FISHERIES RESEARCH & DEVELOPMENT CORPORATION

Genetics IN AQUACULTURE VII

Proceedings

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Contents

Program

SATURDAY, JULY 15

ARRIVALS / PRE-CONFERENCE TOURS

NOTE: Registrations will commence on Sunday at 1700

SUNDAY, JULY 16

ARRIVALS

1700-1900 REGISTRATION at Sheraton (Jupiters)

1800-1930 RECEPTION at Sheraton (Jupiters)

MONDAY, JULY 17

0800 REGISTRATION

0845 OPENING CEREMONY

0900 JOE BAKER, CHIEF SCIENTIST FOR QUEENSLAND

0920 PLENARY BY CHOY HEW "The impact of biotechnology on aquaculture" Uni of Singapore.

1000-1030 Tea and Coffee Break

GENE EXPRESSION, TRANSGENESIS AND MOLECULAR GENETICS
Chair: John Beardmore Uni of Swansea – Wales.

1030 SEUMAS WALKER NZ King salmon Co.
Evaluation of transgenic chinook salmon with enhanced growth.

1050 BOAZ MOAV Te-Aviv Uni – Israel.
Methylation and transgene expression in transgenic common carp.

1110 DAVID PENMAN Uni Sterling – Scotland.
Cytochrome p450 aromatase genes and sexual differentiation in the Nile tilapia *Oreochromis niloticus*.

1130 OLAV VESTRHEIM Norway
Tissue specific transcription of major histocompatibility complex class II in Atlantic salmon (*Salmo salar* L.).

1150 FRANK WONG CSIRO.
Application of SSU rRNA sequence analysis to the identification and detection of the agent of amoebic gill disease in sea-caged Atlantic salmon (*Salmo salar*). FRDC.

1210 TERESA LEWIS Virginia Inst of Mar Sci USA.
Analysis of the VH gene repertoire in outbred and isogenic strains of rainbow trout (*Onchorhynchus mykiss*).

1230 RAFIQUL SARDER Bangladesh.
Identification of MHC class II B genes and their association with specific immune response in the Nile tilapia, *Oreochromis niloticus* L.

1250-1400 Lunch Break

GENE EXPRESSION, TRANSGENESIS AND MOLECULAR GENETICS
Chair: Emmanuel Goyard

1400 KATE WILSON AIMS.
Isolation and expression of a putative reproductive inhibitory hormone from the giant tiger prawn, *Penaeus monodon*.

1420 LASZLO ORBAN
Identification of sex-related genomic markers and genes involved with sex determination in fish.

1440 RUTH PHILLIPS
Identification of interspecific chromosome homologies in salmonid fishes using paint probes.

APPLICATION OF MOLECULAR MARKERS
Chair: Kate Wilson

1500 SHARON APPLEYARD CSIRO.
The application of genetics markers to Fijian Tilapia stock improvement.

1520-1550 Tea and Coffee Break

1550 TAKAHITO SHIKANO
Using microsatellite and RAPD markers to estimate the amount of heterosis in various strain combinations in the guppy (*Poecilia reticulata*) as a fish model.

1610 WILLIAM WOLTERS
Heritability estimations for juvenile growth and disease resistance in channel catfish: Integration of molecular markers and quantitative genetics.

1630 BRAD EVANS CSIRO.
The use of molecular markers for parentage analysis in farmed Australian abalone.

1650 PIERRE BOUDRY
Microsatellite markers as a tool to study reproductive success in the pacific oyster, *Crassostrea gigas* (Thunberg), crossed under controlled hatchery conditions.

1710-1800 POSTER SESSION 1:
GENE EXPRESSION, MOLECULAR MARKERS AND GENE MAPPING

Maria Coimbra. A genetic linkage map for the Japanese flounder, (*Paralichthys olivaceus*).

Roy Danzmann. A microsatellite linkage map of rainbow trout (*Oncorhynchus mykiss*) characterized by large sex-specific differences in recombination rate.

Roy Danzmann. Gametic phase disequilibira as indicators of genomic coadaptation among unlinked chromosomal segments in salmonid fishes.

Gonzalo Gajardo. Multiple-locus heterozygosity, physiological traits and growth in a cohort of the Chilean-Peruvian scallop *Argopecten pupuratus* (Lamarck, 1819).

Lars-Erin Holm. Salgene: gene identification and expression analysis in Atlantic salmon (*Salmo salar*).

Sophie Hubert. Relationship between heterozygosity and growth in families of the Pacific oyster, *Crassostrea gigas*.

Sophie Hubert. Genetics and linkage groups of microsatellite markers in the Pacfic oyster, *Crassostrea gigas* using trisomics.

G. Hulata. Are intersex crayfish (*Cherax quadricarinatus*) genetically females?

G. Hulata. Development of a tilapia artificial center of origin (ACO) and genetic linkage map based on AFLP and microsatellite loci.

G. Hulata. Detection of putative QTLs for body weight and cold tolerance in interspecific tilapia hybrids.

Brownyn Innes. Use of amplified fragment length polymorphisms (AFLPs) as molecular markers in the Pacific oyster (*Crassostrea gigas*). CSIRO.

Patricia Iturra. Characterization of sex chromosomes in salmonid species by in situ hybridization using molecular markers.

Klaus Kohlmann. Biochemical genetics, performance and product quality of tench (*Tinca tinca* L.) strains.

Kagayaki Morishima. Gene-centromere mapping of microsatellite markers in the Loach, *Misgurnus anguillicaudatus*.

Krista Nichols. Quantitative trait loci associated with development rate in clonal *Oncorhychus mykiss* strains.

Marc Noakes. Searching for genes associated with the control of circadian rhythms in the barramundi (*Lates calcalifer*). CSIRO - Animal.

Kazuharu Nomura. Isolation and characterization of (CA)n microsatellites from the Japanese eel, *Anguilla japonica*.

David Penman. Microdissection of *Oreochromis niloticus* putative sex chromosomes and DOP-PCR produces chromosome-specific fish probes.

Takuma Sugaya. Confirmation of mating mode in the kuruma prawn (*Penaeus japonicus*) based on microsatellite DNA markers.

N. Vergara (*Presented by Patricia Iturra*) Use of DNA molecular markers for the identification of species in Chilean salmonid elaborated products.

Quanqi Zhang. Sex identification by male-specific growth hormone pseudogene (GH-Y) in *Oncorhynchus masou* complex and a related hybrid.

1800-1900 IAGA BOARD MEETING

TUESDAY, JULY 18

GENE/GENOME MAPPING

Chair: David Penman

0900 AKIYUKI OZAKI
Analysis of QTLs associated with resistance to viral disease (IPN) in rainbow trout.

0920 KAYO NAKAMURA
Genetic mapping of the dominant albino locus in rainbow trout (*Oncorhynchus mykiss*).

0940 BJORN HOYHEIM
SALMAP: Constructing genetic maps of salmonid fishes.

1000 KATE WILSON AIMS.
International collaboration on genetic mapping of the black tiger shrimp, *Penaeus monodon*: progress update.

1020-1050 Tea and Coffee Break

GENE/GENOME MAPPING

Chair: Andy Beaumont

1050 KARIM GHARBI (*PRESENTED BY ROY DANZMANN*)
Comparative mapping in salmonid fishes: the microsatellite generation.

1110 DANIEL MCGOLDRICK CSIRO
Progress in the mapping of major genes in the Pacific oyster (*Crassostrea gigas*).

1130 PATRICK GAFFNEY
Development of molecular markers for constructing a genetic linkage map of the eastern oyster *Crassostrea virginica*.

1150 GAVIN MCDONALD
Applications of a molecular pedigree analysis to selective breeding in rainbow trout (*Oncorhynchus mykiss*).

1210 GUY PERRY
Thermal tolerance QTL in outbred rainbow trout (*Oncorhynchus mykiss*).

1230-1400 Lunch Break

PLOIDY MANIPULATION

Chair: Hans Komen

1400 JOHN NELL NSW FAI
Tetraploid induction in bivalves by blocking first mitosis with 6-dimethylaminopurine.

1420 BELINDA NORRIS CSIRO
Ploidy manipulation of *Penaeus japonicus* and *Haliotis asinina*.

1440 NOEL WILKINS
Triploid Atlanitic salmon grow better to smoltification than diploids: a comprehensive evaluation at pilot scale.

1500 KATSUTOCHI ARAI
Genetic analyses of the progeny of triploid gynogens induced from unreduced eggs of triploid (diploid female X tetraploid male) Loach.

1520-1550 Tea and Coffee Break

PLOIDY MANIPULATION

Chair: Dosette Pante

1550 FU HONGTUO
Artificial asexual reproduction of loach (*Paramisgurnus dabryanus*) by cell electrofusion.

1630 PIERRE BOUDRY
Aneuploidy and its relationship with growth in different populations of the Pacific oyster (*Crassostrea gigas*, Thunberg).

1650 AKIRA KOMARU
All maternal chromosomes are extruded as two polar bodies in the androgenetic clam *Corbicula leana* - anti tubulin immunofluorescence.

1710-1800 POSTER SESSION 2:

CHROMOSOME MANIPULATION

Kim Brown. Mitochondrial and nuclear inheritance in androgenetic clonal rainbow trout (*Oncorhynchus mykiss*).

Beatrice Chatain. Sex determination in *Dicentrarchus labrax*: no evidence for male or female heterogamety.

Atushi Fujiwara. Identification of eliminated chromosomes in inviable salmonid hybrids.

Gabriel Hoerstgen-Schwark. Sex ratios in tilapia (*Oreochromis niloticus*): interactions between genotype and temperature.

Ryo Ishibashi. All maternal chromosomes are extruded as two first polar bodies in the androgenetic clam *Corbicula leana* - cytocholasin B treatment.

Hiroyuki Nagoya. Growth characteristics and sex reversal of YY amago salmon (*Oncorhyncus masou ishikawae*) produced by androgenesis.

Sei-ichi Okumura. Large larvae of pacific abalone, *Haliotis discus hannai*, obtained from triploid batches.

Pham Anh Tuan. Sex determination and the feasibility of YY male production in the Vietnamese strain of *Oreochromis niloticus* (L.).

Zhaoping Wang. Studies on heterozygosity and growth in triploid Pacific oyster (*Crassostrea gigas*).

Huiping Yang. Increased cell-size as a cause for polyploid gigantism in triploid zhikong scallop, *Chlamys farreri*.

Ziniu Yu. Studies on the karyotypes of two species of sea bream (Sparidae) and six species of flatfishes (Pleuronectidae).

WEDNESDAY, JULY 19

BREEDING AND QUANTITATIVE GENETICS

Chair: Roberto Neira

0900 HANS BENTSEN
Designing Aquaculture mass selection programs to avoid high inbreeding rates.

0920 MATHILDE DUPONT-NIVET
Optimization of mating designs for inference on heritability in fish species.

0940 PETER AMER
Practical Application of an inbreeding control algorithm for salmon.

1000 REMEDIOS BOLIVAR
Response to selection for body weight in Nile tilapia (*Oreochromis niloticus*) using a single-trait animal model.

1020 DOSETTE PANTE
Estimation of additive and non-additive genetic variances for growth in selected populations of rainbow trout.

1040 MASAMICHI NAKAJIMA
Genetic control of the growth in the guppy (*Poecilia reticulata*).

AFTERNOON

1100-1900 TOURS TO BILLABONG AND AIMS (INCLUDES LUNCH).

THURSDAY, JULY 20

BREEDING AND QUANTITATIVE GENETICS
Chair: Brendan McAndrew

0900 GRAHAM MAIR
Genetics status and strategies for improvement of common carp for aquaculture in Karnataka, India.

0920 MODADUGU GUPTA
International network on genetics in aquaculture (INGA): progress and achievements.

0940 GARY THORGAARD
Clonal rainbow trout lines as resources for genetic analysis of complex traits.

1000 STEPHANE BONNET
Conformation and carcass quality traits in seawater adult brown trout: correlated responses to selection for freshwater body length, growth and triploidy x selection interactions.

1020-1050 Tea and Coffee Break

BREEDING AND QUANTITATIVE GENETICS
Chair: Remedios Bolivar

1050 MICHEAL TANCK
Selective Breeding for stress in common carp (*Cypirinus carpio* L.) using androgenesis.

1110 NGUYEN CONG DAN (*PRESENTED BY INGRID OLESEN*)
Genetic studies of farmed tilapia in fresh and brackish water in Vietnam.

1130 JANE SYMONDS
Selective breeding for improved performance of farmed New Zealand chinook salmon.

1150 ROBERTO NEIRA
Studies on carcass quality traits in coho salmon. Phenotypic and genetic parameters.

1210 DOSETTE PANTE
Genetic variation for spinal deformity in Atlantic salmon.

1230 MARC VANDEPUTTE
Growth and survival during yolk-sac resorption in brown trout (*Salmo trutta fario* L.): A quantitative genetic analysis.

1250-1400 Lunch Break

BREEDING AND QUANTITATIVE GENETICS
Chair: Patrick Gaffney

1400 BRAD ARGUE
Selective breeding of pacific white shrimp (*Litopenaeus vannamei*) for growth and TSV resistance.

1420 GREG COMAN
Effect of genotype-environment interaction on the survival and growth of the kuruma shrimp, *Penaeus japonicus*.

1440 EMMANUEL GOYARD
Selection for better growth of *Penaeus stylirostris* in Tahiti and New Caledonia.

1500 PETER CROCOS CSIRO
Comparative growth, survival and reproductive performance of inbred and outbred lines of domesticated shrimp, *Penaeus japonicus*, in Australia.

1520-1550 Tea and Coffee Break

BREEDING AND QUANTITATIVE GENETICS
Chair: Audrey Fernando

1550 CAM MCPHEE QDPI
Improvement in the production of redclaw crayfish (*Cherax quadricarinatus*) through a genetic selection program.

1610 M.G. HUSSAIN
Stock improvement of silver barb (*Barbodes gonionotus* Bleeker) through several generations of genetic selection.

1630 W.S. LAKRA
Fish and shellfish genetics in India: an overview.

1650 N. CHATAKONDI (PRESENTED BY R. DUNHAM)
Commercial evaluation of production and performance traits of channel catfish, *Ictalurus punctatus* X blue catfish, *Ictalurus furcatus* F1 hybrids at industry standards.

1710-1800 POSTER SESSION 3:
BREEDING AND QUANTITATIVE GENETICS

Anthony Gharrett. Preliminary tests of outbreeding depression in hybrids between spatially separated pink salmon (*Oncorhynchus gorbusciia*) populations.

Bjarne Gjerde. Estimates of genetic and phenotypic parameters for body weight and growth recorded at five different ages in Atlantic salmon and rainbow trout.

Emmanuel Goyard. Fetii 1.0 Windows 9x based software for parentage analysis using codominant markers data and optional putative pedigree files.

Mark Henryon. Genetic variation for traits of commercial importance exists in Danish rainbow trout.

Gabriel Hoerstgen-Schwark. Test crosses with clonal lines of tilapia (*Oreochromis niloticus*)

Dean Jerry. There is potential for genetic improvement in the yabby (*Cherax destructor*) due to differences in growth rate, tail width and tail length among natural populations.

Neville Jopson. Fatness measures in a chinook salmon breeding programme.

Klaus Kohlmann. Evaluation of selective breeding results in F3 rainbow trout (*Oncorhynchus mykiss*).

Rupert Lewis. Quanititative evaluation of growth rate between African strains of the Mozambique tilapia (*Oreochromis mossambicus*).

Rupert Lewis. Estimation of the additive genetic variance component of growth rate heritability in the Mozambique tilapia - *Oreochromis mossambicus*.

Graham Mair. Selection for growth and sex ratio in GMT in *Oreochromis niloticus*.

Nigel Preston. Improving the growth rates of farm stocks of *Penaeus japonicus* through selective breeding.

Cheryl Quinton. Analysis of growth and spawning time in diallel crosses of three strains of rainbow trout (*Oncorhychus mykiss*).

M. Rye (*Presented by Ingrid Olesen*). Growth and survival in two complete diallel crosses with five strains of rohu carp (*Labeo rohita*).

Vera Slechtova. Top-crossing with paternal inheritance testing in common carp (*Cyprinus carpio* L.) offspring in two altitudes level.

Kazuo Tabata. Genetic effects on appearance of colour abnormality and reversal of sides estimated from cloned Japanese flounder (*Paralichthys olivaceous* Temminck et Schlegel).

Nguyen Van Hao. Performance of different groups of silver barb (*Barbodes gonlonotus*) in Vietnam.

1900-2300 CONFERENCE DINNER

FRIDAY, JULY 21

WILD AND FARMED GENETIC RESOURCES AND THEIR INTERACTION
Chair: Thomas Cross

0900 STEWART MCCONNELL
The genetic diversity and population structure of the tropical cyprinid *Barbodes goniotus* Bleeker, throughout its species range in SE Asia.

0920 VLASTIMIL SLECHTA
Protein variability in common carp (*Cyprinus carpio*) breeds in the Czech Republic.

0940 RUPERT LEWIS
The conflict between conservation of genetic resources and exploitation - an example from African populations of *Oreochromis mossambicus* (Peters).

1000 RUPERT LEWIS
The paradigm of larval dispersal - an artifact of sample size? Implications for aquaculture.

1020-1050 Tea and Coffee Break

WILD AND FARMED GENETIC RESOURCES AND THEIR INTERACTION
Chair: Nobuhiko Taniguchi

1050 A. WAS
Genetic differentiation among hatchery and naturally originated sea trouts in southern Baltic

1110 NOEL WILKINS
Genetic improvement of ranched Atlantic salmon: a positive interaction between aquaculture and wild fisheries.

1130 THOMAS CROSS
Molecular genetic investigations to assist a ranching programme for Atlantic salmon (*Salmo salar* L.).

1150 CHRIS AUSTIN
The hazards of aquaculture: the rapid extinction of a freshwater crayfish species.

1210 ROBERT WARD
Analysis of microsatellite loci in Western Australian populations of rainbow trout, *Oncorhynchus mykiss*.

1230 KIMBERLY REECE
Molecular genetic analysis of *Crassostrea ariakensis* and related oyster species.

1250-1400 Lunch Break

WILD AND FARMED GENETIC RESOURCES AND THEIR INTERACTION
Chair: Robert Ward

1400 SYUITI ABE
Genetic Variation among Japanese populations of chum salmon inferred from mitochondrial DNA control region sequences.

1420 ZUBAIDA BASIAO
Genetic Variation in three generations of hatchery-bred Nile tilapia *Oreochromis niloticus.*

1440-1520 IAGA GENERAL MEETING

1520-1550 Tea and Coffee Break

1550-1630 POSTER SESSION 4:
WILD AND FARMED GENETIC RESOURCES AND THEIR INTERACTION

Andy Beaumont. Isolation of microsatellite loci from the European flat oyster (*Ostrea edulis*).

Andy Beaumont. Mitochondrial DNA inheritance and hybridization in *Mytilus* spp.

Nelson Diaz. Microsatellite variation in coho salmon, *Oncrhynchus kisutch*, from Chile.

Lachlan Farrington. Allozyme and mitochondrial DNA diversity in Australian rainbow trout (*Oncorhynchus mykiss*).

Audrey Fernando. DNA polymorphisms in colour varieties of the Asian arowana, *Scleropages formosus* by arbitrarily primed polymerase chain reaction

Alexandre Hilsdorf. Mitochondrial DNA diversity in wild and captivity populations of *Brycon opalinus* (Cuvier, 1819) (Characiforme, Characidae, Bryconiae), in the Paraiba do Sul basin, Brazil.

M.G. Hussain. Genetic structure of Hilsa (*Tenualosa ilisha*) populations using starch gel allozymes.

Minoru Ikeda. Genetic differentiation and phylogenetic relationships among populations of Ayu (*Plecoglossus altivelis*), including endangered subspecies, inferred by PCR-RFLP analysis of the mitochondrial DNA D-loop region.

Klaus Kohlmann. PCR-RFLP analysis of the mitochondrial ND-3/4 gene polymorphism in the European and East-Asian subspecies of common carp (*Cyprinus carpio*).

Rupert Lewis. Length variable mitochondrial DNA as a population marker in African populations of Oreochromis niloticus.

Rupert Lewis. British *Porphyra* species - can they be improved?

Ashie Norris. Microsatellite genetic variation between and within farmed and wild populations of Atlantic salmon and the usefulness of these microsatellite markers to determine relatedness and parentage in farmed populations.

Carolyn Smith. Genetic variation among Western Australian pearl oyster, (*Pinctada maxima*) populations: a microsatellite survey. FRDC

Roman Wenne. Variation and transmission of mitochondrial DNA in polish populations of the mussel *Mytilus trosulus.*

Federico Winkler. Genetic variability among naturalized populations of rainbow trout *Oncorhynchus mykiss* (Walbaum) of the northern Chilean rivers Elqui and Limari.

Ziniu Yu. Investigation of genetic variation in populations of two species of bloody clam, *Scapharca broughtonii* and *Tebilarca granosa.*

List of Abstracts

GENETIC VARIATION AMONG JAPANESE POPULATIONS OF CHUM SALMON INFERRED FROM MITOCHONDRIAL DNA CONTROL REGION SEQUENCES

S. Abe[1], S. Sato[2], J. Ando[2], S. Urawa[3], H. Ando[2] and A. Urano[2]

1. Chromosome Research Unit, Faculty of Science, Hokkaido University, Sapporo 060-0810, Japan.
2. Division of Biological Science, Graduate School of Science, Hokkaido University, Sapporo 060-0810, Japan.
3. Genetics Section, National Salmon Resources Center, Sapporo 062-0922, Japan.

Chum salmon (*Oncorhynchus keta*) is the most widely distributed species of salmon in the Northern Pacific Ocean. Its genetic variation at population level has been studied mostly by using allozyme analysis, which, however, is not necessarily adequate for accurate genetic stock identification (GSI). In an attempt to develop a useful molecular marker for chum salmon GSI, we examined the nucleotide sequences of 0.6kb variable portion from the 5' end of mitochondrial DNA control region of about 500 individuals sampled from 12 populations of 6 Hokkaido and 5 Honshu rivers in Japan. Sequence comparison revealed 11 variable sites, defining a total of 12 haplotypes in the examined individuals. All the 12 haplotypes were found in 7 Hokkaido populations, whereas only 4 haplotypes occurred in 5 Honshu populations. Estimated gene diversity also tended to be higher in Hokkaido than Honshu populations. The present findings suggest a greater genetic variation in Hokkaido than Honshu populations and a certain level of genetic differentiation between these geographically distinct populations.

SELECTION FOR GROWTH AND SEX RATIO IN GMT IN *OREOCHROMIS NILOTICUS*

Jose S. Abucay[1] and Graham C. Mair[2]

1 Freshwater Aquaculture Center, Central Luzon State University, Nueva Ecija 3120 Philippines
2 School of Biological Sciences, University of Wales Swansea, Swansea SA2 8PP, Wales, UK

Attempts were made to improve the growth performance and sex ratio (% male) of Genetically Male Tilapia (GMT) produced from novel YY males in the Nile tilapia *Oreochromis niloticus*. Two selection programmes were developed: i) within strain selection for sex ratio and ii) combined within family selection for growth and progeny testing selection for GMT sex ratio in a female synthetic strain.

For the first selection programme several YY males and YY females from different families were progeny tested by crossing to randomly chosen normal XX females and sex reversed XX male genotypes respectively. The YY genotypes that produced 100% male progeny (in large family sizes) were selected to produce the next generation of YY broodstock. Sex ratios produced by YY males increased from 92.3%♂ ± 7.5 in the base population to 97.4 ± 7.7 and 98.3 ± 3.6 in the first and second selected generations respectively. Sex ratios of progeny from YY females were consistently above 99.4%♂ in all generations.

For the second selection programme within family selection for growth of males and females was adopted in a rotational mating scheme based on five original strains of *O. niloticus*. Three generations of selection were completed to date. Selection for sex ratio for growth selected females crossed to YY males was carried out in the first and third generations. Preliminary analysis of growth data for mixed sex progeny in high and low lines and GMT (97.4%♂) produced by crossing high line females to YY males, indicate significant response to selection.

PRACTICAL APPLICATION OF AN INBREEDING CONTROL ALGORITHM FOR SALMON

P.R. Amer[1] and J.E. Symonds[2]

1 AgResearch Invermay, Private Bag 50034, Mosgiel, New Zealand.
2 The New Zealand King Salmon Co. Ltd., Kaituna Research and Development Hatchery, Northbank Road, R.D.5, Blenheim.

Selection methods which reduce rates of inbreeding in livestock improvement programmes provide an alternative to arbitrary limits on numbers of candidates selected from high performing families. This paper describes practical modifications to a published method which uses a dynamic selection rule to maximise the genetic level of selected parents while restricting their average relationship. The method requires a vector of breeding values for total economic merit, and a matrix of additive genetic relationships among individuals as inputs. The output is a vector of optimal genetic contributions of each individual to the next generation. Modifications to the method are required because there is significant probability that the desired genetic contribution of an individual to the next generation can not be achieved in salmon breeding. For example, the individual may not reach sexual maturity within practical time limits for establishment of next generation families. This paper demonstrates that practical application of the algorithm can be achieved by optimising contributions from families, as opposed to contributions from individuals. The dimensions of the optimisation problem are reduced because large numbers of selection candidates per family are effectively eliminated, but also because a constraint on the sex ratio is no longer required. Breeding values within families have to be averaged to approximate the relative genetic potential of animals from that family which might be used as parents. The challenge is then for spawning managers to use individuals within families with the highest possible breeding values given practical limits.

APPLICATION OF LIVESTOCK BREEDING TOOLS TO AQUACULTURE BREEDING PROGRAMMES

Peter Amer

AgResearch Invermay, Private Bag 50034, Mosgiel, New Zealand.

Breeding programmes for commercial farm animals have varied in their success depending partly on the power, cost and convenience of achieving high reproductive potential, as well as the social and organisational structures of the industries. Aquacultural industries would appear to have some advantages for successful breeding programmes in that they may tend to be more vertically integrated across breeding, production, manufacturing and marketing segments, and have much higher reproductive and genetic dissemination capacity, than farmed mammals. A common set of tools used in livestock breeding programmes is presented below;

- Development of breeding objectives for weighting emphasis across traits and for cost benefit calculations
- Variance component estimation to establish the degree of genetic determination of phenotypic traits and relationships among traits
- Breeding value estimation to increase accuracy and simplify selection decisions where information is available on many relatives for many different traits
- Breeding scheme design and selection decision analysis based on quantitative genetic prediction of genetic progress under alternative design scenarios.

The seminar will present each of these tools in principle, and contrast their application in livestock breeding programmes such as sheep and deer with their application in Salmon breeding. A fairly detailed focus on a dynamic selection algorithm which trades-off genetic progress for reductions in inbreeding, and its practical application, will also be provided.

Note for NZKS - While I hope to mention some of the traits measured by NZKS, no indication will be given of actual genetic relationships among them, or their heritabilities etc. I would also like to show that breeding values are used to cull some individuals (2 year old males), to select fish for the next nucleus generation, and to target commercial offspring to appropriate types of production systems.

THE APPLICATION OF GENETIC MARKERS TO FIJIAN TILAPIA STOCK IMPROVEMENT

S.A. Appleyard[1] and P.B. Mather[2]

1. CSIRO Marine Research, GPO Box 1538, Hobart, 7001, Australia.
2. School of Natural Resource Science, Queensland University of Technology, GPO Box 2434, Brisbane, Queensland, 4001, Australia.

Fiji's Fisheries Division has the responsibility for developing tilapia culture in Fiji. The Division recognised the necessity for genetic evaluation, monitoring of stocks and the application of appropriate breeding approaches if tilapia production is to continue to meet market demands. To this end, genetic markers (allozymes and RAPDs) developed in the current study, characterised the levels and patterns of genetic variation in 4 cultured stocks of tilapia (*O. niloticus* 'Chitralada', *O. niloticus* 'Israel', *O. mossambicus* and a Red hybrid stock) across 4 generations. Although marker analyses revealed identical patterns of relationships among the stocks, genetic variability levels differed according to marker type. Allozyme electrophoresis resulted in products at 50 allozyme loci of which 25 were polymorphic among the stocks. RAPD electrophoresis of seven random 10mer primers resulted in products at 95 loci of which 58 putative loci were polymorphic among the stocks. Processes other than gene introgression maintained relatively high levels of genetic variability within most of the stocks. However, low levels of genetic variation and the poorest growth performance across the generations may preclude the use of *O. mossambicus* in future applications. This research established baseline information and genetic protocols for Fijian cultured tilapia and was the first characterisation of genetic diversity in any tilapia stock in the Pacific region. Adopting the genetic approaches outlined in this study and development of an ongoing monitoring schedule will ensure the long-term quality of Fiji's tilapia stocks.

SELECTIVE BREEDING OF PACIFIC WHITE SHRIMP (*LITOPENAEUS VANNAMEI*) FOR GROWTH AND TSV RESISTANCE

B.J. Argue[1], S.M. Arce[1], J.M. Lotz[2] and S.M. Moss[1]

1. The Oceanic Institute, 41-202 Kalanianaole Hwy., Waimanalo, HI. 96795
2. University of Southern Mississippi - Gulf Coast Research Laboratory, 703 East Beach Dr., Ocean Springs, MS 39564

The Oceanic Institute has operated a shrimp breeding program where *Litopenaeus vannamei* has been selected 50% for growth and 50% for survival to Taura Syndrome Virus (TSV). Over eight generations, the genetic correlation between harvest weight and TSV survival was negative (r_G = -0.45 ± 0.09(SE)). Consequently, we decided to select a line 100% for growth and a second line weighted 70% for TSV resistance. Selected shrimp in the growth line were 21% larger than controls (24.2g vs. 20.0g). Half-sib heritability (h^2) estimate for growth was 0.84 ± 0.43(SE) and realized h^2 was 1.0 ± 0.12(SE). Females were 12.7% larger than males but h^2 estimates within gender were similar to the overall estimate. Shrimp averaged 65.1% tail meat with males having a larger percentage than females (65.7 vs. 64.5%). Half-sib h^2 for percent tail was not significant (0.04 ± 0.06(SE)). In the TSV line, there was an 18.4% increase in survival to TSV between selects and controls (46.4 vs. 39.2%). Realized h^2 was 0.28 ± 0.14(SE) and half-sib h^2 for TSV resistance was 0.19 ± 0.08(SE). However, TSV selected shrimp were 4.6% smaller than controls (22.6g vs. 23.7g). The populations were 51.0 and 51.6% female in the growth and TSV line, respectively. Percent female per family ranged from 39.7 to 69.2%, but heritability for sex ratios was not significantly different from zero (-0.002 ± 0.01 and -0.012 ± 0.014(SE)) indicating that we cannot breed for a higher percentage of females. These results indicate that significant improvements in growth and TSV resistance can be made with selective breeding; however, the negative correlation between growth and TSV resistance must be considered when developing breeding plans.

THE HAZARDS OF AQUACUTURE: THE RAPID EXTINCTION OF A FRESHWATER CRAYFISH SPECIES

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The marron, *Cherax tenuimanus*, is a large species of freshwater crayfish restricted to the extreme south west of Western Australia. Allozyme studies of this species conducted between 1979-1982 indicated that two genetically and morphologically distinct forms of marron exist. One form is relatively widespread and is utilised by the local aquaculture industry. The other form is restricted to a single river system, the Margaret River. Samples of marron have been obtained periodically from the Margaret River from 1979 to 1998 and examined for allozyme variation. Over this time period the allozyme data indicate that the widespread form of marron was introduced into the Margaret River in the early-mid 1980s and has been rapidly displacing the endemic Margaret River marron since. At one sampling site there has been 100% changes in allelic frequencies within a 17-year period. The implication of these findings for the management and conservation of wild stocks of aquaculturally important species are discussed.

INTRA- AND INTERSPECIFIC ANDROGENETIC RECOVERY OF NUCLEAR GENOME INFORMATION FROM SALMONID CRYOPRESERVED SPERMATOZOA*

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A series of experiments was conducted in order to test: (i) whether androgenesis may be a mean to recover nuclear genome information originating from salmonid cryopreserved spermatozoa, and (ii) whether induction of androgenetic development of one species is possible if host oocytes from other species (interspecific androgenesis) are used. The success of androgenesis was confirmed using microsatellite DNA analysis. We have obtained 9.4 - 21.0% of androgenetic swim-up larvae in rainbow trout (*Oncorhynchus mykiss*) originating from cryopreserved spermatozoal genomes. The survival considerably decreased to 4.7 - 10.7% after 3 weeks of feeding. Androgenesis in brown trout (*Salmo trutta*) and brook trout (*Salvelinus fontinalis*) yielded, in best treatments, 2.5% and 4.3% of hatched larvae, respectively. Histological examinations of androgenetic individuals showed no differences in gonadal development and phenotypic sex ratio, as compared to controls. There was no difference in efficiency of androgenesis between inbred and outbred sources of rainbow trout spermatozoa, whereas outbred oocytes were much more suitable for irradiation and pressure shock than those of inbred origin. No interspecific androgenetic progeny was obtained among the three genera: *Salmo*, *Salvelinus* and *Oncorhynchus*, despite the fact that 4 of 6 possible control hybrid progenies and intraspecific androgenotes were viable. Interspecific androgenesis within two genera resulted in very low hatching rates: 0.2% of androgenetic Atlantic salmons (*Salmo salar*), developed in brown trout oocytes, and 0.4% of androgenetic Arctic charrs (*Salvelinus alpinus*), developed in brook trout oocytes. This indicates very limited possibility for a restitution of one salmonid species using oocytes from other species, even closely related, by means of homozygous androgenesis.

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MULTIPLE-LOCUS HETEROZYGOSITY, PHYSIOLOGICAL TRAITS AND GROWTH IN A COHORT OF THE CHILEAN-PERUVIAN SCALLOP *ARGOPECTEN PURPURATUS* (LAMARCK, 1819)

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The relationship between individual heterozygosity and six key physiological traits (clearance rate, ingestion rate, absorption efficiency, absorption rate, oxygen consumption, scope for growth, net growth efficiency) was investigated in a laboratory-produced cohort of *Argopecten purpuratus* (Lamarck, 1819). Based on the analysis of 19 loci resolved by starch electrophoresis, six of which were polymorphic (*6-Pgdh, Pgm, Pgi, Idh y ODH*), the cohort exhibited 31.6% of polymorphic loci and a mean heterozygosity of 0.0881. All loci showed a deficiency of heterozygous (D=-028), significantly MDH and ODH. A positive correlation, not significant though (P>0.05), between multi-locus heterozygosity and the standardised physiological rates related to energy uptake and utilisation were found. Although statistically insignificant the fact that more heterozygous individuals did better in energy uptake while at the same time expended similar amount of energy than those more homozygous, is a biological relevant phenomenon which explains why their energy available for growth and/or reproduction was higher. These results are relevant for evolutionary biology and have also practical consequences for aquaculture.

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GENETIC STATUS AND STRATEGIES FOR IMPROVEMENT OF COMMON CARP FOR AQUACULTURE IN KARNATAKA, INDIA

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Common carp, first introduced into India in the late 1950's, is becoming an increasingly important species for low input aquaculture in Karnataka state in southern India due primarily to the relative ease of year round seed production. The local strain of common carp was shown to exhibit precocious sexual maturation and unwanted reproduction under culture. Analysis of hatchery practices demonstrated the likelihood of indirect selection, inbreeding and genetic drift occurring in hatchery populations. Three approaches were investigated as potential solutions: i) the production of monosex populations through the application of exogenous hormones; ii) the production of sterile triploids by application of temperature shocks to fertilised eggs; and iii) the evaluation of the culture performance of a strain newly introduced from Vietnam.

Successful masculinization protocols producing monosex male progeny were developed by oral application of methyldihydrotestosterone (MDHT) and androstenedione. Treated fish are being progeny tested to identify sex reversed XX males which should sire all female progeny. Growth trials of triploid fish indicated slightly but not significantly lower weight at the age of sexual maturation. However, triploids had 9.2 to 13% higher dress-out weights than diploids due to their significantly lower gonadosomal indices. On-station and on-farm growth trials demonstrated faster growth (20-84% and 15% respectively) and delayed sexual maturation of the introduced Vietnamese strain compared to the local common carp strain. These results are discussed in the context of providing long term solutions to the problem of poor seed quality in tropical common carp culture.

GENETIC VARIATION IN THREE GENERATIONS OF HATCHERY-BRED NILE TILAPIA *OREOCHROMIS NILOTICUS*

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Twelve enzymes were assayed in three generations of hatchery-bred *Oreochromis niloticus* maintained at the Binangonan Freshwater Station of SEAFDEC. Starch-gel electrophoresis followed by histochemical staining resolved seventeen loci. Six loci (CK, EST-2, GPI-1, PGM and SDH-1) were polymorphic while eleven were monomorphic. However, PGM was also monomorphic in the first generation fish. No significant deviations from Hardy-Weinberg expectations occurred in the three populations examined. Observed heterozygosity (0.065) in the founder population was higher than the first generation (0.048) and second generation fish (0.047). The mean heterozygosities found in these hatchery populations were lower than the Japanese stock of Nile tilapia (0.091) studied by Basiao and Taniguchi (1984), but similar to the heterozygosity of the Thailand strain (0.048) studied by Macaranas et al. (1995). The genic variability found in the present study is also higher than the Egypt (0.031), Ghana (0.026), and Kenya strain (0.021) reported by Macaranas et al. (1995). The number of broodstock used to produce the 2000 founder fry obtained from Bankok was not known. The first and second generations were produced by the mass spawning of two hundred spawners at a 1 male :1 female sex ratio. The high ratios of Ho/He (0.956 - 1.093) suggest that inbreeding and genetic drift had very little effect on the hatchery populations maintained at the Binangonan Freshwater Station. The effective number of spawners that contribute their genes to the next generation is crucial in maintaining a good level of genetic variability.

DESIGNING AQUACULTURE MASS SELECTION PROGRAMS TO AVOID HIGH INBREEDING RATES

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Most aquaculture organisms are highly prolific. It is often argued that this makes it possible to run aquaculture mass selection programs with high selection intensities. A small number of extremely well performing individuals may be selected to produce a large test population of breeding candidates in the following generation. However, the effective population size depends on the number of breeders selected in each generation rather than the size of the test population, and will be further reduced by selection. High selection intensities may consequently result in rapid accumulation of inbreeding and loss of genetic variability. A series of replicated stochastic simulations was carried out to determine the effect of number of breeders selected (4-100 pairs), number of progeny tested (5-150 per pair) and the magnitude of the heritability (0.1-0.4) on inbreeding and genetic progress through 16 generations of mass selection. It was found that to keep inbreeding rates low (<1% per generation), a minimum of 50 pairs of breeders should be selected and the number of progeny tested per pair should be restricted to between 20 and 150 depending on the heritability. This resulted in a genetic progress of 5-11% per generation. Reducing the number of breeders and testing more progeny per pair increased the rate of inbreeding to as much as 9% per generation. Loss of genetic variability was found to reduce the genetic progress at least as much as what was gained by the increased selection intensities. In addition, a further reduction is likely to occur because of inbreeding depression.

GENETIC VARIATION AMONG WESTERN AUSTRALIAN PEARL OYSTER (*PINCTADA MAXIMA*) POPULATIONS: A MICROSATELLITE SURVEY

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Allozyme studies have indicated no genetic distinction between Western Australian populations of the pearl oyster, *Pinctada maxima*, though recent differences in recruitment dynamics suggest some degree of isolation may exist between populations in WA. The present study has developed microsatellite markers for *P.maxima* and assessed the degree of isolation of pearl oyster stocks used by the Pearling Industry in WA. Approximately 1200 samples were collected across three different age classes and from 5 different locations in WA and from 1 location in the Northern Territory. Several genomic libraries were constructed and screened revealing 124 positive clones containing microsatellites. PCR primers were designed for 34 different loci although only 19 loci could be optimised for fluorescence based PCR. Fifteen microsatellite loci proved to be polymorphic and eight of these polymorphic loci have been assayed for both the adult age class. Preliminary data analysis suggests some differentiation of pearl oyster stocks between locations in Western Australia.

RESPONSE TO SELECTION FOR BODY WEIGHT IN NILE TILAPIA (*OREOCHROMIS NILOTICUS*) USING A SINGLE-TRAIT ANIMAL MODEL

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Within-family selection for improved growth at 16-weeks was undertaken on Nile tilapia (*Oreochromis niloticus*) from 1986-1996. Data from 12 generations of selection were analyzed by a single trait Restricted Maximum Likelihood fitting an animal model. The heritability in the base population was estimated as 0.34. Genetic response for body weight was found with an expected mean increase in body weight of 2.2 g or about 12.4 % per generation. A heritability estimate of 0.14 was obtained based on the regression of mean breeding values on cumulative selection differentials after 12 generations. In close agreement was the realized heritability obtained from regression of selection response (estimated against a random-bred control line) on cumulative selection differentials.

The inbreeding coefficient was 6.3% after 12 generations with an average inbreeding rate of 1.4% per generation. The family rotational mating used to propagate the families was effective in keeping the inbreeding level to a minimum even at a fairly high selection intensity.

Overall, the low inbreeding levels, high selection intensities and the relatively high heritability for body weight at 16-weeks in the population that was used in this selection experiment resulted in substantial response using the within-family selection method.

CONFORMATION AND CARCASS QUALITY TRAITS IN SEAWATER ADULT BROWN TROUT: CORRELATED RESPONSES TO SELECTION FOR FRESHWATER BODY LENGTH GROWTH AND TRIPLOIDY X SELECTION INTERACTIONS

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Correlated responses were evaluated after three generations of selection for freshwater length growth, on diploid and triploid seawater adult brown trout (*Salmo trutta*). Conformation assessed by direct morphological measurements and by geometric analysis of the shape, and quality carcass traits related to bone, muscle and adipose tissues development were more specifically analysed. Control Line (CL: n = 126) and Selected Line (SL: n = 129) were slaughtered at 2.5 years old, after a 15 months seawater rearing period. SL was heavier and longer than CL (mean body weight: 2531.2 g ± 788.6 *vs.* 2023.1 g ± 657.5 and mean body length: 54.8 cm ± 5.1 *vs.* 51.4 cm ± 4.6) corresponding to + 6.7 % of response to selection in length and + 25.0 % of correlated response in body weight.

The magnitude of correlated responses in carcass and visceral weight (+ 24.6 % and + 24.5 %) was similar to the one observed for body weight, suggesting that no modification of visceral adipose tissue weight was induced by the selection for body length growth. The magnitude of correlated response in fillet weight (+ 22.4 %) was smaller than that measured on body weight. This unfavourable correlated response was related to significant higher fillet dressing losses in the SL compared to the CL, with the following decomposition: head weight (+ 29.4 %), vertebral axis weight (+ 29.3 %) and dressing loss weight: skin, fin and perimuscular adipose tissue (+ 27.8 %).

Morphological traits were not affected by selection except for the body width (+ 5.6 %) and the anterior and head lengths (+ 3.8 % and + 14.1 %). Differences in shape between the two lines, tested by geometric analysis, were limited to the head area.

Although triploid characteristics were different than those of diploid, interactions between selection and triploidy were not significant.

Our results demonstrate that selection for freshwater body length growth induces correlated responses in bone and fillet dressing losses weight, which could affect adult carcass quality traits.

MICROSATELLITE MARKERS AS A TOOL TO STUDY REPRODUCTIVE SUCCESS IN THE PACIFIC OYSTER, *CRASSOSTREA GIGAS* (THUNBERG), CROSSED UNDER CONTROLLED HATCHERY CONDITION

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Oysters, like many marine species have a very high fecundity. Previous studies have shown that populations, from both hatcheries and the natural environment, have very low *Ne/N* ratios. These observations reveal high variation in reproductive success. In order to study individual reproductive success under controlled conditions, we used microsatellite markers to quantify parental contributions in *in vitro* crosses (5 males and 5 females) of *Crassostrea gigas,* the Pacific oyster. High polymorphism of the microsatellites (more that 50 alleles per locus) eased the parentage identifications. The results of a cross allowing gametic competition were compared with the results from a second cross where the gametes of the same parents were kept separate for each parental combination until after fertilization. The progeny were then sampled at three stages of development and the parental contributions determined to follow their evolution through time. Despite the fact that equal numbers of gametes were mixed for each male and each female, the contributions of these parents to the resulting progeny was highly unbalanced at both larval and juvenile stages in both crosses. We demonstrated that variation in individual reproductive success is due to both spermatic competition and selective phenomena at early stages.

ANEUPLOIDY AND ITS RELATIONSHIP WITH GROWTH IN DIFFERENT POPULATIONS OF THE PACIFIC OYSTER (*CRASSOSTREA GIGAS*, THUNBERG)

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Cytogenetic abnormalities arising both in mitosis and meiosis are known to be common in bivalves. Here we review result obtained from the observations of somatic aneuploidy in different populations of the commercially important Pacific oyster, *Crassostrea gigas*.

The oysters studied were either (1) produced in IFREMER hatcheries between 1986 and 1999, (some of these were part of the EU funded programme GENEPHYS); or (2) collected from wild populations along the French Atlantic coast. Chromosome numbers were scored from 30 mitotic metaphases in gill tissue per individual studied. Aneuploid cells (2n - X) were observed in all the 13 populations studied. A highly significant negative correlation was observed between the level of aneuploidy and growth, i.e. fast growing animals in each population showed less aneuploidy than slow growing animals. In one of these populations, this negative correlation was established at the individual level by comparison of individual growth performances recorded under common controlled conditions. The transmission of aneuploidy, from parents to their progenies, was investigated; but the inheritance of this phenomenon could not be demonstrated. However, a genetic basis, associated with the geographic origin of the parents, was suggested. The study of the aneuploidy level in six full-sib families, showing contrasting growth performances under common controlled conditions, implied a genetic base for the control of aneuploidy and the aneuploidy-growth relationship.

FETII 1.0 : WINDOWS 9X BASED SOFTWARE FOR PARENTAGE ANALYSIS USING CODOMINANT MARKERS DATA AND OPTIONAL PUTATIVE PEDIGREE FILES

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Fetii 1.0 is a convivial software developed to facilitate the work of geneticists dealing with improvement programs in which the pedigrees are not precisely known (like in many aquaculture programs where selected populations are structured in multiparental batches). It can be used even for hermaphrodite species or when the sex of the parents is undetermined. Data organized in Microsoft® Excel 5.0 files can be used directly by Fetii 1.0 and all the results are given in new Microsoft® Excel sheets.

Its first function is the building of putative pedigree files : the required information is the multiparental origin of the batches and the belonging of the offspring to these batches. It draws a file which gives the probabilities for the offspring to be issued from a given mating.

The main function of Fetii 1.0 is parentage analysis using codominant marker data. It calculates an exclusion matrix which gives (i) for each studied offspring and for each putative mating the percentage of loci which are in concordance with the known genotypes and (ii) for each studied offspring the number of matings for which 100% loci are matching. If some additive information about putative pedigrees is available, it can be used directly to speed up the calculations and to eliminate the non-probable matings which would not have been eliminated by the genotype analysis.

Fetii 1.0 has also a function to look for possible common ancestors between two given individuals which is useful to avoid or limit inbreeding in closed populations.

THE EFFICIENCY OF MULTI-STAGE SELECTION IN THE BREEDING OF RAINBOW TROUT (*ONCHORHYNCHUS MYKISS*) FOR IMPROVED RATE OF GROWTH AT 12 MONTHS OF AGE

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This paper describes the efficiency of multi-stage selection in comparison to random reduction of numbers during a procedure of combined selection for improved rate of growth in rainbow trout (*Onchorhynchus mykiss*) at 12 months of age. The application of a combined selection procedure requires the introduction of a large number of families to obtain a high intensity of selection between families. High fecundity of rainbow trout ensures that a high intensity of selection can be obtained for selection within families. Introduction of a large number of families ($m \cong 50$) with a large individual family size ($n \cong 3000$) places high demands ($N \cong 150\ 000$) on facilities. This requires either random reduction of the number per family or multi-stage selection (W_1, W_2, M_3, M_4) within families, at various stages leading up to the final stage of selection at age 12 months. The distortion of the initial multivariate distribution together with the use of different selection criteria, such as body width during the early stages (W_1, W_2) associated with high numbers, as oppose to body mass during the latter stages (M_3, M_4), complicates the prediction of the efficiency of the multi-stage selection procedure. Contribution from selection at stages W_1 (age 4 months) and W_2 (age 6 months) to the overall gain are small in comparison to stage M_3 (age 9 months) and especially M_4 (age 12 months). Where management requirements are to be met by random reduction in numbers per family at stages W_1,W_2, M_3, M_4, the expected genetic superiority of those selected would then be about 75% of that of fish selected by multi-stage method. We therefor conclude that multi-stage selection procedures should be retained as part of the combined selection program.

MITOCHONDRIAL AND NUCLEAR INHERITANCE IN ANDROGENETIC CLONAL RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Mitochondrial and nuclear inheritance of OSU clonal rainbow trout produced using androgenesis were evaluated using DNA sequencing, AFLP and RFLP. All individuals were produced using sperm from a single sex reversed homozygous female parent crossed with multiple egg sources whose nuclear material was destroyed using Cobalt^{60} gamma radiation. Developing haploid embryos were then heat shocked to prevent the first cleavage restoring a diploid state. Sequencing of 4,716 continuous base pairs was performed on four rainbow trout and one cutthroat trout mitochondrial haplotype spanning the NADH 5 protein through the mitochondrial control region. Total sequence variation between haplotypes ranged from 0.34-6.14% with rainbow trout haplotype variation between 0.34-0.85%. DNA sequence for coded proteins was converted to amino acid sequence using a standard mammalian mitochondrial code to establish conservative and non-conservative amino acid substitutions. Total amino acid variation ranged from 0.60-3.10% with rainbow trout haplotype variation from 0.60-1.12%. Non-conservative amino acid substitutions revealed unique protein sequence's for all mitochondrial haplotypes. AFLP and RFLP techniques were performed on 119 clonal individuals to determine levels of nuclear genetic variation within the line. Low levels of genetic variation were observed among individuals using both AFLP and RFLP with total disparity between individuals not more than 1.0%. This level of genetic variation is comparable to reported genetic variation within inbred lines of mice. Identified variable loci are possibly the result of tetrasomic inheritance. Six (6) polymorphic AFLP loci were randomly chosen for genetic mapping to address this question.

GENETIC VARIABILITY AMONG NATURALIZED POPULATIONS OF RAINBOW TROUT *ONCORHYNCHUS MYKISS* (WALBAUM) OF THE NORTHERN CHILEAN RIVERS ELQUI AND LIMARÍ

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The rainbow trout *Oncorhynchus mykiss* was introduced to Chile starting in 1905. At present it is distributed from the Loa River (23ºS). The broad range of distribution, geographic and climatic, of this species in Chile gives an opportunity to study the effect of artificial introduction on its genetic variability. In the present work, the genetic variability of populations of *O. mykiss* from the Elqui and Limari river basins, in Northern Chile, was analyzed using protein electrophoresis. All populations were in Hardy-Weinberg equilibrium, with an averaged polymorphism of 16% and a mean heterocigosity Hi = 0.048. Eighty five percent of the total genetic variability was explained by intrapopulation genetic variation and the remaining by between populations within rivers and between river basins variation. The mean unbiased genetic distance among populations was D = 0.01. The amount of genetic variability and its distribution within and between populations was similar to that described for wild populations in the native range of distribution of the species, but different to those reported for populations from Southern Chile.

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SEX DETERMINATION IN *DICENTRARCHUS LABRAX* : NO EVIDENCE FOR MALE OR FEMALE HETEROGAMETY

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In order to test the hypothesis of male or female heterogamety in *Dicentrarchus labrax*, three approaches were performed.

A population of juveniles was masculinized through oral administration of methyldeshydrotestosterone. 100% phenotypic males were obtained versus 88% in untreated controls. Twelve of these males were crossed with normal females. Female percentages obtained in these offspring varied from 66 to 87%. No all female offspring suggestive of a simple XX/XY monofactorial system was observed.

Another population of juveniles was feminised through oral administration of estradiol and led to 100% phenotypic females (control group : 30% females). Three of these females were crossed with normal males. Female percentages obtained in these offspring were 55, 63 and 75%. No monosex male offspring was observed that could have led to conclude to a simple ZZ/ZW monofactorial system.

Induced meiotic gynogenesis was performed through pressure shock on eggs of 4 females activated by irradiated homologous sperm (Peruzzi et Chatain, *in press*). Gynogenetic individuals were reared-up to sexing together with control groups constituted of the same crossing but using non irradiated sperm. Female percentages in gynogens oscillated between 30% and 73% and differed significantly from those of control groups (comprised between 72 and 80%). No monosex female issue was observed allowing to conclude in favour of a simple XX/XY monofactorial system.

These results cannot lead to conclude to simple male or female heterogamety but are suggestive of a more complex mechanism controlling sex determination in *Dicentrarchus labrax* possibly under the modulation of environmental factors.

DETECTION OF PUTATIVE QTLS FOR BODY WEIGHT AND COLD TOLERANCE IN INTERSPECIFIC TILAPIA HYBRIDS

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Tilapia culture in temperate climatic regions is highly affected by the species sensitivity to low temperatures. We have used 20 microsatellite markers to search for quantitative trait loci (QTL) associated with cold tolerance and body weight in tilapia. Linkage tests have been conducted in an F_2 family of *Oreochromis aureus* x *O. mossambicus* hybrids. We have found two markers putatively associated with cold tolerance and three markers putatively associated with body weight. Two of those markers are located on the same linkage group, apparently a chromosomal region, which affects growth and survival in tilapia.

A GENETIC LINKAGE MAP OF THE JAPANESE FLOUNDER, *PARALICHTHYS OLIVACEUS*, AND OBSERVATIONS ON SEX RECOMBINATION

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We report the first genetic linkage map of the Japanese flounder constructed with 112 microsatellite markers and 352 AFLP fragments typed on 44 individuals of F1 progeny. The parental male linkage map consisted of 25 linkage groups while the female map consisted of 27 groups, with an average resolution of 8 cM and 6.6 cM, respectively. We have identified linkage among 96% of the 444 markers and the total map length was estimated to be around 1000-1200 cM. This study is the first step towards mapping of single loci and quantitative traits in flounders. The sex differences in recombination were higher in male flounder in contrast to other species, including fish, where this is true for the opposite sex.

EFFECT OF GENOTYPE-ENVIRONMENT INTERACTION ON THE SURVIVAL AND GROWTH OF THE KURUMA SHRIMP *PENAEUS JAPONICUS*

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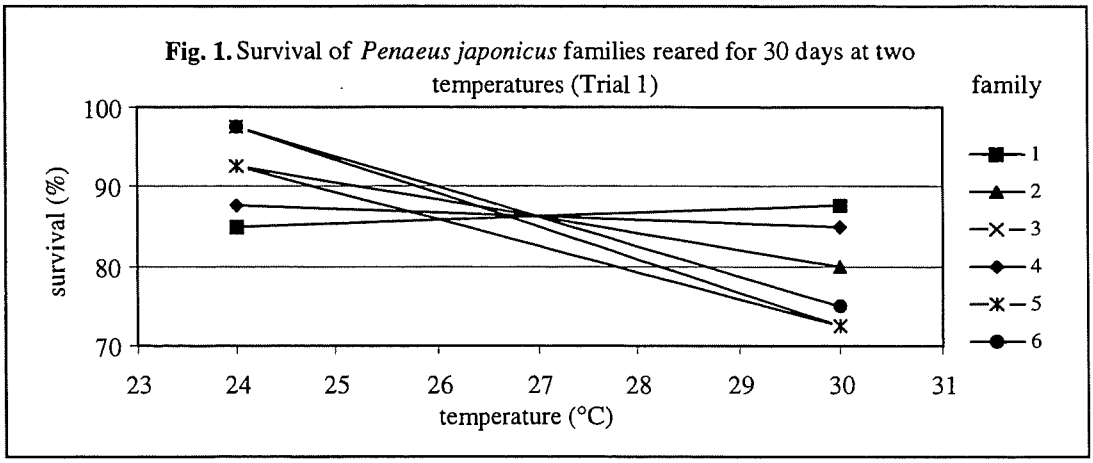
Increased production of livestock can be obtained through the selection of advantageous genotypes in breeding programs. When stocks from breeding programs will be exposed to a range of conditions, it is important to know if there is interaction between the genotypes and the different environments. Genotype-environment interaction occurs when a specific change in the environment does not have the same effect on all genotypes, and can affect the efficiency of selection.

Significant improvements in the growth of *Penaeus japonicus,* a shrimp farmed commercially in Australia, have been demonstrated through genetic selection. The protocols for domestication and selection of this species are now also being applied to another species farmed in Australia, *P. monodon*. Farming of these two species occurs over a large geographic area covering a range of environmental conditions. Presently, it is not known if interactions between genotype and environment need to be considered in the design of breeding programs for these two species. These interactions need to be quantified in order to design breeding programs suitable for the shrimp farming industry.

This study assessed the survival and growth of *P. japonicus* families reared at different temperatures in two trials. Survival was more affected than growth by family-temperature interaction. Interactions for both survival and growth were significant when differences in temperature were larger than 5°C. Changes in the survival ranking of families were found when reared at 24°C compared with 30°C (Fig. 1). The magnitude of these interactions will determine whether different families would be better suited to specific temperatures.



COMPARATIVE GROWTH, SURVIVAL AND REPRODUCTIVE PERFORMANCE OF INBRED AND OUTBRED LINES OF DOMESTICATED SHRIMP, *PENAEUS JAPONICUS*, IN AUSTRALIA

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Recent research advances by CSIRO in shrimp domestication and selective breeding has enabled rapid advances in genetic improvement of farmed shrimp. Over the past 6 years, collaborative research between CSIRO and industry has resulted in successful domestication, captive breeding and demonstrated genetic improvement of *P. japonicus*. Improvements in growth rates through selective breeding have been demonstrated in the laboratory and in commercial farm trials.

In order to assess the benefits of intense selection versus the risk of potential negative effects of inbreeding, a systematic assessment of the effects of inbreeding was made. To make this assessment, we produced comparative families that were of known parentage and otherwise genetically equivalent, but differing only in the known level of inbreeding. Using CSIRO F3 stocks, 4 initial families were used to produce 8 F4 families; 4 from full-sib matings and 4 from rotational matings, resulting in families of known and variable levels of inbreeding. The pedigree for each line was confirmed by DNA fingerprinting. The families were grown to adult size in a controlled-environment tank growout system, during which time the survival, growth and reproductive performance of the families was assessed. The experiment was repeated for the F5 generation to assess the cumulative effects of inbreeding.

There was no difference in the growth performance of the inbred and outbred lines over the 2 generations. Survival was depressed in the second generation of the inbred lines (63% for inbred and 83% for outbred). Reproductive performance was depressed in the second generation of inbred lines (mean 980 nauplii per gram of female for inbred lines, mean 1700 nauplii per gram for the outbred lines.

Establishing and quantifying the effects of inbreeding on production attributes is essential for the optimisation of breeding program design for *P. japonicus* and other farmed shrimp species.

MOLECULAR GENETIC INVESTIGATIONS TO ASSIST A RANCHING PROGRAMME FOR ATLANTIC SALMON (*SALMO SALAR* L.)

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Many western European rivers have been dammed for hydro-electric production, often prohibiting access to spawning areas by anadromous Atlantic salmon. In such cases mitigation hatcheries produce fry for stocking and smolts for ocean ranching. The river Erne in north-west Ireland is one such example. To guide management and to optimise performance of reared salmon, three cohorts of hatchery fish have been investigated using four microsatellite DNA loci (with two other microsatellites or four minisatellite loci often being included). In addition, wild or feral adults returning to the Erne have been investigated, as have salmon from a large hatchery on another catchment more than 200 Km to the south, which, in certain years, provides salmon the Erne hatchery. To consider the Erne in context, wild salmon populations of four rivers entering the same bay (local scale) and in seven large rivers throughout Ireland (regional scale) have also been screened, again for two cohorts, to investigate temporal as well as spatial variation. Genetic variability level in the Erne hatchery salmon is comparable with wild populations, while composition is similar to neighbouring rivers. With wild salmon, temporal stability is evident, whereas significant inter-catchment variation is evident, with some evidence of genetic difference increasing with geographical distance. Management strategies for the river Erne are discussed, as is the distribution of genetic variability within wild Irish salmon populations. Since many of the same loci have been used to study salmon population structure in other regions, comparison on Pan-European and inter-continental scales is also possible.

GENETIC STUDIES OF FARMED TILAPIA IN FRESH AND BRACKISH WATER IN VIETNAM

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A breeding program was started in 1999 for improving survival and growth rate of farmed Nile tilapia in Vietnam. As a base population, 90 females and 50 males from the GIFT population in the Philippines were mated. In addition, 8 groups of fish resulting from mass spawning of GIFT broodfish and 8 groups of fish resulting from indigenous Viet strains were included. The families were reared in two ponds in fresh water (50 progeny per family) and one pond in brackish water (20 progeny per family). The survival rate was high in both fresh water (88 %) and brackish water (86%). A good growth rate resulted in average harvest weight (after ca 190 days) of 175 g in fresh water. In brackish water, average harvest weight was considerably lower (89 g). Heritabilities for growth rate were estimated together with genotype- environment interaction for fish reared in fresh or brackish water. Heritability for growth rate in fresh water was estimated to ca. 0.1, whereas no genetic variation or heritability could be revealed from the data in brackish water. Analyzing data from both fresh and brackish water, significant interaction between genotype and environment was found. Further genetic studies of growth and survival in brackish water are needed. The average harvest weight of the GIFT fish was 32 % higher than the Viet strain in fresh water. In brackish water, the performance of GIFT fish was 17 % higher than the indigenous strain.

GAMETIC PHASE DISEQUILIBRIA AS INDICATORS OF GENOMIC COADAPTATION AMONG UNLINKED CHROMOSOMAL SEGMENTS IN SALMONID FISHES

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Deviations from expected pairwise Mendelian segregation ratios between unlinked molecular markers within full-sib progenies can provide insights into past selective events that have occurred in the evolutionary history of a strain. Under a directional selection regime it may be hypothesized that the most functionally coadapted alleles will increase in frequency within a strain. The possibility that such functionally coadapted alleles may also exhibit increased gametic phase disequilibria was investigated in rainbow trout using known quantitative trait locus (QTL) markers for upper thermal tolerance in the species. Fish were derived from two strains strongly selected for upper and lower thermal tolerance over 3 generations and then bred within lines for another 4 generations.

Disequilibria dynamics are also discussed in relation to the insights such measures can provide on assessing the degree of outbreeding depression among hybridized salmonid genomes. Evidence for segregation distortion may be used to initially to identify regions of chromosomal incompatibility among hybridizing genomes. Assessment of disequilibria among such regions may then be used to determine if significant deviations in disequilibria among full-sibs are related to the parental origins of the strains. Fundamental differences in male/female specific recombination rates among salmonid fishes are expected to strongly influence disequilibrium measurements, and the effect of such rate differences are considered.

ISOLATION AND EXPRESSION OF A PUTATIVE REPRODUCTIVE INHIBITORY HORMONE FROM THE GIANT TIGER PRAWN, *PENAEUS MONODON*

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Achieving reliable spawning of female broodstock of the giant tiger prawn, *P. monodon,* in aquaculture is highly problematic. At present, the only practical means is unilateral eyestalk ablation, which leads to ovulation and spawning over 3-7 days in 60-80% of broodstock. The physiological basis for this effect is removal of the source of a reproductive inhibiting hormone (RIH). RIH belongs to a family of related hormones that also includes crustacean hyperglycemic hormone (CHH) and moult inhibiting hormone (MIH). These hormones are produced in the X-organ and stored in the sinus gland, both of which are located in the eyestalk. Hence unilateral eyestalk ablation removes the source of 50% of the prawn's RIH, leading to ovarian maturation, but it also causes serious physiological effects due to the physical stress and the removal of other key hormones. Hence we are interested in identifying and characterising the RIH from *P. monodon*, with the aim of devising a more specific means to achieve reliable spawning of broodstock in aquaculture.

We have characterised six different CHH/MIH/RIH-like peptides from *P. monodon* by a combination of protein sequencing, gene cloning and Fourier transform mass spectrometry (FTMS). All six can be identified in single sinus glands of an individual prawn by FTMS. One of these peptides is a strong candidate to be the RIH. Here we report high level expression of this protein *in vitro* using the cloned cDNA and functional testing of the activity of this putative RIH using the *in vitro* expressed hormone.

MICROSATELLITE VARIATION IN COHO SALMON, *ONCORHYNCHUS KISUTCH*, FROM CHILE

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Microsatellites previously described for *Oncorhynchus nerka, 0. tschawytscha* and *0. kisutch* were amplified using the PCR with DNA of coho salmon from the IFOP-Coyhaique hatchery, Chile.

The PCR conditions were very similar to those described and cross-amplification occurs for almost all the heterologous microsatellites. Alleles resolution was obtained submitting the amplification products to electrophoresis using Metaphor agarose.

Some of the microsatellites were monomorphic, and in the polymorphic ones the alleles number ranges from 2 to 10.

Microsatellite variation was higher than isozyme variation when the same year class of fish was compared.

EVALUATION OF SELECTIVE BREEDING RESULTS IN F3 RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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The growth and survival in F3 rainbow trout resulting from family selection were compared to the progeny of the original four lines, an outbred group (from which selection started) recreated by diallele cross of the original lines and a second outbred group constructed in the way to resemble the genetic variation of selected fish. The fish belonging to these seven groups were PIT tagged, than evenly divided for three lots and stocked into separate ponds. To mimic different environments, each lot was fed at different levels (low, optimum and high). At the 507th day (from the start of fry feeding) in the optimum fed lot the selected fish were 35.5% heavier than those from the original lines, 7.2% heavier than the second outbred and 10.8% heavier than the first outbred group. The survival rate in the optimum feeding lot was high for all groups. Thus observed genetic gain in growth rate was between 7.2 and 10.8%.

Optimization of Mating Designs for Inference on Heritability in Fish Species

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Microsatellites allow to redraw pedigrees in groups of mixed families. However, genotyping costs are still high and experiments deserve new optimizations based on total number of genotyped offspring. Using stochastic simulations, this paper compares and optimizes three factorial or partly factorial mating designs for inference on heritability :

FF : full factorial : s sires, each mated with s dams (s^2 families)

FD : s sires, each mated with two dams (2s families)

BH : described by Berg and Henryon (1998) : s sires mated with s dams : sire 1 with dams 1,2 ; sire 2 with dams 2,3 ... sire S with dams 1,S (2s families).

A quantitative trait is simulated according to a strictly additive, polygenic model. Two levels for number of genotyped offspring (NO = 300 or 1000) and three levels for true heritability (h^2 = 0.1, 0.25 and 0.5) are considered. For each NO-h^2 combination, all possible couples number of families (NF) / family size were studied to find the one giving the most precise estimation of heritability. Standard error of heritability was calculated over 5000 repetitions in each situation.

In most cases FF designs are less interesting than FD and BH ones. FD designs are more precise than BH designs, except for NO = 1000 and h^2=0.5. Optimum family size is similar for FD and BH designs and both NOs : 3-5 offspring per family for h^2=0.5, 5-8 for h^2=0.25 and 12-20 for h^2=0.1.

THE USE OF MOLECULAR MARKERS FOR PARENTAGE ANALYSIS IN FARMED AUSTRALIAN ABALONE

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The understanding of mating processes in natural and cultured populations is required for a number of ecological and aquacultural questions. Mating and reproductive success are affected by behavioural, ecological and genetic aspects, all of which ultimately determine the transfer of genotypes from generation to generation. Parentage analysis can be used to either estimate the likely pair of parents for each progeny or to determine patterns of inheritance at the population level. Relatively new DNA markers, microsatellites and AFLPs appear to offer the best potential for parentage analysis due to the level of variation available. Microsatellites are likely to be more useful as they are a dominant marker, whereas AFLPs show dominant/recessive inheritance, which would preclude identification of heterozygotes. In order to establish a selective breeding program known pedigreed family lines must be established and their progress compared. The infrastructure required to produce and maintain a large number of family lines in isolation is high. The ability to identify the parents of all progeny from a mixed spawning event would alleviate the need for single pair crossing to produce the pedigree population. Microsatellite and AFLP markers have been developed for the Australian blacklip abalone, *Haliotis rubra*, and have been used to identify contributing broodstock in a Tasmanian abalone farm. The utility of the markers is demonstrated on known family lines. Parentage and relatedness determination is an essential part of the continued expansion of abalone culture worldwide, and the transfer of this technology to other species will also be discussed.

ALLOZYME AND MITOCHONDRIAL DNA DIVERSITY IN AUSTRALIAN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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An examination of allozyme variation at 27 loci has revealed that Australian rainbow trout (*Oncorhynchus mykiss*) show levels of heterozygosity ranging from 0.062-0.087. This is consistent with natural and hatchery stocks from around the world. There is, however, some evidence for founder effects in that mean number of alleles (A_n = 1.370) is lower than various international stocks and there is statistically significant fluctuations in allele frequency between different stocks. There also appears to be reduced mtDNA haplotype diversity and while this is consistent with other studies, it does suggest some genetic bottlenecking during the pre- and post- introductory phases of rainbow trout to Australia. The implications of these findings on the culture of rainbow trout in New South Wales and Victoria are discussed.

DNA POLYMORPHISMS IN COLOUR VARIETIES OF THE ASIAN AROWANA, *SCLEROPAGES FORMOSUS* BY ARBITRARILY PRIMED POLYMERASE CHAIN REACTION

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The Asian Arowana, *Scleropages formosus* (Osteoglossidae) has been listed as endangered, in Appendix 1 by CITES since 1975. Many farms in Southeast Asia have set up captive-bred populations. The genetic variability of this resource remains unknown and with small founder populations and inbreeding, there will be a loss of variability in the long term. Generating DNA fingerprints AP-PCR is one of the two methods being used to assess genetic variation in these varieties. In this study, DNA fingerprints were generated from three colour varieties of the Arowana, the Red, Green and Gold. DNA polymorphisms were amplified by arbitrary primers (5'→3'): *SP/Sf/AP-PCR/1*: TAT GTA AAA CGA CGG CCA GT; *SP/Sf/AP-PCR/2*: TGC CTG TGG GGA ATC C; *SP/Sf/AP-PCR/3*: CGG TCA CTG T; *SP/Sf/AP-PCR/4*: $(GACA)_4$; *SP/Sf/AP-PCR/5*; $(GATA)_3(GACA)_2$; *SP/Sf/AP-PCR/6*: $(GATA)_2(GACA)(GATA)_2$ and *SP/Sf/AP-PCR/7*: $(GATA)_4$. Bands, which can be used to differentiate between the three varieties, were observed in fingerprints generated by three of the seven primers, *SP/Sf/AP-PCR/2, SP/Sf/AP-PCR/4* and *SP/Sf/AP-PCR/5*. Some bands were unique to the Green variety while other bands were present only in fingerprints of the Red and Gold varieties. Similarity Indices between the three varieties ranged from 0.60-0.90. The work is an on-going collaborative effort between NUS and SP.

MOLECULAR GENETIC ANALYSIS OF *CRASSOSTREA ARIAKENSIS* AND RELATED OYSTER SPECIES

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The oyster industry of the US Gulf and Atlantic states has experienced a severe decline due to overfishing and disease. Efforts to reestablish the industry have taken many forms including increased aquaculture production of the native oyster *Crassostrea virginica,* and the use of non-native oyster species. Because of demonstrated disease resistance and apparent hardiness, Asian oysters such as *C. ariakensis* are being examined for use in aquaculture and as a source of germplasm for development of superior oyster strains. Little is known, however, about the distribution or population genetic structure of *C. ariakensis*. This species is thought to be distributed throughout the warm coastal waters of Pakistan, India, China and Japan. Morphological plasticity and possible hybridization with congeneric species can confound identification of this species. We collected *C. ariakensis* samples from Japan and several locations in China. Species identification of these samples using two interspecific molecular typing keys yielded conflicting results. Restriction fragment length polymorphism (RFLP) data based on one key suggested that the putative *C. ariakensis* samples contained three sympatric *Crassostrea* species. Another species identification key did not support these results. Many individuals classified as *C. gigas* using the first key, typed as *C. ariakensis* with the second key. To provide greater resolution, ribosomal ITS-1 sequence data for individuals from each site were compared to sequences of "known" *C. ariakensis* and *C. gigas*. Sequence data confirmed that most individuals in the collected samples were *C. ariakensis*. We found additional restriction enzyme sites useful for distinguishing species.

IDENTIFICATION OF ELIMINATED CHROMOSOMES IN INVIABLE SALMONID HYBRIDS

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Interspecific crossing is a potential means to obtain a hybrid with useful characters in aquaculture. However, such an attempt is often hampered by production of inviable hybrids. In salmonid fishes, uniparental (paternal) chromosome elimination is one of the causes of the hybrid inviability, as shown by our previous cytogenetic analysis at early embryogenesis of inviable hybrids between the female of the masu salmon (Ms, *Oncorhynchus masou*, 2n=66), chum salmon (Cm, *O. keta*, 2n=74), or Alaskan char (Al, *Salvelinus malma*, 2n=82) and the male of the rainbow trout (Rb, *O. mykiss*, 2n=60). In order to understand the underlying mechanism of chromosome elimination, further molecular cytogenetic study was conducted to identify eliminated Rb chromosomes in embryos from each of these crosses, using a combination of replication R-banding at about 400 band level and sequential whole chromosome painting. The number of Rb chromosomes retaining in Ms x Rb, Al x Rb and Cm x Rb hybrids were 17, 15 and 24 on average, respectively. Retained Rb chromosomes, irrespective of size, were nearly common to all the 3 crosses examined. Although the relative value of the frequency of each eliminated Rb chromosome was different among crosses, there was no significant discordance in the order of the frequency of elimination among Rb chromosomes. The present findings clearly indicate a non-random order of chromosome elimination in the inviable hybrids, suggesting the existence of chromosomes to be lost easily (or rapidly after fertilization) or hardly (or later) in the Rb genome.

DEVELOPMENT OF MOLECULAR MARKERS FOR CONSTRUCTING A GENETIC LINKAGE MAP OF THE EASTERN OYSTER *CRASSOSTREA VIRGINICA*

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The diseases Dermo and MSX plague the eastern oyster *Crassostrea virginica*, reducing wild harvests and discouraging the establishment of aquaculture operations in affected waters. A potential solution is the development of genetically improved disease-resistant strains of *C. virginica* that can grow to market size despite disease challenge. One means of accelerating the selective breeding process is to identify genetic markers associated with traits of economic interest such as disease resistance. The goal of this project is to develop polymorphic genetic markers for constructing a linkage map and to eventually identify markers associated with specific traits such as disease resistance. From a small insert genomic DNA library 768 *C. virginica* clones have been partially sequenced, providing ≈ 700,000 bp for marker development. While for the majority of sequences BLAST searches have revealed no homology, a few groups of sequences with retoelement affinities. Both unknown sequences and putative coding regions are being screened for polymorphisms by DGGE analysis of amplified fragments. PCR primers were designed to anneal to regions flanking repeat sequences (micro- and minisattelites). Amplification reactions have been optimised at fourteen repetitive sequence loci for analysis of size variation on an automated DNA sequencer. This set of loci includes four dinucleotide, three trinucleotide and one tetranucleotide microsatellite repeat sequences. Genotypes for allozyme and nuclear DNA loci are being scored for parents and 35-40 F_1 individuals from ten reference families in order to construct a preliminary genetic linkage map.

COMPARATIVE MAPPING IN SALMONID FISHES: THE MICROSATELLITE GENERATION

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Allozyme markers have been extensively used to investigate the genome of salmonids and tentatively infer the chromosomal evolution subsequent to the tetraploidization event in their ancestry. Prevalent among these first generation data was the preservation of linkage group arrangements among species. However, the power to detect chromosomal rearrangements was strongly limited by the scarcity of informative loci within any one species (seldom more than 25 loci). The genome of several salmonid species is currently being mapped by taking advantage of a panel of PCR-based DNA markers which may overcome these limitations. Among those, microsatellites provide a unique opportunity to establish genome wide orthology (i.e. homology) among species and paralogy (i.e. homeology) within species. In this paper, we used outbred pedigrees to construct a microsatellite linkage map for brown trout (*Salmo trutta*) consisting of a framework of 200 anchor markers previously mapped either in rainbow trout (*Oncorhynchus mykiss*) or Atlantic salmon (*Salmo salar*) (see abstract by B. Hoyheim *et al.*). The rationale for the choice of brown trout as reference genome was based upon the assumption that its stable (i.e. immune to Robertsonian polymorphisms) and acrocentric-based karyotype should favor reliable assignment of linkage groups to single chromosome arms. Our findings confirmed and extended allozyme data in that linear linkage relationships were generally conserved among species. In addition, meiotic gynogens were analyzed to map loci in relation to their centromeres, thus revealing whole chromosome arm conservations as well as probable mechanisms of linkage alteration such as apparent Robertsonian translocations and peri/paracentric inversions.

PRELIMINARY TESTS OF OUTBREEDING DEPRESSION IN HYBRIDS BETWEEN SPATIALLY SEPARATED PINK SALMON (*ONCORHYNCHUS GORBUSCHA*) POPULATIONS

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Loss in productivity from outbreeding depression is a potential concern in management and culture of pink salmon, and probably other species. Outbreeding depression can be directly observed from reduced survival and indirectly from changes in traits related to fitness, such as homing/straying or development rate. We have begun examining the results of hybridization between spatially separated populations of pink salmon. In both September 1996 and 1997, control crosses of Auke Creek (Southeast Alaska) pink salmon and hybrid crosses between Auke Creek females and males from Pillar Creek (Kodiak Island) about 1000 km away. Each broodyear, approximately 20,000 control and 20,000 hybrid fish were differentially fin-marked and released. 1998 returns of hybrid (1.53%) and control (1.57%) adult pink salmon were similar. 1999 return rates differed between controls and hybrids; and even when ambiguous marks were counted as hybrid returns, control returns were significantly ($P < 10^{-4}$) higher (5.57%) than hybrid returns (4.55 %). Thorough weekly recovery efforts in nearby (about 1 km) Waydelich Creek revealed similar straying rates (about 1% or less) from Auke Creek by both control and hybrid fish in both years. Although both hybrid and control crosses were made on the same day, times to mid-hatch were 4 to 5 days earlier in both years for the control fish. Reduced survival of 1997 broodyear hybrids and differing development rates between hybrids and controls are consistent with outbreeding depression. Effects observed in F_1 hybrids suggest a role of additive genetic effects. We await the returns of the F_2 crosses.

ESTIMATES OF GENETIC AND PHENOTYPIC PARAMETERS FOR BODY WEIGHT AND GROWTH RECORDED AT FIVE DIFFERENT AGES IN ATLANTIC SALMON AND RAINBOW TROUT

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Body weights were recorded at five different ages, two in freshwater and three while in a net-cage in the sea, in one year-class of Atlantic salmon and one year-class of rainbow trout. The number of fish recorded at each age ranged from 2021 to 2941 offspring of 43 sires and 85 dams in Atlantic salmon and from 1999 to 2859 offspring of 84 sires and 82 dams in rainbow trout. Estimated heritabilites for body weight at each of the five ages ranged from 0.23 to 0.64 in Atlantic salmon and from 0.32 to 0.60 in rainbow trout. Estimated heritabilities for body weight gain between adjacent ages ranged from 0.21 to 0.30 in Atlantic salmon and from 0.26 to 0.51 in rainbow trout. In both species the genetic correlations were medium between the two body weights traits in freshwater and high between the three body weight traits in seawater while the genetic correlations between the body weights in freshwater and the body weights in seawater were medium to low. The genetic correlations between the body weight gains in different periods were medium to low. This strongly indicates genetic variation in the shape of the growth curve in Atlantic salmon and rainbow trout. The significance of the results for a selective breeding program in the two species is discussed.

GENETIC VARIATION FOR A SPINAL DEFORMITY IN ATLANTIC SALMON

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The data were from four year-classes of farmed Atlantic salmon hatched in January 1992, 1993, 1994 and 1995 at Aqua Gen Ltd. two breeding units Sunndal and Hemne, Norway. In the four year-classes, the fish were raised in net cages at three to five different farms. At marketing size after 14-15 months in the sea (2.5 year of age), a deformity score was assigned to each fish based on visual examination; humpback (1) anterior or (2) posterior the dorsal fin, (3) short tail, (4) scoliosis and (5) deformed jaws. In the four year-classes, the number of fish recorded ranged from 15559 to 25464 offspring of 88 to 109 sires and 183 to 226 dams. Scoliosis and deformed jaws was observed at low incidence. The incidence of fish with deformity score 1, 2 and 3 ranged from 1.0 to 25.2 % in the 1992 year-class, from 1.0 to 10.7 % in the 1993 year-class, from 1.6 to 25.2 % in the 1994 year-class and from 1.5 to 4.0 % in the 1995 year-class. Deformed fish were significantly lighter and had higher condition factor than normal fish. A sire and dam model was applied separately to each of the year-classes. The estimated heritability for deformity, on the underlying liability scale, was 0.22, 0.18, 0.23 and 0.06 for the 1992, 1993, 1994 and 1995 year-classes, respectively. This strongly indicates that spinal deformity in Atlantic salmon has a significant additive genetic component. The significance of the results for a selective breeding program is discussed.

GROWTH AND SURVIVAL IN TWO COMPLETE DIALLEL CROSSES WITH FIVE STRAINS OF ROHU CARP (*LABEO ROHITA*)

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To improve rohu through selective breeding, a collaborative research project between CIFA and AKVAFORSK was initiated in 1992. The approach was to bring germplasm of rohu from different rivers in India to CIFA for development of a genetically improved strain for farming. The present results are from two diallel crosses, each with three strains. Both crosses included a Local strain farmed at CIFA, which were crossed with the wild strains of Ganga and Yamuna, and Brahmaputra and Sutlej. At 10-20 grams, samples of fish from each group were individually tagged with Passive Integrated Transponders (PIT) tags. 432 individuals from each diallel were tagged and distributed into three monoculture and two polyculture (rohu, mrigal and catla) earthen ponds. Harvest body weight and survival from tagging to harvest was recorded after a one-year growth period. Estimates of additive genetic, reciprocal and dominance genetic effects (heterosis) were obtained simultaneously for the two diallel crosses. Both in mono- and polyculture average heterosis for harvest body weight was negative. For survival average heterosis was favorable and significantly different from zero in polyculture but zero in monoculture. It is concluded that genetic improvement through crossbreeding of Indian strains of rohu seems to have little practical significance. However, the research project has documented substantial additive genetic variation for both growth rate and survival in rohu (not yet published). Genetic improvement of growth and survival may therefore be obtained through selective breeding.

SELECTION FOR BETTER GROWTH OF *PENAEUS STYLIROSTRIS* IN TAHITI AND NEW CALEDONIA

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The domesticated strain of the peneid shrimp *P. stylirostris* which has been cultivated in French Polynesia and in New Caledonia for 20 years has been used in an experimental mass selection for better growth.

The population under selection was graded once or twice at each generation, which induced a selection rate which fluctuated between 4% and 18% from one year to the next one. At each generation 24 to 32 individuals were used as parents (Ne=21-32) in the selected line and in the control line. The injection of colored elastomer in the two populations allowed an assessment of the genetic progress in earthen rearing ponds : the fifth generation demonstrated an increase of growth rate of 21% when compared to the non-selected control line.

The optimization of the selection schemes and their integration into the production hatcheries is discussed with a special focus on the optimization of the age of grading.

PCR-RFLP Analysis of the Mitochondrial ND-3/4 Gene Polymorphism in the European and East-Asian Subspecies of Common Carp (*Cyprinus carpio* L.)

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Polymorphism within the mtDNA *ND-3/4* gene region was studied by PCR-RFLP analysis among common carp populations belonging to the European (2 farmed and 3 feral strains) and East Asian (Amur wild carp, Vietnamese wild carp and Japanese Koi carp) subspecies. Polymorphism was detected by 9 restriction enzymes and a total of six composite haplotypes were identified. Each East Asian carp population had unique haplotypes, whereas all European carp populations shared a single haplotype that differed from East Asian haplotypes by 0.018 to 0.032 nucleotide substitutions per site. Intrapopulation polymorphism was detected only in Koi and Vietnamese carps. Four enzymes (*Hin*fI, *Alu*I, *Hpa*II and *Taq*I) yielded diagnostic restriction sites for discrimination between European and East Asian maternal lineages. Potential application of these results for monitoring genetic purity of European carp broodstocks is discussed.

INTERNATIONAL NETWORK ON GENETICS IN AQUACULTURE (INGA): PROGRESS AND ACHIEVEMENTS

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The International Network on Genetics in Aquaculture (INGA), established in 1993, as a global forum for collaborative research and training in applied fish breeding and genetics, has made significant contributions to aquaculture genetics and development of improved fish breeds. With the present membership of 13 countries from Asia, Pacific and Africa and eleven advanced scientific institutions, the network has made phenomenal progress in: (i) development of national breeding programs; (ii) initiating two regional research programs for genetic improvement of carps and tilapias; (iii) transfer of germplasm among member countries following strict quarantine procedures and material transfer agreements; (iv) assistance in formation of national genetics networks; and (v) strengthening national research capacity. The paper presents details of the activities and achievements of the network in the last five years.

LENGTH VARIABLE MITOCHONDRIAL DNA AS A POPULATION MARKER IN AFRICAN POPULATIONS OF *OREOCHROMIS MOSSAMBICUS*

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The control region of mitochondrial DNA is routinely used for phylogenetic analyses within species, since its relatively high mutation rate offers greater resolution than coding areas of mtDNA. Evaluation of the control region as a population marker in the Mozambique tilapia *Oreochromis mossambicus*, revealed that the 400bp amplified fragment was length variable between individuals (there was no evidence of length heteroplasmy within individuals). Length variation in mitochondrial DNA has not been reported in this species before, probably because of a loss of genetic variability in the commonly studied Asian founder populations. Length variation diversity in Southern African populations of *O. mossambicus* reflected a similar pattern to microsatellite diversity, prompting our investigation into its utility as a population marker. We investigated the incidence and nature of length variation among wild and captive populations of *O. mossambicus*, and evaluated its potential as a population marker and its utility as a phylogenetic marker by comparison with microsatellite variation, and also with flanking sequence data.

PERFORMANCE OF DIFFERENT GROUPS OF SILVER BARB (*BARBODES GONLONOTUS*) IN VIETNAM

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This paper describes the performance of nine different groups of silver barb (*Barbodes gonionotus*) including wild groups collected from DongNai river (eastern part of south Vietnam), Bassac river, Mekong river, brackish tributaries of Mekong river (Bentre province) and domesticated groups (Tiengiang and Cantho provinces) in earthen pond and rice field. The result shows no difference of performance of silver barb in tested environments ($p>0.05$). The performance among collected groups is quite different ($p< 0.001$). Three groups including Cantho, Dongnai, and CanthoxTiengiang get the best performance. Mekong and BassacxBentre groups stays in the lowest place. The average performance groups are Tiengiang, Bassac, Bentre, and MekongxDongnai groups. Cantho, Dongnai and CanthoxTiengiang could be considered the excellent performance groups in rice field. Otherwise Tiengiang group grew quite good in earthen pond. Only Cantho group demonstrates the interaction of genotype and environment ($p<0.05$).

GENETIC VARIATION FOR TRAITS OF COMMERCIAL IMPORTANCE EXISTS IN DANISH RAINBOW TROUT

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The object of this study was to establish that genetic variation for production traits exists within commercial populations of Danish rainbow trout. Twenty-five sires and 25 dams were mated by a partly factorial design. Each sire was mated with two dams and each dam was mated with two sires, resulting in 50 full-sib families. The families were reared in separate rearing facilities and the fish were assessed for survival, growth rate, food conversion efficiency, and resistance to viral haemorrhagic septicaemia (VHS). REML estimates of additive genetic variation for growth rate and food conversion efficiency were obtained by fitting univariate linear models, while additive genetic variation for resistance to VHS was estimated by fitting a proportional hazards model. The results showed that only 1.2% of the fish died, and it was assumed that additive genetic variation between the fish was not expressed for survival. However, additive genetic variation was detected for growth rate (additive genetic variance = 1109.7 ± 699.0 g, h^2 = 35%), food conversion efficiency (additive genetic variance = 0.0082 ± 0.0035 kg weight gained/kg food fed, additive genetic coefficient of variation = 7.2%), and resistance to VHS (additive genetic variance on the logarithmic time scale = 0.24, h^2 = 14%), and there were positive genetic correlations between each of these traits. These results highlight the potential to successfully implement a breeding program for the genetic improvement of Danish rainbow trout. Genetic variation for production traits does exist within commercial populations of Danish rainbow trout.

THE IMPACT OF BIOTECHNOLOGY IN AQUACULTURE

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As we enter the new millennium, there will be a greater demand for food production due to the increasing world population. Food security will therefore be a major issue facing the mankind. Diminishing agricultural land as well as climatic and environmental changes further compounds this dilemma. Estimates by the United Nations indicate that aquaculture and mariculture will need to be increased several- fold to satisfy the demands.

There are several major platform biotechnologies that could have major impact in increasing the industry 's output. These include

1. The cloning and expression of genes encoding bioactive molecules useful in the industry. Among them are the hormones, growth factors, cytokines and immunostimulants. These genes can be expressed and used directly as feeds, vaccines or as candidates in transgenic studies
2. Gene mapping, RFLP, QTLs and the determination of whole genomic structures of organisms of aquacultural interest to identify important traits in broodstock development and enhancement, for example, the elucidation of pathogenic organisms will allow the designing and production of new vaccines
3. DNA chip technology for elucidating gene function and biomonitoring as a fast way to check fish health, stress level and exposure to pollutants
4. Proteomics for protein and drug discovery. Recent advance in mass spectrometry and bioinformatics has made large-scale protein sequencing feasible and economical. This has enabled many investigators to develop protein data as well as a biological assay to examine cell and protein function upon perturbation by pathogens, environments and other factors.
5. DNA vaccines as a new protocol for immunization against viral and bacterial pathogens.
6. Transgenic and cloning technologies to generate genetically modified organisms with enhanced or beneficial traits.

The presentation will highlight some of the recent developments in fish biology and our own research programs in transgenic technology on the production of faster- growing and disease- resistant fish, DNA micro-array as a means of environmental monitoring and proteomics for novel protein discovery in Singapore and Canada.

IDENTIFICATION OF SEX-RELATED GENOMIC MARKERS AND GENES INVOLVED WITH SEX DETERMINATION IN FISH

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We are studying the molecular basis of sex determination in fish. Fish species with commercial importance (e.g. African catfish, *Clarias gariepinus*) and models (e.g zebrafish, *Danio rerio*) are investigated in our experiments.

For the identification of sex-linked DNA markers were are screening pooled DNA samples of male and female individuals classified according to the histology of their gonad. Two approaches are used: PCR-based assays which either scan the genom randomly at several positions at a time or enrich the differences between the two DNA samples. Putative markers are cloned, sequenced and turned into Sequence Characterized Amplified Regions (SCARs), which can be used for user-friendly molecular sexing at any stage of development. Several examples for such markers will be shown.

Differential gene expression is used during the search for sex-related genes with putative role in sex determination. RNA samples are isolated from male- and female-biased embryo/larvae groups at several developmental stages and cDNA libraries are generated by subtracting them from each other both ways (male-biased from female-biased and vice versa). These libraries are then arrayed onto membranes and analyzed by hybridization to identify the differentially expressed genes. Analysis and characterization of these genes will be demonstrated by several examples.

The role of such markers/genes is tested in closely related species in the hope of identifying conserved DNA sequences and molecular mechanisms related to the early decisions in the formation of fish gonads.

MITOCHONDRIAL DNA DIVERSITY IN WILD AND CAPTIVITY POPULATIONS OF *BRYCON OPALINUS* (CUVIER, 1819) (CHARACIFORME, CHARACIDAE, BRYCONIAE), IN THE PARAÍBA DO SUL BASIN, BRAZIL

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In Brazil, damming of river systems for hydroelectricity generation has affected several native species. *Brycon* is one the main genera among the Neotropical freshwater fishes. In the present study RFLP analysis of mitochondrial DNA was carried out to investigate the population genetic structure of cultured and wild populations of *Brycon opalinus* in the "Paraíba do sul" basin, and ascertain the reproductive success of hatchery-planted fish. mtDNA samples were collected and analysed from 257 specimens sampled in nature and in the hydropower hatchery. An initial screening with 27 restriction enzymes revealed 6 informative enzymes (*Apa* I, *Ava* II, *Eco*R I, *Hinc* II, *Hpa* I, *Nhe* I) which generated 27 haplotypes. Two haplotypes were found exclusively in the hatchery facility, and can be used as a tool to provide information on both the survival and long-term reproductive success of stocked fish. Haplotype diversities were high both in the population from the hydropower plant (h=0.75), and from the wild (h=0.62). Nucleotide diversity among the 27 haplotypes found for the *Brycon opalinus* was 0.825%. The molecular variance analysis (AMOVA) showed the highest observed variance within populations (70,02%) while 15,97% of the total diversity was due to interpopulation variance which suggested that *Brycon opalinus* population is genetically structured according to mtDNA variability. Taken together these data indicate the existence of discrete populations which should be properly managed and used to keep the hatchery's genetic variability in order to reduce the impacts of human activities by restocking programmes.

METHYLATION AND TRANSGENE EXPRESSION IN TRANSGENIC COMMON CARP

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We have demonstrated previously that transgenic common carp with gene constructs containing growth hormone (GH) cDNA showed a higher growth rates as compared to sibling control under non optimal growth conditions (low ambient temperatures and low protein diet) (Hinits and Moav, *Aquaculture* 173: 285-296, 1999).

In this report an "all fish" GH construct (OnH3cGH) was prepared and microinjected into carp zygotes. The GH construct was prepared by fusing the common carp GH cDNA to the sockeye salmon Histone 3 promoter (Chan and Devlin, *Molec. Mar. Biol. Biotech.* 2: 308-318, 1994). Transient expression of the transgene in early carp development indicated that the H3 promoter is one of the strongest known fish promoters. One transgenic adult male carp was used to establish an F_1 transgenic progeny. The transgene was transmitted to 26% of the F_1 progeny. The transgenic individuals were shown to have 66 copies of the above construct per genome.

Expression of the GH transgene was assayed by RT-PCR in non-pituitary tissues of the transgenic individuals. Possible effect of methylation of several CpG sites in the transgene H3 promoter on its expression was tested by a PCR-based methylation assay. The methylated status of certain CpG sites on the H3 promoter implies a possible role for methylation in the inactivation of transgene expression in transgenic common carp.

TEST CROSSES WITH CLONAL LINES OF TILAPIA (*OREOCHROMIS NILOTICUS*)

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Crossbreds and purebreds of four clonal lines were produced and reared communally till the age of 136 days. DNA-markers were used to confirm the clonal nature of fish and to identify the different genetic groups. Purebred clonal lines showed significant differences in body weight. The mean growth performance of all crossbreds was not significantly different from that of the purebreds, but in some crosses significant heterosis effects were observed. Results of communal testing are compared with results of separate testing of genetic groups carried out earlier with regard to estimated genetic effects on body weight.

SALGENE: GENE IDENTIFICATION AND EXPRESSION ANALYSIS IN ATLANTIC SALMON (*SALMO SALAR*)

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The aim of the SALGENE project is to establish a large number of Expressed Sequence Tags (ESTs) in Atlantic salmon and to study the expression of a number of selected genes in different tissues. The collaboration involves four laboratories from Europe and the project is funded by the European Commission. cDNA libraries from nine different tissues have been constructed and sequencing of ESTs is ongoing. The EST sequences will be deposited in Genbank and a physical repository of cDNA clones will be established. Homologous sequences from the databases of a number of clones from the cDNA libraries have already been identified. Information obtained from the sequencing of ESTs will be used for genetic mapping of specifically interesting genes using the reference families from the SALMAP project.

The use of the information from the cDNA libraries from the different tissues obtained in the SALGENE project for construction of microarray chips is a very promising future prospect for studies of the genetic composition of salmonids. The possibility of incorporating several thousand different genes onto a single microarray chip followed by studies of gene expression in different tissues, at different developmental stages, or in individuals exposed to different treatments, will allow the identification of candidate genes for genetically determined traits of specific interest and will allow the description of the ontogeny of gene expression in different tissues.

ARTIFICIAL ASEXUAL REPRODUCTION OF LOACH (*PARAMISGURNUS DABRYANUS*) BY CELL ELECTROFUSION*

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Donor cells were fused with UV-inactivated oocytes by electrofusion. The optimal dosage of UV-inactivation for loach eggs was 180-240mJ/cm^2, which completely destroyed the female pronuclei of recipient oocytes but retained the capacity to support normal development of their cytoplasm. In order to keep one recipient oocyte receive one donor cell and raise efficiency of manipulation, the fusion was carried out in a cone fusion chamber instead of inserting a donor cell into the perivitelline of a recipient oocyte. When long-term cultured blastula cells serially twice fused with UV-inactivated oocytes, fingerlings were gotten at a 1‰ rate. When short-term cultured blastula cells were fused with UV-inactivated oocytes, fingerlings were obtained at a 4‰ rate.

As a special type of cell culture, blastula cells were also preserved at 4°C in different solutions. TC-199 with 10-20% calf serum was the best preservation solution, and can effectively retain the totipotence of blastula cells at least 10 days. Especially, it was firstly found that 4°C preserved gastrula cells had the same totipotence as 4°C preserved blastula cells. When 4°C preserved blastula or gastrula cells were fused with UV-inactivated oocytes, asexual reproduction fish were obtained at a 10% rate.

Normal adults of fusion fish were obtained. Genetic analysis indicated they were originated from donor cells and UV-inactivated female pronuclei didn't take part in the development of fused fish.

A tentative model of artificial asexual reproduction of fish was proposed: single blastula or gastrula were cultured or 4°C preserved for a short-term duration. At the same time, the genetic, biochemical or physiological characteristics were analyzed and identified. If the blastula or gastrula cells met the target conditions, fused them with UV-inactivated oocytes by electrofusion, and got two or more asexual reproduction fish. At their young stage, the sex of half of them was reversed by feeding sex hormone. When they grew to adults, a large quantity of needed fish was conveniently obtained by common sexual reproduction. In fact, they were clone fish originated from one blastula or gastrula if the target gene locus or loci were homologous.

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ANDROGENESIS IN AFRICAN CATFISH (*CLARIAS GARIEPINUS* BURCHELL)

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Fish are ideal objects for genome manipulations: various forms of these techniques are applied routinely in aquaculture. Androgenesis is a single-parent type of inheritance where by definition the genetic material of the progeny originates exclusively from the male gametes, while maternal chromosomes are presumed to be totally excluded from the process. In this publication we present our results of androgenesis in African catfish *(Clarias gariepinus)*. The genome of eggs arranged in batches of 500 was inactivated using Co^{60} gamma ray irradiation, then fertilized with fresh milt. Gamma ray doses ranging from 10 to 40 krad were applied. A heat shock for 2 minutes at 40°C was performed to restore the diploid state of the developing embryos. Heat shocks were applied every third minute from 24 minutes after fertilization until 39 minutes. Random amplified polymorphism of DNA (RAPD) appeared to be the most efficient means of studying the genetic composition of the progeny. RAPD analysis appears to be superior to fingerprinting for this purpose, due to its lower template and time requirement and to its better reproducibility. The highest number of hatched larvae, 41 (8.2%) occurred at 25 krad dose. This dose was used to perform experiments on the timing of heat shock. A heat shock applied at 30 minutes post fertilization yielded the highest number of hatched larvae: 54 (10.8 %). The RAPD analysis of the hatched larvae proved their adrogenetic origin. No fragments of maternal origin were identified in the electrophoretical pattern of the larvae.

SALMAP: CONSTRUCTING GENETIC MAPS OF SALMONID FISHES

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A collaboration with the title: Generation of highly informative DNA markers and genetic marker maps of salmonid fishes (SALMAP), funded by the European Commissions FAIR program (Agriculture and Fisheries), was established aiming at constructing genetic maps of Atlantic salmon (*Salmo salar*), rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*). The project was running from 1997-1999 and involved five European countries and Canada.

Microsatellite PCR-assays were developed in Atlantic salmon and rainbow trout. Approximately 2,300 clones containing microsatellites have been identified from Atlantic salmon and approximately 600 clones from rainbow trout. At the end of Dec. 1999 approx. 1,200 clones from Atlantic salmon and 500 clones from rainbow trout has been sequenced and used for constructing genetic markers. The microsatellite markers were tested in all three species to identify microsatellite markers that show cross-species amplifications and subsequently used for creating comparative maps.

Linkage maps have been constructed for all three species using standard reference families. For Atlantic salmon and rainbow trout maps containing approximately 300 markers each has been constructed. The maps consist mainly of microsatellite markers but other markers such as minisatellites and genes are included. For brown trout 200 markers already mapped in Atlantic salmon or rainbow trout was used for constructing a framework map (see abstract by K. Gharbi et al). In order to map the loci relatively to the centromeres meiotic gynogens have been analysed in all three species.

RELATIONSHIP BETWEEN HETEROZYGOSITY AND GROWTH IN FAMILIES OF THE PACIFIC OYSTER, *CRASSOSTREA GIGAS*

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The relationship between allozyme genotype and growth was investigated in hatchery crosses of *Crassostrea gigas*. Five males and five females from wild populations were crossed and offspring were grown together without grading. The experiment was repeated three times using parents from different wild populations. Three different populations were obtained which were first grown in the hatchery then in a nursery. Populations were sampled at 10 month old and at 21 months old. Seven polymorphic allozymic loci were screened in the juvenile sample (10 months old) and six more were used in the adult sample (21 months old). No correlation was found between heterozygosity and total weight in juveniles. Growth was monitored for the adult sample, so the initial weight, the final weight and growth rate are known for each individual. Some negative correlation between heterozygosity and growth parameters was found in the adult sample. The impact of the environment may explain this unusual correlation. No relationship between genotype and growth was found to occur consistently between populations and between loci.

GENETICS AND LINKAGE GROUPS OF MICROSATELLITE MARKERS IN THE PACIFIC OYSTER, *CRASSOSTREA GIGAS* USING TRISOMICS

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Trisomy (2n+1) is an aneuploid condition where one chromosome is represented by three copies instead of the normal two. Analysis of trisomics may be useful for the chromosomal assignments of markers. Trisomics families in *Crassostrea gigas* are made by crossing diploids with triploids which produce a mixture of normal diploids, triploids, trisomics and other aneuploids. In the present study, putative trisomics with an approximate diploid content were separated with flow cytometry. These individuals were crossed with each other or with normal diploids. Using chromosome count of embryos at the 2cell stage, twenty families were confirmed as trisomic. At one year of age, progeny were again checked for trisomy and were sampled for microsatellite analysis.

Parents from 20 trisomics families were screened with 17 microsatellite markers. Tri-allelism (3 alleles/locus/individual) was observed at 7 loci in 12 trisomic families. Tri-allelism was found only in the putative parents, not in normal diploids. In many cases trisomic progeny were observed at 2 cell stage but not at one year of age. Data obtained from this analysis allowed us to pool three defined previously linkage groups in *Crassostrea gigas* and to add 5 microsatellites which were not previously mapped.

These results indicate that trisomic families can be readily produced and microsatellite markers are useful in trisomic identification.

DEVELOPMENT OF A TILAPIA ARTIFICIAL CENTER OF ORIGIN (ACO) AND GENETIC LINKAGE MAP BASED ON AFLP AND MICROSATELLITE LOCI

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A program aiming at breeding new, synthetic populations of tilapia specifically adapted to temperate climates and saline environments was initiated in 1995 at the Dept. of Aquaculture, ARO, Israel. It is based on ideas from plant breeding (use of interspecific composite crosses is an established practice in plant breeding for achieving wide genetic and phenotypic variability) and takes benefit of the ease of producing interspecific hybrids among tilapias. The multiple species cross (ACO) contains wide genetic diversity and presents opportunities for genes to recombine and interact with other genes originating from different species. An adaptation of this method, termed Multiple Re-Speciation (MRS), was recently applied to develop new cultivars of carnations.

The ACO was produced by the Israeli collaborators by inter-crossing four tilapiine species: *Oreochromis niloticus* [wild type (*On*) and red (R*On*) strains], *O. aureus* (*Oa*), *O. mossambicus* (*Om*), and *Sarotherodon galilaeus* (*Sg*). All hybrids were obtained by natural spawning, except for the *S. galilaeus* x *Oreochromis* sp. F_1 hybrids that were produced by artificial fertilization. All of the different two-way (F_1 hybrids) crosses required to establish the synthetic stock of tilapia (ACO) have been obtained, as well as a set of three four-way-crosses (4WC) derived from five strains in four species and two genera. Full-sibs of the [(*Om* x *Oa*) x (*Sg* x *On*)] 4WC have been successfully bred to enable mixing of the inherited gene blocks; thus, we are ready to start selective breeding for cold tolerance and growth rate in freshwater and for growth rate in saltwater, from this base population. Collaborative work using the 3WC [*Om* x (*Oa* x R*On*)] resulted in mapping 191 AFLP and 26 UNH microsatellite markers to 24 linkage groups. Based on shared microsatellite markers, 12 composite linkage groups have been identified from the combined mapping data previously published and the 4WC family; these likely represent 12 of the 22 tilapia chromosomes.

STOCK IMPROVEMENT OF SILVER BARB (*BARBODES GONIONOTUS* BLEEKER) THROUGH SEVERAL GENERATIONS OF GENETIC SELECTION

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This paper highlights stock improvement of silver barb (*Barbodes gonionotus* Bleeker) using selective breeding technique and the results of generation wise growth performance between selected and non selected progeny groups. The breeding programme was initiated in 1994 involving two wild germplasms obtained from Thailand and Indonesia and existing local stock of Bangladesh. To produce first generation (F1), the three unrelated founder stocks were mated themselves. In 1997, the second generation (F2) trials were made through complete 3X3 diallele crossing experiment to produce nine heterogeneous, outbred genetic groups. For each of the reciprocal crosses, 5 to 8 pairs were mated separately and the best 3 progeny groups were selected to make 18 full sib progeny families were then communally stocked by mixing equal numbers of larvae from each family. During the spawning season of 1998, 20% of the best females and males were mass selected from F2 communal crossbred groups and mated themselves to produce F3 generation. In 1999, mass selection was further made having 10% F3 best matured breeders and used for fourth generation (F4) trials. In each generation, evaluation of growth performance was carried out through comparative trials between selected vs non selected groups. All the data were carefully analyzed and it was estimated that on an average 7.5%, 8.0% and 13.0% additive genetic gains were attained respectively by F2, F3 and F4 selected crossbred groups. Present findings suggest that putative stock improvement of silver barb through several generations of genetic selection might be a model technique for other carp species in Bangladesh and elsewhere in Asia.

GENETIC DIFFERENTIATION AND PHYLOGENETIC RELATIONSHIPS AMONG POPULATIONS OF AYU (*PLECOGLOSSUS ALTIVELIS*), INCLUDING ENDANGERED SUBSPECIES, INFFERED BY PCR-RFLP ANALYSIS OF THE MITOCHONDRIAL DNA D-LOOP REGION

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Ayu (*Plecoglossus altivelis altivelis*), including two ecological forms (amphidoromous and landlocked), is common and a commercially important freshwater fish in Japan. On the other hands, endangered subspecies *P. a. ryukyuensis* inhabits limited area of Ryukyu Islands in southern Japan. We examined PCR-RFLP analysis of the mtDNA D-loop region to reveal genetic variability and differentiation between the last two populations of *P. a. ryukyuensis* in the same island, in comparison with those of the two forms populations of *P. a. altivelis*. The common haplotype was not observed between the two populations of *P. a. ryukyuensis*, which indicates little or no gene flow between them. The haplotype diversities of the two populations of *P. a. ryukyuensis* (0.251 and 0) were far smaller than those of the two forms in *P. a. altivelis* (0.766-0.928), suggesting that strong bottleneck/founder effect occurred on *P. a. ryukyuensis* populations. The value of net number of nucleotide substitution between the two populations of *P. a. ryukyuensis* (0.276%) was larger than that between the two forms of *P. a. altivelis* (0.265%). The phylogenetic tree of 42 haplotypes observed in all populations showed that the divergence of mtDNA in *P. a. ryukuensis* and the two forms of *P. a. altivelis* are not monophyletic, respectively. And several haplotypes in *P. a. altivelis* were related to those in *P. a. ryukyuensis*. This result suggests that the useful maternal line for the restoration of *P. a. ryukyuensis* could be found in *P. a. altivelis*, in case this endangered subspecies is almost extinct unfortunately.

USE OF AMPLIFIED FRAGMENT LENGTH POLYMORPHISMS (AFLPS) AS MOLECULAR MARKERS IN THE PACIFIC OYSTER (*CRASSOSTREA GIGAS*)

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Genetic markers can be used in aquaculture to confirm pedigrees, derive linkage maps, detect quantitative trait loci (QTLs) for commercially important traits, and to deploy marker-assisted selection. Types of markers include allozymes and DNA markers such as restriction fragment length polymorphisms (RFLPs), randomly amplified polymorphic DNA (RAPDs) and microsatellites. Amplified fragment length polymorphisms (AFLPs) are a relatively new type of DNA marker. No prior sequence knowledge of the genome is required in order to develop AFLP markers and multiple loci can be amplified using only one primer combination. While AFLP technology is well established in the plant world, there are few publications of AFLP work in molluscs. We describe the development of AFLP markers in the Pacific oyster using an automated sequencer, and discuss their advantages and disadvantages. Two full-sib families of Pacific oysters from a genetic improvement program were examined and the suitability of AFLP markers for mapping and linkage analysis assessed.

ALL MATERNAL CHROMOSOMES ARE EXTRUDED AS TWO FIRST POLAR BODIES IN THE ANDROGENETIC CLAM *CORBICULA LEANA* - CYTOCHALASIN D TREATMENT

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Our previous studies revealed that *Corbicula leana* and *Corbicula fluminea* reproduce by androgenesis; 1) all maternal chromosomes are extruded as two polar bodies at 1st meiosis; 2) only the male pronucleus is present in the egg cytoplasm; 3) male pronucleus become the metaphase chromosomes of 1st mitosis. We assume that 2nd meiosis can not be observed, because all the maternal chromosomes were extruded. We treated the eggs with cytochalasin D to inhibit the first polar body formation and to examine whether the second meiosis would occur. The eggs treated with CD (1µl/ml) from 10 min after spawning were allowed to develop at 26°C. Eggs were fixed with ethanol, stained with DAPI, and observed under a fluorescence microscope.

Control: Eggs at 20 min amd 30 min were at M-I (91.1%) and at A-I (89.1%) respectively. But at 50 min (52.4%) of eggs were at M-II, at 60 min (67.3%) of eggs had four sets of maternal chromosomes and one male pronucleus. At 80 min four female pronuclei and one male pronucleus expanded (55.2%) and became by 90 min the metaphase chromosomes of the first cleavage (61.7%). The present study clearly indicates that the second meiosis can not practically occur in *C.leana*. However, the system regulating second meiosis still proceeds the "normal" meiotic process schedule.

CHARACTERIZATION OF SEX CHROMOSOMES IN SALMONID SPECIES BY *IN SITU* HYBRIDIZATION USING MOLECULAR MARKERS

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In salmonids, sex chromosomes are not morphologically differentiated in all the species. In rainbow trout (*Oncorhynchus mykiss*) there are morphological differences among the X and Y chromosomes, however, there are populations in which it is not possible to identify the Y chromosome in males. In coho salmon (*O. kisutch*), sex- chromosomes have not been described, although a male heterogamety sex-determining system is present. For these reasons, both species are good models for the study of sex-chromosome evolution.

In previous studies we have demonstrated that the RAPD - SCAR OmyP9 marker is sex inherited and localized it by *in situ* hybridization in sex chromosomes of rainbow trout. Continuing with our objective of contributing to the knowledge of the structure and differentiation of sex chromosomes, the localization of a group of molecular markers in sex chromosomes of rainbow trout and in coho salmon karyotype was studied.

In this presentation we will show the results of the *in situ* hybridization of the following probes: OmyP9, 5S rDNA, a second RAPD-SCAR and a segment of the growth hormone gene (GH2) of coho salmon. We will compare and discuss the localization of these markers in chromosomes of both species and as well as the identification of a subtelocentric pair as the putative sex chromosomes of coho salmon.

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THERE IS POTENTIAL FOR GENETIC IMPROVEMENT IN THE YABBY (*CHERAX DESTRUCTOR*) DUE TO DIFFERENCES IN GROWTH RATE, TAIL WIDTH AND TAIL LENGTH AMONG NATURAL POPULATIONS

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Culture of the freshwater yabby (*Cherax destructor*) forms the basis of a small, but developing, aquaculture industry in Australia. Currently, however, aquaculture of the yabby is primarily based on broodstock sourced from local wild populations. To date there has been very little attempt to domesticate the yabby, or to increase productivity through genetic improvement.

As the precursor to a selective breeding program to increase the productivity of the yabby for aquaculture, we evaluated the relative growth performance of 2100 juvenile yabbies bred from five wild populations for three traits (ie, weight, tail width and tail length). Significant differences were found in the expression of these traits among populations. For example, average weight and tail length at 12 months differed among the fastest and slowest growing populations by up to 44% and 13%, respectively. Differences in growth among populations demonstrates that initial genetic gains can be made by utilizing individuals from the faster growing populations as base generation animals for the selective breeding program.

FATNESS MEASURES IN A CHINOOK SALMON BREEDING PROGRAMME

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Salmon diets with moderate to high fat content can result in high fat percentage in the flesh, with associated quality problems. This paper evaluates two techniques for measurement of fat percentage in chinook salmon and reports their heritabilities and the phenotypic and genetic correlations between them. Fat percentage was measured in 313 salmon fillets using an X-ray computed tomography (CT) scanning technique and a Distell fat meter developed for fish using microwave technology (fat meter). A random subset of 16 samples was analysed using an ether extraction technique to validate the accuracy of the two techniques.

CT and fat meter were both accurate estimates of salmon fat content (R^2 = 0.92 and 0.88; RSD for fat% = 1.21 and 1.54%, respectively). Both traits were highly heritable (h^2 = 0.62 and 0.55 for CT and fat meter, respectively) and the genetic and phenotypic correlations were also high. While CT was the more accurate of the two measures, the portability and convenience of the fat meter makes it more suited for use in a breeding programme. CT offers a suitable auditing system of fat meter accuracy.

ARE INTERSEX CRAYFISH (*CHERAX QUADRICARINATUS*) GENETICALLY FEMALES?

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The red-claw crayfish, *Cherax quadricarinatus*, is a gonochoristic species in which most of the populations consist of individuals that are either males or females. However, cases of intersex individuals, containing both male and female genital openings, were reported in wild and cultivated populations. In the stock cultured in Israel, 1.2% of the population were found to be intersex individuals. An intersex individual which had both male opening at one of its fifth walking legs, and a female opening at the opposite third walking leg, had both an androgenic gland and a testis at the side of the male opening, and an arrested ovary at the side of the female opening. The secondary sexual characteristics, such as the morphology and setation of its pleopods and the red patch on its propodus, were similar to those of normal males. Four groups of females were mated with such intersex individual or with normal males. The F_1 progeny of such groups of females consisted, on average, of 25.2% males in the intersex-mated groups and 50.6% in the normal males-mated groups. Crossings were also made on individual basis, by mating the same females with an intersex individual and later with a normal male. The F_1 progeny resulting from these crosses contained, on average, 23.1% males when the sire was an intersex and 42.4% when it was a normal male. The proportion of intersex individuals in the progeny did not differ significantly between the two types of crossings. A tentative model fitting these results assumes that male is the homogametic sex in this species. A few similar cases of male homogamety have been reported previously in decapod crustaceans. According to this hypothesis, the intersex individuals used for the present study are genetic females with intersexual genital openings and male phenotype. Our finding that removal of the androgenic gland in such intersex individuals caused the degeneration of male sexual characteristics, and the onset of secondary vitellogenesis in the previously arrested ovary, is in accordance with the above hypothesis.

BIOCHEMICAL GENETICS, PERFORMANCE AND PRODUCT QUALITY OF TENCH (*TINCA TINCA* L.) STRAINS

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Tench, a cyprinid species of growing interest for European pond aquaculture, has been genetically characterized by comparing enzyme variabilities of two wild and five cultured strains from Germany and Czech Republic. Based on 23 loci, genetic variabilities were relatively similar among strains (1.2 to 1.3 alleles per locus, 17.4% to 34.8% polymorphic loci and observed heterozygosities from 0.071 to 0.105). The main difference between wild and cultured strains was a lower number of polymorphic loci in most of the cultured strains. The performance of the five cultured strains was evaluated in closed recirculating systems starting from swimming-up larvae till 446 days (first trial) and 452 days (second trial). Although growth was considerably slower than usually observed in ponds significant differences could be found between strains. The ranking of strains for body weight was identical to that for food conversion efficiency. None of the genetic variability measures was correlated to any of the performance traits. Since the evaluation of product quality requires fish of a bigger size (more than 200g wet weight) only the three tench strains from the first performance test could be analysed yet. These strains differed significantly in several flesh parameters. Flesh quality was best in the hybrid line derived from two Czech cultured strains. Our results indicate a great potential of tench for genetic improvement by classical breeding methods.

ALL MATERNAL CHROMOSOMES ARE EXTRUDED AS TWO FIRST POLAR BODIES IN THE ANDROGENETIC CLAM *CORBICULA LEANA* - ANTI TUBULIN LUMUNOFLUORESCENCE

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We previously demonstrated that *C. leana* reproduce by unusual mode, androgenesis. The fertized eggs expel all maternal chromosomes as polar bodies and only the male pronucleus become the metaphase chromosomes of first mitosis. To understand the unusual polar body formation, wholemount eggs stained with monoclonal antibodies against α-tubulin and DAPI were examined. The meiotic spindle was located at the peripheral region of the egg at metaphase-I and its axis was parallel to the egg surface. After segregation of chromosomes at anaphase-1, cytoplasmic bulges formed at both meiotic spindle pole sites. From the apical portion of the bulge, a bundle of astral microtubules radiated toward the bulge base in late anaphase like a half spindle. Maternal chromosomes were all parceled up in two "first polar bodies" and they were eventually discarded. After the polar body formation, only one male pronucleus existed in the egg cytoplasm. The present study showed that the anaphase microtubules originating from a single aster could induce the polar body formation without overlapping of microtubules from the opposing aster.

STUDIES ON THE KARYOTYPES OF TWO SPECIES OF SEA BREAM (SPARIDAE) AND SIX SPECIES OF FLATFISHES (PLEURONECTOIDEI)

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The karyotypes of two species of sea bream, Chrysophry major and Sparus macrocephalus, and six species of flatfish (Pleuronectoidei) in coastal waters of Qingdao, China were examined from renal tissue by air drying method. Ag-NOR banding of the two sea breams were also checked.

Both of the two sea breams have a diploid chromosome number of 48, and their karyotype formulae are 2st+46t, NF=48 and 4m+4sm+2st+38t, NF=56 respectively. The pair of st chromosomes in both species is chromosomes with secondary constrictions, and the result of silver-staining indicates that the regions of secondary constrictions are nucleolus organizer regions (NORs). Polymorphism of NORs is found and phenomenon of NORs union in metaphase figures is generally observed in two species. The phylogenetic relationship of the two types based on characters of neurocranium and of karyotype separately are discussed and outlined accordingly. It is believed that the two types are of divergent evolution in Sparidae.

The karyotype formulae of Paralichthys olivaceus, Pseudorhommbus cinnamomeus, Pseudopleuronectes yokohamae, Kareius bicoloratus and mocrostomus achne is 2n=48, 48t, NF=48; and that of Pleuronichthys cornutus is 2n=48, 12m+2sm+34t, NF=62. The karyotypic variation and evolution of 6 species and several related species are discussed according to the results in this paper and former studies, three types of karyotypes and three ways of karyotypic variation are outlined presumedly.

CYTOCHROME P450 AROMATASE GENES AND SEXUAL DIFFERENTIATION IN THE NILE TILAPIA *OREOCHROMIS NILOTICUS*

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Sex determination in fish is fairly plastic and can be manipulated at a certain stage of early life history (the "labile period"). Hormonal (and genetic) sex manipulation are widely practised in tilapia culture. Many different synthetic steroidal substances have been used to achieve the production of all male tilapia fingerlings. It is now fairly clear that sex steroids are natural sex inducers, but understanding of the decisive steroidal event(s) is poor. Defining the period of sexual differentiation and the labile period using a variety of approaches centred on the role of steroids should help to improve our understanding of this process and lead to better techniques for sex manipulation in aquaculture.

Cytochrome P450 aromatase is a membrane-bound enzyme with a key role in steroid biosynthesis, converting androgens into estrogens. We used two approaches to study the role of aromatase during sexual differentiation in *Oreochromis niloticus:* chemical aromatase inhibition and a molecular genetics approach to study expression of aromatase genes. Our data suggests that the major effects of aromatase on sexual differentiation in this species occur between 11 and 27 days post-fertilisation (dpf), with 13 to 17 dpf being the most sensitive to manipulation. This is in accordance with earlier histological and steroid treatment studies. Expression of the "ovarian" aromatase gene is downregulated during this period in males but not in females, while expression of the "brain" aromatase gene showed no sexual dimorphism. This suggests that the ovarian aromatase gene plays a central role in sexual differentiation.

FISH AND SHELLFISH GENETICS IN INDIA: AN OVERVIEW

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The paper reviews the progress of research in genetic evaluation and improvement of fish and shellfish species in India. This includes studies on genetic characterization, selection, hybridization, sex control, chromosome engineering, gene manipulations, tissue culture and cryopreservation of gametes and embryos. The achievements are highlighted in regard to cytogenetic and molecular profiles of carps (*Catla catla, Labeo rohita, Cirrhinus mrigala*), catfishes (*Heteropneustes fossilis, Clarias batrachus*, shrimps (*Penaeus monodon*) and prawns (*Macrobrachium rosenbergii*), selective breeding in *L. rohita* and *C. catla*, production of monosex and sterile population in exotic species (*Cyprinus carpio, Tilapia mossambica*), gynogenetic and polyploid population in carps, transgenic carp, development of cell lines and sperm banks in endangered mahseer (*Tor putitora*) and Indian major carps. The increased use of molecular markers in genome mapping, construction of genomic libraries from indigenous fishes used in aquaculture, production of improved genetic and transgenic strains and development of simple, reproducible and industrial technologies for sex control and gene banking are the thrust areas for future research.

THE CONFLICT BETWEEN CONSERVATION OF GENETIC RESOURCES AND EXPLOITATION – AN EXAMPLE FROM AFRICAN POPULATIONS OF *OREOCHROMIS MOSSAMBICUS* (PETERS)

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As part of a genetic improvement programme, we undertook molecular genetic analyses of ten strains of *Oreochromis mossambicus* collected throughout its natural range. DNA samples extracted from fin clips were screened for genetic variation at five polymorphic microsatellite loci. Genetic variation within a 400bp amplified fragment of the mitochondrial DNA control region was also assessed. Our results were compared to the performance data of the strains (i.e. exploitation characters), and interpreted in the light of known history of the source populations. From the point of view of conservation of genetic resources, it is tempting to prioritise those strains with greatest genetic diversity, since they have the greatest adaptive potential, and the greatest risk of permanent gene loss. There are clear cases, however, where monotypic strains exhibit the greatest performance, and so provide greatest short-term exploitative benefits. The conflict between potential long-term conservation benefits and certain short-term gains are discussed in the context of a genetic improvement development programme.

BRITISH *PORPHYRA* SPECIES – CAN THEY BE IMPROVED ?

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The improvement of widely cultivated *Porphyra* species by combinations of selection and the introduction of new genetic diversity is hampered by difficulties in identifying and distinguishing the various seasonal and ecotypic forms of the known species. Gross morphology within species is highly variable across environmental gradients, and the morphological simplicity of the genus provides few features for traditional taxonomic classification and phylogenetic analysis.

In this study we used molecular markers to confirm the identity of the putative ecotypes and species, and to assess the potential for improvement of production characters by assessment of levels of genetic diversity. We scored populations of four putative species at twelve allozyme loci, eleven of which were usefully polymorphic. We found striking differences in genetic diversity among species, and uncovered a fifth cryptic species within the *Porphyra laciniata* group. The consequences for exploitation and genetic improvement are discussed.

THE PARADIGM OF LARVAL DISPERSAL – AN ARTIFACT OF SAMPLE SIZE ? IMPLICATIONS FOR AQUACULTURE

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This paper presents as an empirical model some molecular genetic data on the Queen scallop, indicating that large open water populations are self-recruiting, despite an apparently long-lived larval dispersal stage. This conclusion is drawn from a multi-variate discriminant analysis of allele frequencies generated from relatively large sample sizes (approximately 300 individuals in each of 14 samples around the UK), analysed in time (up to three year classes) as well as space. Simulations based on random re-sampling techniques demonstrate that these conclusions are only reached when sample sizes are larger than those commonly used in population genetic studies. A review of such studies suggests that the paradigm of larval dispersal (as opposed to retention) is often inappropriately invoked from studies of relatively low statistical resolution solely in the absence of evidence to the contrary. A general model of local recruitment (hence local adaptation) in marine organisms has profound implications for aquaculture management, particularly regarding sourcing of broodstock, hybridisation models (positive or negative heterosis), and maintenance of the integrity of genetic structure in natural and managed populations.

ANALYSIS OF THE VH GENE REPERTOIRE IN OUTBRED AND ISOGENIC STRAINS OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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The study of antibody responses in prominent aquaculture species such as the rainbow trout impact vaccine development as well as contribute to our paradigm of specific immunity in lower vertebrates. Thus, it is important to identify antibody genes responsible for protective responses. In the mammalian model, hybridoma technology allows for the association of monoclonal antibodies possessing various affinities for antigen with specific VH sequence, gene family utilization, and other molecular events which occur during the specific immune response. The absence of a comparable hybridoma technique for use with piscine B cells has limited the investigation of fish immunogenetics to date. Development of isogenic rainbow trout strains provides a useful model by which antibody responses can be induced to a specific antigen and the resultant molecular events compared to a common germline. Delineation of the VH gene repertoire in these inbred strains and comparison of gene family usage to that identified in outbred strains provides a foundation for further immunogenetic studies in teleost fish. Herein we describe the the strategies we've employed to isolate antibody VH genes, which include immunoselection of antigen-specific B lymphocytes by biomagnetic separation and fluorescence-activated cell sorting, isolation of antibody VH cDNA and genomic DNA, Southern blotting and gene titration, and DNA sequencing. The differences and similarities observed in VH gene repertoire are described. Additionally, the practice of selective breeding to produce disease resistant strains is discussed in the context of identifying possible VH gene repertoire alterations which may influence the overall immunocompetence in these animals.

TOP-CROSSING WITH PATERNAL INHERITANCE TESTING IN COMMON CARP (*CYPRINUS CARPIO* L.) OFFSPRING IN TWO ALTITUDES LEVEL

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The breeding goal was to test weight gain, survival and heterosis effect of common carp crossbreds in low and high altitudes (250 and 750 m, respectively) above the sea level in central European conditions. A newly established Hungarian synthetic mirror carp (HSM) was chosen for testing as a maternal strain. The HSM, as well as wild Amur carp (AC), Ropsha carp (ROP) and Tata carp (TAT) were used as paternal strains. The genetic distance between the maternal strain and the paternal ones according to the Roger´s test was 0.19 for AC, 0.15 for ROP and 0.12 for TAT. The third season of the topcross test up to three-years-old carp was performed in communal stocks of all groups in three ponds in each altitude. The highest significant corrected weight gain in low and high altitude was obtained with HSMxROP cross-bred (1567±271 and 1148±284 g), than with HSMxAC cross-bred (1469±347 and 1035±333 g) and significantly the lowest corrected weights were obtained with HSMxTAT cross-bred (1289±268 and 790±309 g) and HSM purebred (1233±278 and 738±283 g), respectively. Significantly the highest heterosis effect in low and high altitude was obtained for both HSM x ROP (33±17 and 65±28 %) and HSM x AC cross-breds (24±8.5 and 46±14 %), compared to HSM x TAT (9±9 and 10±3 %) cross-bred, respectively, as predicted from the highest genetic distances. Also a HSM x ROP crossbred was very adaptive to the different altitudes, regions and management in pond stations and meteorological conditions.

ESTIMATION OF THE ADDITIVE GENETIC VARIANCE COMPONENT OF GROWTH RATE HERITABILITY IN THE MOZAMBIQUE TILAPIA - *OREOCHROMIS MOSSAMBICUS*

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The genetic contribution to quantified phenotypic variation that exists between and within families of aquaculture species is a useful parameter in structuring future breeding plans. The estimation of distribution and magnitude thereof is imperative to genetic selection strategies and can greatly influence the rate of improvement. The growth rates (increase in total length and body weight) of 5 half-sib families (10 full sib families) from 10 strains each, of the Southern African Tilapia (*Oreochromis mossambicus*), were recorded over 4 growth intervals. Thus a total of 100 families, each with an internal reference group (to control for variation due to a environmental variance) were evaluated in an indoor intensive cage culture system. Based on sib analysis, the observational components of the phenotypic variation in growth rate has been calculated between sires (v^2 s), between dams within sires (v^2 d) and between progeny within dams (v^2 w). Estimates of causal components are based on the observational components and are used to calculate the heritability of an increase in total length and body weight. The genetic and phenotypic correlations obtained from a simultaneous increase in total length and body weight were also derived.

QUANTITATIVE EVALUATION OF GROWTH RATE BETWEEN AFRICAN STRAINS OF THE MOZAMBIQUE TILAPIA (*OREOCHROMIS MOSSAMBICUS*)

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This paper quantifies the variation, generated by an increase in total length (TL) and body weight (BW), among 10 Southern African strains of the Mozambique Tilapia (*Oreochromis mossambicus*). The cumulative variation is a result of individual recordings (TL; BW), taken over 7 growth intervals. 15-20 full sib families per strain were divided between two different culture systems; i) an intensive recirculation system, and ii) a semi-intensive cage system (3 replicates per culture system). An internal reference group was divided among these replicates to reduce the effect of differential environmental variation. *O. mossambicus* is indigenous to the region and strains were collected throughout its natural distribution as well as areas where it has been introduced. Of the 10 strains collected, 6 were obtained from the wild, 2 from semi-domesticated hatcheries and the remaining 2 were domesticated red strains. Results indicate strains most useful to aquaculture, and the prospects for improvement of quantitative traits, which is dependent on the distribution and magnitude of cumulative variation between and within strains.

THE GENETIC DIVERSITY AND POPULATION STRUCTURE OF THE TROPICAL CYPRINID *BARBODES GONIONOTUS* BLEEKER, THROUGHOUT ITS SPECIES RANGE IN S.E. ASIA

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The tropical cyprinid fish, *Barbodes gonionotus* (Bleeker) is commercially important both for wild fisheries and in aquaculture in many countries in S. E. Asia. Its natural species range extends from Java to Northern Laos but has yet to be fully delineated. *B. gonionotus* is one species in a very diverse freshwater fish fauna in the region which includes more than 1000 species in the Mekong system, and more than 900 described species in West Indonesia. This study is one of the very few which describe the population structure of a fish from this region.

We present sequence data from the mtDNA control region and data from 6 microsatellite loci, scored in 8 populations. Results clearly show the influence of the recent geological history of the region in shaping patterns of genetic differentiation in *B. gonionotus*. Two major monophyletic groups representing populations from mainland S.E. Asia and from Indonesia are identified by both marker types, refuting earlier claims that *B. gonionotus* is not native to mainland S. E. Asia. The implications of these results for management and conservation of this species is discussed.

APPLICATIONS OF A MOLECULAR PEDIGREE ANALYSIS TO SELECTIVE BREEDING IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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A molecular pedigree analysis was conducted for a strain development project in rainbow trout using a semi-automated genotyping system. Of 595 progeny derived from crosses within and between three strains, 96 % were assigned back to one parental pair using 12 microsatellite loci. This technique allows for families to be reared together prior to reaching a size that allows for physical tagging. The result is a reduction in both space requirements and between family environmental variance. The pedigree information will be further used to detect quantitative trait loci (QTL) which affect spawning time. The markers to be examined have previously demonstrated linkage to spawning time QTL in families designed for linkage detection. If such associations are to be exploited for marker assisted selection, linkage needs to be demonstrated in breeding stock. The second use of the data will be to determine relative relatedness between the parents of the population using the genetic marker information. We will test if progeny from more closely related parents have reduced growth rates compared to those from more distantly related parents. This technique could also be of use when attempting to design mating strategies for endangered species. Finally, to determine the degree to which pedigree information helps to avoid inbreeding, the relatedness between the most desirable individuals that are spawning on the same day will be examined. This will determine if phenotypic selection without pedigree knowledge would result in inbreeding.

UPDATING OUR PROGRESS IN THE MAPPING OF MAJOR GENES IN THE PACIFIC OYSTER (*CRASSOSTREA GIGAS*)

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Since October 1, 1996 we have been working to generate a coarse 10-20 cm linkage map for use in the genetic improvement of the Pacific oyster (*Crassostrea gigas*). Examination of allele transmission of 24 microsatellite loci in five pedigreed families revealed the not-uncommon segregation of null alleles and the presence of strong selection on marked chromosomal segments. A first analysis (not requiring a genetic linkage map) revealed strong evidence for major genes related to survival, growth and physiology. After learning how to account for null alleles and selection in transmission studies, and with the addition of 35 informative AFLP loci, a genetic linkage map was produced. Our first map from our first family includes 10 microsatellites, 2 allozymes, and 35 AFLP markers and comprises a total of 459 centimorgans. We have now included information from a second family. Using the multiple interval mapping approach together with our linkage map and performance measures in these two mapping families, a genome wide scan and resulting quantitative map for a few major commercial traits has been produced. The genetic architecture (number of major genes, their location, and their genetic effect) has been described for several linkage groups and is discussed in light of classical quantitative genetic theory. The implications for molecular quantitative genetic improvement in oysters and other highly fecund aquaculture species is discussed.

IMPROVEMENT IN THE PRODUCTION OF REDCLAW CRAYFISH (*CHERAX QUADRICARINATUS*) THROUGH A GENETIC SELECTION PROGRAM

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Redclaw crayfish is a commercially important aquaculture species in Queensland, Australia. Current output of the industry is worth $A 1.5M annually, and there is considerable potential for industry expansion in Australia and around the world. Since 1990 the Queensland Department of Primary Industries has been involved in industry development and culture-based research of this species. In 1993 a comparison of strains from discrete river systems was carried out and those with the highest aquaculture potential were identified. These strains were then used in an experimental breeding program where an estimate of heritability (24%) and an improvement in growth rate (9.4%) were achieved. Following that success, commercialisation of the selection program began with 240 females and 120 males from two promising river strains being reciprocally crossed to produce a genetically variable base population. An equal number of commercial strain crayfish from north and south Queensland were also crossed for comparison. The matings were set up in environmentally controlled tanks with a recirculating system. Once females had eggs attached, they were transferred to pens within an earthen pond. Juveniles were grown to approximately 10g in the pens, harvested, sorted and restocked at 10 m^{-2} for growout for 190 days. There was no significant difference in weight at harvest between any of the crosses, however survival of the river crosses (78.8%) was better than the commercial crosses (51.7%). Equivalent yield of the river crosses (5.6 t/ha/yr) was greater than the commercial crosses (4.6 t/ha/tr). A lower proportion of females of the river crosses carried eggs at harvest than the commercial crosses (13.8% v. 9.3%), suggestive of temperature effects on age at reproductive maturity. Selection for rapid growth is being undertaken and early weighings promise selection response in harvest weight in line with prediction.

GENETIC ANALYSES OF THE PROGENY OF TRIPLOID GYNOGENS INDUCED FROM UNREDUCED EGGS OF TRIPLOID (DIPLOID FEMALE X TETRAPLOID MALE) LOACH

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In the loach *Misgurnus anguillicaudatus*, triploid (diploid female x tetraploid male) males were sterile, but triploid females laid both large triploid and small haploid eggs. Gynogens artificially produced from the large eggs exhibited multilocus DNA fingerprints identical or very similar to the somatic cells of the mother. Thus, triploid loach were capable to generate unreduced eggs. Here, we examined reproductive potential of the four-year-old first generation triploid gynogens, and then analyzed genetic nature of the second generation progeny, gynogenetically induced from eggs of the first generation gynogens, using karyotyping, flow-cytometry, DNA-fingerprints, and microsatellite DNA markers.

The first generation triploid gynogens were all-female and they laid three types of gametes; large-sized, small-sized, and newly recognized medium-sized eggs. Rates of large-sized eggs were higher than those reported in the parental generation triploids. When the large-sized eggs were activated by UV-irradiated carp sperm, resultant gynogens had triploid DNA content. However, gynogens induced from the medium-sized eggs gave various aneuploid (1.4 - 1.5 n) chromosome numbers and karyotypes. These aneuploid progeny seemed to be inviable. Gynogens from the small-sized eggs died before feeding, probably due to the expression of haploid syndrome.

Among gynogenetic triploid progeny, some individuals exhibited DNA fingerprints identical to the mother, but the other gave ones slightly differed from the mother, when samples were digested with HinfI and/or Hae III and then hybridized with oligonucleotide probes (GACA)4, (GGAT)4, and 33.15. Analyses using some microsatellite DNA markers also gave different maternal and non-maternal genotypes among gynogenetic triploid progeny. These results showed the occurrence of both clonal and aclonal unreduced eggs in the triploid gynogens, induced from unreduced eggs of original triploid from a hybridization between normal diploid and natural tetraploid loaches.

GENE-CENTROMERE MAPPING OF MICROSATELLITE MAKERS IN THE LOACH, *MISGURNUS ANGUILLICAUDATUS*

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Fifty microsatellite loci have been isolated from a size-selected genomic library of the *loach, Misgumus anguillicaudatus* and then sequenced to design primers for PCR amplification. Then, polymorphic microsatellite loci were screened by observing genotypic distributions among samples from wild populations. Using some full-sib families, Mendelian inheritance was confirmed for each polymorphic locus by examining genotypic segregation. In diploid gynogens which had been produced by inhibiting the second melofic division after fertilization of eggs with UV-irradiated spermatozoa, gene–centromere (G-C) recombination rates were estimated by observing the second division segregation frequencies. Estimated G-C recombination rates ranged between 0.03 and 0.92. These results suggested that microsatellite loci widely distributed in chromosome from centromere to telomere regions.

GROWTH CHARACTERISTICS AND SEX REVERSAL OF YY AMAGO SALMON (*ONCORHYNCUS MASOU ISHIKAWAE*) PRODUCED BY ANDROGENESIS

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This paper describes growth characteristics of YY males of androgenetic amago salmon (*Oncorhyncus masou ishikawae*) and production of 2nd generation YY males by crossing the YY males with hormonally feminized YY females. Androgenetic amago salmon were produced 350 Gray irradiation to unfertilized eggs, followed by hydrostatic pressure shock of 650 Kg/cm^2, 6 min at 7.5 hours after insemination at 10°C. YY females were produced by combination of immersion in water containing 17 α-ethynylestradiol (50μg/l) for two hours twice a week and feeding a diet containing the same steroid(20μg/g) for 30 days.

Survival rate of YY males amago salmon was not different from that of normal amago salmon until 11 months after hatching. Almost YY males matured and died at one year. However, YY females did not mature at one year.

The growth of YY females showed no difference from that of normal females or YY males.

The results of cross experiments suggest that there were problems in quality of the eggs of YY females.

GENETIC CONTROL OF THE GROWTH IN THE GUPPY (*POECILIA RETICULATA*)

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In the guppy (*Poecilia reticulata*), differences of body size were observed among the strains maintained as closed colony in our laboratory. To describe genetic control of growth, heritabilities of body length as a marker of growth were estimated by sib analysis. Furthermore, the cross between S and F strain estimated the number of loci which influencing strain differences of body length. The S strain is small size and F strain is large size. The estimated heritabilities from maternal and paternal half sibs were different between females and males. The heritabilities from maternal half sibs indicated high value constantly, however, these from paternal half sibs indicated low values in females. On the other hand, in males at a stage of after 120 days old, the heritabilities from paternal half indicated high value. The estimated number of loci influencing strain differences was 4.0 in female and 1.7 in male at 180 days old from the cross between females of S and males of F strains. However, these values were 1.8 in female and negative value in male from reciprocal mating. The negative value was caused by lower variance in F2 generation than F1 generation. These results suggest small number of genes were controlling growth of the guppy, and some of them locate on sex chromosome. Especially, the gene(s), which detects the small size of S strain, may be located on Y chromosome.

GENETIC MAPPING OF THE DOMINANT ALBINO LOCUS IN RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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Albinism in animals was generally recessive inheritance but in Japan a dominant oculocutaneous albino (OCA) mutant strain has been isolated in rainbow trout (*Oncorhyncus mykiss*). After confirming that this trait is not arose from tyrosinase gene mutation which causing of OCA1 (tyrosinase negative OCA), we combined amplified fragment length polymorphism (AFLP) technique and bulked segregant analysis (BSA) approach to detect the gene conferring on the dominant oculocutaneous albinism. Four AFLP markers tightly linked to the dominant albino locus were identified. One of these markers was codominant and we have converted this marker into a GGAGT-repeat microsatellite marker *OmyD-AlbnTUF*. Using the pentanucleotide repeat DNA marker, the dominant albino locus has been mapped on linkage group G. These identified markers provide the first step towards for future cloning of the dominant albino gene in rainbow trout in near future.

STUDIES ON CARCASS QUALITY TRAITS IN COHO SALMON. PHENOTYPIC AND GENETIC PARAMETERS

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Phenotypic correlation and heritability estimates of carcass quality traits were obtained from pedigreed populations of two year classes of IFOP-Coyhaique breeding program of coho salmon. Data were recorded at harvest, from 21 month old fishes. 600, 1006 and 1 000 measured fish, randomly chosen from of 86, 95 and 94 FS families sired by 25, 32 and 35 males in three years respectively were analysed. Body weight, carcass weight, dressing percentage, ventral thickness, dorsal fat thickness, intestinal fat weight, muscle fat %, measures of flesh color (tables and fotocolorimetric) and pigment content were the main traits analyzed.

Significant sex effects were found in the majority of carcass traits studied. Heritability estimates for carcass quality traits were medium-high for muscle fat content (0.41) medium for visceral fat (0.31), carcass weight and dressing percentage (0.31 and 0.28), low for visual meat color (0. 14-0.20) and for astaxantine content (0.10). Heritability estimates for fotolorimetric measures of flesh color under a low pigment content food treatment were higher than under a standard pigment content food. All relevant phenotypic parameters and correlations are given.

TETRAPLOID INDUCTION IN BIVALVES BY BLOCKING FIRST MITOSIS WITH 6-DIMETHYLAMINOPURINE

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Reliable production of tetraploid bivalves remains elusive with poor survival of tetraploids beyond the embryo/trochophore stage. Production of tetraploids would provide a means of producing 100% triploids. This would provide consistency and improvement in the triploid product, by eliminating the proportion of diploids usually found in triploids produced by conventional means. It would also guarantee sterility in triploid bivalves, which would allow translocation of non-native species to new culture areas without the risk of spawning. Tetraploid Pacific oysters have been produced in the US by blocking polar body 1 extrusion in strip-spawned eggs from triploids fertilised with sperm from diploids. However reproduction of the technique for producing tetraploids from triploid eggs in Sydney rock oysters has proven difficult.

Commercial application has been identified in NSW for both Sydney rock oyster, *Saccostrea glomerata* (Gould), and Pacific oyster, *Crassostrea gigas* Thunberg, tetraploids for the efficient production of triploids. A mussel, *Mytilus galloprovincialis* Lamarck, was selected in this study as a model on which to hone tetraploid induction techniques because it had previously proven more amenable to ploidy manipulation using similar techniques and chemicals as oysters.

Zygotes of both Sydney rock and Pacific oysters were exposed to 6-dimethylaminopurine (6-DMAP) to block first mitosis, yielding up to 24% tetraploid trochophores. Using a range of 6-DMAP concentrations with Sydney rock oysters, the optimum concentration for this species was experimentally determined to be 600µM 6-DMAP whilst a lower concentration (400µM) was used for Pacific oyster zygotes. Survival to veliger stage was low (<1%) for both species with no tetraploids detected in day 5 larvae. Mussel zygotes treated for 30 min with 600µM 6-DMAP yielded 14% tetraploid larvae on day 5 with only 0.2% survival. Use of 6-DMAP was effective in blocking first mitosis in bivalve zygotes and produced small numbers of tetraploid trochophore larvae, no tetraploid oyster larvae survived beyond this stage whilst tetraploid mussels survived to day 5. A large batch of zygotes has been treated to assess if this method can successfully produce tetraploid mussel spat.

QUANTITATIVE TRAIT LOCI (QTL) ASSOCIATED WITH DEVELOPMENT RATE IN CLONAL *ONCORHYNCHUS MYKISS* STRAINS

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Oncorhynchus mykiss includes many strains with unique physiologies, morphologies, and life histories. Clonal lines of some of these unique strains have been generated and maintained by androgenesis in our laboratory. One of the differences observed among our *O. mykiss* lines is development rate. In this study, we will present a QTL analysis of development rate between two isogenic *O. mykiss* lines. Hybrid clones were produced by crossing Clearwater (CW) River steelhead trout to Oregon State University (OSU) hatchery rainbow trout. Reared at 11°C, the CW line exhibited a faster development rate as measured by accumulated temperature units when compared to the OSU line. Mean time to hatch for CW clones was 292±0.81 degree days and for OSU clones was 342±1.5 degree days. Sperm from OSU X CW hybrid clones was used to produce androgenetic doubled haploids for genetic linkage mapping and QTL analysis. A genetic linkage map will be constructed with genotype information from AFLP, microsatellite, and known gene markers. A QTL analysis will be performed for development rate using composite interval mapping. A previous QTL experiment between our OSU and Swanson River (Alaska) lines revealed one QTL of major effect on linkage group IX. The number and position of QTL identified for development rate in the OSU X CW cross will be compared to these previous results. This study will provide further evidence for QTLs associated with development rate, and will provide the framework for further QTL analyses on meristic values, disease resistance, and seawater adaptability in this cross.

SEARCHING FOR GENES ASSOCIATED WITH THE CONTROL OF CIRCADIAN RHYTHMS IN THE BARRAMUNDI (*LATES CALCARIFER*)

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Circadian rhythms have been observed in many eucaryotes and some procaryotes as oscillations in physiological processes which occur approximately once every 24 hours. Circadian rhythms are identified by three characteristics. These are entrainment by an environmental cue, short-term persistence in continuous darkness and continuity over a range of physiological temperatures. The aim of the present study is to identify the gene, or genes, that control circadian rhythms in Barramundi (*Lates calcarifer*). Conserved circadian clock gene sequences are being used to search for homologous genes in Barramundi. Several regions representing functional protein domains are conserved in the *Clock* gene in human, mouse, rat, chicken, frog and fruit fly. Primers were designed to PCR amplify a PAS region in Barramundi DNA. The similarity of the Barramundi sequence to other species was assessed by BlastN and ClustalW alignments. Since reproduction in many fish is influenced by photoperiod, an understanding of circadian rhythm genes in fish may aid hatchery and broodstock management and enhance breeding strategies.

ISOLATION AND CHARACTERIZATION OF (CA)N MICROSATELLITES FROM THE JAPANESE EEL, *ANGUILLA JAPONICA*

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Seed production and subsequent perfect culture of the Japanese eel will be realized in near future. To develop breeding program for genetic improvement of cultured strains, basic genetic informations are necessary. In this paper, we isolated (CA)n repeats and then studied genetic characteristics of these microsatellite makers for future gene mapping and related QTL analyses.

Genome DNA was isolated from blood cells and then digested with *Hae* III. The DNA fragments ranging between 500 to 800 bp were ligated to pCR-Script SK (+) digested with *Srf* I and each ligation mixture was used to transform competent cells. The genomic library was screened by $(GT)_{10}$ end-labeled oligonucleotides and fifty positive clones were isolated from 4700 recombinant clones. The PCR primers to amplify (CA)n repeats were designed based on the sequence data of clones. Genotypes of microsatellate loci were determined by PCR amplification and 15%PAGE.

Using 20 primer sets synthesized, polymorphic markers (loci) were screened among samples collected from wild and cultured populations. To confirm Menderian segregation of microsatellite markers examined, DNA samples were extracted from 6-day-old hatching fly of full-sib families, which were produced by artificial fertilization between gametes from several single pairs. Ovulation of females was hormonally induced by administration of salmon pituitary gland and DHP, whereas males were matured by HCG. In all families, observed frequencies of microsatellite genotypes were not significantly deviated from the frequencies expected from Mendelism. These results showed that microsatellite makers characterized here are powerful tools for genetic studies of the eel.

MICROSATELLITE GENETIC VARIATION BETWEEN AND WITHIN FARMED AND WILD ATLANTIC SALMON (*SALMO SALAR*) AND THE USEFULNESS OF THESE MICROSATELLITES MARKERS TO DETERMINE RELATEDNESS AND PARENTAGE IN FARMED POPULATIONS

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Genetic diversity between 3 farmed and 4 wild populations of Atlantic salmon from Ireland and Norway were analysed using 15 microsatellite markers. High levels of polymorphism were observed over all populations with the average number of alleles and average heterozygosity at 17.8 and 0.70 respectively. Farmed salmon showed less genetic variability than wild salmon in terms of allelic diversity but not necessarily in terms of overall heterozygosity. Between farmed populations significant differences were observed in expected heterozygosity suggesting that more intensive breeding practices may have resulted in a further erosion of genetic variability. Phylogenetic analysis using either populations or individuals as nodes show a clustering of populations into two groups, farmed and wild. This suggests that founder effects and subsequent selection have had more effect on the genetic differentiation between these strains than geographical separation.

This study also demonstrates how these same microsatellite markers can be used to determine both parentage and relatedness in a mixed aquaculture situation in the absence of physical tags and/or pedigree information. Under a number of different scenarios, both real and simulated, we could assign parentage to offspring with varying degrees of accuracy. The precision of assignment depended not only on the number and variability of the microsatellite markers but also on the number of potential pairings from which to. 15 microsatellite loci were capable of discriminating between related and unrelated individuals in a situation where no pedigree information is known.

PLOIDY MANIPULATION OF *PENAEUS JAPONICUS* AND *HALIOTIS ASININA*

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The use of triploidy as a genetic tool to improve growth rates, or provide genetic containment has been successfully applied to several fish and bivalve mollusc species. The technique is however still in the experimental stage for crustaceans and abalone. Furthermore, chemical or physical treatments used to induce triploid organisms are not 100% effective. The efficiency of induction depends on the dosage, timing and duration of a chosen treatment.

In this study we are examining ways to establish simple and reliable techniques for the induction of polyploidy in commercially important crustaceans and molluscs (ie penaeid prawns and abalone). Ploidy assessment was by Propidium Iodide staining of larvae and analysis with a FACS Calibur Flow-cytometer.

We have induced triploid and tetraploid *Penaeus japonicus* larvae using both heat and chemical shock. Induction rates varied with the type of treatment applied (Table 1).

Table 1. *P. japonicus* induced polyploidy

TREATMENT	DIPLOID	TRIPLOID	TETRAPLOID
Temperature shock	79.5	5.1	15.4
6-DMAP conc 1	52.55	47.45	
6-DMAP conc 2 X 1	27.7	72.3	

Using chemical treatment (6-DMAP) we have successfully induced triploidy in the tropical abalone *Haliotis asinina*. The highest concentration of 6-DMAP and the treatment regime used for this trial resulted in 72.4% triploids within the veliger population (Table 2).

Table 2. Efficiency of induction of triploid *Haliotis asinina* with different concentrations of 6-DMAP

6-DMAP CONCENTRATION	DIPLOID	TRIPLOID
0	100	
1	100	
2	96.8	3.2
3	27.6	72.4

In this paper we discuss the implications of these results for improving the capacity to induce and monitor polyploidy in these species.

LARGE LARVAE OF PACIFIC ABALONE, *HALIOTIS DISCUS HANNAI* OBTAINED FROM TRIPLOID BATCHES

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The growth performances of triploid abalone have been investigated in some species. Since every researchers have remarked the increased growth of triploids by gonad retardation, the growth (or size) of triploid larvae has never been studied. In the present study, we compared the size of triploid and normal diploid larvae in Pacific abalone, *Haliotis discus hannai.*

The triploid larvae were induced by 15 mM caffeine treatment (for suppression of the 1st or 2nd meiotic division) of fertilized eggs obtained from artificial spawning of parental *H. d. hannai.* At 15~16 and 44~46 hours after fertilization, trochophore and veliger larvae in treated and untreated (diploid control) batches, respectively, were sampled and fixed with 10 % formalin seawater. The body area or diameter of the larvae was measured using a video-micrometer (Olympus, VM-30), and compared between treated and control batches. The triploidy status was estimated by nucleoli or chromosome counting.

In the trochophore stage, the measured mean body area of specimens in 10 out of 15 treated batches was significantly larger than that of the specimens from the corresponding control batches. In the veliger stage, the measured mean body area of specimens in all 22 batches was significantly larger than that of the specimens from the control batches. Thus, the phenomenon of increase in the size of triploid larvae was more clearly observed in the veliger stage. We inferred that this phenomenon was caused by polyploid gigantism.

ANALYSIS OF QTLS ASSOCIATED WITH RESISTANCE TO A VIRAL DISEASE (IPN) IN RAINBOW TROUT

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Infectious pancreatic necrosis (IPN) is a highly contagious viral disease which causes excessive mortality among salmonid fish. IPN virus is the prototype of the Birnaviridae virus family: about 60 nm in size, undeveloped, icosahedral animal viruses with bisegmented double-stranded RNA genomes. As a result of extensive studies on IPN, variation in resistance has been found among cultivated varieties of rainbow trout. This variation has facilitated the selection of resistant/susceptible strains to perform linkage analysis.

The recent development of rainbow trout genetic linkage maps has made possible the identification of individual loci controlling quantitative traits such as disease resistance. In this study, we mapped quantitative trait loci (QTLS) for IPN resistance in a segregated population of backcross derived from out-crossing the resistant strain of rainbow trout, RT-201 and the susceptible one, RT-101. Phenotypic scores of 1-dead/susceptible and 0-survivor/resistant for QTL analysis were recorded from the results of artificially induced infection with IPNV. QTL analysis was carried out using a framework linkage map based on microsatellite markers and a software, Map Manager QT.

Two putative QTLs (IPN R/S-1 and -2) affecting disease resistance were detected on Chromosomes A and C designated by Sakamoto et al., suggesting that this trait is polygenic in rainbow trout. These markers offer great potential in marker assisted selection (MAS) and contribute the basis for future cloning of IPN resistance genes. This is the first report of the identification of QTLs associated with disease resistance in fish.

ESTIMATION OF ADDITIVE AND NON-ADDITIVE GENETIC VARIANCES FOR GROWTH IN SELECTED POPULATIONS OF RAINBOW TROUT

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Three sire-dam models were used to estimate the additive, dominance, and additive by additive genetic variance components for body weight at harvest in three populations of rainbow trout in Norway via the tilde-hat approximation to REML. The models were: additive (A), additive plus dominance (A+D) and additive plus dominance plus additive by additive (A+D+A*A), with regression of body weight on individual inbreeding coefficients. Heritability estimates for growth were the same for all populations in the A model and were in good agreement with those found in the literature. The dominance variance estimates, expressed as percentage of total phenotypic variance were 19.8 and 16.6% respectively, for A+D and A+D+A*A models, for population 1; 15.8 and 12.6%, for population 2; and 22.2 and 19.0%, for population 3. The magnitudes of the dominance variances were generally smaller than the additive variance. The additive by additive genetic effect accounted for only 5% of the total phenotypic variance. The dominance variance as a proportion of genetic variance averaged among all populations was 49.6% and 43.4% when calculated under A+D and A+D+A*A models, respectively. The additive by additive variance as a proportion of genetic variance averaged among all populations was estimated at 12%. The inflated values for the dominance variance may be due to confounding with the common environmental effects due to full-sibs. The present study confirmed the presence of dominance genetic variance for body weight in rainbow trout. Future breeding studies should consider the exploitation of the dominance effect in rainbow trout selection programs.

VARIATION AND TRANSMISSION OF MITOCHONDRIAL DNA IN POLISH POPULATIONS OF THE MUSSEL *MYTILUS TROSULUS*

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In comparison with other animal species, doubly uniparental inheritance of mtDNA is a unique and characteristic feature of marine mussels. In addition to one mitochondrial genome inherited from the mother, heteroplasmic mussels contain a second genome inherited from their father. According to the literature, both genomes are highly diverged.

Eleven samples of forty mussels each, from different sites along Polish Baltic coast were collected. Differences between populations were detected using restriction analysis of PCR amplified coding region (ND2-COIII) of 1.2kb in length. Twenty-four haplotypes were identified by digestion with five restriction enzymes (RFLP).

Polymorphism of mtDNA length was studied employing PCR amplification of the major noncoding region. Fourteen length variants in both homo- and heteroplasmic specimens were identified. Heteroplasmy was characterized by the presence of two different length PCR products. Selected PCR products were sequenced. The differences in length can be attributed to the presence of 80-120bp tandem repeats at the 3' end of the region. Length variants were not highly diverged.

The comparison of length and RFLP data was performed and transmission of mtDNA to gametes was studied. The results suggest that length variants can be transmitted through both male and female lines.

MICRODISSECTION OF *OREOCHROMIS NILOTICUS* PUTATIVE SEX CHROMOSOMES AND DOP-PCR PRODUCES CHROMOSOME-SPECIFIC FISH PROBES

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Analysis of meiotic synaptonemal complexes from XX, XY and YY Nile tilapia (*Oreochromis niloticus*) revealed an unpaired region at one end of the chromosome 1 bivalent, which was present only in the XY (heterogametic) genotype. This is the best evidence to date for the identification of the sex chromosomes in this species.

Chromosome microdissection was used to isolate this part of chromosome 1 from a mitotic chromosome spread from an XX *O. niloticus*. Degenerate oligonucleotide primed PCR (DOP-PCR) was then used on this material to amplify and label DNA and thus generate a region-specific probe library. Subsequent chromosome painting on another mitotic metaphase from the same XX individual revealed a preponderance of fluorescent signal on the corresponding part of chromosome 1 that was microdissected. Of the fluorescence located elsewhere in the karyotype, the majority appears to be telomere-associated, probably due to the presence of chromosome 1 telomere DNA in the original microdissected region.

This confirms the origin of the probe and demonstrates that DNA probes specific to the putative X chromosome can be generated using microdissection and DOP-PCR. In subsequent research we will attempt to develop sex-specific DNA probes and primers. The technique may also have application in the analysis of other chromosomal regions in fish species, many of which are difficult to work with due to a lack of distinct chromosome banding patterns.

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THERMAL TOLERANCE QTL IN OUTBRED RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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The existence of putative quantitative trait loci (QTL) for thermal tolerance in outbred salmonids was examined using a pedigree of two commercial rainbow trout (*Oncorhynchus mykiss*) strains unselected for this trait. Half-sib progeny in second-generation (G_2) two-way (two males by two females) diallel lots were subjected to an acute thermal challenge. Physical traits, first-generation (G_1) sires/dams and segregating microsatellite markers were considered in general linear modeling. The trait was expressed both as cumulative thermal exposure (upper thermal tolerance; UTT) and total survival at critical thermal maximum (CTmax). Strong associations were found between alleles at the microsatellite locus *Ssa20.19NUIG* in G_1 sire 93-32-1 and the thermal tolerance of his progeny. Segregants at this locus were associated with a survival time difference of 33.86 minutes at CTmax, or approximately 500 degree-minutes of UTT. Effects of sires, dams and physical correlates were observed in numerous diallel lots. One allele at *Ssa20.19NUIG* in this G_1 sire also exhibited strong effects on UTT when its inheritance was traced from the G_0 grandsire of origin into all of that grandsire's G_2 grandprogeny. Thermal tolerance was also associated with a sex-linked SSR, *OmyFGT19TUF*. The effect of the sex-linked allele appeared to differ depending on whether the family was a purestrain or interstrain cross. This is evidence for the existence of QTL variants for a fitness trait in outbred fish populations, and of a potential environmental modifier (thermal stress) of the sex ratio in rainbow trout, a genetically sex determinate species.

IDENTIFICATION OF INTERSPECIFIC CHROMOSOME HOMOLOGIES IN SALMONID FISHES USING PAINT PROBES

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Whole arm chromosome paint probes were produced for several rainbow trout (*Oncorhynchus mykiss*) chromosomes by microdissection and used on a variety of other salmonid species including Atlantic salmon (*Salmo salar*), brown trout (*Salmo trutta*), lake trout (*Salvelinus namaycush*), brook trout (*Salvelinus fontinalis*), and Arctic char (*Salvelinus alpinus*). Most of the probes highlighted only one chromosome pair, suggesting that the majority of the chromosome pairs in these ancestrally tetraploid fishes are diploidized. However one of the probes highlighted the telomeric region of a second (presumably homeologous) chromosome pair in all of the species. This is consistent with genetic evidence that some duplicate loci near telomeres still share alleles. One of the rainbow trout paint probes prepared from an autosomal chromosome pair paints the long arms of the sex chromosome pair in the three *Salvelinus* species. A paint probe previously made from the short arm of the lake trout Y chromosome (lake Yp probe) paints half of the short arm of a rainbow trout autosomal pair. These results are consistent with separate evolution of the sex chromosomes in *Oncorhynchus* and *Salvelinus*. The lake Yp probe paints interstitial regions on two chromosome pairs in Atlantic salmon, but only one chromosome pair in brown trout. Combined experiments with paint probes and labeled BAC clones for specific genes will be very useful in identifying homeologous chromosome regions within species and in correlating the genetic maps of different salmonid species.

IMPROVING THE GROWTH RATES OF FARM STOCKS OF *PENAEUS JAPONICUS* THROUGH SELECTIVE BREEDING

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Farming of the Kuruma prawn (*Penaeus japonicus*) for live export to Japan is a recent and successful Australian aquaculture industry. Commencing in 1991 the industry now has an annual production of 200 t valued at A$15 million.

Over the past six years, collaborative research between CSIRO and industry has progressively enhanced the growth rates of farm stocks of *P. japonicus*. Selection response and heritability for growth were initially quantified under controlled conditions at the Cleveland Laboratories. The results demonstrated that growth rate is heritable with a gain of 11% per generation in controlled condition tanks.

For the past two production seasons we have monitored the farm production performance of two different genetic lines. One line was second generation (G2), mass selected for growth from commercial production ponds. The other line was a fifth generation high-growth line (H4) originating from controlled-environment heritability experiments at the Cleveland Marine Laboratories.

In the 1998/99 production season there were significant improvements in the growth, survival and total yields of both the selected lines compared to the progeny from wild stocks (G1). Compared to the G1 stocks, there was a mean gain in weight at first harvest of 7 % in the G2 line and 10% in the H4 line. Because larger prawns command a higher price these gains in weight converted to a 21% increase in value for the G2 line and 34 % in the H4 line. In this paper we will report on the performance of the current generations of these lines (G3 & H5) compared to G1 lines in the 1999/2000-production season. We will also discuss the implications of these results on our ability to conserve the current gains and achieve additional genetic gains.

ANALYSIS OF GROWTH AND SPAWNING TIME IN DIALLEL CROSSES OF THREE STRAINS OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*)

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In Ontario, rainbow trout with both fast growth and a spring spawning season would increase production efficiency. The goal of this study was to assess the feasibility of simultaneously improving growth and delaying spawning date through genetic selection. Complete diallel crosses were made from three strains of rainbow trout: one with fast growth and a fall spawning season, a second with intermediate performance in both traits, and a third with slow growth and a spring spawning season. Parents and offspring were PIT-tagged and individual weights, sex, age at maturation, and female spawning dates were recorded. Exact parentage of offspring was determined with microsatellite DNA analysis. Variance components and individual estimated breeding values (EBVs) were estimated using best linear unbiased prediction (BLUP). In general, growth and spawning time of hybrids were intermediate to those of pure strains, but heterosis for growth was detected in some crosses. Early-maturing individuals had the highest instantaneous growth rates from 280 to 650 days old and the highest weights at 2 years old. Heritabilities of weight at two years old and female spawning date were 0.5 and 0.7, respectively. Phenotypic, genetic and environmental correlations between these traits were -0.2, 0.0, and -0.4, respectively. Selection based on BLUP model EBVs could be used to simultaneously increase growth and delay spawning date.

A MICROSATELLITE LINKAGE MAP OF RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) CHARACTERIZED BY LARGE SEX-SPECIFIC DIFFERENCES IN RECOMBINATION RATE

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A genetic linkage map of approximately 300 markers has been constructed for a tetraploid derivative species, the rainbow trout (*Oncorhynchus mykiss*), and is based primarily upon microsatellite markers, but also includes information on some allozyme and ESMPs. The linkage map consists of 29 linkage groups with potential arm displacements in the female map due to male-specific pseudolinkage arrangements. Synteny of duplicated microsatellite markers was used to identify and confirm some previously reported pseudolinkage arrangements based upon allozyme markers. Twenty centromeric regions wer identified with a half-tetrad analysis using gynogenetic diploids. Extreme differences in female:male map distances were observed (Ratio F:M = 3.25:1). Females had much lower recombination rates (0.14:1) in telomeric regions than males, while recombination rates were much higher in females within regions proximal to the centromere (F:M = 10:1). Quadrivalent formations which appear almost exclusively in males are postulated to account for the observed differences.

IDENTIFICATION OF MHC CLASS II B GENES AND THEIR ASSOCIATION WITH SPECIFIC IMMUNE RESPONSE IN THE NILE TILAPIA, *OREOCHROMIS NILOTICUS* L.

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Major histocompatibility complex (MHC) class II B genes in the nile tilapia (*Oreochromis niloticus*) have been identified in this study. The MHC class II B genes were found to be polymorphic in the PCR analysis and DNA sequencing. At least 17 polymorphic loci and 21 distinct haplotypes have been identified in tilapia and the number of loci varied from individual to individual, ranging from 1 to 13. During the study of specific immune response, a number of clonal lines of tilapia having different MHC class II B genotypes were used. Scale grafting between the three clonal lines showed complete allograft rejection and two of the lines demonstrated significantly faster rejection than the other one which presumably due to the involvement of the MHC class II loci those exerted strong alloantigenic effects on the foreign grafts by presenting the foreign peptides to T lymphocytes through the surface of the antigen presenting cells. Thus the association of the MHC class II B genes with the specific immune response could be predicted the relationship between MHC and disease resistance in fish.

USING MICROSATELLITE AND RAPD MARKERS TO ESTIMATE THE AMOUNT OF HETEROSIS IN VARIOUS STRAIN COMBINATIONS IN THE GUPPY (*POECILIA RETICULATA*) AS A FISH MODEL

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To explore the use of microsatellite and random amplified polymorphic DNA (RAPD) markers as a tool for estimating the amount of heterosis in various strain combinations, diallel and reciprocal crosses were performed among 4 domestic strains in the guppy (*Poecilia reticulata*) as a fish model. Salinity tolerance, measured as the survival time after transfer from fresh water to 35ppt seawater, was used to measure the amount of heterosis because the trait is strongly sensitive to inbreeding depression and heterosis. The amount of heterosis, expressed by the ratio between the means of the parents and their F_1 hybrids, did not differ between reciprocal crosses, but the mean values varied from -7.3% to 49.0% among the strain combinations. The Nei's genetic distance measured by microsatellites differed from 0.078 to 0.813 among the strains, and the dissimilarity measured by RAPD differed from 0.125 to 0.320. The amount of heterosis correlated with the Nei's genetic distance ($y=-4.527+56.073x$, $P<0.01$) and also the dissimilarity ($y=-25.816+199.106x$, $P<0.01$). In the strain combinations in which most of the dissimilarities among individuals were higher than the mean dissimilarities within each strain (about 0.1), the amount of heterosis was positive in every pair. In the strain combinations in which some of the dissimilarities were below 0.1, on the other hand, the amount of heterosis was negative in some pairs. These results indicated that the amount of heterosis depend on the genetic differences between the strains, suggesting that microsatellite and RAPD markers are useful for expecting the mean and variance of the amount of heterosis in various strain combinations.

PROTEIN VARIABILITY IN COMMON CARP (*CYPRINUS CARPIO*) BREEDS IN THE CZECH REPUBLIC

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Tissue samples (blood, trunk muscle and liver) were taken from more than 1500 carps belonging to 48 different breeds (including local strains, synthetic breeds, Amur wild carp and breeds related to it). Starch-gel-electrophoretic analysis of products of 37 presumptive structural loci representing 14 protein systems was performed in tissue extracts to describe the protein variability in this economically important fish. Products of 17 loci showed variability - polymorphism exhibiting mostly two, but often three to ten alleles. Differences among the individual breeds studied were found mainly in allelic frequencies at individual protein loci; only in the Amur carp and related breeds, several characteristic alleles were found in some loci allowing it to be distinguished. The results of analyses will be used to describe genetically individual carp breeds in the frame of the Czech governmental programme on gene resources conservation that in commercially important fish species (including also common carp) has supported *in situ* conservation of rare/endangered breeds. Since the genetic relationships among breeds / strains of common carp are very close making it difficult to distinguish genetic differences, our goal is to introduce more sophisticated methods (as DNA variability analysis) of genetic description allowing us more detailed description of individual stocks and in some cases to trace their history and to guide future fish breeding policy.

ISOLATION OF MICROSATELLITE LOCI FROM THE EUROPEAN FLAT OYSTER (*OSTREA EDULIS*)

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The European flat oyster (*Ostrea edulis*) is an important commercial species but stocks and production have declined massively over the last 100 years. The recent infection of most European stocks by the protozoan parasite, *Bonamia ostreae*, has decimated current oyster production. As part of an EU / industry funded CRAFT project to investigate genetic structure of *O. edulis* populations and their potential resistance to *B. ostreae*, a programme to isolate and identify DNA microsatellite markers was undertaken. Here we present sequence data on 24 microsatellites and one minisatellite which were isolated from an *O. edulis* non - enriched partial genomic DNA library. Primers have been designed for a subset of these microsatellite loci and preliminary results are presented for some Scottish populations. Genotype and allele frequency data are used to identify potential genetic differentiation of these stocks which may have remained relatively undisturbed over recent times compared with other European stocks of the native oyster. Apart from their use in stock identification, the development of suitable microsatellite markers will enable their use in gene mapping and to establish linkage with quantitative trait loci (QTL) influencing important traits such as growth, disease resistance, and cold tolerance.

GENETIC IMPROVEMENT OF *LITOPENAEUS VANNAMEI* IN COLOMBIA

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In 1997, Corporación Centro de Investigación de la Acuicultura de Colombia (CENIACUA) in collaboration with Institute of Aquaculture Research Ltd (AKVAFORSK) initiated a national breeding program for *Litopenaeus vannamei* in Colombia. A family and within-family selection scheme was applied with the initial aim of improving growth rate and resistance against Taura Syndrome Virus (TSV). Resistance against white spot syndrome virus (WSSV) was added to the breeding goal in 1999. At present, a total of four batches of full-sib (>200) and paternal half-sib families (>100) have been produced. Growth performances were tested in commercial production farms in Colombia. Disease resistance was studied in controlled challenge test experiments involving simultaneous exposure to TSV and WSSV of a sample of individuals from all families within batch. The paper presents preliminary estimates on genetic parameters such as heritability and genetic correlations for growth, pond survival and disease resistance based on data from the first three batches of families (52, 52 and 70, respectively). Estimated selection responses for the first round of selection (batch 1) is also included.

CONFIRMATION OF MATING MODE IN THE KURUMA PRAWN (*PENAEUS JAPONICUS*) BASED ON MICROSATELLITE DNA MARKERS

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The mating system of Kuruma prawn (*Penaeus Japonicus*) is supposed to be pair mating from observation of their mating habits. However, this suggestion has not been confirmed genetically yet. Thus, we surveyed the mating mode of the prawn by microsatellite DNA markers. Samples were seven copulated natural females and their larvae. We reared progeny from hatching to the postlarval stage. Five microsatellite loci were used; CSPJ2, CSPJ10, CSPJ12, CSPJ14 and CSPJ15 (developed by Moore et al, 1999). At each locus, every progeny has one or two alleles found in their mother. We decided male's genotypes from those of a female and her family at every locus and we could recognize only one combination of genotypes in each family. This result shows that one female copulated to one male and supports that the mating mode of this prawn is pair-mating. This result also suggests that the efficiency of microsatellite markers is "genetic tag" for monitoring of released seeds. The cluster analysis based on ASI (Allele Sharing Index) between individual within/among families was also examined and showed that each family formed single cluster, respectively. This result suggests that microsatellite markers could be useful to reveal the kinship of this prawn.

SELECTIVE BREEDING FOR IMPROVED PERFORMANCE OF FARMED NEW ZEALAND CHINOOK SALMON

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Chinook salmon aquaculture is a growing industry in New Zealand with the potential to increase production over the next few years. In order to improve stock performance an industry based family selective breeding programme was initiated in 1994. The initial aim of the programme was the identification of specific traits for incorporation into a selection index in order to optimise family selection and allow the strategic use of broodstock. At the same time protocols for the commercial implementation of the programme were developed.

Breeding values for thousands of individually tagged broodstock are calculated each year and all individuals are ranked on an index combining breeding values for performance traits. Parents of the next generation families are selected on the index taking into account the relatedness of families because of potentially high levels of inbreeding within the initial commercial hatchery stocks. In 1994 a laboratory was built on the hatchery site with the capability of carrying out PCR analysis and gel electrophoresis. With these tools it is possible to utilise PCR based systems to aid with broodstock selection. Nine microsatellite markers are used to assess family relatedness and significant differences between the stock lines within the programme have been identified. Parentage can also be assigned unambiguously using four microsatellite loci.

GENETIC EFFECTS ON APPEARANCE OF COLOUR ABNORMALITY AND REVERSAL OF SIDES ESTIMATED FROM CLONED JAPANESE FLOUNDER (*PARALICHTHYS OLIVACEUS* TEMMINCK ET SCHLEGEL)

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The genetic effects on the appearance of color abnormality and reversal of sides in Japanese flounder (*Paralichthys olivaceus*) were estimated using clonal lines. Nine second generation clones were induced from first generation cloned fishes of two clonal lines (A and B). After these clones were reared with three controls in the same conditions for 40 days, the rate of color abnormality and reversal of sides in each clone and control was investigated. Induction of cloned flounders was confirmed by evidencing the genetic identity with these adults using ten microsatellite genes. The controls had two peaks of color abnormality, on the contrary clones A and B had one peak respectively. In addition, the degree of color abnormality of clone A was strong, on the contrary clone B was weak. The mean rate of reversal of sides in controls and clone A was 1.0, 1.3%, respectively, while the rate in clone B was 19.9%. These results about the color abnormality and reversal of sides were the same irrespective of the clone from the same adult or a clone from a different adult in any one clonal line. In addition, these characters in both clones imitated the characters in each first generation clone. This confirmed that there are genetic effects on the appearances of color abnormality and reversal of sides. However, the color abnormality was affected by environmental conditions because the degree of color abnormality was biased by the degree of the growth. Further the rate of reversal of sides was also affected by environment because the rate of reversal of sides was 20%.

SELECTIVE BREEDING FOR STRESS IN COMMON CARP (*CYPRINUS CARPIO* L.) USING ANDROGENESIS

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Androgenesis can be used to estimate genetic parameters like heritabilities, breeding values and genetic correlations. We investigated the application of androgenesis in a selective breeding program for high and low stress response in common carp, using plasma cortisol responses to a rapid cold shock as selection criterion. Thirty-three androgenetic families (20 fish/family) from a F1 hybrid cross between wild and domesticated carp were reared in separate tanks and cold shocked at age 3 months. The estimated heritability for stress-related plasma cortisol, using Gibbs sampling and an animal model, was 0.68 (90% Highest-Posterior-Density range 0.47-1.00).

Three families with the highest, and three families with the lowest mean cortisol values were selected, mixed and again cold shocked at age 15 months. Estimated breeding values (EBVs) for cortisol were calculated using Gibbs sampling and an animal model. The two highest and two lowest sires were selected and androgenetically reproduced to create homozygous inbred strains. The regression of the mean plasma cortisol concentrations of the clonal progenies on those of the sires was 0.35. In genetic terms, this regression coefficient can be interpreted as an estimate of the heritability in the broad sense.

Next, these four sires were crossed with two homozygous dams (High and Low) to produce eight F1 hybrids. These were communally reared and subjected to a cold shock. Using microsatellites to identify the parents, the data was examined for general and specific combining abilities of sires and dams. Preliminary results indicate the presence of dominance effects on stress-related plasma cortisol levels.

SEX RATIOS IN TILAPIA (*OREOCHROMIS NILOTICUS*): INTERACTIONS BETWEEN GENOTYPE AND TEMPERATURE

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Different genotypes of Nile tilapia were reared at temperatures ranging from 18°C to 38°C for 10 to 30 days at the beginning of the first feeding stage and thereafter at 28°C till fish reached sexual maturity and gonads were inspected. Control groups of each progeny were kept at 28°C throughout the whole testing period. The progeny groups consisted of genetically all-female progeny with reproducible homozygous and heterozygous genotypes derived from six clonal lines and their crosses as well as all-female progeny from XX males of clonal lines crossed with outbred females, all-male progeny from YY males and mixed sex progeny from inter- and intra population single pair matings. Results obtained so far indicate no effect of temperature treatments on homozygous progeny of clonal lines but on progeny of clone crosses. Long exposure to high temperatures produced males in some crosses. Similarly, at high temperatures, some of the all-female progenies derived from XX males of clonal lines showed portions of males of up to 32% compared to all-female progeny under ambient rearing temperature. A significant increase in the proportion of males was observed also in mixed sex progenies of one population and in population crosses reared at high temperatures, but not in all-male progenies. Low temperatures had no significant effect on the expected sex ratios in most of the progenies. Results are considered in the context of sex determining mechanisms.

CLONAL RAINBOW TROUT LINES AS RESOURCES FOR GENETIC ANALYSIS OF COMPLEX TRAITS

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This paper describes the derivation and characteristics of five homozgyous clonal rainbow trout lines produced by androgenesis and gynogenesis at Washington State University. The lines represent diverse genetic backgrounds including three hatchery (Shasta-type) strains, one Alaskan rainbow source and one Idaho steelhead source. Four are YY male lines and one is an XX female line. The sex chromosome types of the lines were confirmed by progeny testing. The lines were characterized based on differences in karyotype and microsatellite alleles. They show notable differences in development rate, age at sexual maturity and a number of meristic values. Differences are also evident in nonspecific cytotoxic cell response and resistance to the myxozoan parasite *Ceratomyxa shasta*. General approaches for using these lines to facilitate genetic mapping and quantitative trait locus analysis are described.

SEX DETERMINATION AND THE FEASIBILITY OF YY-MALE PRODUCTION IN THE VIETNAMESE STRAIN OF *OREOCHROMIS NILOTICUS* (L.)

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Attempts were made to investigate into sex determination and feasibility of producing novel YY-males in the Vietnamese strain of *Oreochromis niloticus* cultured commonly in Vietnam. To determine the underlying variability in sex ratio, 31 progeny groups, derived from single pair matings of normal broodstock were sexed. Of the 3045 progeny sexed, there were 2076 males (68.2%), with the overall sex ratio being significantly different from 1:1 ($\chi^2 = 402.4$, $P<0.001$). Progeny sex ratios ranged widely from 10.2% to 94% male. The data were highly heterogeneous ($P<0.001$) with over 67.7% of crosses producing sex ratios significantly different from 1:1 ($P<0.001$).

Sex reversed females were produced by using Diethylstiboestrol (DES) treated diets. Female percentage in DES-treatments were from 79.2% to 95.8%, significantly different from the control ($P<0.001$). Of the 12 DES-treated females progeny tested, 7 females produced progeny with sex ratios significantly different from 1:1, similar to 3:1 were proposed as XY-females. These sex reversed females were crossed to normal males to produce novel YY-males.

The elucidation of sex determination and the feasibility of producing novel YY-males in the Vietnamese strain of *O. niloticus* are briefly discussed.

GROWTH AND SURVIVAL DURING YOLK-SAC RESORPTION IN BROWN TROUT (*SALMO TRUTTA FARIO* L.): A QUANTITATIVE GENETIC ANALYSIS

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Some components of the response to selection for growth in the PROSPER program on brown trout (+ 30 % in 3 generations) were investigated. An indirect action of selection on yolk sac resorption efficiency was looked for, and we estimated genetic parameters on growth related traits at the end of endogenous feeding. For this purpose, a diallel cross between selected and control fish was designed, including 200 full-sib families (20 males x 10 females), each of them being represented by 4 individually recorded fish. A positive effect of selection was seen on survival and percent weight gain, and a negative one was shown on final dry weight and dry matter content. No effect was seen on final wet weight. A putative interpretation is that selected individuals have a higher development rate, inducing a higher consumption of yolk reserves by the end of the experiment, and therefore a decrease in wet weight, dry weight and dry matter content. Significant sire heritabilities were seen for final wet weight and percent weight gain (h^2_s = 0,26 ± 0,17 and 0,28 ± 0,19 resp.), but not for other traits. For all traits except percent weight gain, maternal effects were strong, and an important part of them was related to the initial weight of eggs. Significant dominance effects were reported for all traits.

USE OF DNA MOLECULAR MARKERS FOR THE IDENTIFICATION OF SPECIES IN CHILEAN SALMONID ELABORATED PRODUCTS

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Chile is at the moment the second major producer of salmon in the world. During the last years there has been steady increase in demand for Chilean value added trout and salmon exports. The commercialization of salmonid elaborated products, where the morphological external characteristics of the marketed species are removed, it raise the challenge of having methods that allow the unequivocal identification of the salmonid species, used in the products marketed in Chile. These methods should have a genetic base that guarantees their reliability. The development of molecular markers associated to the sex in trout rainbow, carried out by our work group has allowed us to obtain a set of DNA polymorphic markers amplified by PCR that allow the genetic identification of salmonid species. These markers have size and restriction polymorphisms that are useful in the identification of rainbow trout, coho salmon and Atlantic salmon species. Fresh, frozen, smoken and canned products of salmon have been obtained in different supermarket of Santiago's city for their analysis. We will discuss about the feasibility of using these markers to authenticate exportation Chilean salmon products.

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TISSUE SPECIFIC TRANSCRIPTION OF MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II IN ATLANTIC SALMON (*SALMO SALAR* L.)

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Major Histocompatibility Complex (MHC) cl. II is a complex of two glycoproteins (α and β) on the surface of antigen presenting cells, where it presents oligopeptides to the T cell Receptor (TcR) on T-helper cells. The recognition of the MHC cl. II complex and oligopeptide by TcR triggers production of different cytokines to control differentiation of B-cells and phagocytes or to activate cytotoxic T-cells.

In the Atlantic salmon MHC cl. II is encoded by several loci, and this results in transcription of multiple MHC cl. II variants. The aim of this study was to characterise the different MHC cl. II loci and their transcription in different tissues. Multiple tissues from one salmon were dissected, mRNA isolated and cDNA synthesised. A 730 bp PCR product, spanning from the splicing site between exon 1 and exon 2 to 3' UTR in MHC cl. II, was amplified from the cDNA. The fragments were ligated into a vector and transformed into competent cells. Plasmids from 20 clones were isolated from each tissue and sequenced, to obtain clonal sequences. The ratio between the different MHC cl. II transcripts in each tissue was calculated and will be linked to their respective promoter sequence.

The results will be presented at the conference.

TRANSFERRING CHROMOSOME MANIPULATION TECHNIQUES TO THE OYSTER AND SCALLOP INDUSTRY IN CHILE

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Chromosome manipulation and other genetic techniques have shown over the past few years a clear potential for the aquaculture industry to increase their commercial gain through new products or improvement of traditional products for the market. In Chile scallop (*Argopecten purpuratus*) and oyster (*Crassostrea gigas*) aquaculture is a growing industry, with good hatchery facilities, where chromosome manipulation could be introduced.

Up to now most of the genetic knowledge is still located at the Universities and Research Institutions and it is rather difficult for reseachers to get the industry aware of its possible use and receive necessary funding to be able to transfer their experience to them.

An adequate training in chromosome manipulation can only be achieved if the "trainer" has practical experience and biological knowledge of the target species, and the "trainee" has the ability to use a routine light microscope, know how to use basic laboratory equipment and manipulate hazardous chemicals. A good training is required to achieve success, because if the trainee is not able to recognize the difference between the obtained manipulated organisms, whether they are diploids (2n), triploids (3n) or tetraploids (4n), the commercial culture will loose their effort.

Since the aquaculture industry in Chile has not yet recognized the potential of Genetics, including chromosome manipulation, the interaction between research groups and commercial growers is not a common practice. To increase the transference of knowledge towards the productive area, Chile is developing programs with government funding and industrial involvement.

EVALUATION OF TRANSGENIC CHINOOK SALMON WITH ENHANCED GROWTH

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In 1994 a research programme was initiated to evaluate the use of gene transfer technology using a licensed (A/F Protein Inc.) all fish gene construct containing the ocean pout antifreeze protein promoter and the growth hormone cDNA from chinook salmon (opAFP-GHc). The potency of this construct is significant and transgenics 5 to 10 fold larger can be obtained.

Microinjection was employed to introduce the opAFP-GHc construct into fertilised chinook salmon eggs. Over 3 years a total of 34922 eggs were injected and 115 (0.33%) F_0 parents were obtained with increased growth ranging from 1.5 to 5 fold. Crosses using these individuals produced 15 fast growing F_1 lines with inheritance ranging from 0.1% to 47.1%. Stable inheritance through to the F_2 and F_3 generations has also been demonstrated.

Before this technology can be applied in aquaculture it is necessary to establish that growth enhanced transgenic salmon exhibit good health and that the quality of the harvested product is not compromised. Flesh quality assessments have shown that fillets with good colour, firmness and appearance can be obtained from growth enhanced chinook salmon. The fat content of the transgenic salmon has also been determined and line dependant differences in the percentage of fat were found.

STUDIES ON HETEROZYGOSITY AND GROWTH IN TRIPLOID PACIFIC OYSTER (*CRASSOSTREA GIGAS*)

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Heterozygosity and growth were studied in Pacific oyster, *Crassostrea gigas* of a normal diploid group (2n), a cytochalasin B induced triploid (3nCB) and a mated triploid group from diploid female x tetraploid male mating (3nDT). The heterozygosity of 3nCB and 3nDT groups was siginificantly higher than that of 2n control group (72% and 40% higher, respectively). These results supported the statement that polyploid induction increases the heterozygosity in the polyploid individuals. The tissue weight of 3nDT group was significantly bigger than that of 3nCB and 2n groups (13.43% and 25.62% bigger, respectively), and 3nCB group was also significantly bigger than 2n group in tissue weight (10.74% bigger)($p=0.029$). This suggested that the growth of triploids increase with the increase of heterozygosity. But no significant correlations were proved between growth and numbers of heterozygous loci, and no significant difference between the growth of homozygotes and heterozygotes at single locus. Also there was no significant difference among tri-allele heterozygotes, bi-allele heterozygotes and homozygotes at locus Ah_2 in 3nDT group.

Three hypothesis—heterozygosity, energy reallocation and polyploid gigantism—were proposed to explain the phenomenon of the increase in body size or fast growing in triploid shellfish. Results of this study and others suggested that neither heterozygosity nor energy reallocation nor polyploid gigantism was the only factor leading to fast growing in triploids. The increase of body size in polyploids may due to the joint function of heterozygosity, energy reallocation and polyploid gigantism. Among the three factors, heterozygosity may play a much more important role than energy reallocation and polyploid gigantism.

ANALYSIS OF MICROSATELLITE LOCI IN WESTERN AUSTRALIAN POPULATIONS OF RAINBOW TROUT, *ONCORHYNCHUS MYKISS*

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A study has started into the genetics of temperature tolerance in Western Australian stocks of rainbow trout. We report here the results of an initial survey of ten variable microsatellite loci in approximately 60 rainbow trout from each of three Western Australian populations: a cohort from the Pemberton hatchery, an alternate year cohort from the Pemberton hatchery, and a naturalised and isolated population in the Serpentine. Large and significant gene frequency differences were observed between these groups which are attributed to genetic drift and reproductive isolation subsequent to stocking of the Serpentine from New Zealand-derived Pemberton stock some 40 years ago. We compare these results with microsatellite studies of other rainbow trout populations. Matings were carried out between and within one of the Pemberton cohorts and the Serpentine fish in order to examine the genetic basis of temperature tolerance. These results are not yet available; thus far we have genotyped 120 offspring from two mass matings, each of 6 female and 6 male Pemberton fish. We will discuss the ability of the 10 microsatellite loci to determine parentage, and to estimate the actual contribution of each spawner.

GENETIC DIFFERENTIATION AMONG HATCHERY AND NATURALLY ORIGINATED SEA TROUTS IN SOUTHERN BALTIC

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Sea trout (*Salmo truta m. trutta*) is an anadromous salmonid species important for fishery in the Baltic Sea. Sea trout returns for spawning to natal rivers (homing). Number of wild smolts migrating to the sea from rivers in Poland decreased from about 1,5 million ca. 70 years ago to below 100 thousand in recent years. This decrease has been caused by deterioration of environment, regulation of river beds and dams construction. Stocking activities has been performed to enhance naturally occurring populations. Over one million smolts are released to Polish rivers each year. Micrôsatellite DNA was used for studies of genetic differentiation between hatchery produced smolts and wild-caught spawners returned to rivers. Polymorphism at 5 loci: *Ssa*197, *Ssa*171, *Ssa*85, *Str*15, *Str*73 was studied.

GENETIC IMPROVEMENT OF RANCHED ATLANTIC SALMON: A POSITIVE INTERACTION BETWEEN AQUACULTURE AND WILD FISHERIES

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The interaction between aquaculture and wild fisheries is almost always cast in a negative light. It need not be so. Concepts, techniques and protocols in common use in aquaculture can be usefully applied in the management and development of "wild" fisheries.

Wild stocks of Atlantic salmon have been in decline throughout the world in recent years. This decline is most noticeable in the multi-sea winter component of the stocks. Irish salmon stocks have suffered a similar, if not so drastic, decline.

In the River Shannon we have used techniques such as selective breeding, all-female production and coded wire tagging to study and redress the effects of the natural decline. As a result, we have significantly increased the proportion of multi-sea-winter fish returning to the ranched fishery and we have increased the length and weight of grilse salmon both in the offshore commercial net fisheries and in the hatchery stock. Our observation are based on a programme involving more than 500,000 micro-tagged and 300,000 tin clipped smolt releases spanning seven separate mass-spawned lines and 3 generations of line breeding.

In this paper we report our results and discuss how they have enabled us to address the problems of "wild" stock management using aquacultural genetic technology. Such positive interaction as this can help to reduce the negative perceptions of some interests regarding salmonid aquaculture.

TRIPLPOID ATLANTIC SALMON GROW BETTER TO SMOLTIFICATION THAN DIPLOIDS: A COMPREHENSIVE EVALUATION AT PILOT SCALE

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Triploidisation of Atlantic salmon has a number of uses: it avoids sexual maturisation in early maturing stock; it prevents inadvertent genetic interaction with wild stock arising from accidental release of escapes; and it can be used to "lock in" (or gains) in transgenic fish.

Published evaluations of the performance of triploid Atlantic salmon have rarely been scientifically or commercially satisfactory, often lacking proper replication, repitition and controls. The procedure therefore has been slow to be taken up by the industry. In this paper we report the performance and growth in freshwater to smolt stage of cohorts of Atlantic salmon raised from a single batch of eggs. Forty thousand eggs were divided into two aliquots and fertilized to give 20000 all-females (sex inverted XX sire) and 20000 mixed sex (normal XY sire) groups. Each of these was sub-divided to give a diploid control cohort and a cohort which was triploidised by hydrostatic pressure. Each cohort 2N mixed set, 3N mixed sex, 2N all female and 3N all female was sub divided into three separate tanks and ongrown, with periodic sampling, to smolt stage (12 tanks in total).

We show that growth is greater in 3N than in 2N cohorts. We repeated the trial the following year with the same result. We show that 3N growth is better than 2N whether they are grown separately or grown together in the same tank from first feeding. Therefore we have confirmed the superior growth of triploid salmon over their diploid siblings up to smolt stage.

Finally, all cohorts were micro-tagged and released to sea. We report their subsequent recapture in coastal net fisheries and at the hatchery on and two years after release to the wild.

INTERNATIONAL COLLABORATION ON GENETIC MAPPING OF THE BLACK TIGER SHRIMP, *PENAEUS MONODON*: PROGRESS UPDATE

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The black tiger shrimp *P. monodon* is the most important shrimp aquaculture species in South East Asia and Australia. Until now, this US $3 billion industry has relied solely on wild caught broodstock. Domestication of this species and selection of genetically superior animals has been a research priority for a number of years. To enable prawn farmers to take advantage of genetic marker technology as soon as the life cycle of *P. monodon* is reliably closed and genetic selection programs commence, we are coordinating an international effort to develop a linkage map of the *P. monodon* genome.

Type II AFLP (Amplified Fragment Length Polymorphism) markers were generated from four full-sib families with three-generation pedigree information. Linkage analysis has been performed for male specific and female specific markers separately within each family from a total of 19 different AFLP primer pairs, or approximately 190 segregating AFLP markers in each family. Unfortunately, only a small proportion of these markers appear to be common across all families. Hence, in order to combine information from different families to make a consensus map, eleven Type I microsatellite markers are being used to genotype the progeny across all families. The common AFLP markers and microsatellites will provide links to identify the homologous linkage groups between maps. Additional Type I markers, e.g. EPIC (exon-primed intron-crossing) markers will also be mapped across the families as they become available. Progress towards construction of an integrated map will be reported.

Updated information can also be obtained at:

http://www.aims.gov.au/shrimpmap/
http://www. shrimpmap.tag.csiro.au

HERITABILITY ESTIMATIONS FOR JUVENILE GROWTH AND DISEASE RESISTANCE IN CHANNEL CATFISH: INTEGRATION OF MOLECULAR MARKERS AND QUANTITATIVE GENETICS

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Adult channel catfish were stocked into earthen ponds prior to the 1999 spring spawning season and allowed to mate at random. A total of 110 full and half-sib families were selected from four ponds where the broodfish spawned at a high frequency. Tissue samples were taken from all broodfish and offspring parentage of individual spawns was determined by analysis of microsatellite allele inheritance. Juvenile channel catfish were stocked into 800-liter fiberglass tanks and fed a commercial diet to satiation daily. Fish were weighed every 30-days and growth rate, feed conversion, and feed consumption determined from approximately 50 to 110 days of age. Mean family specific growth rates averaged 1.68%/day and ranged from 0.86% to 2.86%/day. Juvenile catfish were also exposed to virulent *Edwardsiella ictaluri* bacteria, the causative agent of enteric septicemia, in aquaria and mean survival calculated 21-days post-exposure. Overall mean family survival was 65.7% and ranged from 6.7% to 100.0%. A genetic linkage map has been constructed with 259 microsatellite loci placed in 34 linkage groups. The linkage map spans 2000 centimorgans of the channel catfish genome, with an average intermarker distance of 9.3 centimorgans. Juveniles will be genotyped with microsatellite markers scanning the genome to correlate phenotype with allele inheritance for identification of important quantitative trait loci. Juvenile catfish from all families will be individually tagged and communally stocked into replicate 0.1-hectare ponds to evaluate feed consumption, growth to marketable size and processing characteristics. Phenotypic and genetic parameters for traits will be estimated from an animal model.

APPLICATION OF SSU RRNA SEQUENCE ANALYSIS TO THE IDENTIFICATION AND DETECTION OF THE AGENT OF AMOEBIC GILL DISEASE IN SEA-CAGED ATLANTIC SALMON (*SALMO SALAR*)

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The genus *Paramoeba* comprises a number of marine amoebic species characterised by the presence of DNA-positive parasomal inclusions located adjacent to the nucleus. *Paramoeba* sp. was identified as the aetiological agent of amoebic gill disease (AGD), which causes significant mortalities of sea-reared Atlantic salmon (*Salmo salar*) farmed in Tasmania, Australia. Outbreaks of AGD caused by immunologically cross-reactive *Paramoeba* isolates have also been reported in farmed salmonids in Ireland, France, USA, and New Zealand. The species *Paramoeba pemaquidensis* was implicated in AGD affecting sea-caged coho salmon (*Oncorhynchus kisutch*) on the west coast of the USA. Complete SSU rRNA gene sequences were respectively determined for *Paramoeba* sp. isolated from infected gills of salmon from Australia (5 isolates) and Ireland (1 isolate), and 4 *P. pemaquidensis* isolates originating from the USA and United Kingdom, including 3 free-living representatives. Alignments over 2104 basepairs of the respective rRNA genes revealed 98-99% sequence similarities among these isolates, suggesting that *Paramoeba* sp. and *P. pemaquidensis* implicated in AGD in several geographically distant countries are homogenous and monophyletic. Results also supported previous findings that *P. pemaquidensis* exists as a widely distributed, free-living marine organism. These *Paramoeba* were phylogenetically unrelated to other amoebae of the class *Gymnamoebia*. Sequence analyses also included other *Paramoeba* species *P. eilhardi* and *P. aestuarina*, and the morphologically similar species *Pseudoparamoeba pagei* to determine relationships within the genus. Development of PCR primers based on unique regions of the SSU rRNA for the specific detection of the AGD pathogen and their application are described.

MITOCHONDRIAL DNA INHERITANCE AND HYBRIDIZATION IN *MYTILUS* SPP.

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The *Mytilus edulis* species complex comprises three commercially important marine mussel species (*M. edulis, M. galloprovincialis and M. trossulus*), with a worldwide distribution. Hybridization between these species occurs to varying degrees in areas of overlap. These mussels also exhibit an unusual mode of mitochondrial DNA (mtDNA) inheritance termed Doubly Uniparental Inheritance (DUI). Females are homoplasmic for the "F" mtDNA type which is transmitted maternally, whereas males are heteroplasmic for this and the paternally transmitted "M" type. Field observations and laboratory experiments suggest that there can be a breakdown of DUI in certain hybrid crosses. In order to investigate this, controlled laboratory crosses have been carried out to produce pure species and hybrid larvae of known parentage from all possible crosses between the three species. Existing and newly developed molecular markers are used to follow the fate of the "F" and "M" mtDNA types through larval development. Results are presented which quantify the resilience of DUI to hybridization. This may be an important factor in the maintenance of genetic integrity for each species across hybrid zones.

INCREASED CELL-SIZE AS A CAUSE FOR POLYPLOID GIGANTISM IN TRIPLOID ZHIKONG SCALLOP, *CHLAMYS FARRERI*

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Increased body size or growth is a general feature for triploid molluscs. It has been observed in almost all molluscs studied so far. The increased body size in triploid molluscs has been considered as an expression of polyploid gigantism. There have been three leading hypotheses attributing polyploid gigantism to energy relocation (from reproduction to growth), increased heterozygosity and cell-size, respectively. We introduced triploid zhikong scallop, *Chlamys farreri*, by inhibiting polar body 1and studied their basic metabolism and cell-size in relation to organ size. The adduct muscle of triploids was 44.28% larger than that of diploids. Triploids showed higher oxygen consumption (0.0262 vs. 0.0195ml/g/hr) and lower NH3-N secretion (2.0437 vs.2.1619mg/g/hr) than diploid at 17°C, the optimal temperature for growth, but the differences were not statistically significant. This result indicates that triploids are not different from diploids in basic metabolism, and polyploid gigantism is probably not caused by metabolic differences. Sections of adduct muscle from triploid and diploid scallops were analyzed with a hematoxylin-eosin stain. Cell-size was estimated by counting the number of nuclei in a given area. Triploids showed significantly larger cells in the adductive muscle than diploids in both horizontal and vertical sections ($p < 0.001$). This result provides the first evidence of increased size of somatic cells in triploid scallops and supports the cell-size hypothesis. The increased cell-size is probably not compensated by a reduction in cell number and therefore, produces polyploid gigantism in the adductor muscle.

INVESTIGATION OF GENETIC VARIATION IN POPULATIONS OF TWO SPECIES OF BLOODY CLAM, *SCAPHARCA BROUGHTONII* AND *TEBILARCA GRANOSA*

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Scapharca broughtonii and *Tegilarca granosa* are two species of bloody clam of commercial importance along coastal waters in China. Based on 22~27 loci genetic variation were investigated in four populations (three from China, one from Korea) of *S. broughtonii* and three populations of *T. granosa*, using horizontal starch gel electrophoresis.

The percentage of polymorphic loci (P.95) ranged from 36.36% to 50.00% in *S. broughtonii*, and were 22.22% (Rongcheng, RT), 25.93% (Qingdao, QT) and 33.33% (Wenchou, WT) respectively in *T. granosa*; The observed heterozygosity per locus were 0.087±0.024 (Qinghuangdao, QH), 0.123±0.038 (Dalian, DL), 0.105±0.023 (Qingdao, QD) and 0.091±0.031 (Pusan, Korea, PK) respectively in *S. broughtonii*, and 0.062±0.031, 0.068±0.026 and 0.097±0.034 in their given order in *T. granosa*. The mean effective number of alleles and expected heterozygosity per locus were also calculated.

Relatively, three Chinese populations of *S. broughtonii* shared more genetic similarity or gene flow than they did with Korea population based on comparison of allele frequencies at loci. Morphological differences between individuals from China and ones from Korea may bore on genetic difference to some extent. Similarly, higher level of gene flow existed between the two northern populations (RT and QT) than that between northern populations and the southern population (WT) in *T. granosa*. Each sample in two species exhibited an overall mean heterozygote deficiency at polymorphic loci.

SEX IDENTIFICATION BY MALE-SPECIFIC GROWTH HORMONE PSEUDOGENE (GH-Ψ) IN *ONCORHYNCHUS MASOU* COMPLEX AND A RELATED HYBRID

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Sex identification is important in aquaculture such as in the production of monosex populations. We have identified genetic sexes by PCR-based male-specificity of growth hormone pseudogene (GH-Ψ) in masu (n=131) and Biwa salmon (n=122), two of the three subspecies of the Oncorhynchus masou complex, and their hybrid Honmasu (n=68). PCR analyses with primers designed from sequences of chinook salmon GH genes amplified GH-I and GH-II fragments in both sexes, but a third GH-Ψ fragment was detected in males. The reliability of sex identification by this GH-Ψ for masu salmon, Biwa salmon and Honmasu was 93.1%, 96.7% and 94%, respectively. The remaining individuals showed deviation from sex-specificity: a few phenotypic males were absent of the third fragment, whereas a few phenotypic females showed the third fragment. Sequences of the third fragment from such females were identical to those from males of the same subspecies which shared about 95% homology with the corresponding GH-Ψ fragment of chinook salmon. This proved that these females were really GH-Ψ-bearing individuals. PCR analyses with primers designed from sequence of Biwa salmon GH-Ψ gave rise to absolutely the same results, which indicated that the absence of GH-Ψ in the phenotypic males were not resulted from the unsuitability of the previous primers. These GH-Ψ-bearing females and GH-Ψ-absent males were more likely to originate from spontaneous sex reversion than from crossing-over between GH-Ψ and the sex determination gene/region.

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