C.M. 1988/F:39.
Mariculture Committee

International Council for the Exploration of the Sea

Report of ICES Working Group on Genetics

Meeting held in Trondheim, Norway, June 21st 1988.

Present: R. Saunders, (Canada); M.L. Koljonen (Finland); E. Quillet, (France); W. Villwock (Germany, Fed. Rep.); G. Naevdal (Norway); A.M. Dos Santos (Portugal); N.P. Wilkins (Ireland), Chairman.

Attending by Invitation: K. Jorstad (Norway); V. Bye (U.K.); R. Lincoln (U.K.).

The Chairman welcomed the members and invited persons to the meeting.

1. The Meeting considered the new terms of reference approved in 1987 (C. Res. 87/2:44; Appendix 1) and the broader role of the Group. There was general agreement that the Group still has a valuable role to play, especially with regard to wild fisheries as distinct from aquaculture, although it was appreciated that aquacultural practice is likely to have an important impact on wild fisheries.

2. It was agreed that as a first step in monitoring developments in aquacultural genetics (Terms of Reference para a), a short resume would be prepared outlining the main topics of the 3rd International Symposium on Genetics in Aquaculture, then meeting in Trondheim. This resume is attached as Appendix 2.

3. Further reports of individual members on genetic activities within their countries (Terms of reference para b) were presented and noted. These are appended as Appendix 3.

4. There was general agreement that the group should undertake, from time to time, a study of one special topic to be the subject of a special meeting/workshop. The topic proposed as the first special topic was GENE MARKERS. It was agreed to recommend that a special 3 day workshop on this topic be held in Helsinki, Finland in 1990, Dr. M.L. Koljonen undertaking the organisation. Material for this topic would be prepared and collated by the group in 1889 in preparation for the Workshop

5. Members of the Group had become aware that another ICES Committee proposed to hold a meeting in Dublin in May 1989 on the genetic and other effects of aquacultural transfers and escapes. There was general dismay that the Working Group on Genetics (sic) had not been informed or consulted about a matter within its terms of reference by the proposed meeting organisers. The Chairman agreed to consult the Secretary General of ICES and inform him that the Working Group on Genetics was willing to cooperate in whatever way it could, in the organising and general advancement of the proposed meeting.

6. The Chairman indicated that with new terms of reference for the group, he felt that now was a good time for the Chairmanship to pass to some other member. The group expressed its thanks to him for his service as Chairman and agreed unanimously to recommend Prof. W. Villwock be nominated as the Working Group's choice as new Chairman. It was agreed that Prof. Villwock's name be forwarded to the Mariculture Committee for ratification.

7. Recommendations:

(1) That a special 3-day workshop on GENE MARKERS be held in Helsinki, Finland in 1990.

(2) That Prof. W. Villwock be proposed as chairman of the Group and be recommended to the Mariculture Committee for appointment.

Professor N.P. Wilkins
Chairman

APPENDIX 1

TERMS OF REFERENCE OF WORKING GROUP ON GENETICS

C. Res 87/

44 The Working Group on Genetics (Chairman: Prof. N. P. Wilkins) will meet in Trondheim from 20-24 June 1988 during the 3rd International Symposium on Genetics in Aquaculture, with the following revised terms of reference:

a) to monitor developments in aquacultural genetics and to identify those aspects which seem of particular significance for aquaculture and for fisheries in general;

b) to discuss, collate and coordinate the reports of the individual members, and monitor the trends they indicate in the application of genetics in aquaculture and fisheries;

c) to advise on the coordination and advancement of appropriate studies in genetics related to aquaculture and fisheries;

d) to evaluate the genetic impact of the introduction and transfer of aquaculture species on natural gene pools of resident species and populations and recommend strategies for such evaluation.

Session on gene markers

Genes (usually enzyme alleles) which are diagnostic or typical for a certain population or a stock can be used as genetic marks of the stock when analysing the stock composition in mixed stock fisheries or when analysing hatchery programmes and stocking success. Stocks can be marked genetically by distributing a specific allele to a population and by changing the allele frequency intentionally (genetic tagging). In some cases, alleles can be used as markers of some other genes or characteristics in the genome, if linkage can be observed. The use of gene marks is most often discussed in connection with studies on the interaction between wild and artificially produced genepools.

The genetic tag has several advantages when compared to traditional tagging (e.g. the Carlin tag): far more individuals can be tagged, the tag does not affect the fitness of the fish - or the probability of being netted. In addition, because of the heritability of the tag, the contribution of the tagged hatchery fish can be monitored in the offspring of the natural stocks. The gene flow between stocks can be measured. The prerequisite for the use of the tag is that it has no linkage to deleterious alleles. The use of a tag should also be well planned because the number of possible tags within a species is limited.

In Norway a enzyme tag (Pgi, a phosphoglucoisomerase allele) for hatchery-reared cod is planned and also the possibility of a mitochondrial tag has been examined. The Norwegians have also found a one-locus system, a phenotypic mark for brown trout, where the homozygotes for the rare allele are small spotted and can be identified by appearance. The appearance of the heterozygote is intermediate. The French have used the natural differences in gene frequencies when studying the effects of brown trout stocking from the Atlantic to the Mediterranean Sea. The Philippines have studied the connections between quantitative characteristics and enzyme genotypes, and some positive correlations have been found.

In conclusion: Several projects are planned but especially from intentional marking, very few results are yet available.

Session on Immunology

The examples presented in the immunological session of the symposium left no doubt that evidence exists of a strong genetic influence on immune system (e.g. disease resistance, histo-compatibility and haemolytic systems). However, very little is yet known about how this influence is effected in fish.

APPENDIX 2

3rd Internationla Symposium on Genetics in Aquaculture

Trondheim, Norway, June 20-24, 1988.

This was the third Symposium on Genetics in Aquaculture, a series commenced in Galway, Ireland in 1982. There were almost 140 registered participants from more than twenty states.

Papers were divided into three general areas encompassing 8 sessions: gene technology (8); Gene markers (3); Immunology (3); Genetic and environmental interactions (4); phenotypic and genetic parameters (3); Selection (7); Ploidy manipulation (5); Fish species (8). In addition there was an extensive poster session in which authors attended and discussed their presentations with the participants.

In general, the symposium reflected the present-day emphasis being placed on new biotechnology especially on fin fish species. Breeding plans and selection were concentrated on salmonid species as appropriate to the venue of the meeting. The following resumes of the sessions were prepared by various members of the Working Group and its invited participants:

Session on gene technology

This topic has evolved considerably since the previous symposium. Particular emphasis was put on the adjustment of gene transfer technology in fish cells. Indeed, the latter have some features (invisible pronucleus especially) that make experiments from mammalian cells difficult to use directly. The technique of micro-injection into the egg cytoplasm is the most commonly used: it has provided transgenic individuals in several fish species. Nevertheless the exact status of foreign DNA sequences in cells is often unknown: up to now, actual evidence that foreign sequences are integrated in the host genome and are transmitted to the progeny are very few. Moreover, the actuality of the expression of foreign genes is a much debated question. Among the different hypotheses to explain the lack of expression (measured at RNA level or by dosages of the final product), the most commonly proposed is that injected sequences most often contain heterologous promoters (viral or mammalian ones) that might not fit the fish cell environment: the isolation and use of piscine promoters might therefore be helpful in understanding and solving that problem.

Other topics of biotechnology were also discussed. One can quote the molecular cloning of physiological substances (growth hormones, vitellogenin,...) that will be helpful for both basic biological studies and for management of broodstock with production purposes.

between chum and chinook salmon. Also methods for sex reversal by immersion of fry in estradiol or testosterone were described.

Session on fish species

The keynote-lecture given by Snorre TILSETH/(Norway) has been one of few contributions that met with the headline, new species for farming. The excellent paper dealt with cod, halibut and wolf fish. It included a short description on the present aquacultural managment status of these three cold-water fishes, as well as their benefit for commercial use as a protein supply in future.

Another convincing contribution was the one given by WILLIAMSON/(USA) on Micropterus salmoides which was shown to be a worthful species for further cultivation. Other papers were by DELABBIO et al./(Canada), on two different stock s of Canadian Arctic Charrr which were raised and kept under saltwater conditions. Of similar value was the paper given by E. QUILLET et al. (France) on European brown trout. Seven different hatchery strains of brown trout were compared under freshwater and saltwater conditions; whether the strains were of landlocked or anadromous strains (brown-versus sea-trout) was not stated. A second paper on brown trout presented enzyme pattern analyses of strains of trout of "Atlantic" versus "Mediterranean" origin.

Two contributions on Tilapias dealt with cold tolerance and salinity tolerance respectively.

An interesting contribution by Langholz et al (F.R.G) was based on extensive data of different populations of Brachydanio rerio, regarded as a favourite candidate for ectoxicological testing procedures. However, all the characters studied exhibited high intraspecific variability suggesting that they may be of limited value as indicator species. The paper by Eknath (India & Phillipines) was a highly interesting and well delivered historical review of carp culture in India.

Breeding Plans

Under the division "Breeding Plans" were treated the following topics:

(a) Genetic and environmental interaction
(b) Phenotypic and genetic parameters
(c) Selection
(d) Ploidy manipulation and performance

Under a) were presented papers on catfish, rainbow trout, Atlantic salmon and Tilapia. In all these species were found indications of genotype and environmental interactions and an overall conclusion was that it is extremely important to carry out selection experiments under well-defined environmental condition and as close to the actual rearing situation as possible.

Under b) were presented three papers, on Atlantic salmon (2)< and Pacific oysters.

In oysters, additive and non-additive genetic variation and also genetic and environment interaction were found, indicating that the productive traits (size and carbohydrate content) may be improved by genetic methods. In salmon new observations on interactions and correlations between productive traits were presented.

Under the headline of Selection was given a keynote lecture on application of breeding schemes and seven experience papers on salmonids (2), oysters (2) and Tilapias (3). Concerning salmonids, clear effects on selection procedures on growth rate were found, but also positive correlated responses were clearly indicated.

In Tilapias were found complicated interaction between growth rate and behaviour, and indirect selection on behaviour traits may be effective foer growth rate improvement. Also within-family selection may be effective providing that intra-group competition can be minimized.

In oysters, clear responses to selection were found, but it was also emphasized that evaluating and utilizing of stock differences are very important for an effective selection program.

Under the headling of Ploidy Manipulation and Performance were presented four papers, all on fish. Production of androgenetic diploid rainbow trout is now possible by using sperm from tetraploid males. Thus genotypes may be recovered from cryopreserved sperm, which is a ocnsiderable improvement for the establishment of gene banks. Another important aspect of chromosome engineering is production of viable triploids of hybrids, and an example was given of a successful triploid hybrid

APPENDIX 3

REPORTS ON CURRENT GENETIC PROGRAMMES IN MEMBER STATES

Reports prepared by:-

Canada - R. Saunders

Finland - M.L. Kolyonen

France - E. Quillet

Ireland - N.P. Wilkins

Norway - K. Jorstad

Poland - K. Goryczko

CURRENT GENETIC STUDIES ON FISHES IN FINLAND
Report for the ICES Working Group on Genetics June 1988
compiled by Marja-Liisa Koljonen

I) Finnish Game and Fisheries Research Institute, Fisheries Division, P.O. Box 202, SF-00151 Helsinki, Finland

1) Electrophoretic studies on whitefish, M. Heinonen

Genetic polymorphism and its relation to taxonomy, systematics and management was investigated in whitefish from the Lake Saimaa area. Comparisons with earlier estimates of genetic distances throw doupt on the supposed species status of four Lake Saimaa whitefish (Heinonen 1987, Heinonen 1988).

2) Karyological analyses of whitefish, K. Juntunen

The chromosomes obtained from Pokeweed-mitogen blood leucocyte cultures were studied for several whitefish forms from northern Finland and for Coregonus peled Gmelin from the USSR. In C. peled the diploid chromosome number (2n) was 76 and NF 98, and in the Finnish whitefish (C. pallasi Valenciennes) 2n was 80 and NF 102 (Juntunen 1987).

3) Registry of valuable fish stocks in Finland,
I. Kallio-Nyberg

A questionnaire has been issued to Finnish fish specialists (350) with a view to compiling registers of valuable and threatened fish stocks. The whitefish register has been completed and contains information on 181 whitefish stocks, on their origin, the degree to which they are endangered, the threats to the stocks and their management (Kallio-Nyberg and Koljonen 1988).

4) The diversity of quantitative characteristics of Atlantic salmon, I. Kallio-Nyberg

The dynamics of the age and size composition of maturing salmon has been analysed during the periods 1930-1944 and 1976-1986 for three Atlantic salmon stocks. The variation in the age and size of the spawners in different year classes appears to follow the patterns predicted by a life history model based on the developmental plasticity of age and size at maturity (Kallio and Koljonen 1986, Kallio and Pruuki 1987, Kallio-Nyberg and Pruuki 1988).

5) Electrophoretic markers for the whitefish species pair C. peled and C. pallasi, M-L. Koljonen

Diagnostic genetic markers have been developed for brood stock management (Koljonen et al. 1988).

6) Electrophoretic studies on brown trout in northern Finland, M-L. Koljonen

An electrophoretic survey of brown trout stocks in northern Finland is being conducted for breeding and management purposes. Some of the results have been published (Koljonen and Sarjamo 1987).

7) Electrophoretic studies on Atlantic salmon, M-L. Koljonen

Monitoring is in progress of allele frequencies in smolts and spawners of salmon stocks in the Bothnian Bay.

8) Gynogenesis in rainbow trout, M-L. Koljonen, T. Mäkinen

Experimental studies on gynogenesis in rainbow trout were conducted in spring 1988.

9) Electrophoretic studies on grayling (Thymallus thymallus), J. Koskiniemi

An electrophoretic study on Finnish grayling stocks is in progress. Preliminary results have been published (Koskiniemi 1987).

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

1) Evolution of coregonid fish, J. Vuorinen

Studies are being made of the population genetics, evolution and taxonomy of coregonids. They include the amount and distribution of genetic variation in vendace and whitefish, differentiation of species hybrids by electrophoresis, and the resolution of problems encountered in the taxonomy of holarctic coregonid fishes (with J. D. Reist, R. A. Bodaly and others).

2) Genetic variation of Atlantic salmon, J. Vuorinen

The populations studied include landlocked salmon in Finland, and both landlocked and anadromous populations in the River Namsen, Norway (with O. K. Berg), and the largest western European river, the Tana (with K. Elo, University of Turku).

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

1) Mitochondrial DNA analyses of some Salmonidae in Finland, K. Partti-Pellinen, M. Hakumäki, T. Palva*
(* recent address: Dept. of Molecular Genetics, Swedish University of Agricultural Sciences, Box 7003, Uppsala, Sweden)

The purpose of this type of genomic analyses is to monitor and identify some Finnish Salmonidae. Whitefish, grayling and char stocks have been studied from breeding populations of

the Finnish Game and Fisheries Research Institute. The mitochondrial DNA is isolated from fish liver by alkaline extraction and then digested with restriction endonuclease. The fragments obtained are separated by electrophoresis, alternative end labelling can be used. The size determination of mtDNA fragments is performed with a microcomputer analysing system in combination with standards.

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CANADA

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E. M. Donaldson, I. I. Solar, F. Piferer, T. J. Benfy and I. J. Baker

Production of all-female stocks of Pacific salmonids

Two methods for the production of monosex (female) stocks are currently being tested at commercial salmon hatcheries in B. C. These are: direct feminization using estrogen treatment during early ontogenesis and indirect femi[illegible] by using "female sperm" produced by fish which are genoty[illegible]c/phenotypic males. Recently, significant progress has be[illegible]ade in the optimization of estrogen treatment for the production of largely female stocks of coho and chinook salmon by direct feminization. It has been shown that in the period just before and around hatching time the fish are most sensitive to estrogen treatment. Immersion treatments at the time of hatching and one week later are being tested at 9 commercial salmon rearing stations under Experimental Study Certificate issued to the Biol. Sci. Branch by Health and Welfare, Canada.

In the production of monosex stocks by the indirect method, two alternatives have been considered and tested: the production of females by the use of sperm from masculinized genotypic females is now being widely used for the culture of chinook salmon in B. C.; tests are also being conducted of the induction of gynogenesis followed by hormonal masculinization, thereby shortening to one generation the time required to produce "female sperm".

Productiion of sterile Pacific salmonids

The production of neutered Pacific salmonids for aquaculture is being studied using hormonal and a range of other genetic techniques. For details on genetic sterilization see the paragraph on Chromosome Set Manipulation. Most of the recent research on hormonal sterilization at this laboratory has focused on coho and chinook salmon. Current studies are underway an the application of hormonal sterilization procedures developed for coho salmon at 16 commercial mariculture facilities in B. C. The main objective is to investigate doses and form of administration, appropriate time to cease treatment, the interaction of hormonal treatment with seawater adaptation of zero-age coho smolts and the minimization of treatment induced physiological effects. In chinook salmon, higher doses than those required for coho salmon have been used successfully to produce sterile groups which after two years of net-cage rearing have shown good growth and survival.

Chromosome set manipulation

A study of the reproductive physiology of triploid Pacific salmonids has been completed. Triploid females show no endocrine evidence of a reproductive cycle, and remain immature throughout their lives. Triploid males, on the other hand, are no different from

diploids with respect to gonadotropin and sex steroid levels, development of secondary sex characteristics, precocious maturation and post-spawning mortality. However, they produce very dilute milt comprised of aneuploid spermatozoa and, thus, are genetically sterile.

To sterilize fish for aquaculture by induced triploidy, it will be necessary to make all-female triploids. This has been done by treating mixed-sex triploid coho salmon with 17 β -estradiol at the normal time of sex differentiation. The productiion characteristics of sterile coho salmon produced in this way are being evaluated at a commercial salmon farm. All-female triploids can also be produced by inducing triploidy in eggs fertilized with homogametic milt from sex-reversed diploid females. To test this, gynogenetic diploid coho salmon have been masculinized with 17 α -methyltestosterone. These fish should produce homogametic milt when they mature.

Biological Sciences Branch, Fish Culture Research Section, Salmon Genetics Program, Pacific Biological Station, Dept. of Fisheries and Oceans, Nanaimo, B. C.

Ruth Withler, Debra Tuck, W. Craig Clarke and Henrik Kreiberg

A genetic analysis of variation in growth, survival, age-at-maturity and flesh color was conducted among and within four strains of coho salmon(each represented by four pairs of paternal halfsib families) reared under net pen conditions. Adult length and weight measurements were sexually dimorphic and differed among strains. Flesh color and levels of precocious maturation varied among strains, but there was no effect of strain on freshwater of saltwater survival. Heritabilities and genetic correlatiions were calculated among traits and indicated that selection for increased size and flesh pigmentation, but not for increased survival, would be successful.

A genetic analysis of variation in growth, survival and age-at-maturity is being conducted among and within six strains of chinook salmon (each represented by five pairs of paternal halfsib families) reared in net pens at five sites along the coast of B. C. Survival and growth rate in fresh water (to 140 days) differed among stocks and was variable among families within stocks. The heritability of freshwater survival was uniformly low, whereas the heritability of smolt weight ranged from 0.0 to 0.9 among stocks. The saltwater phase of the study is underway and there is evidence accumulating for strong effects of both site and strain on saltwater growth, survival and age-at-maturity.

Inheritance of the red and white flesh color phenotypes in chinook salmon from the Quesnel River, B. C. has been shown to be either a threshold trait with very high heritability or a Mendelian trait controlled by at least two loci. This study demonstrated that chinook salmon may differ markedly from other salmonids studied to date in displaying a dichotomous flesh color variation that is under the control of a few major genes. In addition, quantitative genetic variation likely contributes to the range of flesh pigmentation observed within the red fleshed phenotype. Aquacultural production of the more valuable red-fleshed phenotype will depend primarily on the choice of a broodstock that possesses the appropriate (red) flesh color genotypes.

The effect of pooling milt from several male salmon before application to eggs during hatchery fertilization on the contribution to

fertilization by the contributing males was investigated using chinook salmon from the Big Qualicum River Hatchery. In control crosses, milt from each male was individually applied to determine male fertility, whereas in experimental crosses an equal volume of milt from each of three males was mixed and applied to eggs. Electrophoresis was used to establish paternity among the resulting progeny. In crosses made with pooled milt, the contributioin to fertilization ranged between 1 and 75% for participating males. The contribution of a male was not correlated with his fertiility in control crosses nor with his spermatocrit value and varied depending on which other males contributed to the milt mixture. This study has shown that the use of pooled milt will accelerate the loss of genetic variatiion in hatchery-maintained salmonid populations and should be avoided.

A study to investigate the response to selection for increased size under hatchery production and sea ranching conditions is being conducted on Quinsam River coho salmon. Second generation offspring from a selected (for increased adult length) and a control line of coho at the Quinsam River Hatchery will be released in May 1988. Length measurements made on returning adults (November 1989) will be evaluated to determine if adult length is a heritable trait under these rearing conditions, as judged by the response to selection.

Mortality of an unknown etiology occurs among chinook salmon alevins incubated in the Chehalis River Hatchery. The soft water conditions in the hatchery and an, as yet, unidentified pathogen are apparently responsible for the mortality. A genetic analysis of the variation in survival under Chehalis Hatchery conditions revealed that the Capilano(Big Qualicum) River stock of chinook salmon was more resistant to mortality than was the Harrison River stock. Within stocks, and in interstock hybrids, the influence of the male parent on survival exceeded that of the female parent. For the genes that control resistance to this mortality, expression of the alleles inherited paternally may occur sooner in alevin development than expression of the maternal alleles. Thus, in hatcheries with suboptimal incubation conditions, both the selection of an appropriate stock and the choice of parents within a stock, may be important determinants of culture success.

A preliminary examination of genetic variability in susceptibility to bacterial kidney disease (BKD) revealed no significant differences among five strains of coho salmon. Heritible variation in time-to-death was observed within three strains of coho from southern B. C., but not within two northern strains. A more extensive study of the heritability in one northern and one southern stock, each represented by 20 pairs of halfsib families, is now underway. The possible relationship between transferrin genotype and BKD susceptibility in these stocks is also under investigation.

The genetic control of smoltification in chinook salmon that display different life history patterns in the wild is being investigated. Chinook juveniles from coastal B. C. populations generally migrate seaward in their first spring, and perform well in aquacultural operations in which transfer to saltwater takes place after three or four months of freshwater rearing. Juveniles from interior populations generally do not migrate seaward until their second spring, and require photoperiod and thermal manipulation to perform well when transfer to saltwater takes place in the first spring. The genetic differentiation and genotype-environment interactions under two photoperiods for control of smoltification are being investigated in factorial crosses within and between an interior and a coastal chinook salmon population.

A pilot-scale selection program to increase smoltification success and adult (harvest) weight in coho salmon has been initiated. The baseline population for establishing both a control and a selected line has been created using pure and hybrid families from three coho salmon stocks (Robertson Creek, Quinsam River and Kitimat River). The freshwater and saltwater performance of 120 single pair families will be evaluated at three saltwater rearing sites and the selected and control lines will be established in November 1^39.

Biological Sciences Branch, Salmon Dynamics Section, Pacific Biological Station, Dept. of Fishewries and Oceans, Nanaimo, B. C.

Terry Beacham, Clyde Murray and Wally Barne

Quantitative genetics of pink and chum salmon

Two 5 X 5 factorial crosses were conducted on each of two pink and two chum salmon stocks and the embryos and alevins were reared at low, medium and high water temperatures until fry emergence. Additive genetic, maternal, dominance and common environmental effects were estimated for a number of developmental characters including survival, timing of hatching and emergence and alevin and fry size.

Two large-sized and two small-sized pink salmon males were mated to each of four females and the resulting juveniles from the 16 families were reared to sexual maturity. Adults from the F_1 generation were then crosed in three 3 X 3 factorial crosses, with the juveniles reared till near sexual maturity. Heritibility of weight at different ages was examined in both experiments, as well as maternal, dominance and common environmental effects in the factorial crosses.

Four males were mated to eight females in a nested breeding design in five populations of pink salmon. The resulting juveniles were reared till near sexual maturity. Heritibility of weight at different ages was examined, as well as the genetic correlation between weight and sexual maturity.

Ten males were mated to 20 females in a nested breeding design in a chum salmon population. The embryos and alevins were reared at low, medium and high temperatures until the fry had emerged and subsequently grown to about 50 mm fork length. Heritibility and genetic correlations were examined for a number of morphometric and meristic traits.

Biological Sciences Branch, Salmon Production Section, Sockeye Salmon Program, Pacific Biological Station, Dept. of Fisheries and Oceans, Nanaimo, B. C.

Chris Wood and Chris Foote

The Section is conducting experiments with juvenile sockeye and kokanee salmon and their hybrids at the Rosewall Creek Experimental Hatchery which may be of benefit to the aquaculture industry.

Specifically, egg-to-fry development, growth rates, osmoregulatory capability and rheotactic behavior are being monitored and, hopefully, maturation success in freah and saltwater. Preliminary results indicate that the growth rate of kokanee and hybrid kokanee-sockeye is very similar to that of pure sockeye (in fresh water) and that all types osmoregulate well at three months after ponding (under a suitable photoperiod). There have been no problems with disease after one year. Thus, to the extent that broodstock would be more easily obtained from wild kokanee than wild sockeye stocks, kokanee may be a preferred fish for "sockeye" culture.

Biological Sciences Branch, Salmon Production Section, Salmon Stock Assessment Program, Pacific Biological Station, Dept. of Fisheries and Oceans, Nanaimo, B. C.

Brian Riddell and Doug Swain

Quantitative Genetic Study of Morphological Variation in Coho Salmon

Because selection acts on the whole phenotype rather than on characters in isolation, a knowledge of the additive genetic covariances among traits is needed to understand the constraints on selection and to predict its course. We are determining genetic covariance matrices for four populations of coho salmon (interior wild, south coastal wild, north coastal wild and south coastal hatchery). The traits examined are important in local adaptation and differentiate interior from coastal and wild from hatchery stocks. This work will have important implications regarding the transplanting of stocks between regions (or from hatcheries to the wild), and the effect of hatchery culture on the underlying genetic architecture of populations.

Behavioral and Physiological Comparison of Hatchery and Wild Coho

Aggressive behavior and tolerance to environmental stress (high temperature, low oxygen) will be compared between several hatchery and wild populations. Both wild-caught fry and the hatchery-reared offspring of wild parents will be used in order to distinguish between environmental and genetic effects.

Atlantic Salmon Federation, Salmon Genetics Research Program, St. Andrews, N.B.

Gerry Friars and John Bailey

Selection

Response to selection for grilse length in Atlantic salmon parents was reported to have produced significant correlated responses for growth in fresh water (ICES AnaCat Report 1986). Observations on the same control and select lines was carried out in a combined experiment involving extension of photoperiod in sea cages. The growth advantage in the select line over the control line was 1.2 cm (44.2 vs 45..4) in fork length by 27 weeks in sea cages. Hence, a significant economic advantage has been realized in selection for size.

Family - photoperiod interaction

Response to a 16 hour photoperiod, as opposed to natural daylength (12 to 9 hours), during autumn growth of parr in the hatchery, was more pronounced in families that were small with respect to weight and length at the start of the treatment period. Consequently, selection for growth appears to need to be considered in combination with specific environments.

Correlations between freahwater and saltwater growth

Simple correlations between full-sib family means, when family sizes are large, are indicative of the direction of correlated responses expected when selection is applied to the alternative trait. In general, such relationships indicate that genetic ability for fast growth in fresh water is positively related to early phases of growth after smolts have been placed in sea cages. However, these relationships get weaker as the period of growth, in saltwater, becomes farther removed from the freshwater phase in terms of time. Consequently, selection may need to be applied to both freshwater and seawater performance if simultaneous gains are to be attained in both phases of growth.

Selection criteria for sea ranched and cage reared Atlantic salmon

Data on Atlantic salmon, collected between 1974 and 1987, were examined to identify traits amenable to selection for improved hatchery performance and increased market value in both sea ranching and sea cage culture.

Differences in length and weight at three, six or 15 months post-hatch were not significant ($p > 0.05$) among stocks of salmon produced in 1978 and 1979. Half sib heritability estimates for length and weight were low to intermediate (0.1 to 0.4) and intermediate to high (0.4 to 0.9) in 1979. Genetic, environmental and phenotypic correlations between length and weight at the three sampling times were positive and high(0.7). The proportion of salmon which smolt at 1+ years of age was estimated in 179 full sib families sampled during the first November post-hatch. The heritability estimate for the proportion of parr which smolt at 1+ years was 0.85 ± 0.34. Correlations between lengths in July and November were significantly ($p< 0.001$) greater than zero. Correlation between mean length in July and the proportion of 1+ smolts was negative.

Full sib families from four domestic strains of salmon were reared in sea cages in southern New Brunswick. Heritibility estimates for length at 10 and 15 months post-smolt ranged from 0.28 to 0.67 and from 0.28 to 0.57, respectively. Phenotypic and genetic correlations between length measurements at 10 and 15 months were positive and high in all strains examined. Significant ($p< 0.05$) year-class and strain effects were found for length, day of return and percent return among grilse and multiple-sea-winter salmon returns for three year-classes of sea ranched salmon. Salmon parr from 42 full sib families became infected during a severe furunculosis epizootic. Survival ranged from 18 to 98%, yielding a full sib heritibility estimate of 0.32 ± 0.06 for tolerance to furunculosis. Linear selection indices designed to increase mean parr length at six months post-hatch, percent 1+ smolts and length at 15 months post smolt were developed for sea ranching and cage culture conditions in southern New Brunswick.

Ministry of Natural Resources, Stock Assessment and Genetics Unit, Research Section, Fisheries Branch, Maple, Ontario

Peter Ihssen

Genetics of temperature tolerance and low oxygen toleraance in rainbow trout

Rainbow trout that have been selected for high and low tolerance to high temperature are being tested for tolerance to low oxygen. A significant correlation between tolerance to high temperature and tolerance to low oxygen concentrations has been observed. Similarly, fish selected for low tolerance to high temperature had lower tolerance to high oxygen concentrations.

Chromosome manipulation in salmonids

Gynogenetic diploids and triploids of brook trout, brown trout, brook x brown hybrids and rainbow trout have been investigated for survival and growth under hatchery conditions. This work has been in collaboration with Drs I. M. McMillan and L. McKay of the Animal and Poultry Science Dept., Univ. of Guelph. The brown x brook hybrid, although a desirable fish (good growth, exceptional flesh quality) has not been used extensively in aquaculture because of its poor early survival. Selection experiments are in progress to improve early survival. Gynogenetic diploids have been used to compare the gene-centromere maps for different salmonids.

Inheritance of seasonal spawning time in rainbow trout

Rainbow trout strains available for aquaculture have spawning times ranging from September to April. For continuous production throughout the year, it is desirable to develop strains that spawn during May, June, July and August. Using controlled light cycles to obtain spawn during these months involves considerable additiional expense because brood stocks have to be maintained in lightproof enclosures under carefully controlled light cycles. Experiments are in progress to assess the feasibility of selecting rainbow trout for spawning outside their normal spawning season.

Genetic impacts of planted fish, transplanting and domestication

Work is continuing to assess genetic impacts of fish culture and various fisheries management strategies. For some transplanted stocks and broodstocks maintained in hatcheries, considerable erosion of genetic variability has been observed. Methods have been instituted, such as rotational line crossing, to minimize adverse genetic impacts of fish culture. Genetically marked stocks (lake trout) have been developed to assess the genetic contribution of planted fish to natural populations.

Huntsman Marine Science Centre, Aquaculture Section, St. Andrews, New Brunswick,

Julie Delabbio and Brian Glebe

Arctic char is currently under investigation as a potential mariculture species. In 1986, three stocks of Arctic char (two

[illegible] were subjected to 96 hour saltwater (37.5 ppt) challenge test over a three month period (April - June). During the test the landlocked char stock had high mortality but both anadromous stocks, although showing effects of stress, had inconsequential mortality.

Subsequently, the two anadromous char stocks were grown out in ambient seawater over a nine month period and their saltwater performance was compared with that of Atlantic salmon held under similar environmental conditions. Results indicate significant differences in saltwater tolerance in the two anadromous char stocks, one stock having less than 30% survival while the other demonstrated greater than 60% survival. Growth within char stocks on average was less than that of Atlantic salmon. However, one char stock developed a bimodal weight distribution during saltwater rearing and final mean weight of the upper modal group was 25% greater than that of the Atlantic salmon.

Aquaculture Genetics Program, Dalhousie University, Halifax, Nova Scotia

Roger W. Doyle and Gary F. Newkirk

Fish Genetics

The application of breeding programs to fish is being studied with consideration of the experimental and biological problems of fish. Methods to reduce the phenotypic variance through physical or statistical control as well as consideration of the behavioral problems are being developed. Methods such as size specific selection rather than age specific selection, within family selection (avoiding common environment and maternal effects) and variance reduction by culling at the start of observation periods are being tested. Circulus spacing is being used to estimate instantaneous growth rates for strain comparisons under extensive culture conditions. The potential for using molecular tags for pedigree control in extensive aquaculture systems will be explored. Research is done in Canada with hybrid Tilapia and in S. E. Asia with several commercial species.

Selection for growth in European oysters

The breeding program of the European oyster, Ostrea edulus, has been restricted to the work of one student, Padermsak Jayarabhand. He is testing families produced from parents that were selected by within family selection for weight at two years of age. Field tests are in progress. He is also assessing the effect on the variance in size following growth under crowded conditions. Commercial use of the selected stocks developed so far is pending the establishment of a commercial hatchery.

Department of Fisheries and Oceans, Halifax, Nova Scotia and St. Andrews, New Brunswick

John A. Ritter, editor

Report of the Working Group on Broodstock Development and Conservation for the Southern New Brunswick Aquaculture Industry

This report reviews the current status and recommended strategies for broodstock development and conservation for the

aquaculture industry in southern New Brunswick. The report was prepared following a number of meetings and discussions among researchers and other officials of the Department of Fisheries and Oceans and the New Brunswick Department of Fisheries together with other researchers and members of the southern New Brunswick salmon aquaculture community. The following genetics considerations are addressed in the report: (1) inventory of existing stocks; (2) choice of foundation stock; (3) selection goals; (4) breeding and propagation; (5) rerquirements to access wild stocks; and (6) risks to wild stocks. Further details may be had by contacting John A. Ritter, Department of Fisheries and Oceans, P. O. Box 550, Halifax, Nova Scotia.

NORWAY

INTRODUCTION

Genetics methods have traditions back to around 1960 in Norwegian fisheries research when work on blood groups for population identification of cod was initiated by the Institute of Marine Research (Dag Møller). For such investigations electrophoresis of enzymes and other proteins soon came in use, and in later years also mitochondrial DNA studies have been applied.

Aquaculture (fish farming) started in the late 60-ties, and around 1970 work was initiated on quantitative genetics for improvement of productive traits of farmed salmonids. This work has been conducted continuously since then, and in the meanwhile also work on chromosome engineering has been started. In recent year work on gene technology has been initiated with the prime target of being used for genetic improvement of farmed fish.

In the following overviews these topics are dealt with:

Identification of population units and sibling species by gene markers

Genetic improvement of salmonids - classical quantitative genetics

Chromosome engineering

Gene technology

IDENTIFICATION OF POPULATION UNITS AND SIBLING SPECIES

At Biological Station, University of Trondheim (Jarle Mork), the following projects are carried out:

- Population structure and evolution of various gadoid fish species studied by electrophoretic methods
- Studies on potential homing in marine fish (cod, plaice) by tagging/transplantation experiments
- Biochemical genetic identification of fish eggs

At Department of Fisheries Biology, University of Bergen, a program for studies on species identification, species validity and intraspecies variation of redfishes (Genus Sebastes) is undertaken in cooperation with the Institute of Marine Research.

Genetic studies on cod and herring stocks have been continued, including analyses of all new yearclasses as well as the spawning population of Arctic cod. The last mentioned work is mainly focused on yearclass variation and identification of subpopulations by using protein electrophoresis and restriction fragment analysis of mtDNA.

The same institutions are cooperating on studies of genetic composition of natural and stocked cod populations, and their actual and potential interaction in several stocking areas in different part of the Norwegian coast. A central part of these investigations is use of genetically tagged offspring from a broodstock homozygotous for a rare P6I-allele, developed by the Institute of Marine Research.

In a cooperative project between the same institutions, a morphological genetic marker in trout (fine spottet) has been developed for studies on genetic interactions between farmed and wild stocks of salmonids.

At the University of Oslo, investigations on the population structure of Iceland scallops have been undertaken.

Studies on polymorphic variation on wild salmon are carried out by the Directorate of Nature Management, Trondheim.

QUANTITATIVE GENETICS

A large scale program for genetic improvement of salmonids is initiated by the fish farmers assosiations (Fiskeoppdretternes Salslag, Trondheim). The breeding program is carried out at Kyrksæterøra (NFA), about 100 km south of Trondheim, and the improved fish material is transferred to the fish farming industry via multiplying stations in each county.

Institute of Aquaculture Research (AKVAFORSK), under the Agriculture

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

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

Non-additive genetic variations are found to contribute to the variation of traits connected to fish quality (fat in flesh, intestine fat, flesh colour, belly thickness etc.) in rainbow trout. Such studies are started also for Atlantic salmon. Datatomography was found to be of considerably help in the registrations of body composition in fish.

Immunological factors which may be connected to genetically determined desease resistence are identified and tested for genetic variation and covariation with productive traits and actual resistence. Also the connection between "stress" and immuneresponse is studied. These studies are carried out in cooperation with Department of Animal Breeding, Agricultural University of Norway, and Department of Microbiology and Immunology, Veterinarian University of Norway.

A project for studying the ironbinding proteins (transferrins) and their effect on disease resistence has been started. In vitro-tests on fish patogenes will be carried out, and also cell lines and modell fish will be used for studies on gene regulations.

At Department of Aquaculture, Institute of Marine Research, Bergen, research on quantitative genetics on Atlantic salmon are continued by studying the performance of a high number of sibgroups at different commercial fish farms under different environmental condition. These studies are closely connected to studies on environmental impact by fish farming, field studies on fish health and studies for determination of optimal densities in net pens. Genetic/environmental interactions, mostly as different expression of genetic variations, were indicated in these studies.

In cooperation with Department of Biotechnology, University of Bergen, studies on genetic variation in immune response have been undertaken.

Studies on growth rate variation connected to genetic variation in trypsine-like isozymes in Atlantic salmon are carried out at the Aquaculture Research Station, Matredal, under the Institute of Marine Research.

CHROMOSOME ENGINEERING

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

GENETECHNOLOGY

Isolation of genes coding for growth hormones, prolactine, insuline or disease resistence have been undertaken by several laboratories with the double aim of basic studies of such mechanisms and of transferring "valuable" genes between and within species. Both Atlantic salmon and modell fish (zebrafish) are used for such investigations.

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

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

APPENDIX

Laboratory for Biotechnology, University of Bergen
P.O. Box 3152, Årstad, N-5029 BERGEN

Marine Genetics
c/o Laboratory for Biotechnology (address above)

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

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

Department of Biotechnology, Norwegian Technical University
N-7034 TRONDHEIM

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

Department of Physiology
(same address)

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

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

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

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

Norsk Hydro, Research Center, N-3901 PORSGRUNN

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

FRANCE

Genetic studies in France concern two main groups of aquatic organisms that are of economic importance : Salmonids and Molluscs. For other species, studies are much limited.

1. SALMONIDS

1.1. Genetic differenciation between populations (Lab. 1)

Studies have been carried out on natural and domestic populations of brown trout. A map of french populations has been drawn, which displays clear genetic differenciation between Atlantic and Mediterranian stocks. The hatchery strains that were examined form a group of highly related populations, all originating from the Atlantic drainage area. Evidences for interbreeding between native and domesticated fishes released for restocking were found in several Mediterranian rivers.

1.2. Quantitative genetics

- Evaluation of species (Lab. 1, 3, 4) : a comparative evaluation of brown trout and atlantic salmon in sea water cages has shown that brown trout could be considered as an interesting alternative species for production of large size salmonids.

- Evaluation of strains (Lab. 1, 3, 4) : a comparison of different strains of brown trout (French and Danish populations) displayed a large variation for growth and sexual maturation rate in fresh and sea water. As a general rule, domesticated stocks perform better than wild or hybrid ones, though a consistent between group variation remains among domesticated groups.

- Choice of a method od selection for growth (Lab. 1) : family selection has proved to be efficient in fish selection. Nevertheless, it is very expensive (many tanks are required) and it might be not possible to initiate such breeding schemes for all species in all countries. More over, the theoretical superiority of family selection upon mass selection relies on two hypotheses (1) equality of intensities of selection in both cases, (2) lack of environmental variance between families, which may not be considered in practice and change appreciably the relative efficiency of the two methods. Therefore, efforts are made to re-estimate interest and efficiency of mass selection

whose gain will be optimized, in groups where genetic variation has been enlarged and phenotypic variation reduced. Brown trout has been chosen as a model for this study.

- Selection for disease resistance (Lab. 1, 5) : a program has just been initiated in order to analyse genetic basis of resistance to bacterial diseases (furonculosis).

1.3. Chromosome engineering

The efforts made to define techniques of induction of gynogenesis or polyploidy will tend to reduce, most of the techniques being availables, at least in rainbow trout.

Researches will now turn into the following topics :

- the adjustment of the techniques of production of monosex and triploid sterile fish availables in rainbow trout to the other species reared in aquaculture (brown trout and atlantic salmon) (Lab. 1, 3).

- the analysis of the performances of homozygous endomitotic gynogenetics and tetraploids, and of their potential interest in future breeding schemes for genetic improvement (Lab. 1, 2).

1.4. Gene transfer (Lab. 1)

A technique of gene transfer by micro-injection of plasmids into egg cytoplasm has been developed on rainbow trout, and proved to be efficient. Analysis of second generation individuals strongly supports the fact that the foreign DNA sequences are integrated in the host genome. Nevertheless, no expression of foreign genes could be detected until now, when heterologous promoters are used.

2. MOLLUSCS

2.1. Population genetics (Lab. 6)

Electrophoretic markers are used to analyse population genetics of Ostreidae.

The European flat oyster (Ostrea edulis) is the main species currently studied (samples from hatcheries, and natural populations from Atlantic and Mediterranian areas - France, Morocco, Yugoslavia) but studies are also performed on C. gigas, C. angasi and O. stentina, as well as on populations of Pteriidae (Pinctada margaritifera, P. mazatlanica, P. radiata and P. maculata).

2.2. Chromosome engineering (Lab. 7)

The main purpose of current experiments is the production of triploid shellfish, either by direct inhibition of meiotic divisions after fertilization, or indirectly by crossing tetraploid and diploid parents.

- direct production : treatments with cytochalasin B were tested on four species : C. gigas, O. edulis, Chlamys varia and Ruditapes philippinarum ; triploids were produced in any case, though the rate of triploidy still remains between 50 to 70% (triploidy is assessed by counting chromosomes at very early stages).

- production of tetraploids : cytochalasin B and high pressure shocks were tested ; both treatments proved to be efficient (up to 18% of tetraploids in O. edulis, and 25% in C. gigas after high pressure treatment, and about 30% of tetraploids in R. philippinarum with cytochalasin B).

2.3. Evaluation of species and hybridization (Lab. 7)

Attempts will be made to produce hybrids (eventually triploid hybrids in order to improve survival, as it has been described in fishes) between different species of oysters (C. gigas and O. edulis) and clams (R. decussatus and R. philippinarum). In both cases, the purpose is to produce hybrids with good performances for commercial production (growth rate and disease resistance).

LIST OF LABORATORIES

(1) - Laboratoire de Génétique des Poissons - C.R.J.
INRA - 78350 JOUY-EN-JOSAS

(2) - Station d'Hydrobiologie
INRA - SAINT PEE SUR NIVELLE
64310 ASCAIN

(3) - IFREMER
Laboratoire de Zootechnie des Salmonidés
Station d'aquaculture - Centre de Brest
BP 70
29263 PLOUZANE

(4) - SEMII F.A. (Station expérimentale marine IFREMER-INRA)
Le Drennec
BP 17
29237 SIZUN

(5) - Laboratoire d'Ichthyopathologie
INRA - Route de Thiverval
78850 THIVERVAL GRIGNON

(6) - Laboratoire de Zoogéographie - Génétique
Université Paul Valéry
BP 5043
34032 MONTPELLIER CEDEX

(7) - IFREMER
Station de Génétique et Pathologie des Mollusques
Mus du Loup
17390 LA TREMBLADE

Ireland

Salmon

Work continued on the production of sex-reversed, all female and triploid stocks of Atlantic salmon at a pilot scale level. Triploidy levels up to 80% were induced by heat shocks and monitored by direct karyological examination. Mosaic individuals occurred commonly among heat-treated embryos; they were less frequent in samples examined at the fingerling stage. Mortality in heat-shocked samples was greater than in controls. Mortality occurred mainly in the egg phase, but was continued at levels higher than those of controls up to three months post first-feeding. It is hypothesised that the increased mortality occurs predominantly among mosaic fish.

A small batch of triploidised all female salmon was tagged by microtag and released as smolts. Tag returns may indicate mortality levels in this group on release to the wild.

Studies are continuing on these topics with a view to determining optimum conditions for use with Irish farmed salmon.

Studies have been completed on the normal karyotype and G-banded karyotype of salmon. Robertsonian polymorphism is widespread with modal 2N values of 56 and 58. The G-banding results have been applied to elucidate the karyotype in the species and the overall results are being prepared for publication - University College Galway (Wilkins/Nolan).

Molluscs

Preliminary experiments on triploidy induction by heat shocks on clams have been initiated. This work is at an early stage and levels of triploidy in excess of 50% have been achieved.

Cooperative studies on European & Baltic populations of mussels were continued between Dr. E. Gosling, Galway & Dr. P. Bulnheim, Germany and the results have been published (Helgo. Meeresunters 42, 113-129, 1988). - Regional Technical College, Galway (Gosling/Nolan).

POLAND

Sex control in rainbow trout.

The masculinized females (300 fish) were produced by the metyltestosterone treatment applied to the progeny of functional males (genetic females) obtained as a result of an experiment completed in the year 1986. These fish will be distributed in 1988 to enable the whole female market fish production in Northern Poland.

Gynogenesis

Experiments aimed to induce artificial gynogenesis in sea trout and rainbow trout started in autumn 1986 (s.t.) and in spring 1987 (r.t.). Sea trout ova were inseminated with brook trout sperm previously sterilized by UV light, and then treated with heat shock (28^0 C). Survival rate at the beginning of external feeding was 14%. It is supposed that the method applied was effective as in autumn 1987 the whole treated population consisted of sea trout only - no one 'tiger' fish was found. Similar result was obtained with rainbow trout. Ova of xantoric (yellow) female were inseminated with previously UV irradiated sperm obtained from the "wild" coloured male. Inseminated eggs were heat shocked. Survival rate up to swim-up stage was 8%. All fish are xantoric (yellow) what may suggest that they are of maternal genotype only. Control group (eggs and milt of these same experimental fish normally fertilized) consisted of "wild" coloured fish only.

Induced poliploidity

The experiments on induced poliploidization in rainbow trout were continued in 1987 in cooperation with the Academy of Agriculture in Warsow. To induce poliploidity the thermal shock was applied to the two groups of ova. The first one consisted of eggs fertilized by normal sperm, the second were eggs fertilized by sperm obtained from masculinized females. The poliploidity ratio in experimenttal groups was 80% (amount of DNA in erythrocytes nuclei as well as nuclei diameter was measured).

No significant differences in experimental and control fish growth rate during the first season was observed.