BARD Workshop: Aquaculture Genetics – status and prospects
February 20-23, 2006 – Eilat, Israel

ABSTRACTS

What history may tell us about the future of aquaculture genetics

William K. Hershberger

Director, USDA/ARS, National Center for Cool and Cold Water Aquaculture, Leetown, WV 25430, USA

BHershberger@ncccwa.ars.usda.gov

Historically, genetics has not been a major part of aquatic sciences education or practices. However, it is interesting to note that one of the first animals used for genetic research after rediscovery of Mendel's results was a Poecileid, i.e., the guppy. Furthermore, the use of genetic principles and practices for improvement of aquatic species raised under controlled or semi-controlled conditions has not become a standard component of aquaculture production, contrary to the situation with other agricultural plants and animals. A number of explanations for the lack of utilization of otherwise accepted genetic approaches will be explored. In addition to the seeming reticence to employ the standard genetic approaches in aquaculture, realization of the purported promise of some of the more recently developed molecular genetic tools has been slower than anticipated. In addition to the difficulties experienced with deployment of this technology, the costs and the need for larger multidisciplinary teams to develop the tools for reliable analyses have raised challenges not previously experienced in the field. These factors have led to increased efforts to utilize cooperative approaches to major genetic problems that need to be solved. It would appear that future employment of modern genetic analytical tools to improve aquaculture production will be enhanced and, in fact, further assured via cooperative research ventures.

State of the art in selective breeding of aquaculture species

Bjarne Gjerde

AKVAFORSK, Institute of Aquaculture Research, P.O. Box 5010, N-1432 Ås, Norway

bjarne.gjerde@akvaforsk.no

The first selective breeding programs for aquaculture species that also used sib information in the selection decisions were established in the early 1970's for Atlantic salmon and rainbow trout. Presently there are a total of about 60 such programs for about 20 different species in the world. However, still less than 5% of the world aquaculture production is from genetically improved stocks and most of these programs practise selection for a narrow breeding objective (e.g. growth only). An exception is Atlantic salmon for which close to 100% of the production is from improved stocks and selection is practised for a much broad breeding objective including as many as 6-10 different traits (growth, sexual maturity, disease resistance, carcass quality, deformities) in some of the programs. A sustainable selective breeding program needs reliable genetic parameters and economic values for all traits of economic and strategies importance, not only for the traits actually selected for. In published literature there are in particular few reliable estimates of genetic correlations among the different types of traits, and for economic important species no objective study has been performed on the derivation of economic values. Studies on the design of cost effective simple (mass selection) and advanced (use of also sib information) nucleus selective breeding programs for aquaculture species are few and more should be undertaken that take into account the high fecundity and reproductive characteristics of the species and being performed at a predefined and acceptable rate of inbreeding. Important are studies on the effects of new selection algorithms and mating design, new and emerging technologies like DNA-markers for both parental assignment and marker assisted selection, new technologies that can record more of the traits selected for on the live breeding candidates, of low but significant genotype by environment interaction for traits in a competitive market for genetic material, and studies on procedures to obtain unbiased estimates of genetic changes. At the multiplier level additional studies are needed on selection and mating strategies to fully capitalize both on the additive and non-additive genetic effects and how additional strategies like ploidy and sex manipulation may be used to further increase the productivity of the commercial fry. A socio-economic challenge is the need for some form of legal or biological protection measures to assure a fair share of the revenues from genetic improvement to investors and for further research and development of the programs, while at the same time having access to genetic resources for further development of the programs.

Genetics and genomics - integration of breeding and molecular genetics programs

Jeffrey T. Silverstein*, Gregory M. Weber, Caird E. Rexroad III, and Roger L. Vallejo

USDA/ARS, National Center for Cool and Cold Water Aquaculture,Leetown, WV 25430, USA

* jsilvers@ncccwa.ars.usda.gov

At the National Center for Cool and Cold Water Aquaculture (US Department of Agriculture, Ag. Research Service) in Leetown, WV, we have a rainbow trout broodstock development program now entering the 2nd generation of family based selective breeding using expected breeding values (EBVs). Our major breeding objectives are faster growth and resistance to *Flavobacterium psychrophilum*, the causative agent of bacterial coldwater disease. For these traits we have developed assays to evaluate phenotypic performance. In addition to our breeding program, our molecular genetics team, in a worldwide collaboration, is developing microsatellite markers linkage maps with the intent of identifying QTL's and using them in marker or gene assisted selection (MAS or GAS). There are several possible approaches to take with regard to the types and numbers of markers to develop, the strategies for using molecular information, and methods for employing the markers in a selective breeding program. This paper describes the choices we have made concerning QTL identification for traits of high, low and unknown degrees of heritability. These traits are cortisol response to stress ($h^2 \approx 0.4$), feed intake ($h^2 \approx 0.1$) and resistance to *Flavobacterium psychrophilum* (h^2 not yet determined). In order to identify QTLs in a relevant commercially important rainbow trout line we are making crosses from within our resource population. The development of breeding/research family crosses, choice of markers for genome scanning, and planned steps to implementation of these results are described.

Practical genetics in Israeli mariculture: history, present status and prospects

Sergei Gorshkov

Department of Genetics and Physiology, National Center for Mariculture,
Israel Oceanographic & Limnological Research Ltd., P.O. Box 1212, Eilat 88112, Israel

gorshkov_s@ocean.org.il

The aim of this review is to show the progress and achievements made to date in selective breeding of marine cultured fish in Israel. The National Center for Mariculture (NCM) was the first scientific organization which determined that a long-term selective breeding program is the key strategy for genetic improvement of commercially important marine fish. The main objective of the program was (and still is) the development of genetically improved strains of commercially important marine fish, and the immediate practical goal was to improve the profitability of national mariculture. The growth improvement in the gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*) was (and still is) considered to be the most important objective. Due to specific reproductive constraints in sea bream, mass selection was found to be an effective practical tool for improving culture performance. Today, the commercial selective breeding program for sea bream consists of several different lines, mass selection for growth conducted in industrial sea cages and terminal crossbreeds for sales. Up to four generations or cycles of mass selection have been completed for some lines. A method for genetic protection of improved sea bream strains from unlicensed reproduction using a recessive deleterious Mendelian mutation (named "ebony") was developed. Interspecific hybridization, chromosome set manipulations, cytogenetic and sex control techniques were also applied; some of these are shown to be promising tools for short-term genetic improvements. In general, the NCM selective breeding program for sea bream showed that genetic gain for growth and faster economic return can be achieved within "reasonable" time spans (10-12 years). The commercial selective breeding program is under the supervision of the Genetics and Physiology Department at the NCM. Presently, most of the Israeli commercial mariculture is using genetically improved strains of sea bream and new domesticated strains of sea bass.

Lessons for aquaculture breeding from livestock

Joel I. Weller

Department of Cattle and Genetics, Institute of Animal Sciences, Agricultural Research Organization, The Volcani Center, Bet Dagan 50205, Israel

weller@agri.huji.ac.il

Investment in breeding is unique because genetic gains are eternal and cumulative. They are never "used up", and never "wear out". The rate of genetic gain for milk production in dairy cattle has been about 1% per year for the last 20 years, and the economic value of each gain is cumulative on all previous gains. However, nearly all of the gain is transferred to the national economy. Very little stays with farmers or commercial breeders.

After initial start-up costs, annual costs will tend to be constant. The main cost elements in traditional breeding programs are data recording (useful to farmers for herd management), keeping nonproductive animals (males) for future breeding, progeny testing of candidate males and data analysis. Generally, in traditional breeding programs, total direct costs are small relative to the value of genetic gain. Once a genetic gain is obtained, it is never lost, but its value must be discounted in future years. With a discount rate of 0.08, a profit horizon of 20 years, first returns after 5 years, nominal annual genetic gain of $10/animal; total returns to profit horizon will be $325.8/animal. Unlike genetic gains, costs are not cumulative. Using the same parameter values at the "break even point" (total costs equal to total gains) nominal annual genetic gain will equal 0.3 of nominal annual costs. Thus a breeding program is profitable even if annual costs are three-fold the nominal value of the annual genetic gain!

Compared to breeding programs for other species, livestock breeding programs are limited by the facts that the generation interval is long, and each animal is very costly; most traits of interest (milk production) can only be measured on females; female fertility is very limited, while male fertility is nearly unlimited; and nearly all economic traits are quantitative, and heritability of most traits is low. Most current breeding programs for dairy cattle are based on the progeny test design. Since the 1950's rates of genetic gain have increased due to better pedigree information, more traits recorded and more accurate recording, and better statistical methods (BLUP, animal model, test day model).

The genetic gain possible by trait-based selection is limited, because only additive genetic variance is utilized, dominance and epistasis are not. Selection is based only on animals that express the economic traits or their relatives. Trait-based selection is inefficient for low heritability traits or for traits with negative genetic correlations, and is not useful for crossbreeding where objective is to obtain heterosis.

From the beginning of modern breeding programs selection in dairy cattle focused on milk production. From 1985 breeding goals moved towards improving protein yield. The Scandinavian countries selected for production together with health and fertility, while the North American countries selected for conformation together with production. In recent years, selection objectives

were broadened to include “functional herdlife”, fertility, and health traits. The main reasons behind this shift were quotas and/or price constraints, and increasing concerns associated with the deterioration of the health and fertility of dairy cows.

Modern technology complements traditional breeding, but does not replace it. To date nearly all progress in animal breeding has been obtained by traditional trait-based methodology. There is no substitute for accurate data and pedigree recording.

A review of ploidy manipulations in aquaculture: The Israeli experience

Shmuel Rothbard

YAFIT R&D Laboratory, Fish Breeding Center, Gan Shmuel 38810, Israel

rot_bard@gan.org.il

The technology of ploidy manipulation was introduced to Israel at the beginning of 80's (last century), about 20 years ago. The Israeli fish centers conducted intensive research in this field during these two decades, to improve existing methods. The chromosome-set technology was adapted for 10 fish species and varieties, including two marine fish. Presently, though the methodology achievements are remarkable, practical implementation was possible only for the common carp. In this economically important species, extensively cultured in Israel, the aim was to integrate gynogenesis and androgenic sex-reversal to generate neomales with special genetic traits. Carp neomales are capable to serve as parents for production of fast-growing all-female population. This paper describes ploidy manipulation progress and activities as has been carried out in four Israeli fish centers. Nevertheless, there are some objectives that should be considered for future prospects: (a) Implementation of the existing know-how to produce tilapia YY-males as broodfish for production tilapia male-monosex; (b) Dissemination and promotion of exportation to foreign markets of the advantageous female triploid grass carp and black carp; (c) Continuation of genetic work with marine fish to develop breeds that could be advantageous for mariculture; (d) Reconsideration of usage ploidy-manipulated koi as a genetic tool that can be utilized to facilitate gene-mapping in carps.

Practical use of cytogenetics in fish biology and aquaculture

Galina Gorshkova

National Center for Mariculture, Israel Oceanographic & Limnological Research Ltd.
P.O. Box 1212, Eilat 88112, Israel

gorshkov_g@ocean.org.il

The genetic mechanisms responsible for speciation, natural selection and the dynamics of wild populations of the Pacific salmon were studied. In many salmonid species, intraspecific chromosomal polymorphism was found to be a widespread genetic phenomenon that affects survival of different generations and thus the dynamics and abundance of these populations. Our recent cytogenetic studies were done with commercially important fish and included research on taxonomy, interspecific hybridization, chromosome set manipulations, chromosomal disorders and sex control. Cytological analyses performed on imported sturgeon stocks provided reliable species-specific identification and we made recommendations concerning conservation of these stocks. Subsequently, the use of combined cytometric and karyological techniques for genetic management of cultured sturgeon stocks was proposed. We did karyotyping of two endemic Barbinae (Cyprinidae) species inhabiting the Lake Kinneret basin. This group of fish has a polyploid origin and a complicated taxonomy. Therefore, new karyological data can help for a better understanding of the taxonomy and phylogeny of these fish. The genetic laboratory at NCM was the first to develop practical techniques for chromosome set manipulation of sea bream and sea bass. As a result, triploid and gynogenetic forms were mass-produced and tested for culture performance. For verification of ploidy levels in chromosomally manipulated forms, we used karyological analyses of embryos or fry and cytometrical analyses of erythrocytes on fingerlings. Karyological tests were carried out on females and males of different sea bass strains and revealed sex-associated differences in their karyotypes. Cytological examination was also applied to study the chromosomal aberrations in early embryogenesis. In the white grouper, we found that chromosomal disorders could adversely affect embryonic survival. These findings make possible a survival prognosis of larvae belonging to different progenies. Karyological variation found in cultured strains of sea bream was suggested as attributing to species-specific chromosomal polymorphism. In the future, we hope to be able to identify cytogenetic differences responsible for the important phenotypic mutations (named “ebony” and “yellow”) found in local cultured strains of sea bream. We are collaborating with other fish genetics laboratories (EC) on a major initiative to map the sea bream genome. Detailed chromosomal maps will allow researchers to identify genetic variation in commercially important traits such as growth, survival and disease resistance.

Use of molecular tools for research and improvement of aquaculture stocks

Eric M. Hallerman

Department of Fisheries and Wildlife Sciences, Virginia Polytechnic Institute and State University, Blacksburg, VA 24061-0321, USA

ehallerm@vt.edu

Development of molecular genetic markers provides aquaculture with tools for a number of research and practical applications. Genetic marking of experimental groups allows their evaluation in the same rearing units, increasing statistical power within limited research infrastructure. Parentage can be inferred for individuals in mixed-progeny groups, quantifying the contributions of individual parents, and supporting the estimation of sire and dam effects. Building upon parentage assignment, walk-back selection entails retention of the best members of each family as broodstock for the next generation. Molecular markers can be used to detect the segregation of quantitative trait loci, and knowledge of such linkages can be used for marker-assisted selection. Purposeful genetic marking can be used to identify proprietary stocks, marketed products, and fish out-planted or escaping into natural ecosystems. Although each application has been demonstrated, genetic markers are not routinely utilized in commercial aquaculture. Limited practical application can be explained by the limited development of broodstocks for most aquaculture species, the small size and limited scope of most aquaculture operations, and the costs of genetic screening.

Transgenic fish – where we are and where do we go?

Rex Dunham

Department of Fisheries and Allied Aquacultures, Auburn University, Auburn, Alabama 36849, USA

dunhara@auburn.edu

Transgenic fish and recently shellfish have been produced that exhibit accelerated growth rates, increased disease resistance, altered body shape and composition, altered coloration, expression of anti-freeze proteins, are bioreactors producing medical compounds and potential sterility. Several species have been studied, and common carp in China, tilapia in Cuba, salmon and trout in the US are nearing government approval and commercialization as food fish. Transgenic ornamental fish have already been commercialized in the US and Asia.

Transgenesis appears to have the potential to enhance growth and disease resistance to a greater extent than other genetic enhancement programs; however, there appears to be a highly variable response by species with species having relatively slow growth benefiting the most. Maximum attainable size was quadrupled in mud loach by introducing growth hormone genes. However, little data is available in experimental units that simulate commercial aquaculture conditions. Initial data indicates that the ultimate aquaculture genotypes will likely result from utilizing a variety of genetic enhancement programs.

Growth enhanced transgenic fish have altered body composition and muscle structure. Analysis of these changes, actual and theoretical, indicates that there are no adverse changes in regards to food safety, and in fact, in some cases desirable changes in flesh quality. The primary danger revolves around the introduction of proteins that would cause allergic reactions to transgenic fish flesh, but no such gene transfers have been accomplished. Based on these analyses, it appears that the concerns about transgenic fish flesh for human consumption are decreasing in the international community.

The primary impediment to commercialization of transgenic fish is the concern about potential environmental impacts. Fitness traits examined to date in aquaculture species of transgenic fish indicate a lowered fitness of these fish, although more data is needed. However, despite this data, government approval of various transgenic fish will be slow, and this is the impetus for the current research on transgenic sterilization, which if successful, would eliminate almost all potential environmental impact. Constructs have been studied that have shown promise to disrupt embryonic development or gamete maturation in fish. Triploidy is also a potential method to sterilize production animals of transgenic fish of some species, but still requires fertile brood fish and the polyploidy can reduce the extent of the transgenic enhancement.

During the last decade extensive research has been accomplished in fish genomics. Integration of this vast genetic information has the potential to greatly impact transgenic research and development in the future.

Production of recombinant hormones and growth factors for use in aquaculture

Bruria Funkenstein

National Institute of Oceanography, Israel oceanographic & Limnological Research Institute, Tel-Shikmona, P.O. Box 8030, Haifa 31080, Israel

bruria@ocean.org.il

Recent progress in recombinant DNA technology has provided a valuable means for the production of large quantities of fish hormones and growth factors that otherwise required the sacrifice of enormous numbers of fish for obtaining only small amounts of the native protein. Fish hormones and growth factors produced by recombinant DNA technology for potential use in aquaculture include growth hormone (GH), insulin-like growth factor I (IGF-I), insulin-like growth factor II (IGF-II), the gonadotropin follicle-stimulating hormone (FSH, formerly known as GtH I) and luteinizing hormone (LH, formerly known as GtH II), prolactin, somatolactin, parathyroid hormone related protein (PTHRP), myostatin and others. These hormones and growth factors were produced from a large variety of fish species currently used in the aquaculture or mariculture industry. Among these are the common carp, Indian major carp, tilapia, the gilthead sea bream, red sea bream, tuna, barramundi, coho salmon, rainbow trout, turbot and eel.

Production of the recombinant proteins in general involves cloning of the cDNAs coding for the desired peptides in appropriate expression vectors. The choice of vectors depends on the expression system employed (bacterial, insect, yeast or mammalian cells). An important consideration in the choice of vector and system depends on specific characteristics of the peptide in question; whether it requires post-translation modifications such as glycosilation, the formation of homo- or heterodimer for biological activity.

Potential uses of recombinant hormones and growth factors in aquaculture can vary from enhancing growth rate (GH) to developing quantitative assays such as radioimmunoassay (RIA) and enzyme linked-immunosorbent assay (ELISA) for determination of plasma and tissue levels of the specific peptides under various physiological conditions such as stress, reproduction, adaptation to salinity, nutritional state, etc.

Factors that should be considered when producing and employing recombinant hormones: cost effective methods of production, efficient methods for renaturation (folding) of the recombinant peptides, appropriate assays to determine their biological activity, extending their half-life, methods of delivery.

Genomics and genetics: Molecular variation, bioinformatics and functional genomics, implementation into the NCCCWA breeding program

Caird E. Rexroad* III, Yniv Palti, Roger Vallejo, and Jeffrey T. Silverstein

USDA/ARS National Center for Cool and Cold Water Aquaculture Leetown, WV 25430, USA

* CRexroad@ncccwa.ars.usda.gov

The USDA/ARS National Center for Cool and Cold Water Aquaculture is working to integrate molecular genetic technologies into a selective breeding program aimed at the genetic improvement of rainbow trout for aquaculture production efficiency. To date, much of our efforts have focused on the development of a diverse arsenal of genome tools and reagents which will be required to undertake this task. This includes the : 1) characterization of microsatellite genetic markers for genetic mapping, linkage disequilibrium mapping, and population genetic studies; 2) identification of expressed sequence tags to support candidate gene approaches and high-throughput functional genomics; and 3) construction of bacterial artificial chromosome libraries to conduct physical mapping, fine mapping, integration of cytogenetic and genetic maps, and large scale sequencing projects. The addition of these resources enhances our research programs and facilitates the development of new selective breeding strategies.

Genetic mapping approaches traditionally target traits which are expensive or difficult to measure or require sacrificing fish. Loci influencing natural killer cell-like activity, temperature tolerance, spawning date, body weight, resistance to infectious pancreatic necrosis virus (IPNV), embryonic development rate, and albinism have been identified in rainbow trout. We have elected to target three traits due to their relevance to aquaculture production: stress tolerance (crowding stress measured by cortisol response), resistance to the bacterial pathogen *Flavobacterium psychrophilum*, and feed efficiency. Our approach includes the combination of genomics with traditional breeding strategies to identify and characterize loci (and eventually genes) responsible for genetic variation in NCCCWA broodstock. Identification of such loci will allow for use of marker/gene assisted selection in a multi-trait selection program where population genetic parameters are evaluated to achieve a balance of maximizing genetic gains without sacrificing genetic diversity to the point of negative impact.

Employing molecular genetics in selective breeding programs enables the accumulation of vast amounts of molecular, phenotypic, and pedigree data for a broodstock population. Integration of these data into a meaningful breeding strategy is an essential task which requires the development of specialized bioinformatic tools. New molecular genetic and bioinformatic technologies must constantly be evaluated for their potential in increasing efficiency of selective breeding.

Informatic analysis of genomic resources for comparative genomics in catfish

Zhanjiang Liu*, Huseyin Kucuktas, Ping Li, Puttharat Baoprasertkul, Peng Xu, Eric Peatman, Shaolin Wang, Benjaporn Somridhivej, Jason Abernathy, Wonkyo Lee, and Samiran Nandi

Department of Fisheries and Allied Aquacultures and Program of Cell and Molecular Biosciences, Aquatic Genomics Unit, Auburn University, Auburn, Alabama 36849, USA

* liuzhan@auburn.edu

The completion of the Human Genome Project marks the start of the post-genomics era. However, with aquaculture species, genome research is still at the early stages of structural genomics. Recent rapid progress has laid a foundation for exponential growth and development of genome resources and information in aquatic species. Scientists are mandated to make rapid transitions between studies on a single gene to thousands of genes, from development of a few molecular markers to markers with genome coverage, from Northern blot or RT-PCR to high-density microarrays, and from working with clones to entire genome mapping. Such advances have led to the emergence of informatic sciences which in turn will be used to dissect genes and their functions, not only within a species, but also across species. The power of comparative genomics has yet to be realized, which will provide many clues about evolution, and facilitate functional studies. Progress made in the last five years in catfish genomics will be reviewed in the areas of molecular marker development, construction of framework linkage maps, development of genomic resources, and analysis of genomic organization and composition. Results of informatic analysis of genomic resources such as expressed sequence tags (ESTs) and BAC end sequences (BES) will be presented in relation to comparative genomics.

Mapping genes and QTLs in aquaculture species

Gary H. Thorgaard[a,*], Krista M. Nichols[b], and Ruth B. Phillips[c]

[a] *School of Biological Sciences and Center for Reproductive Biology, Washington State University, Pullman, WA 99164-4236, USA*

[b] *Departments of Biological Sciences & Forestry and Natural Resources, Purdue University, West Lafayette, IN 47907, USA*

[c] *Department of Biological Sciences and Center for Reproductive Biology, 14204 NE Salmon Creek Ave., Washington State University, Vancouver, WA 98686-9600, USA*

* thorglab@wsu.edu

Mapping genes and quantitative trait loci (QTLs) is of both fundamental and applied interest in aquaculture species. Such studies can provide fundamental information about rates and patterns of evolutionary change and may provide tools which can be used in marker-assisted selection. More detailed information is available on genetic maps and DNA sequences in some non-aquaculture species (e.g., fugu,, zebrafish, medaka) than in aquaculture species and there is interest in being able to extrapolate from those species to the aquaculture species. Information on the rate of change in the order of genes on chromosomes across fish species is only just beginning to become available. Gene order should largely be conserved within species and studies on map order within a species, with a few exceptions, can be extrapolated to other populations. The degree to which QTLs are conserved among populations within species and among species is only now beginning to be studied. Ideally, it would be desirable to be able to extrapolate information about QTL locations from experimental populations to breeding populations, but our ability to do that successfully remains to be determined.

Aspects of comparative genomics in the gilthead sea bream, *Sparus aurata*

Georgios Kotoulas* and the BRIDGE-MAP Consortium

Hellenic Centre for Marine Research, Heraklion, Crete, Greece

* kotoulas@imbc.gr

Comparative genomics is increasingly applied for studying vertebrate genome evolution, by comparing conservation of sequences, syntenies and gene orders. It may establish links between conservation of structural and functional information across species and facilitate finding genes in evolutionary conserved genome regions.

For organisms where the complete genome is available, comparative approaches have been very efficient. Nevertheless, more species amenable to such kind of analysis are needed to serve as operational stepping stones between genomes. To this end, economically important fish species are priority candidates due to their societal interest, and to considerable information produced on different aspects of their husbandry, physiology, biology and pathology. However for these species, comparative genome studies become difficult as there is a huge lack of molecular tools.

Here are presented the results of the European project BRIDGE-MAP, which brings on the gilthead sea bream (*Sparus aurata*) a key species for the Mediterranean Aquaculture. The gilthead sea bream belongs to the species rich family Sparidae, within the order Perciformes, that also comprises Fugu rubripes and teatraodon nigroviridis that have fully sequenced genomes.

The resources and information produced comprises a linkage map based on about 200 markers, a radiation hybrid panel that allows easy mapping for thousands of markers and genes, a radiation hybrid map comprising about 1000 molecular markers and genes, cDNA libraries, Expressed Sequence Tags (ESTs), microsatellite markers, and optimized protocols for chromosome set manipulation for gynomito- and andromito-genesis, as well as a 6X coverage BAC library.

Applications will take advantage of comparative genomics of the gilthead sea bream against model fish species to combine and narrow the candidate genes and QTL approaches.

Genetic basis of sex determination in fishes: searching for master key regulator genes in the sex determination pathway in tilapias

Andrey Shirak[a,*], Eyal Seroussi[a], Noam Zilberman[a], Gideon Hulata[a], Micha Ron[a], Avner Cnaani[b], and Thomas D. Kocher[b]

[a] *Institute of Animal Science, Agricultural Research Organization, Volcani Center, PO Box 6, Bet Dagan 50250 Israel*

[b] *Hubbard Center for Genome Studies, University of New Hampshire, Durham, NH 03824, USA*

* shirak2@bezeqint.net

Recent results show a striking similarity in the dynamics of expression of gonad differentiation regulators in zebrafish and mammals. Candidates for the role of master key regulators (MKRs) in tilapia were selected based on three concepts established in the literature for non-mammal species. These three concepts are: (1) MKRs are DM-proteins or their close downstream/upstream partners in the sex determination pathway; (2) MKRs are genes working just upstream of aromatase in the sex determination pathway, or the aromatase itself; and (3) MKRs are homologs of mammal genes which are close partners of SRY ("missing" in non-mammal vertebrates), mostly belonging to the SOX family. Coding sequences of putative genes were searched in cichlid (TIGR) and general (NCBI) databases, and in tilapia gonad EST library (RBEST). Primers in two adjacent exons were designed based on predicted exon-intron boundaries for each of 11 selected genes. Amplified segments of the targeted genes in two purebred tilapia species were sequenced. Seven SSLP and four SNP-based markers were identified in the candidate genes for master key regulators of sex determination and mapped to the tilapia genetic map using genotype data of 76-90 individuals of the F_2 mapping family. The mapping positions of the selected genes relative to previously reported QTL regions for sex determination are discussed.

Regulated sex control in commercially important fishes – a physiological perspective

William L. Shelton

Zoology Department, University of Oklahoma, Norman, Oklahoma 73019, USA

wshelton@ou.edu

The management of fish reproduction has resulted in major advancements in the commercial culture of fishes. Artificial propagation has stabilized seed-stock production and reproductive manipulations in induced sex reversal and ploidy manipulation have provided mechanisms to improve yield. The design and effectiveness of these manipulations are regulated by various physiological factors. The effectiveness of protocols for the induction of gynogenesis, triploidy and tetraploidy is improved through knowledge of physiological effects on important parameters. Application of the Developmental Rate based on mitotic interval (τ_o) incorporates standardization relative to temperature. Timing of shock with reference to species-specific τ_o relationship is effective in clarification and optimization of treatments. Such standardization is important to any late (endomitotic - Em) shock induction, but also in polar-body (Pb) induction for many species. Hormonally induced sex reversal also must be applied relative to an efficacious treatment protocol, developed relative to a window of gonadal lability during the genetically directed chronology and physiological influenced differentiation. Size and/or age are important modifying parameters which can be affected by various growth-altering environmental factors, such as temperature and density-dependent effects. Considering these influences that affect physiological rates relative to reproductive manipulations provides a more in-depth understanding of protocol effectiveness.

The effect of rearing temperature on sex differentiation in European sea bass (*Dicentrarchus labrax*)

Benny Ron[a,*], Sergei Gorshkov[a], Galina Gorshkov[a], Gilad Heinisch[a], Iris Meiri[a], Hagit Kvitt[a], Isachar Ben-Atia[a], Adi Peduel[a], Keren Bressler[a], Mirit Gada[a], Sigal Lutzky[a], Constantinos Mylonas[b], Hanna Rosenfeld[a], and Amos Tandler[a]

[a]*National Center for Mariculture, Israel Oceanographic and Limnological Research, P.O. Box 1212, Eilat 88112, Israel*

[b]*Institute of Aquaculture, Hellenic Center of Marine Research, P. O. Box 2214, Iraklion, Crete 71003, Greece*

* ronbenny@ocean.org.il

The European sea bass *Dicentrarchus labrax*, a commercially important marine finfish, is a gonochoristic species characterized by a pronounced sexual growth dimorphism in favor of females. Sea bass females reach market weight much earlier than males and, by this time, females may weigh 40% more than males. Therefore, the ability to manipulate sex by increasing the proportion of females in cultured sea bass stocks has obvious economic advantages. Our recent studies demonstrated that sex differentiation in sea bass might be under the control of complex genetic, environmental and their interactions. Specifically, significant maternal and paternal effects on the proportion of females among the progeny were found. It was also found that different sea bass strains varied significantly in traits such as sex ratio, growth, survival and body shape abnormalities. One of the main environmental factors influencing gonadal development and sex differentiation in sea bass is temperature. Therefore, the objective of this work was to study the effect of different temperature regimes on sex differentiation in *D. labrax* during the first 100 days post-hatching and to identify when plasticity of gonadal sex differentiation occurs. As there were indications that the genetic makeup of the population may influence response to temperature, we examined the influence of temperature on sex differentiation in two Mediterranean strains of *D. labrax*. The results for both strains demonstrate that exposure to a high temperature (21°C) during early development has a strong masculinizing effect. Conversely, exposure to a low temperature (13°C) resulted in a population with a significantly higher percentage of females. The present study indicated that sex differentiation is temperature-sensitive from 10 to 90 days post hatching and the male pathway of gonadal development can be prevented in *D. labrax* by exposing the fish to low temperatures during the larval and nursery stages. Our results demonstrated that low temperature treatments have short-term negative effects on growth during the early stages. Growth compensation occurs, however, later on, with the females reaching market size earlier than the males. Pronounced sexual growth dimorphism in *D. labrax* can be used as an ideal model for investigating the physiological mechanisms of this phenomenon.

As a first step in the development of molecular tools for analyzing the central growth regulators, we have cloned and characterized the related cDNAs encoding for seabass IGF-I and IGF-II. The latter as well as the Genbank-extracted sequence of seabass GH were used to establish real-time,

fluorescence-based quantitative PCR assays and thus measure the mRNA levels of the respective genes. These tools enabled us to verify the expression patterns of pituitary GH and liver IGFs in two size-graded sea bass groups. Following 4 consecutive gradings (at 2, 4, 5 and 7 months post-hatching), the extremely-small (S) and large (L) sea bass groups consisted of highly skewed sex ratios, with male dominancy in the S group and female dominancy in the L group. PCR-amplified fragments for GH and IGFs were detected in the respective pituitary and liver tissues sampled from both L and S groups at 150, 200, 250, and 300 days post-hatching (dph). The IGF-I transcript levels were constantly higher (~2-fold and ~3-fold for the S and L samples respectively) than those of IGF-II, suggesting a more important role for IGF-I during the study. The more significant differences between the L and the S groups were observed at 200 dph, during which the levels of both GH and IGF-I were significantly higher in the L samples. Interestingly, a dramatic change in the growth rate of the L population coincided with the period of 200 dph as well. Our preliminary findings highlight the 200 dph as a critical stage contributing to the asymmetric growth in sea bass L and S groups. It is expected that once immunological tools necessary to profile the concurrent growth factors are perfected, the interactions within the GH-IGFs axis will be better understood.

Genomic approaches to identifying sex-determining genes in tilapia

Thomas D. Kocher*, Bo-Young Lee, and Avner Cnaani

Hubbard Center for Genome Studies, University of New Hampshire, Durham, NH 03824, USA

* tdk@cisunix.unh.edu

Variation in sex determination mechanisms among the different tilapia species has been recognized for over 40 years. We have looked for associations between microsatellite DNA markers and sex in families from different species and strains of tilapia. We found that at least two different linkage groups are involved in sex determination in this group of fishes. In two species, *O. niloticus* and *T. zillii*, we found evidence for male heterogamety with a major sex-determining locus on linkage group 1 (LG1). In two other species, *O. aureus* (Israeli strain) and *O. karongae*, we found evidence for female heterogamety with a major locus for sex determination on LG3. In *O. aureus* (Egyptian strain) and in *O. mossambicus*, loci associated with sex determination were found on both LG1 and LG3, and a complex mechanism of sex determination was detected. Physical mapping by fluorescence *in situ* hybridization (FISH) suggests that LG3 corresponds to the largest chromosome pair, and that there is recombination suppression in the sex determination region. The sex-determining region in *O. niloticus* has been mapped to an 11cM region between markers *GM201* and *UNH995* on LG1. A BAC contig containing *UNH995* was identified and several BACs in the contig were end- or shotgun sequenced. BLAST analysis of these sequences identified *Tetraodon* chromosome 5 as the homolog of tilapia LG1. Additional SNP and microsatellite markers were developed from published cichlid ESTs and the order of these markers is consistent between tilapia and *Tetraodon*. We have narrowed the sex-determining region to a 2.6cM interval which corresponds to a 400 kb region of *Tetraodon* chr5. We are completing genetic and physical maps across this region in order to identify the gene(s) responsible for sex determination in this species.

Traditional and phylogenetic approaches in the diagnosis and identification of pathogens in mariculture

Angelo Colorni*, Ariel Diamant, Hagit Kvitt, and Michal Ucko

Israel Oceanographic and Limnological Research, National Center for Mariculture, P.O. Box 1212, Eilat 88112, Israel

* angelo@ocean.org.il

Traditionally, the most common approach to diagnosis of microbial fish pathogens has relied on *in vitro* isolation of the microorganism and the information provided by its phenotypic features. However, viruses are generally highly species-specific and established cell lines do not necessarily show cytopathic effect, many species of bacteria are difficult or impossible to culture *in vitro*, while parasitic microorganisms often have a complex life cycle that requires propagation in live hosts. An increasing number of microbial pathogens are identified today by molecular methods, without the need for isolation. A PCR direct method for detection and identification of *Mycobacterium marinum* based on the 16S rRNA gene sequence was successfully developed already 13 years ago at NCM. Comparison of the 16S rRNA sequence of *Streptococcus iniae* isolates revealed that, despite phenotypic, biochemical and pathogenetic similarities, marine and freshwater isolates were different strains. With time, however, it has become clear that 16S rRNA gene sequences alone are often insufficient to detect variation within bacterial species, and today other specific loci are also being employed. More recently, on the basis of *hsp*65 gene in addition to 16S rRNA gene, Israeli *M. marinum* isolates in marine and freshwater fish were found to belong to two distinct strains, and both were different from Israeli *M. marinum* clinical (human) isolates. Specific 18S rDNA probes for detection of elusive life stages of two myxosporean parasites, *Kudoa iwatai* and *Enteromyxum leei*, in sea bream and sea bass are being employed in studies conducted over the last few years at NCM. By using whole-genome structures rather than single gene sequences, two fingerprinting techniques - Amplified Fragment Length Polymorphism (AFLP) and Randomly Amplified Polymorphic DNA (RAPD) - have provided a generally higher level of precision in genotyping. However, while the AFLP method revealed broad polymorphism among *S. iniae* isolates, the RAPD method did not provide additional information. These examples show that not all regions of the DNA are equally useful in diagnosis and genotyping and therefore there is no single "best" molecular method. Molecular strategies have provided a phylogenetic approach to determining identification and taxonomic position by grouping closely related organisms that share a relatively recent ancestry into clusters. Although the question remains of how much genetic diversity is permissible in a discrete cluster for its members to be regarded as a single taxon, the ability to place a microorganism in a given taxon on the basis of its evolutionary development is of importance: if known members of the same family do not have a "clean bill" concerning their pathogenicity, any related organism may be justifiably regarded as a potential offender. Traditional methods and molecular methods provide different levels of information: only their combination offers a comprehensive insight into the microorganism's nature.

Genetic perspective on stress and disease resistance in aquaculture

Avner Cnaani

Hubbard Center for Genome Studies, University of New Hampshire, Durham, NH 03824, USA

acnaani@unh.edu

Despite continuous progress and improvements in aquaculture technologies and husbandry techniques, fish diseases remains a major limiting factor in the fish culture industry. Like all vertebrates, the fish immune response is a combination of two systems, the innate (non-specific) immune system and the acquired (specific) immune system. However, unlike mammals and birds, the fishes' innate system is more developed and has a larger role in the organism immune response than the acquired system. Thus, the innate immune response is thought to have a major role in disease resistance of fish. The aquaculture environment exposes fish to repeated acute stress, which lead to physiological responses that have suppressive effects on growth, reproduction and immune capacity. The strong link between stress and susceptibility to diseases in farm animals has long been acknowledged, and parameters of high and low stress response, as reflected in innate immunity and blood biochemical components, were found to be associated with disease resistance in fish. Only few studies on genetic aspects of immune response were conducted. The estimated heritability of several parameters of the innate immune response was mostly moderate. Quantitative trait loci were found for resistance for several diseases and for different responses to stress conditions. Gene expression studies showed that hundreds of genes are involved in the fishes' physiological and immunological response to stress. This wide response to stress is controlled by a few major genes on the top of the pathway, which activate a cascade of reactions, having a significant effect on the overall health of the fish. Selective breeding for disease resistance fish is an attractive prevention strategy and several studies have reported progress in this field. However, the biological pathways of stress response and disease resistance are not well characterized, and their genetic basis and control are still poorly understood. An extensive research is still needed for a better understanding of these pathways, and this should be a collaborative effort of researchers from different fields: genetics, immunology, pathology, physiology and endocrinology.

Marker-assisted breeding for viral disease resistance in Japanese flounder (*Paralichthys olivaceus*)

Takashi Sakamoto[a,*], Kanako Fuji[a], Kazunobu Kobayashi[a], Osamu Hasegawa[b], Akiyuki Ozaki[c], and Nobuaki Okamoto[a]

[a] *Department of Aquatic Biosciences, Tokyo University of Marine Science and Technology, Tokyo 108-8477, Japan*
[b] *Kanagawa Prefectural Fisheries Research Institute, Kanagawa 238-0237, Japan*
[c] *National Research Institute of Aquaculture, Fisheries Research Agency, Mie 516-0193, Japan.*

* takashis@s.kaiyodai.ac.jp

DNA marker technologies can be used for genetic improvement through selection of favorable traits, such as disease resistance. These traits are generally modeled as being controlled by many genes of small additive effects, which are known as quantitative trait loci (QTL). Construction of a genetic linkage map, based on DNA markers at a large number of sites in the fish genome is necessary to identify QTL controlling traits of disease resistance. By identifying markers associated with high performance QTL in different strains or species, it may also be possible to successfully improve the performance of such traits in other strains through introgression of the desired QTL. One of the goals of selective breeding programs is to integrate genetic marker information from pedigreed brood stock into successful management and culture. Such an approach, termed marker-assisted selection (MAS) and/or marker-assisted gene introgression (MAI), is expected to increase genetic response by affecting efficiency and accuracy of selection.

Japanese flounder (*Paralichthys olivaceus*) is an economically important food fish, widely cultured in Asian countries such as Japan, Korea and China. Lymphocystis disease (LD) is causes by LD virus (family Iridoviridae), and has become widely spread in these countries and seriously damaged fish farms. There is no effective treatment for LD or a commercially available vaccine. To solve this problem, we have initiated a genetic linkage study in search for markers associated with LD resistance. As a first step, we have constructed a primary genetic linkage map in Japanese flounder (*Paralichthys olivaceus*) using microsatellite markers. A second generation genetic linkage map consisting of approx. 500 markers have been constructed and more markers are currently added to the map. Linkage analysis of LD resistance was conducted in a backcross progeny (n=136) produced by crossing a susceptible male with a (susceptible x resistant) hybrid female. One major locus (*Poli.9-8TUF*) for LD resistance was detected on linkage group 15 of the Japanese flounder genetic linkage map.

To introduce the trait and marker information linked to LD resistance into a commercial strain, we performed a cross between a resistant strain and a commercial strain, and generated F_1 hybrid families. The LD resistant Japanese flounder stock produced by MAI was tested on commercial fish farms. The results of the field tests on F_1 hybrid families demonstrated that LD resistance was successfully transmitted to the commercial strain. Our results show that MAI could be useful for genetic improvement through selection of favorable traits.

Food size rainbow trout selective breeding: Current and future prospects

Scott E. LaPatra[a,*] and Richard H. Towner[b]

[a] *Clear Springs Foods, Inc., Research Division, P.O. Box 712, Buhl, Idaho 83316, USA*

[b] *GenTec Consulting, Payette, Idaho 83661, USA*

* scottl@clearsprings.com

Rainbow trout (*Oncorhynchus mykisss)* are a valuable aquaculture production species in the United States. Production of food size rainbow trout averaged 25,000 mt per year in the U.S. from 1988 – 2002. Clear Springs Foods, Inc. is one of the largest producers of aquacultured rainbow trout producing 10,000 mt annually. Privately held by an employee owned trust, Clear Springs is a vertically integrated company from brood stock through egg production, feed manufacturing, farm operations, processing and distribution. Clear Springs also has a significant commitment to research and development. Selective breeding of rainbow trout is an important component. The current goals of the selective breeding program are to improve growth and disease resistance. In order to improve these traits data are recorded on thousands of individuals each year. Growth data is collected at various ages to determine which families and which individuals within each family have the best growth. In order to improve disease resistance a portion of the progeny from each family are exposed to specific pathogens in a standardized challenge test. Currently each family is tested for survivability to infectious hematopoietic necrosis virus (IHNV) and *Flavobacterium psychrophilum* the causative agent of bacterial coldwater disease (CWD) and rainbow trout fry syndrome (RTFS).

Selection to improve growth began when the breeding program was initiated. In 1991 the average weight was 660 grams at 328 days of age. The average weight of the odd year generation group was 921 g at 301 days of age in 2003. The average weight of the even-year group increased from 620 g at 328 days in 1992 to 866 g at 301 days in 2004. The selection to improve IHN resistance was started with the 1994 generation. Using a standardized challenge test the IHN mortality has decreased 25.8% and 29.7% in the odd and even year generation groups, respectively. Growth is a moderately heritable trait that can be changed rapidly and economically with traditional quantitative genetic techniques. Disease resistance has a much lower heritability and is more difficult to change. Improved knowledge of specific and general disease resistance mechanisms in trout would aid the industry to improve their stocks for the future.

Recent advances in genetic improvement and genomics of *Morone sp*.

Mark E. Westerman[a,*], Charlene R. Couch[b], Amber F. Garber[b], Caird Rexroad III[c], and Craig V. Sullivan[b]

[a] *Kent SeaTech Corporation, 11125 Flintkote Ave, Suite J, San Diego, CA 92121 USA*

[b] *Department of Zoology, Campus Box 7617, College of Agriculture and Life Sciences, North Carolina State University (NCSU), Raleigh NC 27695-7617 USA*

[c] *USDA/ARS, National Center for Cool and Cold Water Aquaculture, P.O. Box 100, Kearneysville, WV 25430 USA*

* mwesterman@kentseatech.com

The striped bass, *Morone saxatilis*, has been a prized foodfish since colonial times in the United States. In 1670, one of the first public schools in North America was established with income from coastal striped bass fisheries. Although these fisheries were in severe decline by the 1980s, the decline provided market opportunities for aquaculture of striped bass and hybrid striped bass (HSB; *M. chrysops* x *M. saxatilis*). NCSU researchers and North Carolina farmers pioneered commercial production of HSB in freshwater ponds during the late 1980s, and these successes were quickly extended to intensive, tank based aquaculture systems. The U.S. HSB industry has traditionally been and is still dependent on fingerlings produced in ponds from HSB fry obtained directly from river-caught wild broodfish during the spring spawning season. Annual U.S. production of HSB is currently approaching 13 million pounds (5900 metric tons), with pond culture accounting for ~60% of total production and intensive systems the remaining 40%.

Over the last 15 years, scientists at NCSU and the University of Maryland conducted studies on the reproductive biology of *Morone* species, describing hormonal regulation of the gametogenic cycle and cytological changes in the maturing gonads. These capabilities led to establishment and domestication of striped bass and white bass broodstocks drawn from diverse geographic lineages at the NCSU Pamlico Aquaculture Field Laboratory. Currently 4th (striped bass) through 7th (white bass) generation broodfish are routinely spawned with fertility, fecundity, and rates of progeny survival equivalent to those of mature wild fish captured from local spawning grounds. These developments set the stage for selective breeding in these species and a landmark meeting entitled, "Workshop on Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry" (October 2003). At this meeting the "National Program for Genetic Improvement and Selective Breeding for the Hybrid Striped Bass Industry" was established as a cooperative venture between scientists in academia, government, and industry.

The national HSB breeding program currently emphasizes combinations of direct (mass) selection and walkback selection based on identification of superior broodstock by genotyping of superior performing progeny. Preliminary common garden trials using DNA markers for pedigree tracking have revealed a strong paternal component underlying growth of striped bass and HSB. To support detailed genetic analysis of striped bass (*M. saxatilis*) and white bass (*M. chrysops*), 153 microsatellite loci have been isolated from repeat-enriched striped bass DNA libraries. Of these,

147 markers amplified in striped bass (average 4.7 alleles per locus) and 133 in white bass (average 2.2 alleles per locus). 121 markers were amplified successfully in hybrid striped bass. Four additional repeat-enriched have been produced and screened leading to development of an additional 345 microsatellites markers for striped bass. A subset of these markers (n=71) was genotyped on samples from two striped bass broodstock populations for characterization with respect to polymorphism, heterozygosity, Hardy-Weinberg equilibrium (HWE), linkage disequilibrium (LD) and usefulness in population genetic applications. These markers have been posted on GenBank and will form the basis for the first genetic linkage map for a *Morone* sp.