Microsatellite loci.
STATUS: Three year project finishing in December 2001.

Study 22
LABORATORY/RESEARCHERS: Aquaculture Development Centre, National University of Ireland, Cork / T. Cross and D. O'Leary. Biochemistry Department, National University College, Cork / T. McCarthy.
SPECIES: Cod, *Gadus morhua.*
PROJECT FUNDING: Forbairt (Irish Science Council).
OBJECTIVE: To evaluate GMPD in fish.
DESIGN: Glycosylase mediated polymorphism detection (GMPD) detects some 90 % of single base substitutions in humans. The method is being transferred to cod which will then be screened from throughout the range
METHODOLOGY: GMPD for rapid partial sequencing.
STATUS: Three year study from November 1998.

LATVIA

Study 1
LABORATORY/RESEARCHERS: Latvian Fisheries Research Institute / O.P. Vasin.
SPECIES: Atlantic salmon.
PROJECT FUNDING: In house.
OBJECTIVE: Genetic monitoring of main hatchery and wild populations.
METHODOLOGY: Allozymes (in polyacrylamide).
STATUS: Long term study started 1984.

NORWAY

Study 1
LABORATORY/RESEARCHER: Department of Fisheries and Marine Biology / University of Bergen (DFMB), T. Johansen and G. Nævdal. Institute of Marine Research (IMR) Bergen. Møreforskning, Ålesund.
SPECIES: Redfish, Genus *Sebastes*.
PROJECT FUNDING: IMR and the University of Bergen.
OBJECTIVE: Study the genetic variation between morphologically similar species, and the population structure within the species.
DESIGN: Extensive sampling has taken place throughout the distibution areas of the redfish species, with main emphasise on Icelandic and Greenland waters in collaboration with Institute of Marine Research, Reykjavik and Møreforsking, Ålesund. Samples have also been exchanged with Canadian, Spanish and German researchers.
METHODOLOGY: Gel electrophoresis and isoelectric focusing (allozymes). RAPD in collaboration with IMR.
STATUS: Studies on haemoglobins and allozymes by electrophoresis and isoelectric focusing have been going on since 1987; the last years with main 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. Data are now being prepared for publication.
COMMENTS: The project has revealed a relative simple species and population structure for redfish species in the eastern North Atlantic, while the picture seems very complicated in Greenland waters. The Giant redfish at the Reykjanes Ridge deviate from the other redfish groups. If funding is possible the studies will be continued in the Iceland - Greenland area through extensive international cooperation, with main emphasise on the deep sea and Oceanic S. mentella in the Irminger Sea.

Study 2
LABORATORY/RESEARCHER: Department of Fisheries and Marine Biology and University of Bergen (DFMB) / G. Nævdal.
SPECIES: Mesopelagic fish species.
PROJECT FUNDING: Own funding.
OBJECTIVE: Identify morphologically similar species and study the structure of the more common mesopelagic fishes (*Maurolicus mülleri, Benthosema glaciale, Notolepis rissoi kroyeri*).
DESIGN: Samples from Norwegian fjords and offshore waters are being analysed.

Study 3
LABORATORY/RESEARCHER: Institute of Marine Research / Ø. Skaala. University of Stirling, Scotland / J.B. Taggart, A. Teale and K. Glover.
SPECIES: Seatrout *Salmo trutta.*
PROJECT FUNDING: Electricity industry and Norwegian Research Council.
OBJECTIVE: Compare performance of families and stocks of anadromous trout.
DESIGN: Compare performance in natural habitat and in hatchery.
METHODOLOGY: Identify families by microsatellites.
STATUS: Families produced.

Study 4
LABORATORY/RESEARCHER: IMR, Norway/ Ø. Skaala
SPECIES: Brown trout, *Salmo trutta.*
PROJECT FUNDING: Statkraft.
OBJECTIVE: Study genetic effects of stocking in Lake Halnefjord.
DESIGN: Compare genetic composition in recipient and donor stocks.
METHODOLOGY: Allozymes.
STATUS: Genotyping completed.

Study 5
LABORATORY/RESEARCHER: Department of Aquaculture, Institute of Marine Research (IMR), Bergen / G. Dahle.
SPECIES: Different marine species.
PROJECT FUNDING: Norwegian Research Council.
OBJECTIVE: Investigate possible genetic markers for identification of marine species in seafood - authentication.
DESIGN: Analyse several amplified mtDNA fragments, and produce a RAPD library from many different species, both economically important and others.
METHODOLOGY: PCR amplification of different mtDNA fragments, RFLP, and RAPD.
STATUS: Project to be finished in 1999.

Study 6 (1998)
LABORATORY/RESEARCHER: Institute of Marine Research (IMR), Bergen / K.E. Jørstad.
SPECIES: European lobster (*Hommarus gammarus*).
PROJECT FUNDING: IMR, Norwegian Research Council.
OBJECTIVE: Genetic comparison of cultured and wild lobsters.
DESIGN: Sampling of wild and recaptured cultured lobsters. Comparison with samples of wild stock at Kvitsøy and nearby regions.
METHODOLOGY: Starch gel electrophoresis, polymorphic enzymes.
STATUS: Preliminary report 1997.
COMMENTS: The work is part of a large-scale lobster enhancement project.

Study 7 (1998)
LABORATORY/RESEARCHER: Institute of Marine Research (IMR), Bergen / K.E. Jørstad.
SPECIES: Mainly herring (*Clupea harengus*).
PROJECT FUNDING: IMR, Ministry of Foreign Affairs, Norway.
OBJECTIVE: Year class 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 8 (1998)
LABORATORY/RESEARCHER: Institute of Marine Research (IMR), Bergen / K.E. Jørstad.
SPECIES: European lobster (*Hommarus gammarus*).
PROJECT FUNDING: IMR, Norwegian Research Council.
OBJECTIVE: Estimate genetic impact from Scottish lobster/import.
DESIGN: Collection of lobster samples from Scotland and compare with samples from recipient areas in Norway.
METHODOLOGY: Allozyme and microsatellite DNA analyses.
STATUS: Initiated spring 1998.
COMMENTS: Partly in co-operation with A. Ferguson, Belfast.

Study 9
LABORATORY/RESEARCHERS: The Norwegian College of Fishery Science, University of Tromsø / S.E.Fevolden with two Norwegian and several other European partners.
SPECIES: Rainbow trout (*Oncorhynchus mykiss*).
PROJECT FUNDING: EC FAIR-CT95-0152.
OBJECTIVE: To establish a protocol for the selective breeding of finfish for increased tolerance to stress and to assess whether stress tolerance is an advantage under aquaculture conditions in terms of growth, disease resistance and reproductive performance.
METHODOLOGY: Selection based on post-stress plasma levels of cortisol and lysozyme.
STATUS: Four year project started in 1996.

Study 10
LABORATORY/RESEARCHER: The Norwegian College of Fishery Science, University of Tromsø / S.E. Fevolden.
SPECIES: Atlantic cod (*Gadus morhua*).
PROJECT FUNDING: Norwegian Research Council 127357/122.
OBJECTIVE: To increase the knowledge and understanding of the macro- and microgeographic diversity at the synaptophysin locus of the species. The following subgoals are given: (i) to verify whether the typical coastal cod *Syp* I

allele frequencies found in Troms and Finnmark are also typical of waters further east along the Kola Peninsula, and further south along the Norwegian coast; (ii) to study the stability of microgeographic genetic diversity within specific fjords; and (iii) to investigate thermal preferential between the various *Syp* I genotypes found at different temperature regimes offshore and inshore, and at different depths within the same region.
METHODOLOGY: The synaptophysin locus (*Syp* I).
STATUS: Three year project started in 1999.

Study 11
LABORATORY/RESEARCHERS: Norwegian College of Fishery Science, Norwegian College of Veterinary Medicine, Norwegian Institute of Fisheries and Aquaculture / S.E. Fevolden, K.H. Røed and J.S. Christiansen.
SPECIES: Capelin (*Mallotus villosus*).
PROJECT FUNDING: Norwegian Institute of Fisheries and Aquaculture.
OBJECTIVE: To find/develop microsatellite primers for capelin that could be used to study population variability in the North Atlantic.
STATUS: Pilot project started in 1999.

Study 12 (1998)
LABORATORY/RESEARCHER: Norwegian Institute for Nature Research (NINA) / K. Hindar.
SPECIES: Atlantic salmon.
PROJECT FUNDING: Directorate for Nature Management, Norway and NINA.
OBJECTIVE: Establish baseline information about the population genetic structure of Atlantic salmon in Norway.
DESIGN: Samples from all over Norway to analyse spatial and temporal variation in gene frequencies.
METHODOLOGY: Allozymes.
STATUS: Ten-year project to be completed 1998.

Study 13 (1998)
LABORATORY/RESEARCHER: Norwegian Institute for Nature Research (NINA) / K. Hindar. In collaboration with two UK and one Irish group.
SPECIES: Atlantic salmon and brown trout.
PROJECT FUNDING: EU AIR3 94 2484.
OBJECTIVE: Quantify and understand hybridisation between Atlantic salmon and brown trout, especially in the light of an increasing tendency of escaped farmed salmon to hybridise with trout.
DESIGN: Index samples from Ireland, Scotland and Norway including undisturbed and "genetically polluted" rivers; behavioural studies of spawning; estimates of fitness components in artificially produced hybrids.
METHODOLOGY: Genetic markers (allozymes, nuclear and mitochondrial DNA); feeding history markers (natural and synthetic pigments); constructed spawning arenas; rearing and release studies.
STATUS: 27 month study completed December 1996.

Study 14 (1998)
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 15 (1998)
LABORATORY/RESEARCHER: Norwegian Institute for Nature Research (NINA) / K. Hindar and K. Kvaløy.
SPECIES: Atlantic salmon.
PROJECT FUNDING: Research Council of Norway.
OBJECTIVE: Analyse genetic variation in extinct and re-established populations based on microsatellite DNA isolated from dried scales.
DESIGN: Study of populations for which good scale samples exist.
METHODOLOGY: PCRable microsatellite DNA.
STATUS: Four-year project started in 1998.

Study 16
LABORATORY/RESEARCHER: University of Trondheim, Biological Station / J. Mork.
SPECIES: Indifferent.
PROJECT FUNDING: Institutional.
OBJECTIVE: Interactive MS-DOS simulation program (DGS-9D.EXE) for general simulation of evolution in multiple populations simultaneously.
DESIGN: Simultaneous handling of combined genetic effects from random genetic drift, gene flow (model-independent), and selection (additive effects) at multiple loci in a genetically freely 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 currently in use at several university sites.

Study 17
LABORATORY/RESEARCHER: University of Trondheim, Biological Station / M. Giæver.
SPECIES: Blue whiting (*Micromesistius poutassou*).
PROJECT FUNDING: The Norwegian Research Council, grant NF 113606/122.
OBJECTIVE: To enlighten the genetic population structure in the blue whiting, with special emphasis on the north-eastern parts of its distribution range (the Norwegian Sea and the Barents Sea).
DESIGN: Genotyping of a large number of individuals from a tight sampling net in the relevant areas, during and outside the spawning season.
METHODOLOGY: Allozymes and minisatellites.
STATUS: Project end reported in 1998. Ph.D. dissertation in 1999.
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 characterisation of this north-eastern blue whiting.

Study 18
LABORATORY/RESEARCHER: University of Trondheim, Biological Station / J. Mork.
SPECIES: Cod (*Gadus morhua*).
PROJECT FUNDING: Institutional.
OBJECTIVE: Study of the long term stability of haemoglobin, allozyme and DNA markers allele frequencies in a local population of cod, and test for correlation between genotype and growth/survival.
DESIGN: Bi-annual sampling (research vessel) of about 200 specimens from a local cod population with no commercial exploitation.
METHODOLOGY: Collection of biological data (length, age, sex etc.), and genotyping for polymorphic haemoglobins and tissue enzymes.
STATUS: Haemoglobin analysis started in 1974 and is ongoing; allozymes from 1980 and ongoing.
COMMENTS: DNA mini- and microsatellites included from 1998 (back-tracking analyses possible as well).

Study 19
LABORATORY/RESEARCHER: Biological Station, University of Trondheim / J. Mork. Collaborations with University College, Cork, Ireland / T. Cross and P. Galvin. University of Wales, Swansea, U.K. / G. Carvalho and C. Turan. 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 1992-1998.
METHODOLOGY: Allozymes, haemoglobins, DNA mini- and microsatellites.
STATUS: Haddock, cod and blue whiting allozymes analysed (>3000 specimens each). Norway pout ca 1700 specimens and herring ca 800 specimens analysed for isozymes. DNA mini- and microsatellite pilot studies commenced for most of the gadoid 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 20
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 / R. Millner.

SPECIES: Cod, hake, blue whiting and poor cod.
PROJECT FUNDING: EU FAIR CT95 0282 (4 years: 1996-2000).
OBJECTIVE: To develop and calibrate a set of molecular markers for use in detection and characterisation 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 correlation between genetic structures and species-specific traits in biology.
METHODOLOGY: Allozymes, DNA mini- and microsatellites, cDNA, transcribed sequences, mtDNA.
STATUS: Cod, hake, blue whiting and poor cod allozyme analyses completed. DNA mini- and microsatellite analyses are ongoing for blue whiting.
COMMENTS: For all samples, individual biological records (sex, length, weight, maturity stage, age) as well as sampling information (vessel, date, gear, fishing depth) are collected.

POLAND

Study 1
LABORATORY/RESEARCHER: Sea Fisheries Institute, Gdynia / E. Wlodarczyk and R. Wenne.
SPECIES: Sea trout (*Salmo trutta*).
PROJECT FUNDING: Polish Committee for Scientific Research, 5P06 D00714.
OBJECTIVE: To study population genetic structure of the sea trout in Poland.
METDOLOGY: RFLP analysis of PCR amplified mtDNA segments (ND-1 and ND-5/6).
STATUS: Completed.

Study 2
LABORATORY/RESEARCHER: Sea Fisheries Institute, Gdynia / A. Was, R. Wenne
SPECIES: Sea trout (*Salmo trutta*).
PROJECT FUNDING: Institutional.
OBJECTIVE: To study population genetic structure of the sea trout in Poland.
METHODOLOGY: Microsatellites, PCR, silver staining.
STATUS: Ongoing.

Study 3
LABORATORY/RESEARCHER: Marine Biology Center, Polish Academy of Sciences, Gdynia / M. Pempera, A. Burzynski and R. Wenne. School of Biological Sciences, University of Wales, Swansea, UK / D.O.F. Skibinski.
SPECIES: Mussel, *Mytilus trossulus.*
PROJECT FUNDING: Polish Committee for Scientific Research: 6P04C 004 11.
OBJECTIVE: To characterize length heteroplasmy of mitochondrial DNA and population variation in Baltic.
METHODOLOGY: PCR amplification and restriction analysis of two regions of mtDNA, sequencing of the major noncoding region.
STATUS: Ongoing.

Study 4
LABORATORY/RESEARCHER: Marine Biology Center, Polish Academy of Sciences, Gdynia / B. Smietanka, R. Wenne, collaboration. Zoological Museum, Helsinki University, Finland / R. Vainola.
SPECIES: Mussels *Mytilus.*
PROJECT FUNDING: Institutional, Committee for Scientific Research: 6P04C 004 11.
OBJECTIVE: To compare European populations.
METHODOLOGY: PCR amplification of mtDNA female and male genomes, restriction analysis.
STATUS: Ongoing.

Study 5
LABORATORY/RESEARCHER: Inland Fisheries Institute, Salmonid Research Department, Rutki / K. Goryczko, S. Dobosz, collaboration with: Institute of Freshwater Ecology and Inland Fisheries, Berlin, Germany / K. Kohlmann, Warsaw University of Agriculture / A. Zynczynski.
SPECIES: Rainbow trout.
PROJECT FUNDING: Committee for Scientific Research and Institutional.
OBJECTIVE: To improve the breeding value of rainbow trout.
METHODOLOGY: Each family is reared separately until the end of the first season, then the fishes are tagged (PIT tags), number of families culled is 60, fishes are reared in one pond until sexual maturity.

Study 6
LABORATORY/RESEARCHER: Inland Fisheries Institute, Salmonid Research Department, Rutki / K. Goryczko, S. Dobosz, H. Kuzminski.
SPECIES: Rainbow trout.
PROJECT FUNDING: Institutional.
OBJECTIVE: To protect genetic diversity in a valuable strain maintained by stocking (Vistula sea trout gene bank).
METHODOLOGY: Samples of 50 g of fertilised eggs from each wild female spawned were taken, incubated and reared at SRL. Random samples of 1991 and 1993 year generations presmolts were PIT tagged (1200 and 600 fish respectively). Smoltification, growth and age at first, maturity are monitored.

Study 7
LABORATORY/RESEARCHER: Institute of Oceanography, University of Gdansk, Gdynia / K. Blicharska, M. Wolowicz, collaboration with: Observatoire Oceanologique, Universite Pierre et Marie Curie, CNRS-INSU, Villefranche sur Mer, France / C. Thiriot-Quievreux and Netherlands Inst. Ecol., Center for Estuarine and Coastal Ecology, Netherlands/H. Hummel.
SPECIES: Bivalves: *Mytilus trossulus, Macoma balthica, Cerastoderma glaucum* and *Mya arenaria.*
PROJECT FUNDING: Institutional.
OBJECTIVE: To study influence of pollution on populations at the genetic level.
METHODOLOGY: Analysis of colhicin-treated mitotic chromosomes from somatic tissue, silver stainig, allozymes.

Study 8
LABORATORY/RESEARCHER: Chair of Genetics and Cytology, University of Gdansk, Gdansk / J. Laszczuk, J. Sell, T. Sywula.
SPECIES: Isopod *Saduria.*
PROJECT FUNDING: Institutional.
OBJECTIVE: To characterize genetic polymorphism in recently established populations in Baltic.
METHODOLOGY: Allozymes.

Study 9
LABORATORY/RESEARCHER: Biological Station, University of Gdansk, Gorki Wschodnie / E. Mulkiewicz, M. Zietara and E.F. Skorkowski.
SPECIES: *Saduria enthomon* (Isopoda), *Palaemoneles varians* (Decapoda).
PROJECT FUNDING: Institutional.
OBJECTIVE: To characterize genetic polymorphism of LDH.

Study 10
LABORATORY/RESEARCHER: Institute of Maritime and Tropical Medicine, Gdynia / B. Szostakowska, P. Myjak. Gdansk Dept. of Microbiology, Technical University / J. Kur.
SPECIES: *Anisakis, Histerocelacium, Contracaecum.*
PROJECT FUNDING: Institutional.
OBJECTIVE: To construct molecular diagnostic markers for species identification.
METHODOLOGY: Allozymes, nuclear DNA, PCR, RFLP.

Study 11
LABORATORY/RESEARCHER: Chair of Biochemistry, University of Gdansk, Gdansk / M. Zmijewski, G. Klein, B. Lipinska.
SPECIES: Sea bacteria, *Vibrio harveyi.*
PROJECT FUNDING: Institutional, Committee for Scientific Research.
OBJECTIVE: To characterize gene coding heat shock protein HSP - DnaK and DnaJ and to study role of its products.
METHODOLOGY: Molecular cloning, Northern blotting, sequencing, transcription analysis.

Study 12
LABORATORY/RESEARCHER: Department of Basic Fishery Sciences, Olsztyn University of Agriculture and Technology / M. Luczynski. Sea Fisheries Institute / M. Wyszynski.
SPECIES: Herring (*Clupea harrengus*).
PROJECT FUNDING: Institutional.
OBJECTIVE: Baseline studies of genetic population structures in the Polish Baltic Sea coastal waters.
METHODOLOGY: Allozymes.
STATUS: Completed.

Study 13
LABORATORY/RESEARCHER: Department of Basic Fishery Sciences, Olsztyn University of Agriculture and Technology / M. Luczynski and collaborators. Sea Fisheries Institute, Gdynia / R. Bartel. Fish Farm "Aquamar" / Marczynski.
SPECIES: Salmon (*Salmo salar*).
PROJECT FUNDING: Institutional, Polish Committee for Scientific Research.
OBJECTIVE: To assess genetic polymorphism in hatchery population.
METHODOLOGY: Allozymes.
STATUS: Ongoing.

PORTUGAL

Study 1
LABORATORY/RESEARCHER: Laboratory of Molecular Population Genetics, Center for Marine Sciences. Universidade do Algarve / R. Castilho. 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. Three year project, end December 1999.

Study 2
LABORATORY/RESEARCHER: Laboratory of Molecular Population Genetics, Center for Marine Sciences / M.L. Cancela, R. Streiff and M. Castro. University of Birmingham, UK / A. Birley.
SPECIES: *Nephrops norvegicus.*
PROJECT FUNDING: EU C1/96.064.
OBJECTIVE: Obtention and characterization of microsatellites from nephrops and use of this markers to determine a new methodology to assess population size.
METHODOLOGY: Development of genetic markers, microsatellite DNA loci.
STATUS: Two year project started May 1998.

Study 3
LABORATORY/RESEARCHER: Laboratory of Molecular Population Genetics, Center for Marine Sciences. Universidade do Algarve / R. Castilho;
SPECIES: Black-scabbard fish (*Aphanopus carbo*).
PROJECT FUNDING: CCMAR.
OBJECTIVE: Study of the genetic variability in the Atlantic.
METHODOLOGY: RFLPs on mtDNA.
STATUS: Ongoing.

Study 4
LABORATORY/RESEARCHER: Laboratory of Molecular Biology and Biotechnology, Center for Marine Sciences / M.L. Cancela, T. Guillemaud and F. Almada. University of Acores-Azores Islands, CITMA-Madeira island ISPA-Lisbon. Marine Laboratory of Guia -Lisbon.
SPECIES: Marine Fishes (Lipophrys pholis and Coris julis).
PROJECT FUNDING: Praxis EMG/1957/95.
OBJECTIVE: Obtention and characterization of microsatellites from littoral fishes within different geographical locations. Assessment of their genetic variability.
METHODOLOGY: To develop microsatellite and mitochondrial DNA markers suitable to establish genetic variability and perform phylogenetic studies.
STATUS: 3 year project, Started May 1997.

Study 5 (1997)
LABORATORY/RESEARCHER: Lab. de Citogenetica, ICBAS / I. Malheiro. University of Porto / C. Thiriot. Observatoire Oceanologique de Villefarnce-sur-Mer, University P. et M. Curie, CNRS-INSU.
SPECIES: *Ostrea edulis, Crassostrea angulata, C. gigas, C. virginica* and *C. sikama.*

OBJECTIVE: Chromosome analysis to study the cytogenetic organisation of different species of oyster; detection of the response of aneuploidy and the possible transmission of this phenomenon to the next generation; relationships between the presence of aneuploidy and development.
METHODOLOGY: Karyotyping from brachial 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 6 (1997)
LABORATORY/RESEARCHER: Dept. of Fisheries Technology, IPIMAR / A.M. Teia dos Santos. Instituto de Investigaciones Marinas (S - Head of project). Federal Research Centre of Fisheries (D). University de Santiago de Compostela (S). Rowett Research Institute (UK).
SPECIES: Sardine and squid.
PROJECT FUNDING: EU-FAIR (accepted).
OBJECTIVE: To develop DNA-based diagnostic techniques adequate to identify species of aquatic organisms (fish, shellfish and molluscs) in products of which other techniques, such as protein methods are inappropriate.
METHODOLOGY: Various techniques to isolate and distinguish DNA sequences such as RFLP, SSCP, specific probes and sequencing.
COMMENTS: This project has the aim to set up a DNA computer database for the identification of fishery products.

Study 7 (1997)
LABORATORY/RESEARCHER: Dept. of Fisheries technology, IPIMAR / 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/RESEARCHERS: Laboratory of Genetics, Ichthyology, Universitat de Girona, Girona / J.L. García-Marín, C. Pla, M.I. Roldán, M.Sanz, M. Cortey and J. Viñas.
SPECIES: *Salmo trutta.*
PROJECT FUNDING: AGF98-0636 (Spain).
OBJECTIVE: Use of the mitochondrial D-loop region as genetic marker on the study of the biogeography and management of Spanish populations.
METHODOLOGY: Amplification of D-loop by PCR and sequencing.
STATUS: Two years project:1998-2000.

Study 2
LABORATORY/RESEARCHERS: Laboratory of Genetics, Ichthyology, Universitat de Girona, Girona / C. Pla; J.M. Pujolar; J. Viñas and M.I. Roldán.
SPECIES: Bluefin tuna.
PROJECT FUNDING: Project 95/010 (DG-XIV,CEE).
OBJECTIVE: Genetic analysis to study the stock structure of juvenile bluefin tuna in the Mediterranean.
METHODOLOGY: Protein electrophoresis and DNA amplification and sequencing.
STATUS: Two years project:1996-1998.

Study 3
LABORATORY/RESEARCHERS: Laboratory of Genetics, Ichthyology, Universitat de Girona, Girona / C. Pla, J.M. Pujolar and J. Viñas.
SPECIES: *Coryphaena hippurus* and *C. equiselis.*
PROJECT FUNDING: Project 95/073 (DG-XIV,CEE).
OBJECTIVE: Genetic analysis to study the stock structure of dolphin fish in the Mediterranean and Central-East Atlantic.

METHODOLOGY: Protein electrophoresis and, DNA amplification and sequencing.
STATUS: Two years project:1996-1998.

Study 4
LABORATORY/RESEARCHERS: Department of Ecology, Comunidad Autónoma de Madrid, Madrid and Laboratory of Genetics, Ichthyolgy, Universitat de Girona, Girona / A. Almodovar, A. Machordom, C. Pla, J.L. García-Marín and N. Sanz.
SPECIES: *Salmo trutta.*
PROJECT FUNDING: Project SC95-005 (Spain).
OBJECTIVE: Genetic and ecological analyses to study the stock structure of brown trout in the Central System in Spain.
METHODOLOGY: Protein electrophoresis and DNA amplification and sequencing.
STATUS: Three years project:1995-1998.

Study 5
LABORATORY/RESEARCHERS: Grupo Genética Molecular, Dpto. Genética-Fac., Ciencias, Granada.
SPECIES: *Sparidae.*
PROJECT FUNDING: DIGICYT PB96-1402.
OBJECTIVE: Analysis functional and evolution of satellite DNA in order to clarify the phylogeny of *Sparidae.*
METHODOLOGY: Satellite DNA.
STATUS: 1997-2000.

Study 6
LABORATORY/RESEARCHERS: Grupo Genética Molecular, Univ. Granada and CICEM, Aguas del Pino.Huelva.
SPECIES: *Perkinsus atlanticus* and clams.
PROJECT FUNDING: JACUMAR.
OBJECTIVE: To get a molecular method for genetic diagnosis of *Perkinsus atlanticus* in clams.
METHODOLOGY: Ribosomal DNA.
STATUS: 1998-1999.

Study 7
LABORATORY/RESEARCHERS: Departamento de Biologia, Universidad de Malaga, Celular y Genetica, Malaga / M. Carmen Alvarez.
SPECIES: *Sparus aurata* and other sparids.
PROJECT FUNDING: AIR 3-CT94-1926.
OBJECTIVE: A comprehensive genetic study of cultured and wild stocks of gilthead sea bream (*Sparus aurata*) and genetic assessment of several related species as candidates for aquaculture.
METHODOLOGY: Microsatellites, allozymes, mitochondrial DNA.
STATUS: Three and a half year project, ended 1998.

Study 8
LABORATORY/RESEARCHERS: Departamento de Biologia, Universidad de Malaga, Celular y Genetica, Malaga./Instituto Espanol de Oceanografia (Mazarron, Murcia) / M. Carmen Alvarez and A. Garcia.
SPECIES: *Dicentrarchus labrax.*
PROJECT FUNDING: CICYT MAR-1831-CO2-01.
OBJECTIVE: To study the influence of genetic factors and the temperature on the growth of the sea bass.
METHODOLOGY: Histology, fish rearing, measuring growth parameters, microsatellites.
STATUS: Three year project, ended 1998.

Study 9
LABORATORY/RESEARCHERS: Departamento de Biologia, Universidad de Malaga, Celular y Genetica, Malaga / M. Carmen Alvarez.
SPECIES: *Dicentrarchus labrax.*
PROJECT FUNDING: FAIR CT97 3886.
OBJECTIVE: Assessment of procedures for the development of a European standardised multi-site testing programme: Application to seabass.
SPECIES: *Dicentrarchus labrax.*
STATUS: Two year project Concerted Action ending 1999.

Study 10
LABORATORY/RESEARCHERS: Departamento de Biologia Funcional, Area de Genética, Universidad de Oviedo. / J.A. Sánchez Prado, G. Blanco Lizana and E. Vazquez Menendez.
SPECIES: Turbot.
PROJECT FUNDING: DGICYT PB94/1348.
OBJECTIVE: To develop microsatellite primers for this species and chromosomal manipulation.
METHODOLOGY: Microsatellite DNA loci. Polyploidy.
STATUS: Three year project started 1995.

Study 11
LABORATORY/RESEARCHERS: Departamento de Biologia Funcional, Area de Genética, Universidad de Oviedo / G. Blanco Lizana, J.A Sánchez Prado and E. Vazquez Menendez.
SPECIES: Atlantic salmon.
PROJECT FUNDING: EU-96-036.
OBJECTIVE: To use microsatellite in this species to search for inter population variability throughout the range.
METHODOLOGY: Microsatellite DNA loci.
STATUS: Two year project started 1997.

Study 12
LABORATORY/RESEARCHERS: Departamento de Biologia Funcional, Area de Genética, Universidad de Oviedo. / G. Blanco Lizana, J.A. Sánchez Prado and E. Vazquez Menendez.
SPECIES: Atlantic salmon.
PROJECT FUNDING: DGE-UE-98-0017.
OBJECTIVE: To use microsatellite in this species to search for inter population variability in Spanish population.
METHODOLOGY: Microsatellite DNA loci.
STATUS: Two year project started 1997.

Study 13
LABORATORY/RESEARCHERS: Departamento de Biologia Funcional, Area de Genética, Universidad de Oviedo. / J.A. Sánchez Prado, G. Blanco Lizana and E. Vazquez Menendez.
SPECIES: *Salmo trutta.*
PROJECT FUNDING: DGE-PB-97-1299.
OBJECTIVE: To use microsatellite variation as genetic marker on the study of the diversity and management of Spanish populations.
METHODOLOGY: Microsatellite DNA loci.
STATUS: Three year project started 1998.

Study 14
LABORATORY/RESEARCHERS: Departamento de Biologia Funcional. Area de Genética, Universidad de Oviedo / J.A. Sánchez Prado, G. Blanco Lizana and E. Vazquez Menendez.
SPECIES: *Salmo trutta.*
PROJECT FUNDING: Project CN-94-131-D1.
OBJECTIVE: Genetic analyses to study the stock structure and stocking effects on brown trout populations of Navarra rivers, Spain.
METHODOLOGY: Protein electrophoresis and microsatellite variation.
STATUS: Six year project started 1994.

Study 15
LABORATORY/RESEARCHERS: Departamento de Biologia Funcional, Area de Genética, Universidad de Oviedo / J.A. Sánchez Prado, G. Blanco Lizana and E. Vazquez Menendez.
SPECIES: *Salmo trutta.*
PROJECT FUNDING: Project CN-96-135-B1.
OBJECTIVE: Genetic analyses to study the stock structure and stocking effects on brown trout populations of León rivers, Spain.
METHODOLOGY: Protein electrophoresis and microsatellite variation
STATUS: Three year project started 1996.

Study 16
LABORATORY/RESEARCHERS: Departamento de Biologia Funcional, Area de Genética, Universidad de Oviedo / J.A. Sánchez Prado, G. Blanco Lizana and E. Vazquez Menendez.
SPECIES: *Salmo trutta.*

PROJECT FUNDING: Project CN-98-179-B1.
OBJECTIVE: Genetic analyses to study the stock structure and stocking effects on brown trout populations of Vizcaya rivers, Spain.
METHODOLOGY: Protein electrophoresis and microsatellite variation.
STATUS: Three-year project started 1998.

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: Allele and haplotype frequencies are used to study spatial and temporal genetic diversity among salmon populations.
METHODOLOGY: Allozymes and DNA.
STATUS: Ongoing, long term study.

Study 2
LABORATORY/RESEARCHER: SLU- Dept of Aquaculture / J. Nilsson.
SPECIES: Arctic charr.
PROJECT FUNDING: EC, Swedish Council for Forestry and Agricultural Research.
OBJECTIVE: Develop sustainable aquaculture of Arctic charr, develop breeding plan for Arctic char in European aquaculture.
DESIGN: Genotype- environment interactions are studied using family structured breeding populations replicated and reared in different fish-farms. Importance of variation in genes with potential effects on economically important traits is studied in domesticated strains as an attempt to obtain useful genetic markers for breeding.
METHODOLOGY: Quantitative and molecular genetics.
STATUS: Third year.

Study 3
LABORATORY/RESEARCHERS: Institute of Freshwater Research, Fisheries Board of Sweden / L. Edsman and B. Ekstrand.
SPECIES: Noble crayfish (*Astacus astacus*), Signal crayfish (*Pacifastacus leniusculus*).
PROJECT FUNDING: Carl Tryggers Foundation.
OBJECTIVE: Mapping genetic variation in the native noble crayfish-genotypical diversity for biologically relevant crayfish management and policy.
DESIGN: Samples of noble crayfish populations from different geographical regions in Sweden are collected and tested for genetic differences in the laboratory.
METHODOLOGY: PCR, RFLP in mtDNA, microsatellites.
STATUS: Just started, first report describing methodology for sampling and for getting a PCR product ready.

Study 4
LABORATORY/RESEARCHERS: National Board of Fisheries, Institute of Freshwater Research, Laboratory of Fish Genetics / T. Järvi, E. Petersson, J. Dannewitz and L. Johansson. Division of Population Genetics, Stockholm University / N. Ryman.
SPECIES: Atlantic salmon, brown trout.
PROJECT FUNDING: N. Bd. Fish., Swedish Council for Forestry and Agricultural Research, EC-FAIR.
OBJECTIVE: Reveal any ecological effect of releasing domesticated salmon and trout on wild conspecifics. The study includes quantitative genetics and paternity studies based on nuclear microsatellites and ND1 in mtDNA.
DESIGN: Breeding programs for experiments in aquariums and in nature.
METHODOLOGY: RFLP/mtDNA Microsatellites.
STATUS: A breeding program started -98 and series of experiments have been effectuated to reveal genetic contribution to life-history traits.

Study 5
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: Ongoing, long term study.

Study 6
LABORATORY/RESEARCHERS: Salmon Research Institute / H. Jansson and T. Öst. 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) incidence and direction of hybridisation between Atlantic salmon and brown trout in Swedish rivers, 2) factors that promote hybridisation, and 3) genotypes, survival and fertility of progeny from hybrids backcrossed to the parental species.
DESIGN: Hybrid frequencies are assessed annually in different habitats. The maternal species of the hybrids is determined. First generation hybrids and backcross individuals are used in crossing experiments. Parts of the project are performed in cooperation with T. Järvi, E. Petersson and B. Ragnarsson / National Board of Fisheries.
METHODOLOGY: Allozymes and mitochondrial DNA.
STATUS: Three year study 1997-1999. Final report 2000 / 2001.

Study 7
LABORATORY/RESEARCHERS: Department of Zoology, Uppsala University / J. Dannewitz. Institute of Freshwater Research, National Board of Fisheries / E. Petersson.
SPECIES: Atlantic salmon (*Salmo salar*).
PROJECT FUNDING: Institutional funding and private funding.
OBJECTIVE: To investigate the influence of egg size on future growth, survival and life history adoption in Atlantic salmon. To test new methods for releasing hatchery produced Atlantic salmon.
DESIGN: The experiments will be conducted in natural and semi-natural streams.
METHODOLOGY: Microsatellites will be used as markers in the parentage-studies.
STATUS: One field experiment has been initiated. The laboratory work will start in autumn 1998.

Study 8
LABORATORY/RESEARCHER: Division of Population Genetics, Stockholm University / N.Ryman.
SPECIES: Brown trout (model organism).
PROJECT FUNDING: Swedish Natural Science Research Council (NFR).
OBJECTIVE: Long-term genetic/ecological study of natural brown trout populations in a protected area in northern Sweden. The aim is to illuminate how natural populations function genetically. Several issues have been addressed using the data collected so far, and the results will be of practical significance for fish conservation in general. For instance, theory developed at the Division for estimating effective population size when generations are overlapping has been applied to empirical data accumulated within the project.
DESIGN: The same natural and introduced populations are sampled annually. Data on age, sex, length, weight are collected for every individual. Tissue samples (muscle, liver, eye) is collected for every individual.
METHODOLOGY: Allozyme and, partly, mtDNA analyses. Theory development, statistical analyses.
STATUS: Ongoing, long term study.

Study 9
LABORATORY/RESEARCHERS: Division of Population Genetics, Stockholm University / N.Ryman and L. Laikre
SPECIES: Brown trout (model organism).
PROJECT FUNDING: Foundation for Strategic Environmental Research (MISTRA).
OBJECTIVE: The release of hatchery fish into the wild (stocking) is practised extensively within the field of fishery management. Stocking may result in a series of genetic interactions between the hatchery-bred fish and natural populations. The genetic integrity of wild populations is threatened not only when releasing fish with an exotic genetic background - loss of genetic variation may occur also when the released fish originate from, or belong to, the recipient population (so-called supportive breeding). Nevertheless, the genetic effects of breeding-release activities on the genetic composition of natural populations are poorly understood. The aim of the project is to produce information that makes it possible to reduce or eliminate the harmful effects on biodiversity on the gene level that are potentially inherent to stocking activities. Anadromous brown trout populations from the Baltic Sea (Gotland) will be used as a model system.
DESIGN: Theory development and computer simulations. Biochemical analyses of brown trout tissues samples collected from populations at Gotland.
METHODOLOGY: Theory development, primarily allozyme analysis.
STATUS: Ongoing.

Study 10
LABORATORY/RESEARCHER: Division of Population Genetics, Stockholm University / N.Ryman.

SPECIES: Brown trout (model organism).
PROJECT FUNDING: Swedish Natural Science Research Council (NFR).
OBJECTIVE: Studies of molecular genetic markers have added relatively little to the understanding of the genetic basis for variation in phenotypic traits. Here the existence of genetically determined phenotypic differences between populations of brown trout that are divergent at electrophoretically detectable protein loci is investigated.
DESIGN: Genetically tagged individuals from two stocks exhibiting behavioural and ecological differences have been introduced into a drainage system previously void of brown trout. In the common environment the presence of phenotypic differences among different groups of offspring is expected to reflect genetically determined dissimilarities between the original stocks.
METHODOLOGY: Allozyme analyses, statistical evaluation of genetic and morphological/ecological data.
STATUS: Ongoing, long term study.

Study 11
LABORATORY/RESEARCHER: Division of Population Genetics, Stockholm University / N.Ryman.
SPECIES: Brown trout (model organism).
PROJECT FUNDING: Swedish Natural Science Research Council (NFR).
OBJECTIVE: Release of genetically modified organisms poses a potential threat to wild populations. Important information on the spread of genes can be obtained through the study of gene introgression via organisms, which are not genetically altered. By not using "real" transgenic organisms risks are avoided and costs minimised.
DESIGN: Two genetically different stocks of brown trout have been translocated into a natural lake system. The introgression of genes from these stocks to naturally occurring brown trout populations is studied.
METHODOLOGY: Allozyme analyses, computer simulations, statistical evaluations.
STATUS: Ongoing, long term study.

Study 12
LABORATORY/RESEARCHER: Division of Population Genetics, Stockholm University / L. Laikre.
SPECIES: Brown trout (model organism).
PROJECT FUNDING: County administrative board of Värmland (Länsstyrelsen, Värmland).
OBJECTIVE: To address the problems of monitoring biological diversity at the gene level using natural brown trout populations in the Province of Värmland.
DESIGN: Biochemical analyses of tissue samples collected from selected brown trout populations.
METHODOLOGY: Primarily allozyme analysis, statistical evaluations, computer simulations.
STATUS: Ongoing.

Study 13
LABORATORY/RESEARCHER: Division of Population Genetics, Stockholm University / L. Laikre.
SPECIES: Brown trout (model organism).
PROJECT FUNDING: Erik Philip-Sörensens Foundation.
OBJECTIVE: Information regarding the temporal dynamics of alleles at genetic marker loci in natural populations is exceedingly sparse. Typically, population genetic investigations include sampling at one particular occasion only. This fairly limited knowledge of the extent of temporal variation of DNA markers influences the interpretation of observed spatial patterns; it is largely unclear if they are stable over time. In this project temporal shifts of mtDNA haplotypes in natural brown trout populations in the Province of Jämtland is studied. The amount of genetic drift over several consecutive cohorts (year classes) is quantified and provides the basis for estimating female effective size in these populations. The extent of mtDNA haplotype frequency change is compared with the corresponding allele frequency changes at allozyme loci for the same populations and cohorts.
DESIGN: Tissue sample collections from natural brown trout populations over several years. Biochemical analyses of the samples followed by data analyses involving application of theoretical developments provided by other projects at the Division.
METHODOLOGY: Primarily mtDNA analyses using PCR and restriction enzyme analysis, statistical evaluations, computer simulations.
STATUS: Ongoing.

Study 14
LABORATORY/RESEARCHERS: Salmon Research Institute / T. Öst and H. Jansson.
SPECIES: Pike-perch (*Stizostedion lucioperca*).
PROJECT FUNDING: National funds.
OBJECTIVE: To develop genetic markers to study interpopulation variability among Swedish populations.
METHODOLOGY: Allozymes and DNA.
STATUS: Just started.

Study 15
LABORATORY/RESEARCHERS: National Board of Fisheries, Institute of Freshwater Research, Laboratory of Fish Genetics / T. Järvi and L. Johansson. Division of Population Genetics, Stockholm University / L. Laikre.
SPECIES: Anadromous brown trout.
PROJECT FUNDING: N. Bd. Fish. and EUGFJ.
OBJECTIVE: Develop tourism on sustainable sport-fishing based on biological principles. The study includes population ecological and genetic analyses on sea trout from eleven Gotland streams.
DESIGN: Sampling by electrofishing for the assessment of population dynamics and genetic variation within and between streams.
METHODOLOGY: RFLP/ND1 in mtDNA and nuclear microsatellite analyses.
STATUS: Terminated in 1999.

Study 16
LABORATORY/RESEARCHERS: National Board of Fisheries, Fishery Experimental Station, Kälarne / J. Henricson and T. Andersson. Salmon Research Institute / H. Jansson.
SPECIES: Atlantic salmon (landlocked River Gullspång stock), brown trout.
PROJECT FUNDING: National Board of Fisheries.
OBJECTIVE: To develop and manage captive breeding programs for conservation (living gene banks).
METHODOLOGY: Establishment and breeding of endangered stocks in fish culture according to breeding programmes designed to maintain genetic diversity.
STATUS: Long term project.

Study 17
LABORATORY/RESEARCHERS: National Board of Fisheries, Fishery Experimental Station, Kälarne / J. Henricson and T. Andersson. With partners within the Board of Fisheries.
SPECIES: Atlantic salmon.
PROJECT FUNDING: The hydro-power industry and EU.
OBJECTIVE: Nationwide preservation of salmon stocks based on deep freezing of sperm.
METHODOLOGY: Males from approx. 18 rivers are collected each autumn and sperm taken for deep freezing in liquid nitrogen (cryopreservation, pellet method).
STATUS: 5 years of sampling. Long term storage in "frozen gene bank".

Study 18
LABORATORY/RESEARCHER: SLU- Dept of Aquaculture / J. Nilsson.
SPECIES: Atlantic salmon.
PROJECT FUNDING: Swedish Council for Forestry and Agricultural Research.
OBJECTIVE: Analysis of population structure of Baltic salmon (*Salmo salar*).
METHODOLOGY: mtDNA RFLP and microsatellite loci.
STATUS: One year project (1999).

Study 19
LABORATORY/RESEARCHERS: SLU- Dept of Aquaculture / J. Nilsson. Salmon Research Institute / H. Jansson and T. Öst. Cooperation with Finnish and Estonian research groups.
SPECIES: Atlantic salmon.
PROJECT FUNDING: Swedish Council for Forestry and Agricultural Research.
OBJECTIVE: Phylogeography of Eastern Atlantic and Baltic salmon (*Salmo salar*).
METHODOLOGY: MtDNA RFLP and sequencing.

Study 20
LABORATORY/RESEARCHERS: SLU- Dept of Aquaculture / J. Nilsson and J. Carlsson.
SPECIES: Brown trout.
PROJECT FUNDING: EU structural funds.
OBJECTIVE: Reveal patterns of fine-scale genetic variation in the R. Ammerå system.
METHODOLOGY: Microsatellite loci.
STATUS: Fourth year.

UNITED KINGDOM, NORTHERN IRELAND

Study 1
LABORATORY/RESEARCHER: School of Biology and Biochemistry, The Queen's University of Belfast / A. Duguid, P. Prodöhl and A. Ferguson.

SPECIES: Brown trout (*Salmo trutta*).
PROJECT FUNDING: Fisheries Society of the British Isles Studentship 1998 - 2001.
OBJECTIVE: To determine the extent of population structuring within and among brown trout in large freshwater lakes in Scotland.
DESIGN: Population sampling of major lake systems. Population genetic analysis.
METHODOLOGY: Allozymes, mtDNA RFLPs, microsatellites, specific nuclear gene RFLPs and sequencing.
STATUS: Started October 1997.

Study 2
LABORATORY/RESEARCHER: School of Biology and Biochemistry, The Queen's University of Belfast / R. Hynes, P. Prodöhl and A. Ferguson.
SPECIES: Brown trout (*Salmo trutta*).
PROJECT FUNDING: Internal University funds.
OBJECTIVE: To determine the extent of population structuring and postglacial colonisation patterns for brown trout in Britain and Ireland.
DESIGN: Sampling of unstocked freshwater and anadromous populations. Population genetic analysis.
METHODOLOGY: MtDNA RFLPs and sequencing; transferrin and LDH sequencing.
STATUS: Ongoing.

Study 3
LABORATORY/RESEARCHER: School of Biology and Biochemistry, The Queen's University of Belfast / P. Prodöhl, R. Hynes and A. Ferguson. In collaboration with the Salmon Research Agency of Ireland / P. McGinnity.
SPECIES: Atlantic salmon (*Salmo salar*).
PROJECT FUNDING: Internal funding.
OBJECTIVE: To determine the survival at sea and homing abilities of Atlantic salmon of native, farmed and hybrid parentage.
DESIGN: Four groups were reared in common environment in hatchery and released to sea at smolt stage. Adults sampled in coastal drift nets and on return to freshwater in fixed traps. Parentage is being determined by DNA profiling.
METHODOLOGY: Standard fisheries measurements, microsatellite and minisatellite DNA profiling.
STATUS: Due for completion June 1999.
COMMENTS: Follow on from juvenile freshwater project, which was funded by EU-FAIR.

Study 4
LABORATORY/RESEARCHER: School of Biology and Biochemistry, The Queen's University of Belfast / P. Prodöhl and A. Ferguson. Salmon Research Agency of Ireland / P. McGinnity.
SPECIES: Atlantic salmon (*Salmo salar*).
PROJECT FUNDING: Internal funds used to support initial work.
OBJECTIVE: To determine the genetic impact of hybridisation between wild and farmed Atlantic salmon on native populations through an assessment of the performance of second generation hybrids and backcrosses.
DESIGN: The freshwater and marine performance of F2 hybrids and backcrosses of F1 hybrids to both wild and farmed stocks is being assessed.
METHODOLOGY: Standard fisheries measurements, microsatellite DNA profiling.
STATUS: Ongoing.
COMMENTS: Further funding being sought.

Study 5
LABORATORY/RESEARCHER: School of Biology and Biochemistry, The Queen's University of Belfast / P. Prodöhl and A. Ferguson. IMR, Bergen, Norway / K. Jorstad. Shellfish Research Laboratory, Galway, Ireland / J. Mercer. Aristotle University of Thessaloniki, Greece / C. Triantaphyllidis.
SPECIES: European lobster (*Homarus gammarus*).
PROJECT FUNDING: EU FAIR; IMR internal funds.
OBJECTIVE: To develop microsatellite and mitochondrial DNA markers and to optimise screening conditions to enable high-resolution studies of European lobster genetics. To determine the contribution of ranched individuals in mixed wild and ranched harvests and the potential genetic impact of stock management and enhancement on natural populations. To elucidate the breeding structure in various European lobster populations.
DESIGN: Population samples are being obtained from throughout the native range. Eggs from "berried" females are being examined for parentage. Genetic impact of ranching is being assessed using genetic tags.
METHODOLOGY: Standard fishery measurements, microsatellites, mtDNA RFLPs.
STATUS: Ongoing.

Study 6
LABORATORY/RESEARCHER: School of Biology and Biochemistry, The Queen's University of Belfast / P. Prodöhl and A. Ferguson. Danube Delta Institute, Romania / R. Suciu.
SPECIES: Sturgeons (*Acipenser, Huso*).
PROJECT FUNDING: Royal Society.
OBJECTIVE: To determine the genetic population structure of endangered sturgeon species of the Lower Danube.
DESIGN: Biopsy tissue samples will be obtained from ascending individuals in the lower part of the river. Individuals will be given ultrasonic tags to determine final spawning locations.
METHODOLOGY: Microsatellites, mtDNA RFLPs.
STATUS: Ongoing.

Study 7
LABORATORY/RESEARCHER: School of Biology and Biochemistry, The Queen's University of Belfast & Agriculture and Environmental Science Division, Department of Agriculture for Northern Ireland / D. Booth, P. Prodöhl and W.W. Crozier.
SPECIES: Atlantic salmon (*Salmo salar*).
PROJECT FUNDING: DANI (Department of Agriculture for Northern Ireland).
OBJECTIVE: To quantify current genetic diversity within and among Northern Irish populations of Atlantic salmon prior to the salmonid enhancement project, and to examine their spatial and temporal stability.
DESIGN: Population sampling of all major River systems in Northern Ireland, Analysis of available scale sets, Standard fisheries measurements, relatedness and population genetic analysis.
METHODOLOGY: Microsatellite profiling, mtDNA RFLPs.
STATUS: Started October 1998.

UNITED KINGDOM, SCOTLAND

Study 1
LABORATORY/RESEARCHER: Molecular Genetics Section, Fish Cultivation Team, FRS Marine Laboratory, Aberdeen / E. Verspoor. In collaboration with others.
SPECIES: Monkfish.
PROJECT FUNDING: Scottish Office, Atlantic Salmon Trust, INTAS (pending).
OBJECTIVE: To investigate population structuring of the species in the Northeast Atlantic.
DESIGN: Screening of geographical samples stratified by age.
METHODOLOGY: Microsatellites.
STATUS: Started January 1999.

Study 2
LABORATORY/RESEARCHER: Molecular Genetics Section, Fish Cultivation Team, FRS Marine Laboratory, Aberdeen / E. Verspoor (project leader). Department of Zoology, University of Aberdeen. Scottish Agricultural College, Edinburgh.
SPECIES: *Salmo salar.*
PROJECT FUNDING: Scottish Office, NERC, Scottish Salmon Growers Association
OBJECTIVE: To develop the scientific basis for the application of molecular markers to the selective breeding of Atlantic salmon.
DESIGN: Research into three areas of molecular marker development and application: pedigree analysis, assessment of genetic diversity, and assessment of breeding merit using QTLs.
METHODOLOGY: Microsatellites; allozymes, mtDNA and minisatellites.
STATUS: Ongoing.

Study 3
LABORATORY/RESEARCHER: Molecular Genetics Section, Fish Cultivation Team, FRS Marine Laboratory, Aberdeen and Department of Cell and Molecular Biology, University of Aberdeen / E. Verspoor, P.J. Wright and N. Haites.
SPECIES: *Ammodytes marinus; Melanogrammus aeglefinus.*
PROJECT FUNDING: Scottish Office.
OBJECTIVE: To identify optimal molecular markers for marine fish population structure studies.
DESIGN: To identify variation in the coding and non-coding regions of the DNA of growth hormone and tranferrin genes and compare their utility in resolving population subdivisions.
METHODOLOGY: Minisatellites, cDNA libraries, DNA sequencing.
STATUS: Ongoing.

Study 4
LABORATORY/RESEARCHER: Molecular Genetics Section, Fish Cultivation Team, FRS Marine Laboratory, Aberdeen / E. Verspoor. Centro Ictiológico de Arredondo, Spain / C. Garcia de Leaniz.
SPECIES: *Salmo salar.*
PROJECT FUNDING: EU, Scottish Office, British Council.
OBJECTIVE: To gain insight into the genetic consequences of deliberate or inadvertent transfers of salmon from one river to another.
DESIGN: Transplantation and monitoring of genetically marked groups of fish using common garden experiments
METHODOLOGY: MtDNA, allozymes, minisatellites.
STATUS: Ongoing.

Study 5
LABORATORY/RESEARCHER: Molecular Genetics Section, Fish Cultivation Team, FRS Marine Laboratory, Aberdeen / E. Verspoor. In collaboration with others.
SPECIES: *Salmo salar.*
PROJECT FUNDING: Scottish Office, Atlantic Salmon Trust, INTAS (pending).
OBJECTIVE: To investigate into the phylogenetics and phylogeography of Atlantic salmon across the species range.
DESIGN: Collation of published and unpublished genetic data; selected sampling of new locations; synthetic analysis of data.
METHODOLOGY: Microsatellites, mtDNA, allozymes.
STATUS: Ongoing.

Study 6
LABORATORY/RESEARCHER: Molecular Genetics Section, Fish Cultivation Team, FRS Marine Laboratory, Aberdeen / C. Cunningham (project leader).
SPECIES: *Gyrodactylus salaris.*
PROJECT FUNDING: EU, Scottish Office.
OBJECTIVE: To resolve taxanomic groups at the specific and intraspecific level to facilitate pathogen detection.
DESIGN: Analysis of DNA sequence variation among parasites associated with different hosts and the same hosts in different geographical regions focusing on ribosomal and mitochondrial DNA.
METHODOLOGY: DNA sequencing, RFLP analysis.
STATUS: Ongoing.

Study 7
LABORATORY/RESEARCHER: Molecular Genetics Section, Fish Cultivation Team, FRS Marine Laboratory, Aberdeen / C. Cunningham.
SPECIES: Various.
PROJECT FUNDING: Scottish Office.
OBJECTIVE: To develop novel, rapid, sensitive methods for the detection of pathogens in fish tissues.
DESIGN: Sequencing of pathogen DNA and development of species specific PCR detection method.
METHODOLOGY: DNA sequencing, RFLP analysis.
STATUS: Ongoing.

Study 8
LABORATORY/RESEARCHER: Molecular Genetics Section, Fish Cultivation Team, FRS Marine Laboratory, Aberdeen / M. Snow and C. Cunningham.
SPECIES: Rhabdovirus.
PROJECT FUNDING: Scottish Office, EU.
OBJECTIVE: To determine if different species specific or geographically distinct strains of Rhabdoviruses exist in relation to VHS outbreaks in marine species.
DESIGN: Culture of Rhabdoviruses from different species and locations and sequencing of genes.
METHODOLOGY: DNA sequencing.
STATUS: Ongoing.

Study 9
LABORATORY/RESEARCHER: Gatty Marine Laboratory, University of St. Andrews, St. Andrews. Molecular Genetics Section, Fish Cultivation Team, FRS Marine Laboratory, Aberdeen / I. Johnstone and A. McLay.
SPECIES: *Salmo salar, Salmo trutta.*
PROJECT FUNDING: NERC, Scottish Office, British Council.
OBJECTIVE: To determine the effect of incubation temperature on early growth and muscle development.
DESIGN: Comparison of parameters among genetically tagged families and populations reared under controlled hatchery conditions and under ambient conditions in the wild.

METHODOLOGY: Microsatellites.
STATUS: Ongoing.

Study 10
LABORATORY/RESEARCHER: FRS Marine Laboratory, Aberdeen / A. McLay.
SPECIES: *Salmo salar.*
PROJECT FUNDING: Scottish Office.
OBJECTIVE: To assess family and population variation in maturation timing.
DESIGN: Comparison of two synchronously spawned, genetically tagged stocks of salmon in a common controlled rearing environment.
METHODOLOGY: Minisatellites; hormone assays.
STATUS: Ongoing.

Study 11
LABORATORY/RESEARCHER: Gatty Marine Laboratory, School of Environmental & Evolutionary Biology, University of St Andrews, St Andrews, / C.D. Todd, K. Wolff and M.G. Ritchie.
SPECIES: *Lepeophtheirus salmonis* (on *Salmo trutta, S. salar*).
PROJECT FUNDING: NERC, U.K. (1997-2000).
OBJECTIVE: (1) Development of molecular markers for population analyses of sea lice infesting wild and farmed salmonids around the Scottish coasts. (2) Quantification of interactions between wild and farmed stocks in terms of infestation dynamics.
DESIGN: Confirmation of marker heritability in laboratory cultures. Screening of parasites from wild and farmed stocks. Time-series analyses of specified populations.
METHODOLOGY: DNA sequencing, RAPD, SCAR, microsatellites.
STATUS: Ongoing.

Study 12
LABORATORY/RESEARCHER: Behaviour, Speciation & Genetics Research Group, University of St Andrews etc / M.G. Ritchie, J. Graves and A.E. Magurran.
SPECIES: Various Mexican Goodeid species.
PROJECT FUNDING: NERC, UK.
OBJECTIVE: (1) To determine levels of genetic differentiation among lineages of goodeid, which differ in the extent of female-controlled sexual selection. (2) To determine the genetic heterogeneity of captive populations of endangered species.
DESIGN: Collection of samples from the wild and captive populations. Development of microsatellite DNA markers and DNA sequencing for genetic analysis.
METHODOLOGY: DNA sequencing, microsatellites, behavioural analysis.
STATUS: Ongoing.

Study 13
LABORATORY/RESEARCHER: Fish Muscle Research Laboratory and Gatty Marine Laboratory, School of Environmental & Evolutionary Biology, University of St. Andrews, St. Andrews. Marine Laboratory, Scottish Office Agriculture. Fisheries Laboratory, Aberdeen. Department of Zoology, University College, Galway, Ireland. Matre Aquaculture Research Station. Havforskingsinstitutett, Norway / I.A. Johnston.
SPECIES: *Salmo salar.*
PROJECT FUNDING: EU.
OBJECTIVE: Minimising the interaction of cultured and wild fish: a comprehensive evaluation of the use of sterile, triploid, Atlantic salmon.
DESIGN: Sampling of fish to assess muscle growth throughout development.
METHODOLOGY: Histology, microscopy and image analysis.
STATUS: Ongoing.

Study 14
LABORATORY/RESEARCHER: Department of Zoology, University of Aberdeen, Aberdeen / P. Boyle and E. Greatorex. In collaboration with others.
SPECIES: *Loligo forbesi* plus others.
PROJECT FUNDING: EU.
OBJECTIVE: To identify molecular markers, which can resolve population structure.
DESIGN: Development of microsatellite loci; screening of wild samples from different geographical areas.
METHODOLOGY: Microsatellite cloning, DNA sequencing, PCR primer development.
STATUS: Ongoing.

Study 15
LABORATORY/RESEARCHER: Department of Zoology, University of Aberdeen, Aberdeen and FRS Marine Laboratory, Aberdeen / N. Bailey, P. Boyle and L. Noble.
SPECIES: *Nephrops norvegicus.*
PROJECT FUNDING: Scottish Office, Aberdeen University.
OBJECTIVE: To identify molecular markers, which can resolve population structure.
DESIGN: Development of microsatellite loci; screening of wild samples from different geographical areas.
METHODOLOGY: Microsatellite cloning, DNA sequencing, PCR primer development, mtDNA RFLPs.
STATUS: Ongoing.

Study 16
LABORATORY/RESEARCHER: FRS Freshwater Fisheries Laboratory, Pitlochry / A. Youngson, J. Taggart plus others.
SPECIES: *Salmo salar.*
PROJECT FUNDING: Scottish Office, MAFF.
OBJECTIVE: To assess within population variation in spawning success, offspring survival and distribution in fish ascending the Girnock Burn, Scotland.
DESIGN: Biopsy of mature fish passing through the Girnock trap, sampling of spawning redds above the trap, electrofishing of post-hatch juveniles.
METHODOLOGY: Minisatellite DNA fingerprinting.
STATUS: ongoing

Study 17
LABORATORY/RESEARCHER: Atlantic Salmon Trust, Pitlochry. FRS Freshwater Fisheries Laboratory, Pitlochry / J. Webb, A. Youngson and J. Taggart.
SPECIES: *Salmo salar.*
PROJECT FUNDING: Atlantic Salmon Trust, Scottish Office.
OBJECTIVE: To study competition among families in relation to parentage, redd location, fry densities and patterns of dispersal and survival in the Baddoch burn, Scotland.
DESIGN: Planting out of families at the eyed ova stage into the burn in artificial incubators followed by sampling of fry and juveniles by electrofishing and in a downstream trap; monitoring of returning adults.
METHODOLOGY: Minisatellite DNA fingerprinting.
STATUS: Ongoing.

Study 18
LABORATORY/RESEARCHER: School of Environmental and Evolutionary Biology and Behaviour, Speciation & Genetics Group, University of St Andrews, St Andrews / A. Magurran, J. Graves and J. Evans.
SPECIES: *Poecilia reticulata.*
FUNDING: PhD studentship University of St Andrews.
OBJECTIVE: The development of microsatellites for the analysis of paternity and for population structure.
DESIGN: Use enriched technique to isolate microsatellite sequences, design primers and test for polymorphism.
STATUS: Ongoing.

Study 19
LABORATORY/RESEARCHER: Genetics and Reproduction Research Group, Institute of Aquaculture University of Stirling, Stirling / B. McAndrew and Adams.
SPECIES: *Salmo salar.*
PROJECT FUNDING: Commercial/NERC.
OBJECTIVE: Inheritance of disease resistance.
DESIGN: 200 families will be monitored under commercial conditions and resistant and susceptible individuals will be identified to family level. Full sib families will be challenged and results correlated with commercial results.
METHODOLOGY: Parentage analysis using microsatellites and controlled disease challenges.
STATUS: Ongoing.

Study 20
LABORATORY/RESEARCHER: Genetics and Reproduction Research Group, Institute of Aquaculture University of Stirling, Stirling / B. McAndrew and N. Bromage.
SPECIES: Atlantic Halibut.
PROJECT FUNDING: Private/BBSRC.
OBJECTIVE: To describe differences in growth and other features in male and female halibut. Identify sex-determination mechanism and develop microsatellite markers for broodstock management.
DESIGN: Compare growth and performance under controlled conditions of farm produced halibut fry.

METHODOLOGY: Chromosome set manipulation, microsatellites.
STATUS: Ongoing.

Study 21
LABORATORY/RESEARCHER: Genetics and Reproduction Research Group, Institute of Aquaculture University of Stirling, Stirling / N. Bromage.
SPECIES: Tilapia / rainbow trout.
PROJECT FUNDING: EC Training and Mobility Grant.
OBJECTIVE: To follow chromosome pairing during meiosis in triploid fish to identify possible sites of sex specific markers.
DESIGN: Follow gonadal maturation in experimentally derived populations during multiple (tilapia) and single (rainbow trout) gonadal maturation cycles.
METHODOLOGY: Supranemal chromosome complex and DNA probing.
STATUS: Ongoing.

Study 22
LABORATORY/RESEARCHER: Genetics and Reproduction Research Group, Institute of Aquaculture University of Stirling, Stirling / D. Penman and B. McAndrew.
SPECIES: *Puntius gonionotus.*
PROJECT FUNDING: DFID Fish Genetics Programme.
OBJECTIVE: Development of monosex culture in Puntius species.
DESIGN: Investigation of the sex determination systems of Puntius species; production and evaluation of monosex female *P. gonionotus.*
METHODOLOGY: Chromosome set manipulation, DNA fingerprinting.
STATUS: Ongoing.

Study 23
LABORATORY/RESEARCHER: Genetics and Reproduction Research Group, Institute of Aquaculture University of Stirling, Stirling / D. Penman.
SPECIES: Common and major carp species.
PROJECT FUNDING: DFID Fish Genetics Programme.
OBJECTIVE: Genetic improvement of Indian and common carp for aquaculture.
DESIGN: Investigation of the present status of *Catla catla* in Karnataka state and development of a genetic improvement programme. Investigation of early sexual maturation and unwanted reproduction of common carp in Karnataka state and development of solutions.
METHODOLOGY: MtDNA, allozymes, microsatellites, chromosome set manipulation.
STATUS: Ongoing.

UNITED KINGDOM (1997)

Study 1
LABORATORY/RESEARCHER: School of Ocean Sciences, University of Wales, Bangor / A. Beaumont and M.D.R. Portilla.
SPECIES: *Mytilus edulis.*
PROJECT FUNDING: CONACYT (Mexico) and UWB (PhD programme).
OBJECTIVE: To investigate the potential genetic effects of the artificial selection of fast growing larvae in hatchery culture.
DESIGN: Series of laboratory matings (mass matings and single family crosses) with subsequent selection for fast and slow growing larvae and eventual allozyme electrophoresis of juveniles.
METHODOLOGY: Larval rearing, allozyme electrophoresis and some DNA analysis.
STATUS: Ongoing - preparation of papers.

Study 2
LABORATORY/RESEARCHER: School of Ocean Sciences, University of Wales, Bangor / A. Altun, A. Beaumont and J. Latchford.
SPECIES: *Mytilus edulis* and *Crassostrea gigas.*
PROJECT FUNDING: Mustafa Kemal University, Turkey & UWB (PhD programme).
OBJECTIVE: To develop gene transfer technologies suitable for bivalves.
DESIGN: Development of suitable insert DNA and its transfection by electroporation of eggs.
METHODOLOGY: Electroporation, cloning, genomic DNA library.
STATUS: Ongoing.

Study 3
LABORATORY/RESEARCHER: School of Ocean Sciences, University of Wales, Bangor / F. Carissan and A. Beaumont. Plymouth Marine Laboratory / R. Pipe. ZENECA Laboratories, Brixham / T. Hutchinson.
SPECIES: *Mytilus edulis* and *Hediste* (Nereis) *diversicolor.*
PROJECT FUNDING: Welcome Trust Ecotoxicology Studentship (PhD programme).
OBJECTIVE: To investigate genetic variability in relation to immunocompetence.
DESIGN: Individuals characterised on the basis of their immunocompetence and correlated to allozyme genotype at enzyme loci.
METHODOLOGY: Immunocompetence measured on the basis of variation in numbers of different blood cell types and their phagocyte capacity in the face of challenge. Allozyme electrophoresis at enzyme loci.
STATUS: Ongoing.

Study 4
LABORATORY/RESEARCHER: School of Ocean Sciences, University of Wales, Bangor / K. Abey, A. Beaumont and J. Latchford.
SPECIES: *Cerastoderm edule*, the cockle.
PROJECT FUNDING: NERC & UWB (PhD studentship).
OBJECTIVE: To investigate population genetic variation over species range.
DESIGN: Develop microsatellite Markers and test on samples from various populations.
METHODOLOGY: Create DNA library, search for and sequence suitable microsatellite Markers. Develop primers and use with PCR to investigate population genetic variation.
STATUS: Ongoing.

Study 5
LABORATORY/RESEARCHER: Molecular Ecology and Fisheries Genetics Laboratory, Department of Biological Sciences, University of Hull, Hull / 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 Ongoing programme of studies designed to assess the impact of selective fishing on levels of population biodiversity.

Study 6
LABORATORY/RESEARCHER: Molecular Ecology and Fisheries Genetics Laboratory, Department of Biological Sciences, University of Hull, Hull / G.R. Carvalho and G. Adcock. British Antarctic Survey, Cambridge / P.G. Rodhouse.
SPECIES: Squid (*Illex argentinus*).
PROJECT FUNDING: Natural Environment Research Council, UK.
OBJECTIVE: To determine the impact of fishing intensity on genetic diversity.
DESIGN: Preserved samples of *I. argentinus* collected from Falkland waters between 1988-1996 will be examined to compare genetic structure over a period that the intensity of fishing has changed markedly, and there have been documented crashes in population size.
METHODOLOGY: Microsatellite analysis of preserved samples will be undertaken to assess levels of genetic diversity and temporal patterns of allele frequencies. Genetic data will be compared with information on the intensity of exploitation, catches landed and estimates of population size.
STATUS: The project will commence in April 1997, and continue for an initial 9 month period.
COMMENTS: This Study will provide one of the first to compare genetic structure in an exploited fishery over a period of major change in the intensity of harvesting. Data will provide some indication of whether the current low levels of genetic diversity are characteristic of relatively unexploited populations, or related to fishery-induced reductions in population size.

Study 7
LABORATORY/RESEARCHER: Molecular Ecology and Fisheries Genetics Laboratory, Department of Biological Sciences, University of Hull, Hull / G.R. Carvalho and C. Turan.
SPECIES: Atlantic herring (*Clupea harengus*).
PROJECT FUNDING: Overseas post-graduate studentship (Turkey) + in-house funding.
OBJECTIVE: To develop novel molecular Markers for stock discrimination of herring.
DESIGN: To develop novel genetic markers in widely-separated populations of herring from the North Sea (esp. Norwegian fjords), Baltic and Canadian waters using novel approaches (Polymerase chain reaction (PCR) based analysis of mitochondrial and nuclear DNA.
METHODOLOGY: PCR-based analysis of mtDNA (ND genes), allozymes and microsatellites, morphometrics and meristics.
STATUS: April 1994 - April 1997.
COMMENTS: All practical studies are now complete, and final stages of analysis are underway. Genetic differentiation (allozymes) between Norwegian fjord herring and coastal stocks, and all samples and Baltic herring. Significant genetic differentiation detected between previously genetically homogeneous samples using microsatellites (e.g. Icelandic herring). Paper in press in *J. Mar. Biol. Assoc. U.K.* (late 1997).

Study 8
LABORATORY/RESEARCHER: School of Biological Sciences, University of Wales Swansea, Swansea / Dr D.O.F. Skibinski.
SPECIES: Mussels (*Mytilus*).
PROJECT FUNDING: NERC.
OBJECTIVE: To analyse growth and gene flow in mussel populations.
DESIGN: Allozyme, nuclear DNA and mitochondrial DNA analysis of diverse populations and species.
METHODOLOGY: As above.
STATUS: Ongoing.

Study 9
LABORATORY/RESEARCHER: School of Biological Sciences, University of Wales Swansea, Swansea / Dr D O F Skibinski.
SPECIES: Aquatic animals.
PROJECT FUNDING: NERC.
OBJECTIVE: To analyse causes of genetic diversity in aquatic animals.
DESIGN: Use of allozyme database.
METHODOLOGY: Statistical and simulation analyses of database.
STATUS: Ongoing.

Study 10
LABORATORY/RESEARCHER: School of Biological Sciences, University of Wales Swansea. Swansea / Dr D O F Skibinski.
SPECIES: Tilapia.
PROJECT FUNDING: ODA.
OBJECTIVE: To produce improved strains for aquaculture in Africa and the Far East.
DESIGN: Selective breeding and chromosome manipulation.
METHODOLOGY: DNA and transgenic technology.
STATUS: Ongoing.

UNITED STATES OF AMERICA

Study 1
LABORATORY/RESEARCHER: Department of Biological Sciences, University of Wisconsin-Milwaukee, Milwaukee, Wisconsin / R.B. Phillips.
SPECIES: Salmonids; rainbow trout; zebrafish.
OBJECTIVE: Research involves the application of molecular and cytogenetic techniques to problems in evolution, conservation and aquaculture of salmonid fishes. Projects related to aquaculture include isolation of genes important in disease resistance in salmonids (Mhc, Nramp), and mapping of the rainbow trout genome.
METHODOLOGY: Molecular cytogenetic techniques; chromosome mapping.
STATUS: Ongoing.

Study 2
LABORATORY/RESEARCHER: NOAA./NMFS/Northwest Fisheries Science Center, Genetics Program, Seattle, Washington / S. Grant and R. Waples.
SPECIES: Pacific salmon, anadromous trout and other fish, including anchovies and sardines; mammals.
OBJECTIVE: Research includes: characterisation of genetic diversity in salmonids of the Pacific Northwest and in marine fishes and mammals; identification and definition of population units for management and conservation, and for use in mixed-stock analysis in fisheries; and investigation of markers for quantitative trait loci.
METHODOLOGY: Protein, DNA and quantitative trait methods are used to characterise genetic diversity in marine fishes and mammals. An extensive protein (allozyme) population baseline has been developed over several years, and continues to be extended and updated, to provide a basis for defining populations units.
STATUS: Ongoing.

Study 3
LABORATORY/RESEARCHER: US Geological Survey, Biological Research Division, Leetown Science Center, Kearneysville, West Virginia / T. King.
SPECIES: Atlantic salmon, lake sturgeon, hard clams (*Mercenaria mercenaria*).
OBJECTIVE: Population genetics and stock identification studies are being conducted on Atlantic salmon. In addition, lake sturgeons are being analysed for population differences. Hard clam populations are being studied to determine any genetic contributions of hatchery stocks to wild populations for stock enhancement.
METHODOLOGY: DNA; Microsatellite DNA seems to be most effective for allelic polymorphisms.
STATUS: Ongoing.

Study 4
LABORATORY/RESEARCHER: NOAA, NMFS, Northeast Fisheries Science Center, Milford Laboratory, Milford, CT / S. Stiles, J. Choromanski and James Hughes.
SPECIES: Bay scallop (*Argopecten irradians*); tautog (*Tautoga onitis*).
OBJECTIVE: Genetic investigations that comprise various approaches for increasing growth and survival rates of the commercially valuable bay scallop, *Argopecten irradians*, are in progress. Projects include mass-selection, inbreeding, population genetics and strain/field evaluations. Information is being developed on population genetics of tautog.
METHODOLOGY: Quantitative genetics of selected traits, cytogenetics, population genetics (allozymes - joint project with the Institute of Oceanology, Qingdao, China / Q-Z Que; DNA and transgenic studies with University of Connecticut, Storrs, Connecticut / T. Chen, J. Crivello and L. Strausbaugh).
STATUS: Ongoing.

Study 5
LABORATORY/RESEARCHER: Rutgers University, Haskin Shellfish Research Laboratory, Port Norris, NJ / X. Guo.
SPECIES: Oysters (*Crassostrea virginica, C. gigas*), hard clam (*Mercenaria mercenaria*) and sea scallops (*Placopecten magellanicus*).
OBJECTIVE: Genetic technology involves selection for disease resistance and superior growth in shellfish for aquaculture. Studies in basic genetics include sex determination, genome evolution, and induced polyploidy in shellfish.
METHODOLOGY: Selective breeding; cytogenetics - triploid, tetraploid, gynogenetic and aneuploid shellfish for aquaculture; molecular genetics - molecular markers, genome mapping; artificial gynogenesis; FISH probes.
STATUS: Ongoing.

Study 6
LABORATORY/RESEARCHER: NOAA, NMFS, Southwest Fisheries Center, Marine Mammal Division, Population Genetics, La Jolla, California / A. Dizon.
SPECIES: Marine mammals, turtles, and fish.
OBJECTIVE: Genetic methods are used to examine population structure of marine mammals, turtles, and fish, in phylogenetic studies for taxonomy and systematics and in stock identification and stress analyses of marine mammals.
METHODOLOGY: Genetic and forensic analyses, molecular genetic techniques; mathematical tools for data interpretation in developing models.
STATUS: Ongoing.

Study 7
LABORATORY/RESEARCHER: NOAA, NMFS, Southeast Fisheries Science Center, Population Genetics, Charleston, South Carolina / C. Woodley.
SPECIES: Scombrids (tunas), sharks, scianids, marine mammals, sea turtles, and oysters.
OBJECTIVE: Molecular genetic techniques are being used to examine population structure of bottle nose dolphins and other marine mammals, and for stock identification of various species.

METHODOLOGY: Molecular genetic techniques - DNA (RFLPs, DNA sequencing, mitochondrial DNA markers); stress proteins.
STATUS: Ongoing.

Study 8
LABORATORY/RESEARCHER: University of Delaware, College of Marine Studies, Lewes, Delaware / P. Gaffney.
SPECIES: Oysters - *Crassostrea virginica., C. gigas* and *C. ariakensis.*
OBJECTIVE: Efforts to improve the productivity of Atlantic coast oyster populations include investigation of non-native species, evaluation of growth and disease resistance, and development of disease resistant oysters. Researchers in the mid-Atlantic US are collaborating on a number of projects involving growth and disease of oysters.
METHODOLOGY: Protein and DNA analyses (microsatellite and single nucleotide polymorphisms), and genome mapping.
STATUS: Ongoing.

Study 9
LABORATORY/RESEARCHER: The University of Connecticut, Biotechnology Center and Department of Molecular and Cell Biology, Storrs, Connecticut /T. Chen, J. Crivello & L. Strausbaugh.
SPECIES: Bay scallops (*Argopecten irradians*).
OBJECTIVE: To develop biochemical and molecular markers, to evaluate genetic diversity and to improve the performance (growth and survival) of bay scallops in aquaculture.
METHODOLOGY: Classical and modern genetic methods have the potential to dramatically improve the aquaculture industry. Studies focus on two general types of molecular genetic approaches: Type I (Randomly Amplified Polymorphic DNAs) and Type II (polymorphisms in known DNA sequences, often flanking regions and introns) markers.
Strategies are being pursued to artificially tag scallops by using transgenic technology to introduce either anonymous DNA or fluorescent reporters into the genome. (Collaboration with Milford Laboratory geneticists).
STATUS: Ongoing.