ISGA XII

INTERNATIONAL SYMPOSIUM ON GENETICS IN AQUACULTURE XII

Santiago de Compostela, Spain
June 21st-27th, 2015

Contents

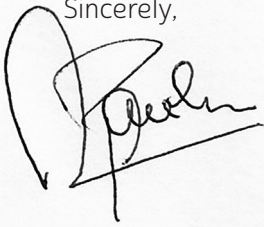# WELCOME LETTER FROM THE CHAIRMAN

Dear Colleagues,

It is a great pleasure to welcome all of you to the 12th International Symposium on Genetics in Aquaculture that it is being held at the University of Santiago de Compostela. I hope that your time here in Santiago de Compostela will be productive both for updating your knowledge in the field and for establishing new friendships and scientific collaborations.

I wish to thank invited speakers who are presenting us updating reviews on classical and emerging genetic fields applied to aquaculture, and to all of you who have submitted poster and oral presentations, which represent the substrate for productive discussions on the different species and areas involved in the Symposium. I would like to thank all of our sponsors, without whom this meeting could not be held. And of course, my best gratefulness to all members of the Scientific Committee, they provided valuable insights and support for the meeting organization, and, especially to my colleagues of the Organizing Committee for their continuing support and dedication to craft this exciting program and all the logistics for enabling an optimal development of the meeting.

An increasing number of genetically improved aquaculture species are now commercially available throughout the world. Major improvements have been achieved through enhanced reproduction, nutrition, and disease diagnosis procedures, and the application of genetics to breeding programs. Genetic research is becoming more and more important as aquaculture develops. The combination of a variety of genetic methodologies, including classical breeding, biotechnology, genomics and genetic engineering, is the support for selecting the best genotypes to improve production. A great advance has been accomplished in genomics in the past 20 years and an increasing variety of genomic tools are now available to apply to breeding programs. It is time to take advantage of the vast effort invested in genomics in the last years and to incorporate this information to breeding programs.

Finally, a call of attention on the potential risks of aquaculture progress and its potential impact on wild populations should be done. There is an increasing concern regarding to the impact of escapees from aquaculture facilities that can not only threaten natural populations and fisheries, but also produce severe unbalances in natural ecosystems with unpredictable consequences. An effort should be made to walk towards a sustainable aquaculture to the future.

I wish you have a great time, enjoy the Galician hospitality, jump the fires in Saint John´s night, and return back home with a good remainder of this Spanish region. I hope you can enjoy the beautiful ancient town of Santiago and through the scheduled excursions delight the Galician landscape and seafood. Have safe travel back to your home and we look forward to see you soon.

Sincerely,

Paulino Martínez

Committees

Organizing Committee

ISGA XII Congress

PAULINO MARTÍNEZ PORTELA

Professor of Genetics

CHAIRMAN OF THE ISGA XII CONGRESS
Departament of Genetics. Faculty of Veterinary. University of Santiago de Compostela. Campus de Lugo. 27002 Lugo. Spain.
Phone: + 34 982 822428
Email: paulino.martinez@usc.es

ANA MARÍA VIÑAS DÍAZ

Associate Professor

Departament of Genetics. Faculty of Biology. CIBUS Building University of Santiago de Compostela 15782 Santiago de Compostela .
Phone: + 34 881 816918
Email: anamaria.vinas@usc.es

BELÉN GÓMEZ PARDO

Assistant Professor

Departament of Genetics. Faculty of Veterinary. University of Santiago de Compostela. Campus de Lugo. 27002 Lugo. Spain.
Phone: + 34 982 822428
Email: belen.gomez@usc.es

LAURA ELENA SÁNCHEZ PIÑÓN

Professor of Genetics

Departament of Genetics. Faculty of Veterinary. University of Santiago de Compostela. Campus de Lugo. 27002 Lugo. Spain.
Phone: +34 982 822428
Email: lauraelena.sanchez@usc.es

ROSA FERNÁNDEZ OTERO

Head of Technology Promotion and Transfer Area

Centro Tecnológico del Mar - FUNDACIÓN CETMAR
Phone: +34 986 247047 **Ext:** 207
Email: rfernandez@cetmar.org

CARMEN BOUZA FERNÁNDEZ

Associate Professor

Department of Genetics. Faculty of Veterinary. University of Santiago de Compostela. Campus de Lugo. 27002 Lugo. Spain.
Phone: +34 982 822428
Email: mcarmen.bouza@usc.es

JESÚS FERNÁNDEZ MARTÍN

Researcher

Department of Animal Genetic Improvement National Institute for Agricultural and Food Research and Technology (INIA) Crta. A Coruña Km. 7,5 E-28040 Madrid Spain.
Phone: +34 91 3471487
Email: jmj@inia.es

BEATRIZ VILLANUEVA GAVIÑA

Researcher

Department of Animal Genetic Improvement National Institute for Agricultural and Food Research and Technology (INIA) Crta. A Coruña Km. 7,5 E-28040 Madrid Spain.
Phone: +34 91 3476807
Email: villanueva.beatriz@inia.es

TECHNICAL SUPPORT

MÓNICA ÁFRICA OTERO OBARRIO

Technical support

Department of Genetics. Faculty of Veterinary. University of Santiago de Compostela. Campus de Lugo. 27002 Lugo. Spain.
Phone: +34 982 822428
Email: monicaafrica.otero@usc.es

SONIA GÓMEZ FERNÁNDEZ

Technical support

Department of Genetics. Faculty of Veterinary. University of Santiago de Compostela. Campus de Lugo. 27002 Lugo. Spain.
Phone: +34 982 822428
Email: sonia.gomez@usc.es

MARÍA PÉREZ

Technical Technology Transfer

Technical Technology Transfer
Centro Tecnológico del Mar - FUNDACIÓN CETMAR
Phone: +34 986 247047
Email: mperez@cetmar.org

Scientific Committee

ISGA XII Congress

UTHAIRAT NA-NAKORN

Thailand

Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Jatujak, Bangkok, Thailand.
Email: ffisurn@ku.ac.th

ROBERTO NEIRA

Chile

Departamento de Producción Animal, Facultad de Ciencias Agronómicas, Universidad de Chile, PO Box 1004, Santiago, Chile
Email: rneirar@gmail.com

ZHANJIANG LIU

USA

The Fish Molecular Genetics and Biotechnology Laboratory, 203 Swingle Hall, School of Fisheries, Aquaculture and Aquatic Sciences, and Program of Cell and Molecular Biosciences, Auburn University, Auburn, AL, 36849, USA
Email: liuzhan@auburn.edu

BJARNE GJERDE

Norway

Nofima, P.O. Box 5010, 1432 Ås, Norway
Email: bjarne.gjerde@nofima.no

GIDEON HULATA

Israel

Institute of Animal Science, Agricultural Research Organization, The Volcani Center, PO Box 6, Bet Dagan 50250, Israel
Email: vlaqua@volcani.agri.gov.il

DEAN JERRY

Australia

Aquaculture Genetics Research Program, School of Marine and Tropical Biology, James Cook University, Townsville, 4811, Queensland, Australia
Email: dean.jerry@jcu.edu.au

WILLIAM S DAVIDSON

Canada

Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby BC, V5A 1S6, Canada
Email: wdavidso@sfu.ca

DAVID PENMAN

United Kingdom

Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling, Scotland, FK9 4LA, UK
Email: d.j.penman@stir.ac.uk

MIGUEL ANGEL TORO

Spain

Department. Livestock, ETS Engineers Agronomists, UPM, University City, 28040 Madrid, Spain
Email: miguel.toro@upm.es

Program

ISGA XII - THE INTERNATIONAL SYMPOSIUM ON GENETICS IN AQUACULTURE XII 21st-27th JUNE 2015 IN SANTIAGO DE COMPOSTELA, SPAIN

SUNDAY, 21st JUNE 2015

19:30-21:30	**REGISTRATION AND WELCOME COCKTAIL** (Assembly Hall of the **Law Faculty**, Campus Sur, University of Santiago de Compostela)

MONDAY, 22nd JUNE 2015

08:00-08:30	**REGISTRATION**		
08:30-08:45	**WELCOME** Presentation of the ISGA 2015 Symposium (**Prof. Paulino Martínez**) Representatives of the Galician Government and EU Administration, and the Rector of the University of Santiago de Compostela		
08:45-09:00	**[S1]** **HISTORY OF AQUACULTURE AND APPLIED GENETICS IN SPAIN (Prof. Paulino Martínez)**		
09:00-09:45	**Opening Conference Prof. John Woolliams (SPONSORED BY Red de Excelencia de Biotecnología en Acuicultura, Aquagenomics-Net - Ministerio de Economía y Competitividad)**		
	[S2] GENOMIC SELECTION: FROM LIVESTOCK TO AQUACULTURE APPLICATIONS		
SESSION BREEDING PROGRAMS 1 MODERATOR: Prof. Paulino Martínez	09:45-10:00	**[01]**	CURRENT STATUS OF SELECTIVE BREEDING IN EUROPEAN AQUACULTURE. **Janssen K.**, Chavanne H., Berentsen P., Komen H.
	10:00-10:15	**[02]**	EVOLUTION OF *C. virginica* BREEDING IN CHESAPEAKE BAY, USA: FROM MASS TO FAMILY SELECTION. **Moss Small J.**, Kube P., Allen Jr. S.K.

SESSION BREEDING PROGRAMS 1 MODERATOR: Prof. Paulino Martínez	10:15-10:30	**[03]** ADVANCED ANIMAL BREEDING IN AQUACULTURE: USING GENOME-WIDE MOLECULAR BREEDING VALUES FOR RAPID ANIMAL IMPROVEMENT IN THE SILVER-LIPPED PEARL OYSTER. **Zenger K.R.**, Jones D.B., Raadsma H.W., Khatkar M.S., Moser G., Taylor J.T., Toole P., Jerry D.R.
10:30-10:50	**COFFEE BREAK**	
SESSION BREEDING PROGRAMS 2 MODERATOR: Prof. John Liu	10:50-11:05	**Fishboost EU Project (Dr. Anna Sonesson)** **[S11]** FISHBOOST- AN EU-FP7 PROJECT TO ADVANCE EUROPEAN AQUACULTURE BREEDING FOR SIX FINFISH SPECIES
	11:05-11:20	**[04]** A COMPARISON OF CLASSIC SELECTION AND MATING METHODS FOR AQUACULTURE BREEDING PROGRAMMES. **Saura M.**, Villanueva B., Fernández J., Toro M.A
	11:20-11:35	**[05]** GENETIC IMPROVEMENT OF YELLOW PERCH I: PROGRESS AND PROSPECTS. **Wang H-P.**, O'Bryant P., Yao H., Rapp D., Shen Z. and Li Y.
	11:35-11:50	**[06]** GENETIC GAINS ACHIEVED OVER 10 YEARS OF SELECTIVE BREEDING FOR RESISTANCE TO AMOEBIC GILL DISEASE IN ATLANTIC SALMON *(Salmo salar).* **Evans B.S.**, Kube P.D., Taylor R.S., Elliott N.G.
	11:50-11:05	**[07]** GENETICS OF THERMOTOLERANCE IN RAINBOW TROUT, *Oncorhynchus mykiss.* **Dupont-Nivet M.**, Crusot M., Rigaudeau D., Labbé L., Quillet E.
	11:05-11:20	**[08]** GENETIC VARIATION IN DEVELOPING ATLANTIC SALMON *Salmo salar* TO HYPOXIA TOLERANCE. Andrewartha S.J., Hamilton M., **Elliott N.G.**, Frappell P.B.
	11:20-11:35	**[09]** HERITABILITY OF COPING STYLES IN FARMED EUROPEAN SEABASS. **Allal F.**, Ferrari S., Horri K., Vidal M.-O., Ruelle F., Vandeputte M., Chatain B., Bégout M.-L.
12:35-13:45	**LUNCH**	
SESSION BREEDING PROGRAMS 3 MODERATOR: Dr. Dean Jerry	13:45-14:15	**Short review** **[S5]** ASIAN SEABASS GENOME PROJECT: A STATUS REPORT **(Prof. Laszlo Orban)** **[S6]** CURRENT STATUS OF THE ASIAN SEABASS BREEDING PROGRAM **(Prof. Gen Hua Yue)**

SESSION BREEDING PROGRAMS 3 MODERATOR: Dr. Dean Jerry	14:15-14:30	**[010]** GENOTYPE-ENVIRONMENT INTERACTIONS OF CHANNEL CATFISH, *Ictalurus punctatus,* ♀ X BLUE CATFISH, *Ictalurus furctatus*, ♂ HYBRIDS-CHANGING CULTURE ENVIRONMENTS-CHANGING CLIMATE. **Dunham R.A.**, Alsaqufi A., Youssef N., Makhubu N.P., Su B., Peatman E.
	14:30-14:45	**[011]** GENOTYPE-BY-ENVIRONMENT INTERACTION FOR UNIFORMITY OF GROWTH IN RAINBOW TROUT *(Oncorhynchus mykiss).* Sae-Lim P., **Kause A.**, Janhunen M., Vehviläinen H., Koskinen H., Gjerde B., Lillehammer M., Mulder H.A.
	14:45-15:00	**[012]** META-ANALYSIS METHODOLOGY FOR SUMMARIZING GENOTYPE-BY-ENVIRONMENT INTERACTIONS ACROSS AQUACULTURE SPECIES. **Kause A**.
	15:00-15:15	**[013]** GENETIC PARAMETERS AND GENOTYPE X ENVIRONMENT INTERACTIONS FOR GROWTH IN THE RED ABALONE *(Haliotis rufescens).* **Winkler F.**, Farías W., Brokordt K., Herbinger C.
	15:15-15:30	**[014]** ESTIMATES OF HERITABILITY AND GENOTYPE BY ENVIRONMENT INTERACTIONS IN THE PURPLE FRESHWATER PEARL MUSSEL *Hyriopsis cumingii.* Li Q., **Bai Z.**, Han X., Luo H., Dong S., Li J.
	15:30-15:45	**[015]** HERITABILITY AND GXE INTERACTIONS OF DISEASE RESISTANCE TO SUMMER SPAT MORTALITIES IN THE PACIFIC OYSTER *Crassostrea gigas* USING BAYESIAN MODEL. **Enez F.**, Puyo S., Boudry P., Lapègue S., Gonzalez-Araya R., Guémené D., Chapuis H., Haffray P.
15:45-16:05	**COFFEE BREAK**	
16:05-17:30	**BROKERAGE/POSTER SESSION**	
17:30-19:00	**ROUND TABLE/WORKSHOP (GBS VS SNP-CHIPS).** **MODERATOR: DR. ROSS HOUSTON**	
	RAD-SEQ - **Dr. Luca Bargelloni.** Universitá degli Studi di Padova (IT) AFFYMETRIX CHIP - **Dr. Alastair Hamilton**. Landcatch Natural Selection Ltd (UK) ILLUMINA CHIP - **Dr.Cindy Taylor Lawley**. Manager, Market Development Illumina, Inc. (USA) GENOMAGIC - **Dr. Gil Ronen**, CEO, NRGene (ISR)	

TUESDAY, 23rd JUNE 2015

SESSION ENVIRONMENTAL RISK MODERATOR: Dr. Béatrice Chatain	08:30-08:45	**Aquatrace EU Project (Dr. Luca Bargelloni)** **[S12]** THE DEVELOPMENT OF TOOLS FOR TRACING AND EVALUATING THE GENETIC IMPACT OF FISH FROM AQUACULTURE: "AQUATRACE"
	08:45-09:00	**[016]** EVALUATION OF THE ERROR RATE AND SOLUTIONS ASSOCIATED TO DOUBLE DIGESTION RAD GENOTYPING BY SEQUENCING IN THREE EUROPEAN MARINE AQUACULTURE SPECIES. **Maroso F.**, Hermida M., Pardo B.G., Martínez P., Bargelloni L.
	09:00-09:15	**[017]** IDENTIFYING PARALLEL AND NON-PARALLEL GENOMIC CHANGES BETWEEN INDEPENDENT PAIRS OF WILD/DOMESTIC ATLANTIC SALMON POPULATIONS USING A HIGH DENSITY SNP ARRAY. **López M.E.**, Correa K., Di Genova A., Moore J-S., Perrier C., Bernatchez L., Gilbey J., Soto C., Bassini L., Maass A., Neira R., Figueroa R., Lhorente J.P., Yáñez J.M.
	09:15-09:30	**[018]** IDENTIFICATION OF SPECIES-SPECIFIC SNP MARKERS IN TILAPIAS USING DOUBLE-DIGEST RAD SEQUENCING (ddRADseq). Syaifudin M., Bekaërt M., Taggart J.B., Hulata G., D'Cotta H., Baroiller J.F., **Penman D.J.**, McAndrew B.J.
	09:30-9:45	**[019]** A GENOMIC APPROACH TO THE GENETIC MANAGEMENT OF AQUACULTURE-BASED STOCK ENHANCEMENT IN A MARINE REEF FISH, THE RED SNAPPER *Lutjanus campechanus.* Norrell A.E., Jones K.L., **Saillant E.A.**
	09:45-10:00	**[020]** ECONOMIC AND ENVIRONMENTAL IMPACTS OF GENETIC IMPROVEMENT IN FISH FARMING DEPEND ON LIMITING FACTORS. **Besson M.**, Vandeputte M., Aubin J., van Arendonk J.A.M., de Boer I.J.M., Quillet E., Komen H.
	10:00-10:15	**[021]** MICROSATELLITE ASSESSMENT OF GENETIC DIVERSITY OF WILD AND CAPTIVE POPULATIONS FOR RESTOCKING THE MIGRATORY CURIMBA *(Prochilodus argenteus)* IN THE SÃO FRANCISCO RIVER (BRAZIL). Lima A.P.S., Oliveira K.K.C., **Coimbra M.R.M.**
	10:15-10:30	**[022]** CAN SELECTIVE BREEDING FOR GROWTH OR FILLET YIELD DECREASE ENVIRONMENTAL IMPACT OF FISH FARMING? A GILTHEAD SEA BREAM *(Sparus aurata)* CASE STUDY. **Haffray P.**, Acosta Alba I., Cariou S., Bruant J.S., Bugeon J., Vandeputte M., Aubin J.

10:30-10:50	COFFEE BREAK	
	10:50-11:05	**Short review**
		[S7] BREEDING PROGRAMS IN FISH AQUACULTURE: HISTORICAL CONTEXT AND PERSPECTIVES **(Prof. Roberto Neira)**
	11:05-11:20	**[023]** GENETIC PARAMETERS FOR THE OPERCULUM AND JAWS DEFORMITIES IN LARVAE OF GITLHEAD SEABREAM, Sparus aurata. **Batargias C.**, Fragoulis S., Loukovitis D., Tzokas K., Koumoundouros G.
SESSION BREEDING PROGRAMS 4	11:20-11:35	**[024]** EFFECTS OF STRAIN ON GROWTH PERFORMANCES OF TRIPLOID THAI WALKING CATFISH, CLARIAS MACROCEPHALUS GÜNTHER, 1864. Chatchaiphan S., Srisapoome P., **Na-Nakorn U.**
MODERATOR: Dr. Marc Vandeputte	11:35-11:50	**[025]** GENETIC PARAMETERS FOR UNIFORMITY OF HARVEST WEIGHT IN THE GIFT STRAIN OF NILE TILAPIA ESTIMATED USING DOUBLE HIERARCHICAL GENERALIZED LINEAR MODELS. **Marjanovic J.**, Mulder H.A., Khaw H.L., Bijma P
	11:50-12:05	**[026]** RESPONSE TO SELECTION FOR HARVEST WEIGHT IN A FAMILY BASED SELECTION PROGRAM OF GILTHEAD SEABREAM *(Sparus aurata).* **Thorland I.**, Kottaras L., Refstie T., Dimitroglou A.,Papaharisis L., Rye M.
	12:05-12:20	**[027]** THE EFFECT OF PARENTAL RELATEDNESS ON THE FITNESS IN NEXT GENERATION OF THE GUPPY *Poecilia reticulate.* Ismail M.S., Nakajima T., **Nakajima M.**
12:20-13:35	LUNCH	
	13:35-13:55	**Short review**
		[S8] SELECTIVE BREEDING IN NILE TILAPIA: ACHIEVEMENTS AND FUTURE DIRECTIONS. **Komen H.**, Benzie J.
SESSION BREEDING PROGRAMS 5	13:55-14:10	**[028]** GENETIC PARAMETERS IN ATLANTIC SALMON FOR GROWTH RATE AND CARCASS QUALITY TRAITS RECORDED AT THE SAME BODY WEIGHT OR THE SAME AGE. **Kristjánsson Ó.**, Gjerde B., Lillehammer M., Jónasson J.
MODERATOR: Dr. Beatriz Villanueva	14:10-14:25	**[029]** NEGATIVE GENETIC CORRELATION BETWEEN RESISTANCE AGAINST Piscirickettsia salmonis AND HARVEST WEIGHT IN COHO SALMON (Oncorhynchus kisutch). **Yáñez J.M.**, Barría A., Dufflocq P., Oyarzún M., Neira R., Newman S., Lhorente J.P.

SESSION BREEDING PROGRAMS 5 MODERATOR: Dr. Beatriz Villanueva	14:25-14:40	**[030]** PARENTAGE ASSIGNMENT IN SALMON USING HIGH DENSITY SNP PANELS: A SIMULATION STUDY. **Grashei K.E.**, Kjøglum S., Moen T., Ødegård J.
	14:40-14:55	**[031]** SHRIMP BROODSTOCK MANAGEMENT FOR THE CONTROL OF GENETIC DIVERSITY AND INBREEDING. **Pérez-Enríquez R.**, Robles-Cota C., Peiro-López J., Haffray P.
	14:55-15:10	**[032]** ESTIMATES OF GENETIC VARIABILITY AND INBREEDING IN SELECTED POPULATIONS OF EUROPEAN SEA BASS.**Hillen J.**, Carr A., Hellemans B., Ogden R., Taggart J., Vandeputte M., Vergnet A., Volckaert F.A.M., Aquatrace consortium, Coscia I
	15:10-15:25	**[033]** GENETIC SIGNATURES OF SELECTION AND ASSOCIATION ANALYSIS OF THE DOMESTICATION EVENT IN SOUTH AFRICAN ABALONE, Haliotis midae. **Rhode C.**, Dale-Kuys R., Vervalle J., Bester-Van der Merve A., Roodt-Wilding R.
	15:25-15:40	**[034]** GENOME-WIDE SNPS PROVIDE INSIGHTS INTO FINE-SCALE POPULATION STRUCTURE AND VARIABILITY IN THE FIJIAN BLACK-LIP PEARL OYSTER Pinctada margaritifera. **Lal M.M.**, Southgate P.C., Jerry D., Zenger K.R.,
	15:40-15:55	**[035]** GENETIC STRUCTURE BASED ON MICROSATELLITES AND COLOR VARIANCE ANALYSIS FOR A BRIGHT RED CLAM, Paphia amabilis (PHILIPPI, 1847) Yu F., Li Q.Z., Wu X.X., Luo B., Zhu J., **Wang Y**
15:55-20:15	**BOAT TRIP AND VISIT TO MUSSEL FACILITIES**	

WEDNESDAY, 24th JUNE 2015

SESSION GENOMES AND GENETIC ARCHITECTURE 1 **(SPONSORED BY NRGENE)** MODERATOR: Prof. Rex Dunham	8:30-9:00	**Invited Conference**
		[S3] GENOMIC ADVANCES AND APPLICATIONS IN AQUACULTURE **(Prof. John Liu)**
	09:00-09:15	**[036]** SECOND-GENERATION LINKAGE MAPS REVEAL ERRORS IN THE ASSEMBLY OF THE PACIFIC OYSTER *(Crassostrea gigas)* GENOME AND FACTORS AFFECTING MAP LENGTHS AND MARKER ORDERS. **Hedgecock D.**

SESSION GENOMES AND GENETIC ARCHITECTURE 1 **(SPONSORED BY NRGENE)** MODERATOR: Prof. Rex Dunham	09:15-09:30	**[037]** IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISM MARKERS ASSOCIATED WITH BACTERIAL COLD WATER DISEASE RESISTANCE AND SPLEEN SIZE IN RAINBOW TROUT. **Liu S.**, Vallejo R.L., Palti Y., Gao G., Marancik D.P., Wiens G.D.
	09:30-09:45	**[038]** A HIGH DENSITY GENETIC LINKAGE MAP FOR RAINBOW TROUT *(Oncorhynchus mykiss)* CONTAINING 47,839 SNPS. **Baranski M.**, Palti Y., Moen T., Grove H., Guangtu G., Lien S., Liu S., Rexroad C.
	09:45-10:00	**[039]** GENE MAPPING IN THE SENEGALESE SOLE *(Solea senegalensis)*. Rodríguez M.E., Portela-Bens S., Merlo M.A., Cross I., **Rebordinos L.**
	10:00-10:15	**[040]** GENETIC LINKAGE MAP AND QTL ANALYSIS OF GROWTH RELATED TRAIT IN PACIFIC BLUEFIN TUNA. **Uchino T.**, Hosoda E., Nakamura Y., Sekino M., Fujiwara A., Yasuike M., Sugaya T., Tanaka Y., Kumon K., Sano M., Sakamoto T.
	10:15-10:30	**[041]** MARKERS FOR RESISTANCE TO WHITE SPOT SYNDROME VIRUS IN BLACK TIGER SHRIMP *(Penaeus monodon)* AND CORRESPONDANCE TO cSNPS IN WHITE SHRIMP *(Litopenaeus vannamei)*. **Robinson N.**, Baranski M., Gopikrishna G., Vinaya Kumar K., Shekhar M.S., Gopal C., Panigrahi A., Balasubramanian C.P., Ashok Kumar J., Rajendran V., Moghadam H., Aranguren F., Salazar M., Gitterle T., Vijayan K.K.
	10:30-10:45	**[042]** EXTENT OF GENOME-WIDE LINKAGE DISEQUILIBRIUM IN FARMED ATLANTIC SALMON *(Salmo salar L.)* USING HIGH-DENSITY GENOTYPES. **Yáñez J.M.** , Correa K., López M.E., Lhorente J.P., Figueroa R., Bassini L., Di Genova A., Maass A.
	10:45-11:00	**[043]** USE OF NEXT-GENERATION SEQUENCING IN THE PACIFIC OYSTER TO DISCOVER AND GENOTYPE SNP MARKERS FOR BUILDING THIRD-GENERATION LINKAGE MAPS. **Arias-Pérez A.**, Hedgecock D.
11:00-11:20	**COFFEE BREAK**	

	11:20-11:40	**Short review**
		[S9] A REVIEW OF SHELLFISH BREEDING PROGRAMS **(Prof. Standish Allen)**
SESSION GENOMES AND GENETIC ARCHITECTURE 2 **(SPONSORED BY GRUPO PESCANOVA)** MODERATOR: Prof. Roberto Neira	11:40-11:55	**[044]** BLUNT SNOUT BREAM, *Megalobrama amblycephala,* GENOME REVEALS THE EVOLUTION AND ADAPTATION TO HERBIVOROUS DIET. **Liu H.**, Wang W.
	11:55-12:10	**[045]** GENOME SEQUENCING OF THE TURBOT *(Scophthalmus maximus;* Pleuronectiformes) A FLATFISH OF HIGH AQUACULTURE VALUE. Figueras A., Corvelo A., Robledo D., Hermida M., Pereiro P., Gómez J., Carreté L., Bello X., Marcet-Houben M., Forn-Cuní G., Abal-Fabeiro J.L., Pardo B.G., Taboada X., Fernández C., Alvarez-Dios J. A., Gómez-Tato A., Viñas A., Maside X., Gabaldón T., Novoa B., Bouza C., Alioto T., **Martínez P.**
	12:10-12:25	**[046]** GENOME SEQUENCING OF 12 PUFFERFISHES. **Hosoya S.**, Tasumi S., Kobayashi H., Kikuchi K.
	12:25-12:40	**[047]** DE NOVO GENOME ASSEMBLY OF THE AFRICAN CATFISH *(Clarias gariepinus).* **Kovács B.**, Barta E., Pongor S. L., Uri Cs., Patócs A., Orbán L., Müller T., Urbányi B.
12:40-13:50	**LUNCH**	
SESSION GENOMIC SELECTION AND MAS **(SPONSORED BY ILLUMINA)** MODERATOR: Prof. Laszlo Orban	13:50-14:05	**[048]** ACCURACY OF POPULATION-WIDE AND WITHIN-FAMILY GENOMIC SELECTION IN ATLANTIC SALMON. **Sonesson A.K.**, Meuwissen T.H.E., Baranski M., Moghadam H., Lillehammer M., Norris A., Bakke H., Lund V.
	14:05-14:20	**[049]** GENOMIC SELECTION FOR BCWD RESISTANCE IN RAINBOW TROUT USING RADSNP AND SNP GENOTYPING PLATFORMS, SINGLE-STEP GBLUP AND BAYESIAN VARIABLE SELECTION MODELS. **Vallejo R.L.**, Leeds T.D., Liu S., Gao G., Welch T.J., Wiens G.D., Palti Y.
	14:20-14:35	**[050]** WITHIN-FAMILY GENOMIC SELECTION IN AQUACULTURE BREEDING PROGRAMMES. Saura M., Villanueva B., Fernández J., **Toro M.A.**
	14:35-14:50	**[051]** DEVELOPMENT OF GENOMIC RESOURCES AND WHOLE GENOME PREDICTION IN PACIFIC WHITE-LEG SHRIMP (LITOPENAEUS VANNAMEI). **Jerry D.R.**, Raadsma H.W., Khatkar M.S.., Prochaska J., van der Steen H.., Jones D.B., Zenger K.R..

SESSION GENOMIC SELECTION AND MAS **(SPONSORED BY ILLUMINA)** MODERATOR: Prof. Laszlo Orban	14:50-15:05	**[052]** QTL-SELECTION CONTRIBUTES TO INCREASED RESISTANCE TO CARDIOMYOPATHY SYNDROME (CMS) IN ATLANTIC SALMON *(Salmo salar* L.). **Kjøglum S.,** Moen T., Korsvoll S.A., Ødegård J., Santi N.
	15:05-15:20	**[053]** GENOTYPING-BY-SEQUENCING USING CUSTOM ION AMPLISEQ™ TECHNOLOGY AS A TOOL FOR GENOMIC SELECTION IN ATLANTIC SALMON *(Salmo salar).* **Baranski M.,** Jowdy C., Moghadam H., Norris A., Bakke H., Sonesson A., Meuwissen T., Lillehammer M., Lund V.
	15:20-15:35	**[054]** EFFECT OF IMPUTED MARKER GENOTYPES ON ACCURACY OF GENOMIC SELECTION IN AQUACULTURE POPULATIONS. **Vela-Avitúa S.**, Ødegård J.
	15:35-15:50	**[055]** CANDIDATE GROWTH GENES IDENTIFIED BY QTL FINE MAPPING IN BIGHEAD CARP *Aristichthys nobilis.* Sun Y.H., Liu H.Y., Feng X., Yu X., Fu B.D., **Tong J.**
	15:50-16:05	**[056]** ESTIMATES OF HERITABILITY FOR DISEASE RESISTANCE TO SRS USING GENOMIC RELATIONSHIPS PREDICTED USING HIGH DENSITY SNP DATA IN ATLANTIC SALMON AND RAINBOW TROUT. **Martínez V.**, Santi N., Odegard J., Moen T.
	16:05-16:20	**[057]** A GENOME-WIDE ASSOCIATION STUDY FOR SEX DETERMINATION IN ATLANTIC SALMON. **Covelo-Soto L.**, Morán P., Kent M.P., Saura M.
16:20-16:40	**COFFEE BREAK**	
16:40-17:45	**BROKERAGE/POSTER SESSION**	
17:45-19:15	**ROUND TABLE (TECHNOLOGICAL TRANSFER). MODERATOR: MS. ROSA FERNÁNDEZ (CETMAR)**	

Ms. Ana Riaza, Stolt Sea Farm S.A. (ES)
Dr. Marine Herlin, Culmarex S.A. (ES)
Dr. Pierrick Haffray, SYSAAF, Syndicat des Sélectionneurs Avicoles et Aquacoles (FR)
Dr. Anna Kristina Sonesson, Nofima (NO)
Mr. Courtney Hough, Federation of European Aquaculture Producers and European Aquaculture Technology Platform (EU)

THURSDAY, 25th JUNE 2015

SESSION FUNCTIONAL GENOMICS 1 MODERATOR: Dr. David Penman	8:30-9:00	**Invited Conference**
		[S4] INTEGRATING EPIGENETICS INTO AQUACULTURE RESEARCH **(Dr. Francesc Piferrer)**
	09:00-09:15	**[058]** THE EFFECTS OF EARLY LIFE STRESS ON THE EPIGENOME AND TRANSCRIPTOME OF ATLANTIC SALMON *(Salmo salar).* **Moghadam H.**, Tveiten H., Robinson N., Andersen Ø., Burgerhout E., Johnsen H.
	09:15-09:30	**[059]** GENOME-WIDE ANALYSIS OF DNA METHYLATION OF ATLANTIC SALMON IN RESPONSE TO STRESS. Covelo L, Reyes D., González R., Pérez-Figueroa A., Morán P., **Vidal R.**
	09:30-09:45	**[060]** GENE EXPRESSION PROFILES DEFINING HOST RESISTANCE TO INFECTIOUS PANCREATIC NECROSIS VIRUS IN ATLANTIC SALMON FRY. **Houston R.D.**, Taggart J.B., Bishop S.C., Bron J.E., Bekaert M.B.
	09:45-10:00	**[061]** GENE EXPRESSION PROFILE ANALYSIS OF MANILA CLAM *(Ruditapes philippinarum)* HEMOCYTES AFTER A *Vibrio alginolyticus* OR *Perkinsus olseni* CHALLENGE USING AN IMMUNE-ENRICHED OLIGO-MICROARRAY. **Moreira R.**, Romero A., Milan M., Bargelloni L., Novoa B., Figueras A.
	10:00-10:15	**[062]** COMPARATIVE ANALYSIS OF MICRORNAs TRANSCRIPTOME EXPRESSION IN CHITRALADA, RED STIRLING AND IN CROSSBRED NILE TILAPIA *(Oreochromis niloticus)* USING HIGH THROUGHPUT SEQUENCING. **Herkenhoff M.E.**, Bovolenta L.A., Dias M.A.D., Hilsdorf A.W., Pinhal D.
	10:15-10:30	**[063]** FROM TILAPIA'S COMPARATIVE TRANSCRIPTOME ANALYSIS TO CHARACTERIZATION OF NUTRIENT TRANSPORTERS. Rozenberg P., Ronkin D., Nitzan T., Seroussi E., Doron-Faigenboim A., **Cnaani A.**
	10:30-10:45	**[064]** TRANSCRIPTOME AND MICRO-RNA ANALYSIS REVEALS NOVEL INSIGHTS INTO DEVELOPMENT OF INTERMUSCULAR BONE IN TELEOSTS. **Gao Z.X.**, Wang W.M., Yi S.K., Wan S.M., Chen B.X.
	10:45-11:00	**[065]** STRESS SPECIFIC GENE EXPRESSION PATTERNS IN RELATION TO EARLY LIFE STRESS IN THE GILTHEAD SEA BREAM *(Sparus aurata).* **Sarropoulou E.**, Tsalafouta A., Sundaram A.Y.M., Papandroulakis N., Oulas A., Leithaug M., Gilfillan G.D., Kotoula G., Pavlidis M.

11:00-11:20	COFFEE BREAK	
SESSION SEX CONTROL 1 MODERATOR: Dr. Francesc Piferrer	11:20-11:40	**Short review**
		[S10] RECENT ADVANCES IN ANALYSING SEX DETERMINATION IN FISH **(Dr. David Penman)**
	11:40-11:55	[066] INCIPIENT TRANSITION OF A SEX DETERMINING GENE IN *Takifugu* PUFFERFISH. Ieda R., Hosoya S., Tasumi S., Suzuki S., **Kikuchi K.**
	11:55-12:10	[067] SEX CHROMOSOME EVOLUTION AND MECHANISM FOR SEX DETERMINATION AND REVERSAL REVEALED BY WHOLE-GENOME SEQUENCING AND METHYLATION SEQUENCING IN HALF-SMOOTH TONGUE SOLE *Cynoglossus semilaevis.* **Chen S.L.**, Zhang G.J., Shao C.W., Huang Q.F., Liu G. , Song W.T., Sha Z.X., Xie M.S., Liu Y., Wang N., Yang C.G., Hu Q.M., Schartl M., Tang Q.S., Wang J.
	12:10-12:25	[068] EPIGENETIC CHANGES OF SEX GENES INDUCE SEX REVERSAL IN BARRAMUNDI *Lates calcarifer.* **Domingos J.A.**, Budd A.M., Banh Q.Q., Zenger K.R., Jerry D.R.
	12:25-12:40	[069] FAST TURNOVER OF SEX DETERMINATION AND GENOMIC INCOMPATIBILITIES IN HYBRIDIZING SCULPINS (Cottus). **Cheng J.**, Nolte A.W.
12:40-13:50	LUNCH	
SESSION SEX CONTROL 2/ BIOTECHNOLOGY MODERATOR: Dr. Elena Sarrapoulou	13:50-14:05	**Parasite EU project (Dr. Ángel González)**
		[S13] BIOBANKING: THE PRESENT AND FUTURE OF FRESHWATER AND MARINE SAMPLING COLLECTION
	14:05-14:20	[070] INVESTIGATING THE GENETICS OF SEX DETERMINATION IN EUROPEAN SEA BASS *(Dicentrarchus labrax)* USING RAD-SEQ. Palaiokostas C., Bekaert M., Taggart J.B., Gharbi K., McAndrew B.J., Chatain B., **Penman D.J.**, Vandeputte M.
	14:20-14:35	[071] DOES EARLY GROWTH PLAY A ROLE IN THE SEX DETERMINATION OF EUROPEAN SEABASS *Dicentrarchus labrax?* **Vandeputte M.**, Horri K., Allal F., Ferrari S., Vidal M.O., Ruelle F., Bégout M.L., Chatain B.

SESSION SEX CONTROL 2/ BIOTECHNOLOGY MODERATOR: Dr. Elena Sarrapoulou	14:35-14:50	**[072]**	GENE EXPRESSION ANALYSIS AT THE ONSET OF SEX DIFFERENTIATION IN TURBOT *(Scophthalmus maximus)* AT DIFFERENT REARING TEMPERATURES. **Robledo D.**, Ribas L., Cal R., Sánchez L., Piferrer F., Martínez P., Viñas A.
	14:50-15:05	**[073]**	FEMALE SPECIFIC MARKERS AND ATTEMPTS OF ALL-FEMALE PRODUCTION IN HALF-SMOOTH TONGUE SOLE *Cynoglossus semilaevis.* **Zhang Q.**, Wang X., Yu H., Wang Z., Qi J., He Y.
	15:05-15:20	**[074]**	INVENTING TETRAPLOID BREEDING FOR ANIMALS USING THE EASTERN OYSTER *C. virginica* AS THE MODEL. **Allen Jr. S.K.**, Kube P., Small J.
	15:20-15:35	**[075]**	ENVIRONMENTAL DNA (eDNA): A NEW FORENSIC TECHNIQUE TO DETECT PATHOGENS IN FARMED FISH. **Gomes G.B.**, Miller T.L., Hutson K.S., Jerry D.R.
	15:35-15:50	**[076]**	A BAC TRANSGENIC ANALYSIS OF THE asip1 LOCUS REVEALS DEVELOPMENTAL MECHANISMS OF DORSO-VENTRAL PIGMENTATION IN FISH. **Cal L.**, Gómez-Marín C., Gómez-Skarmeta J.L., Cerdá-Reverter J.M., Kelsh R.N., Rotllant J.
	15:50-16:05	**[077]**	VERIFICATION OF ISOGENIC NATURE OF CLONAL LINES IN THE ATLANTIC SALMON *(Salmo salar)* THROUGH ddRADseq. **Oral M.**, Taggart J.B., McAndrew B.J., Penman D.J., Fjelldal P.G., Hansen T.
16:05-20:00	**VISIT TO STOLT SEA FARM S.A. FACILITIES**		
21:00-24:00	**GALA DINNER (Not Included in the registration fee)**		

FRIDAY, 26th JUNE 2015

SESSION FUNCTIONAL GENOMICS 2 MODERATOR: Prof. Laura Sánchez	09:15-09:30	**[078]**	TISSUE-SPECIFIC TRANSCRIPTOMES OF *Mytilus galloprovincialis* REVEAL NEW FUNCTIONS. **Moreira R.**, Canchaya C., Novoa B., Posada D., Figueras A.
	09:30-09:45	**[079]**	APPLICATION OF RNA-SEQ IN INVESTIGATING A MAJOR PARASITIC DISEASE OF TURBOT *(Scophthalmus maximus),* ENTEROMYXOSIS. **Ronza P.**, Robledo D., Losada A.P., Bermúdez R., Pardo B.G., Martínez P., Quiroga M.I.

SESSION FUNCTIONAL GENOMICS 2 MODERATOR: Prof. Laura Sánchez	09:45-10:00	**[080]** EXPLORING THE GENETIC BASIS OF RESISTANCE TO PANCREAS DISEASE IN ATLANTIC SALMON *(Salmo salar).* **Gonen S.**, Baranski M., Thorland I., Norris A., Grove H., Arnesen P., Bakke H., Lien S., Bishop S.C., Houston R.D.
	10:00-10:15	**[081]** ANNOTATION OF *Seriola lalandi* REFERENCE TRANSCRIPTOME OF LARVAE AND DIFERENTIAL GENE EXPRESSION BETWEEN NORMAL AND SKELETAL DEFORMED INDIVIDUALS. **Patel A.**, Hernandez E., Barra V., Martinez V.
	10:15-10:30	**[082]** ALLELE SPECIFIC EXPRESSION ON LIVER AND HEAD KIDNEY OF *Salmo salar* WITH DIFFERENTIAL SUSCEPTIBILITY TO THE CHALLENGE WITH Piscirickettsia salmonis. **Dettleff P.**, Martínez V.
	10:45-11:00	**[083]** POPULATION GENETICS AND TRANSCRIPTOMICS OF MANILA CLAM *(Ruditapes philippinarum)* AND CARPET-SHELL CLAM *(R. decussatus)*: IMPLICATIONS FOR AQUACULTURE. **Saavedra C.**, Milan M., Cordero D., Leite R., Peña J.B., Delgado M., Liu B., Ruesink J., Cancela L., Bargelloni L., Patarnello T.
	10:45-11:00	**[084]** IDENTIFICATION OF BACTERIAL COMMUNITY COMPOSITION IN TILAPIA BIOFLOC SYSTEM UNDER DIFFERENT ENVIRONMENTAL CONDITIONS USING PCR-DGGE TECHNIQUE. **Suloma A.**, Mabroke R.S, Tahoun A.M., Zidan A.N., El-Menofy W., Adam M. and El-Shafiey M.H.M.
10.45-11.05	**COFFEE BREAK**	
SESSION BREEDING PROGRAMS 6 MODERATOR: Prof. Uthairat Na-Nakorn	11:20-11:35	**[085]** GENETIC VARIATION IN PACIFIC OYSTERS *(Crassostrea gigas)* FOR RESISTANCE TO *Ostreid herpesvirus-l.* **Kube P.D.**, Dove M.C., Cunningham M., Kirkland P.D., O'Connor W.A., Elliott N.G.
	11:35-11:50	**[086]** A FIRST STEP FOR SUSTAINABLE BREEDING PROGRAMMES IN PIKEPERCH *(Sander lucioperca)* THROUGH THE EVALUATION OF THE GENETIC VARIATION IN DOMESTICATED BROODSTOCKS AND NATURAL POPULATIONS. Tsaparis D., Kyriakis D., Ekonomaki K., Darivianakis S., Fontaine P., **Tsigenopoulos C.S.**
	11:50-12:05	**[087]** RECORD OF LACK OF INTERMUSCULAR BONES IN SPECIMENS OF *Colossoma macropomum* (Characiformes): UNUSUAL PHENOTYPE TO BE INCORPORATED INTO GENETIC IMPROVEMENT PROGRAMS. Perazza C.A., Menezes J.T.B., Silva L.A., Pinaffi F.V., Ferraz J.B.S., **Hilsdorf A.W.S.**

12:05-13:00	**General Assembly of International Association of Genetics in Aquaculture, closing ceremony, awards and selection of venue for ISGA XIII**
13:00-14:10	**LUNCH**
17:00-19:00	**VISIT TO SANTIAGO DE COMPOSTELA**

SATURDAY, 27th JUNE 2015

| 9:00-19:00 | **GALICIAN TOURIST TOUR** |

Invited communications

THE HISTORY OF AQUACULTURE AND BREEDING PROGRAMS IN GALICIA AND SPAIN

Martínez P.

Departamento de Genética, Facultad de Veterinaria, Universidad de Santiago de Compostela, Lugo, 27002, Spain

Spain, as part of the Iberian Peninsula, has a long coastal line (~5,000 km) and its surface is crossed by a dense hydrographic network, all in all providing a rich marine and continental fauna, which explains the tradition of Spaniards for seafood (41 kg/person/year; 8th world ranking). Although Galicia (NW Spain) represents only 6% of the Spanish territory, it includes 25% of the Spanish coast, and thus, it is by large the main seafood producer region. Galician Rías, originated by continental subsidence and the subsequent seawater invasion of fluvial valleys, represent one of the most productive ecosystems in the world. A great variety of mollusk, fish and crustacean are part of Galician "marisco", the most appreciated in Spain. Seafood production has always represented a key area of Galician economy.

The first activities related to aquaculture in Spain come from Romans, who constructed the first nurseries for growing fish, mollusk and crustaceans. Later, in the Middle Ages, monasteries and abbeys developed the first aquaculture technology. The "Monasterio de Piedra", founded in 1865, was the first fish farm in Spain devoted to stocking, education and research. By the same time, the richness of Galician Rías attracted an investment for improving seafood collection and especially, for their safety storage either in salt or as canned food. The Galician rafts ("bateas") were established by the mid XX century, representing now part of its sea landscape. Fish culture in Galicia began with trout in the 60's, and the first marine fish cultured was the turbot. This is a highly appreciated flatfish species, and although its current production is around 10,000 tons in Spain, its economic value is close to mussel (200,000 tons).

Flatfish, and especially the turbot, are the species where genetic technology has been mostly developed. Breeding programs in this turbot run since 90's, and the three main companies are now in the fifth generation of selection. Growth has been the main target, but resistance to pathologies and sex control have also involved an important effort. Genomic resources in the turbot include dense genetic map and microarrays, and recently the whole genome. The architecture of the main turbot traits has been addressed through QTL and candidate genes analysis, and gene expression patterns have been investigated in response to the main pathogens. Breeding programs also exist for seabream and seabass (growth rate), and within mollusk, a first breeding program was developed for resistance to bonamiosis, and in Mediterranean mussel the basis for decreasing toxin accumulation has been evaluated.

Keywords: Aquaculture, history, Spain, flatfish, genetics

[§] Corresponding author. Tel: +34 982822425: Fax: +34 982822428
E-mail address: paulino.martinez@usc.es

GENOMIC SELECTION: FROM LIVESTOCK TO AQUACULTURE APPLICATIONS

Woolliams J.A.

The Roslin Institute, University of Edinburgh, Easter Bush, Midlothian EH25 9RG, U.K.

Genomic selection, both in concept and in the underlying technology, has been very liberating for breeders, particularly for livestock. We have been able to break free from the idea that selection accuracy was static, determined by the physical structure of a breeding scheme e.g. the number of candidates and family structure, and at least consider whether the only boundary to achieving perfect accuracy was how big a training set could be accumulated, and whether the limit to precision breeding was the phenotypes we could collect. However implementation of genomic selection has proved challenging, except perhaps in dairy cattle, where the cost and structure of progeny testing offered both a ready incentive and a low-risk implementation route. Nevertheless all major livestock sectors are now at varying stages of implementation and the approaches are being adapted for plant and forest breeding. Implementation in aquaculture has been hampered by the efforts required to develop both the genomic tools, training sets and applications that are cost-effective. This has meant that only the most advanced sectors such as salmon breeding has been able to take make use of the technology. One barrier has been the cost of developing the huge genomic training sets required to deliver high accuracy across the population, and the challenge for breeders is to drive down the unit cost of genomic information. However the use of sequencing technology combined with imputation has brought about massive genomic data sets, with training populations of several hundred thousand, all with full sequence and phenotype data. It re-opens the question of how close can breeders get to perfect accuracy for the full range of economically important traits. However delivering precision breeding in a species requires us to have a much deeper understanding of the correlated responses that come from our selection procedures, and would allow us to better address issues such as genotype by environment interactions. The recent emergence of large 'encode' type projects for livestock, e.g. such as the international consortium FAANG, offer the vision that the biological priors necessary to make predictions of such genetic correlations may also be feasible within the decade.

Corresponding author. Tel.: + 44 131 651 9100; Fax: + 44 131 651 9105
E-mail address: john.woolliams@roslin.ed.ac.uk

GENOMIC ADVANCES AND APPLICATIONS IN AQUACULTURE

Liu Z.J.

Auburn University, Auburn, Alabama, USA

The channel catfish genome has been completely sequenced. In this presentation, progress related improvements of whole genome sequence assembly, scaffolding, and construction of chromosome-level sequence builds will be presented. With the draft or reference genome sequences, the relationship of whole genome variations and performance or production traits can be dissected using RNA-Seq, bulk segregant RNA-Seq (BSR-Seq), or genome-wide association studies (GWAS). We have devoted much effort towards understanding the catfish disease resistance and low oxygen tolerance using genomic approaches including genome sequencing, assembly and annotation, expression after bacterial infection and low oxygen challenge. Earlier research focused on a large set of known genes using microarrays, whereas our most recent studies have adopted the next generation sequencing approaches such as RNA-Seq, BSR-Seq, and GWAS for the analysis of candidate genes involved in disease resistance and low oxygen tolerance. Harnessing genomic techniques, especially GWAS work using the high density 250K SNP array, and ongoing work for the development of the 675K SNP array, and BSR-Seq promise both a better understanding of teleost disease resistance and low oxygen tolerance, and the potential for practical applications in aquaculture.

[§] Corresponding author: Tel: 334-844-4784
E-mail address: liuzhan@auburn.edu

INTEGRATING EPIGENETICS INTO AQUACULTURE RESEARCH

Piferrer F.§, Anastasiadi D., Díaz N., Ribas L.

Dept. Renewable Marine Resources, Inst. Marine Sciences (CSIC), Barcelona, 08003, Spain

Epigenetics is an exciting and fast developing area of biology that deals with the study of changes in gene expression and function that, nevertheless, do not involve alterations of the genotype. These changes can be heritable through cell mitoses and meiosis and thus through individual generations. Three major epigenetic mechanisms that can activate or suppress gene expression are recognized: DNA methylation, modification of chromatin structure through changes in histone proteins, and regulatory processes mediated by small RNAs. Epigenetic changes are being actively studied in some areas of biology such as developmental biology and for its implications in human health, notably in cancer. However, less attention has been paid in other fields, including evolutionary biology, ecology and, pertaining to our interest, animal production. Since epigenetic processes have been found to be responsible for the integration of both biotic and abiotic factors, epigenetics can help to understand how organisms respond a particular environment. In addition, epigenetic variation may explain the phenotypic variation in production-relevant traits observed in farmed populations. Thus, epigenetic variation can be considered in the light of selection programs to increase animal production. Particularly interesting is the link between conditions during early development, where many heritable epigenetic marks are established, and gene function later in life since proper management of these early conditions may contribute to better growth and health of farmed animals. Because of their external fertilization, aquatic animals in general and fish in particular are excellent research subjects where to study epigenetic changes. These various aspects of epigenetics will be discussed. Among others, an example will be provided on how modifications of a major environmental cue such as temperature is integrated through an epigenetic mechanism, ultimately determining phenotypic sex and growth in a production fish. *Supported by Spanish government grant AGL2013-41047-R ("EPI-FARM") to F.P.*

Keywords: *DNA methylation, histone modifications, miRNAs, epigenetic marks, transgenerational effects, animal farming*

§ Corresponding author. Tel.: +34-932309567 Fax: +34-93209555
E-mail address: piferrer@icm.csic.es

ASIAN SEABASS GENOME PROJECT: A STATUS REPORT

Orbán L.* & The Asian Seabass Genome Consortium**

Temasek Life Sciences Laboratory and Agri-Food & Veterinary Authority (both Singapore) have been working on the improvement Asian seabass lines through marker-assisted selection. Under the umbrella of a research program supported by the National Research Foundation of Singapore, we have started to develop advanced platforms for Asian seabass *(Lates calcarifer)* and Mozambique tilapia *(Oreochromis mossambicus)* with the aim of developing genomic selection for both species.

In my presentation, I will describe the sequencing, assembly and annotation of the Asian seabass genome (700 Mb). I will compare the usefulness of different technologies for generating high quality reference genomes (de novo sequencing) from fish. In addition, I will also describe selected set of genomic platforms that have been developed based on the information obtained from the genome assembly. A few examples will also be presented for the use of these platforms to understand more about the genetic diversity of Asian seabass, as well as its response to different nutritional conditions and pathogens.

In parallel with the studies described above, we have also been working on the genome of the Mozambique tilapia (1 Gb) using a more affordable approach. At the end of my talk, I will provide a brief status report for that genome as well.

* Corresponding author. Tel: +65 68727413; Fax: +65 68727007
E-mail address: laszlo@tll.org.sg
** The Asian Seabass Genome Consortium consists of over 40 scientists from the following organizations: Temasek Life Sciences Laboratory, Singapore; Comparative Genome Centre, Murdoch University, Perth, Australia; Georgikon Faculty, University of Pannonia, Keszthely, Hungary; Institute of Cytology of the Russian Academy of Sciences, St. Petersburg, Russia; South African National Bioinformatics Institute, University of the Western Cape, Bellville, South Africa; Pacific Biosciences, Menlo Park, CA, USA; Theodosius Dobzhansky Center for Genome Bioinformatics, St Petersburg State University, St Petersburg, Russia; Genome Research Centre, The Chinese University of Hong Kong, Hong Kong; University of East Anglia, Norwich Research Park, Norwich, UK; The Genome Analysis Centre, Norwich, UK; Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, USA; James Cook University, Townsville, Australia; Central Institute of Brackishwater Aquaculture, Chennai, India; National Bureau of Fish Genetic Resources, Kochi, India; CSIR-Institute of Genomics and Integrative Biology, New Delhi, India

CURRENT STATUS OF THE ASIAN SEABASS BREEDING PROGRAM

Yue G.H.[1], Orbán L.[1] & Lim H.S.[2]

[1] *Temasek Life Sciences Laboratory, 117604 Singapore. Email: genhua@tll.org.sg*
[2] *Agri-Food and Veterinary Authority of Singapore, Singapore*

The Asian seabass (*Lates calcarifer*) is an important marine foodfish and has been cultured for food in Southeast Asia since the 1980s. The aquaculture industry of this fish is growing at a rapid rate. In Singapore, with the strong support from Singapore government, we started a breeding program for Asian seabass to improve its growth performance, disease resistance and meat quality in 2004. After two generations of family-based selection in mass crosses with molecular parentage analysis, we have increased the growth of Asian seabass by over 40% as compared to the control. Using progeny test, we have identified brooders resistant to the big belly disease and established one line of Asian seabass resistant to the disease. Using QTL mapping, we identified DNA markers associated with growth and meat quality traits, and established one line for quick growth and another line for high content of omega-3s in flesh. In addition, we have developed a large number of genomic resources to facilitate the breeding program, such as DNA markers, a molecular parentage system, linkage maps, BAC libraries, transcriptomes, draft genome sequence and genotyping by sequencing platform based on NEXTseq 500. In this presentation, I will summarize our traditional and molecular breeding program, and the achievements of this breeding program, as well as the future directions of research.

Keywords: Asian seabass, breeding, QTL, genome, MAS

[§] Corresponding author: Tel: (65) 6872 7405
E-mail address: genhua@tll.org.sg

BREEDING PROGRAMS IN FISH AQUACULTURE: HISTORICAL CONTEXT AND PERSPECTIVES

Neira R.[§a]**, Yáñez J.M.**[b,c]**, and Lhorente J.P.**[c]

[a]*Facultad de Ciencias Agronómicas, Universidad de Chile, Santa Rosa 11315, Santiago, Chile*
[b]*Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, Chile*
[c]*Aquainnovo, Puerto Montt, Chile*

Two thirds of the world farmed food fish produced are finfish species, with 87.4% of it produced inland, mainly carps and tilapias. Although finfish species grown from marine culture represent only 12.6% of the total farmed finfish production by volume, their value (around US$24 billion) represents 27% of the total value of all farmed finfish species and are mostly the carnivorous species, Atlantic and Pacific salmons and rainbow trout. Historical aspects and perspectives of breeding programs of these mentioned species is the main focus of this short review. Although differences within and between fish populations attracted the attention of fish culturist over the past two millennia, was not only since the early 70's of the last century that breeding programs started to use the theoretical basis for selection and outbreeding developed and used with great success in livestock breeding since the 30's and 50's. As the fish culture industry started its significant growth in the 80's, genetic improvement of fish linked to production also began. Out of 104 genetic improvement programs identified in aquaculture in 2010, 86 were in finfish species, 28 of them in tilapias, 30 in salmonids and 8 in carps. Most of these programs include selection for several traits, including growth traits, carcass quality traits and sexual maturity traits. A retrospective review is done looking how traditional selection schemes has been applied to fish breeding and indicating which are the main challenges today to applied genetic improvement of fish. Selection for increased resistance to diseases based on controlled challenge tests is now also included when molecular genetics tools are available. Examples of these are genomic selection using a SNP arrays through fish genotyping and looking for associations between marker alleles and phenotypes and the use of a major QTL explaining most of the genetic variance for resistance to IPN in Atlantic salmon populations. These technologies are now being applied by salmon breeding programs in the Norwegian and Chilean salmon industry.

§Corresponding author. Tel.: +56 22 978 5754
E-mail address: rneirar@gmail.com

SELECTIVE BREEDING IN NILE TILAPIA: ACHIEVEMENTS AND FUTURE DIRECTIONS

Komen H.[a] and Benzie J.[b]*

[a] *Animal breeding and genomics group, Department of animal sciences.*
Wageningen University, Wageningen, 6700 AH, the Netherlands
[b] *WorldFish, Jalan Batu Maung, 11960 Bayan Lepas, Penang, Malaysia.*

We present a review of the current state of selective breeding programs for Nile tilapia (*O.niloticus*). The main traits of interest have been harvest weight, growth rate and body traits. There is now ample evidence of sustained gains of 5~10% per generation, over several generations. Using phenotypic production data from various sources using strains of GIFT origin, we calculated thermal growth coefficients, which show that fish from 12-15 generations of selection grow 4-5 times faster than unselected strains. Extrapolation of these responses in growth rate shows that by generation 30, Nile tilapia might reach harvest weights of 1 kilo and more in less than 4 months. Selection for growth thus far has not been accompanied by any undesirable correlated response in production traits. Responses in fillet yield have been small, and inconclusive. In contrast, there seems to be a correlated response in shape, with later generations of Nile tilapia becoming more rotund. We also show recent results from reproduction experiments that indicate, with a note of caution, positive genetic correlations between production traits and numbers of eggs spawned. A major concern remains the low production efficiency that is often observed in the field. Problems occur during reproduction, with increased time needed to produce families, and during grow-out in less optimal conditions where farmers are faced with high mortalities and unexpected slow growth. We discuss possible reasons for this, and review the current status of our knowledge on genotype by environment interaction. We conclude that future research should focus on the use of bio-economic models to predict the impact of selective breeding on smallholder farmers, and on understanding feed efficiency as the main driver for economic and environmental profitability.

Keywords: Nile tilapia, selective breeding, production efficiency, GIFT, GxE

*These authors have contributed equally to this work
§ Corresponding author. Tel.: + 31317482465
E-mail address: hans.komen@wur.nl

A REVIEW OF SHELLFISH BREEDING PROGRAMS

Allen Jr. S.K.

Aquaculture Genetics and Breeding Technology Center, VIMS, Gloucester Point, Virginia, USA

Genetic improvement for shellfish species can broadly be divided into three major activities: selection for disease resistance, selection for production traits, and triploidy. Moreover, the scope of these activities is wide, ranging from primary research to selection experiments to full scale breeding programs, with publication inversely related to this order of activities. The most attention has been given to disease resistance, the penultimate motivation for breeding programs. Nearly all attempts have realized gains in selecting for resistance, including MSX-disease, JOD, and Dermo-disease in the Eastern oyster, *Crassostrea virginica*, summer mortality and OsHV-1 in *C. gigas*, QX and winter mortality in *Saccostrea glomerata*, bonamiosis in *Ostrea edulis*, and Withering syndrome in abalone. Until recently, selection programs for *C. gigas* have concentrated on production traits, as few diseases afflicted this species. Now, with the onset of OsHV-1 induced mortalities, several programs have incorporated selection against this virus into their programs, notably New Zealand, Australia, and France, although in the latter case, there is a disconnect between research and industry use, such that, many commercial hatcheries have instigated their own breeding programs. Conversely, some programs that began with selection for disease resistance have realized signicant gains and now adopted production traits, such as, in the Eastern oyster. Overall, however, actual breeding programs that have maintained a continuity of effort for multiple generations are rare in molluscan species, primarily because the vast majority of seed is still obtained from wild collection, not through hatchery production. The small constituency of users of hatchery seed makes it difficult to support sustained funding of breeding programs. The use of triploids, in oysters in particular, has become an important mode of instantaneous genetic improvement world-wide. While triploids in multiple shellfish species have been induced for experimental purposes, commercial triploids are largely restricted to *C. gigas* and produced by tetraploid x diploid crosses in the hatchery. In nearly all cases, triploids yield a significant quantum improvement over their diploid counterparts and often confer higher survival, implying greater resistance to disease. Breeding of tetraploids themselves is a recent activity and innovation in this field has come from alternative methods for producing them. That tetraploid breeding needs to go hand in hand with diploid breeding has been demonstrated in places like France, Australia, and mid-Atlantic USA.

Keywords: shellfish, breeding, polyploidy, disease resistance

Corresponding author. Tel.: +1-804-684-7710; Fax: +1-804-684-7717
E-mail address: ska@vims.edu

RECENT ADVANCES IN ANALYSING SEX DETERMINATION IN FISH

Penman D.J.

*Institute of Aquaculture, School of Natural Sciences, University of Stirling,
Stirling FK9 4LA, Scotland, United Kingdom*

For many (but not all) aquaculture species, control over sex ratio, maturation and reproduction is of importance to achieve optimal production. Fundamental to much of this is an understanding of sex determination, which is very diverse in these organisms. This diversity, including gonochorism and hermaphroditism, genetic sex determination and environmental sex determination (sometimes both GSD and TISD in the same species), and genetic influences ranging from a single master locus to polygeny, left scientists with some difficulty in starting analysis of sex determination in a "new" species. Recent advances in genomics technologies have provided much more rapid and efficient ways to analyse sex determination. Some of these approaches and their applications will be reviewed, along with other recent approaches to controlling sex ratio and maturation in farmed species.

§ Corresponding author: Tel: +44 [0]1786 467901
E-mail address: d.j.penman@stir.ac.uk

FISHBOOST- AN EU-FP7 PROJECT TO ADVANCE EUROPEAN AQUACULTURE BREEDING FOR SIX FINFISH SPECIES

Sonesson A.K.

Nofima, Ås, 1430, Norway

In Europe, there is large diversity among the aquaculture breeding programs and some breeding programs contain only the very basic components. Hence, there is a large potential to increase efficiency and profit by domestication and genetic improvement of farmed finfish. The main challenge of the EU-FP7 project FISHBOOST is to realise this potential into economic and social acceptable breeding schemes, and advance these for each of the six target species. Acknowledging the different capacities of the species, the aim of FISHBOOST is: 'To improve the efficiency and profitability of European aquaculture by advancing selective breeding to the next level for each of the six main finfish species through collaborative research with industry'. FISHBOOST considers the main components of breeding programs for Atlantic salmon, common carp, European seabass, gilthead seabream, rainbow trout and turbot. 26 RTD, SME and NGO participants in Europe working in aquaculture breeding collaborate from 2014 until 2019 in this comprehensive research project. A mixture of low- and high-technological advances depending on current capacities of the species are developed to advance each species' breeding program to the next level. For the six finfish species, the project develops recording protocols for defining new traits for the breeding goal on production efficiency and disease resistance, heritability and genetic correlations are estimated and the genomics field are being further developed through improved RAD sequencing protocols, QTL mapping, and genomic selection methodologies. Recommendations on optimized breeding programs for the six finfish species are based on economic assessments and perceptions of producers and European representative organizations. Results are disseminated to the public, aquaculture producers and the research community through a comprehensive scheme. For more details: www.fishboost.eu.

Keywords: aquaculture breeding, finfish species, EU project

[§] Corresponding author. Tel.: +47 93098047
E-mail address: anna.sonesson@nofima.no

THE DEVELOPMENT OF TOOLS FOR TRACING AND EVALUATING THE GENETIC IMPACT OF FISH FROM AQUACULTURE: "AQUATRACE"

Bargelloni L.[a]§, AquaTrace Consortium, Nielsen E.E. [b]

[a] *Department of Comparative Biomedicine and Food Science,*
University of Padova, Legnaro, 35020, Italy
[b] *Section for Population Ecology and Genetics, National Institute of Aquatic Resources,*
Technical University of Denmark, Silkeborg, DK-8600, Denmark

The genetic changes associated with domestication in aquaculture pose an increasing threat to the integrity of native fish gene pools. Consequently, there is a bourgeoning need for the development of molecular tools to assess and monitor the genetic impact of escaped or released farmed fish. In addition, exploration of basic links between genetic differences among farmed and wild fish and differences in important life-history traits with fitness consequences are crucial prerequisites for designing biologically informed management strategies. To respond to such a challenge, the EU-funded project "AquaTrace" was set up, gathering 22 academic and industrial partners from 10 European countries. Started in November 2012, AquaTrace just passed its midterm turning point. Already accomplished tasks are an industrial survey, which involved 29 breeding companies and provided detailed information on existing selective breeding programs in Europe, an in-depth overview of existing knowledge on AquaTrace target species, and an extensive sampling of farmed and natural populations for three major cultured fish species, turbot (>1,500 specimens), European sea bass (>4,000), and gilthead sea bream (>3,500). On these samples, cutting-edge genomic methods (ddRAD and NGS) are currently being applied for the development of high-powered, cost-efficient, forensically validated and transferable DNA based tools for identifying and tracing the impact of farmed fish in the wild. Preliminary data show a large set of SNPs discovered and genotyped on 1,500-2,000 individuals. Controlled experiments with wild and farmed fish and their hybrids have been performed with salmon and brown trout as model organisms using advanced "common garden" facilities. These experiments will elucidate the fundamental consequences of introgression by pinpointing and assessing the effects on fitness of specific genomic regions.

Generated insights will form the basis of a risk assessment and management recommendations including suggestions for mitigation and associated costs. This information and the developed molecular tools will be available as open-access support to project participants and external stakeholders including the aquaculture industry. The project is expected to facilitate technology transfer to the aquaculture sector by promoting better tailored breeding practices and traceability throughout production chain. Overall this initiative will support the development of sustainable European aquaculture.

Keywords: Genetic impact, introgression, traceability, SNPs, common garden

§ Corresponding author. Tel.: +39-049-8272506; Fax: + 39-049-8272973
E-mail address: luca.bargelloni@unipd.it

BIOBANKING: THE PRESENT AND FUTURE OF FRESHWATER AND MARINE SAMPLING COLLECTION

González A.F.[a][§], Pascual S. [a], Outeiriño L.[b], Vello C.[b]

[a] *Instituto de Investigaciones Marinas (CSIC). Eduardo Cabello 6, 36208 Vigo*
[b] *Grupo 3 Comercial Hospitalaria, Parque Empresarial Porto do Molle, 36350 Nigrán*

Currently, a major trend in environmental research consists in addressing global phenomena around the study of large series of samples organized with well-defined and detailed criteria, with the specific information required in each case. The availability of traceable samples and associated information of high quality has been a plea that has been at the root of many of the most important advances in marine science. Typically, the collection of significant numbers of marine samples and quality information involves a major effort in planning, construction, and finally operation; it is very time and resource consuming constraining the accurate development of the marine research. Therefore, the promotion and implementation of biobanks facilitate the access to quality samples (and associated data) by researchers who present a project with the appropriate scientific organization and with the proper ethical and legal safeguards. This represents an essential milestone in shortening the time that normally elapses between research and the application of its results, improving also the effectiveness of research. To enhance this effort and the desired impact, constant coordination and collaboration between biobanks and conceptually related initiatives is required, and within each of them among the different professionals involved in processing the samples and their associated information: identification of the extractions (samples), sampling itself, processing, storage, distribution, transfer, use of samples and associated information, and overall management of these proceedings. The main distinguishing feature of biobanks, as currently understood, with respect to the classical concept of a collection of samples and associated data (collection of a research group, institutional collection, or private collection), is its commitment to transferring samples and associated information to research groups in an open, transparent, and partnering way for the benefit and improvement of high-quality science. This distinguishing feature is unambiguously reflected in the current definition of a biobank drawn up by the OECD as a Biological Resource Centre. The biological biobanks scheme and infrastructure we developed is expected to provide a number of outcomes that will boost the development of a network of biobanks, first in parasitology and later in other scientific fields.

Keywords: Biobanks, Parasitology, Freshwater and Marine studies.

[§] Corresponding author. Tel.: + 34986231930 (Ext 183); Fax: + 34 986292762
E-mail address: afg@iim.csic.es

Oral presentations

CURRENT STATUS OF SELECTIVE BREEDING IN EUROPEAN AQUACULTURE

Janssen K.[a, §], Chavanne H.[b], Berentsen P.[c], Komen H.[a]

[a] *Animal Breeding and Genetics Group, Wageningen University, Wageningen, 6708 PB, The Netherlands*
[b] *Università degli Studi di Padova, Department of Public Health, Comparative Pathology and Veterinary Hygiene, Legnaro, 35020, Italy*
[c] *Business Economics Group, Wageningen University, Wageningen 6706 KN, The Netherlands*

For the EU funded FP7 research projects Aquatrace and Fishboost, surveys were conducted among breeding companies of the six main species cultured in Europe: Atlantic salmon, rainbow trout, European seabass, gilthead seabream, common carp and turbot. The objectives were to describe the main characteristics of selective breeding in European aquaculture and to determine its market share in total production. The market share was estimated by comparing the egg or juvenile production originating from breeding companies to the European total egg or juvenile production (in 2012).

For Atlantic salmon all breeding companies performed family selection. Main traits in the breeding goal were growth, processing yield, product quality and disease resistance. Most salmon has been selected for about ten generations. The market share was 93-95%. For rainbow trout most breeding companies performed family selection; selected traits commonly included growth, morphology, processing yield, disease resistance and reproduction. The number of selected generations in mass selection ranged up to 20 and in family selection up to 14. The market share was 65-68%. Most breeding companies of European seabass and gilthead seabream have integrated their breeding program with production, mainly selecting on growth and morphology. Some of the companies that performed family selection also selected on disease resistance, processing yield, product quality or feed efficiency. For European seabass the number of selected generations ranged from two to eight and for gilthead seabream from one to five. The market shares were 43-56% for European seabass and 60-66% for gilthead seabream. In turbot two companies performed family selection and one performed mass selection. The number of selected generations was three to five. The market share was 100%. In common carp genetic improvement was largely based on crossbreeding of different lines. Important traits are scaling pattern, health status, sexual maturity and general appearance.

It is concluded that, based on the volume of fish production, in total over 75% of the European aquaculture production originates from selective breeding, but there is much variation between species. Most breeding companies perform family selection and growth, morphology, disease resistance, processing yield and product quality are most commonly selected traits.

Keywords: Breeding programs, market share, aquaculture, genetics, Europe

§ Corresponding author. Tel.: + 31 317 483581
E-mail address: kasper.janssen@wur.nl

EVOLUTION OF *C. virginica* BREEDING IN CHESAPEAKE BAY, USA: FROM MASS TO FAMILY SELECTION

Moss Small J.[a][§], Kube P. [b], Allen Jr. S.K.[a]

[a]*Aquaculture Genetics and Breeding Technology Center, VIMS, Gloucester Point, VA, USA,*
[b]*CSIRO Agriculture Flagship, Hobart, Tasmania*

The old story about the dramatic loss of natural populations of the eastern oyster, *Crassostrea virginica*, in the Chesapeake Bay, USA has been replaced by a new one: the extraordinary growth of oyster aquaculture. Harvests from oyster culture now exceed those from the wild fishery. At the heart of this development is the oyster breeding activity at the Aquaculture Genetics and Breeding Technology Center (ABC) established at the Virginia Institute of Marine Science in 1997. The initial breeding strategy undertaken targeted disease resistance based on mass selection. Genetically distinct lines derived from the mid-Atlantic were developed for resistance to the protozoan parasite *Haplosporidium nelsoni* (causing MSX disease). To combat Dermo disease, caused by the protozoan *Perkinsus marinus*, Louisiana populations with natural resistance were introgressed. By 2006, 15 lines were being tested by ABC and several were being used by a now growing industry. Because of a steep salinity gradient in the Chesapeake, a salinity-specific selection strategy was necessary to counter the environment x genotype interaction. In 2008, the 15 lines were consolidated to four "Superlines" based on genetic orgin. "Superlines," now disease resistant compared to wild stocks, were subjected to mass selection for fast growth until 2012. Today, these lines are the basis of 95% of total production in the Chesapeake, almost 90% of that is used for triploid oyster production. Following a collaborative project with CSIRO to establish a bio-economic model and associated economic weights from 2011-2013, ABC adopted a family breeding program to enable a more refined breeding approach. Heritabilities for traits deemed important by the Virginia oyster industry – survival, total weight, width index (roundness), cup index (shell depth) and meat yield – were found to be sufficiently high to suggest that substantial improvement in *C. virginica* is likely using family selection. Progeny testing of 130 families produced each year occurs at four field locations (two low salinity/low diease, two moderate salinity/high disease), spanning 8-24ppt. After completion of field testing, estimated breeding values (EBVs) of genetic gains associated with those traits described above are produced using morphometric and pedigree data from the extant populations and past relatives.

[§] Corresponding author. Tel.: + 1 804 684 7955; Fax: + 1 804 684 7717
E-mail address: + jamoss@vims.edu

ADVANCED ANIMAL BREEDING IN AQUACULTURE: USING GENOME-WIDE MOLECULAR BREEDING VALUES FOR RAPID ANIMAL IMPROVEMENT IN THE SILVER-LIPPED PEARL OYSTER.

Zenger K.R.[a, §], Jones D.B.[a], Raadsma H.W.[b], Khatkar M.S.[b], Moser G.[c], Taylor J.T.[d], Toole P.[d], Jerry D.R.[a]

[a] Centre for Sustainable Tropical Fisheries & Aquaculture, and the College of Marine and Environmental Sciences, James Cook University, Townsville QLD, Australia
[b] Reprogen - Animal Bioscience, Faculty of Veterinary Science, University of Sydney, Camden NSW, Australia
[c] Dairy Futures Cooperative Research Centre (CRC), Bundoora, Victoria, Australia
[d] Atlas Pearls and Perfumes, Pertokoan Sanur Raya 18-19, Jalan Bypass Ngurah Rai Sanur, Denpasar Selatan, 80227 Bali

Aquaculture of the silver-lipped pearl oyster, *Pinctada maxima*, is an important tropical aquaculture industry which produces the highly valuable South Sea pearl. Advanced selective breeding programs in the species are limited by the inability to accurately and rapidly identify high-performance animals for selection as parents in structured breeding programs. Although traditional animal improvement methods have had some success with simple traits (i.e. animal growth), they are inefficient for the complex highly valued pearl traits (e.g. size, colour, lustre and shape), which are polygenic (many genes of small effect), hard to measure and have a low to moderate heritability (h^2 from 0.14 to 0.34).

Our research over 8 years in *P. maxima* has produced a suite of thousands of genome-wide single nucleotide polymorphism (SNP) markers, genetic linkage maps, and characterized the quantitative genetic basis of commercially important shell growth and pearl quality traits. Furthermore, using 2,114 pearl grading records from 11 half sib families, 16 putative quantitative trait loci (QTL) accounting for 20.7% to 46.1% of pearl phenotypic variation and 32 genetic associations with effect sizes ranging from 0.1 to 4.3 SD were identified in eight commercially important shell growth and pearl quality traits.

Our research has provided novel insights into the genomic architecture of complex commercial traits and the genome structure of pearl oysters. It provided evidence that key traits have sufficient quantitative genetic variation for genetic selection programs to be effective and sets the foundations for research into genomic selection for difficult to measure traits that will enable increased selection intensity of elite animals at very young ages. Use of genomic selection in the species will be highly efficient leading to reduced generation interval and circumnavigates current limitations associated with sole reliance on phenotypic selection in the pearling industry and long generation intervals. This is the next step in the ongoing research program.

Keywords: Pinctada maxima; Pearl quality; Shell growth, Quantitative genetics, Genomic selection.

§ Corresponding author. Tel.: + 61-7-4781 6532; Fax: + 61-7-4781 4585
E-mail address: kyall.zenger@jcu.edu.au

A COMPARISON OF CLASSIC SELECTION AND MATING METHODS FOR AQUACULTURE BREEDING PROGRAMMES

Saura M.[a§]**, Villanueva B.**[a]**, Fernández J.**[a]**, Toro M.A.**[b]

[a] *Departamento de Mejora Genética Animal, INIA, Madrid, 28040, Spain*
[b] *Departamento de Producción Agraria, ETS Ingenieros Agrónomos, Madrid, 28040, Spain*

As a consequence of the high reproductive capacity of fish species, very large family sizes can be produced in aquaculture breeding programmes. Under this scenario and using stochastic simulations, we compared classic selection methods, including individual, within family, family and sib selection (and some combinations of them), in terms of genetic gain and inbreeding. Ten generations of selection practised on populations composed by 50 families of 100 (or 200 for sib selection scenarios) full-sibs were simulated. We also considered three mating systems: random, assortative and dissasortative. Different scenarios were analyzed by varying selection pressure (p = 2%, 20%), heritability (h^2 = 0.1, 0.4) and common environmental effect (c^2 = 0.0, 0.4). All results presented are those for generation 10. The method that performed the worst was sib selection, as it gave, in general, low responses (about 70% lower than those obtained from individual selection for c^2 = 0.4) and the highest levels of inbreeding (F > 0.9). It is therefore important to implement some control measures on the rate of inbreeding when this selection method is applied in practice. Unacceptable levels of inbreeding were also observed for individual selection with c^2 = 0.4. Performing family selection followed by within family selection could be a valuable alternative as reduces the levels of inbreeding substantially with a small change in gain when compared with individual selection. For c^2 = 0.4, this two-step approach (selecting the best 25 families in the first step) inbreeding levels were reduced by more than 70%, while gain was increased (h^2 = 0.4) or reduced (h^2 = 0.1) by about 7%. This approach could also have the advantage of saving resources. The effect of the mating system on both genetic gain and inbreeding was minimal in most cases. The highest difference between assortative and random mating was observed for sib selection with h^2 = c^2 = 0.4, where the genetic gain obtained with the former mating strategy was 8% higher than the gain obtained with random mating.

Keywords: selection methods, selection response, inbreeding, mating system

[§]Corresponding author. Tel.: +34 91 347 8786; Fax: +34 91 357 2293
E-mail address: saura.maria@inia.es

GENETIC IMPROVEMENT OF YELLOW PERCH
I: PROGRESS AND PROSPECTS

Wang H-P. [§], O'Bryant P., Yao H., Rapp D., Shen Z. and Li Y.

*Aquaculture Research Center, and Aquaculture Genetics and Breeding Laboratory,
The Ohio State University South Centers, Piketon, OH, 4566, USA*

Yellow perch *Perca flavescens* is an important aquacultural and ecological species in the Great Lakes Region and the Midwest USA. Two of the most important factors that currently constrain the expansion of the yellow perch aquaculture industry are that yellow perch are small and grow slowly. As a part of the effort to enhance growth and aquaculture production of yellow perch, we have undertaken selective breeding programs using different strategies. Genetic tool development: More than 200 microsatellites have been developed using methods of microsatellite-enriched library construction and EST database mining. Eight microsatellite markers were optimized for parentage analyses for the breeding program. Parentage assignments were performed using the exclusion-based approach implemented in the program CERVUS 3.0. We have completed restriction-site associated (RAD)/DNA sequencing of eight strains of yellow perch to develop single-nucleotide polymorphisms (SNPs) and identify genomic diversity of those strains for further improved perch growth. We have also completed RNA sequencing of neo-males, regular males and regular females in yellow perch to identify pathways associated with sex dimorphism and sex determination.

Selective breeding for growth: A commercial-scale (more 100 families/year) for selective breeding for improving growth has been conducted using a marker-aided cohort selection (MACS). So far, four rounds of selection have been conducted. In the first 4-round selection, a marker-aided cohort selection has been developed and tested to establish an effective selection method, which was designed to be easily adapted by industry, maximize genetic gain and minimize loss of genetic variation for the breeding program.

Selective breeding for all-female population: Neo-male broodstocks and genetically fast-growing monosex female populations were created using genetically improved fish. Growth performance of all-female populations *vs.* regular mixed-sex populations has been evaluated.

Selective breeding for utilization of soybean diet: Five phases of an experiment were conducted for two years in this study. Phenotypic relationship between residual feed intake and body weight variations was determined using compensatory feeding regimes. Indirect criteria to improve residual feed intake of SBD in fish in yellow perch has been developed.

Genetic mapping: Two mapping families have been established and their growth is being monitored in tanks for QTL mapping.

[§] Corresponding author
E-mail address: wang.900@osu.edu

GENETIC GAINS ACHIEVED OVER 10 YEARS OF SELECTIVE BREEDING FOR RESISTANCE TO AMOEBIC GILL DISEASE in ATLANTIC SALMON *(Salmo salar)*

Evans B.S.[a, §], Kube P.D.[b], Taylor R.S.[b], Elliott N.G.[b]

[a] *Tassal Operations, Hobart, 7000, Australia*
[b] *CSIRO Agriculture Flagship, Hobart, 7000, Australia*

Genetic selection is being used to assist in the management of amoebic gill disease (AGD) in Atlantic salmon in Tasmania, Australia. This disease is caused by an external gill parasite and causes death if untreated. Freshwater bathing has been used to manage this disease, with regular treatments being required throughout the marine grow-out. Treatments are costly and add significantly to production costs. Selection for resistance is now reducing the number of treatments required. Here we present a summary of the progress of selection for AGD resistance over 10 years of selection in the Tasmanian population.

Data have now been collected for 10 year classes, initially as part of a research project and then as part of an operational selective breeding program. In total, measurements have been taken from 43,866 individuals representing 1,713 families, 797 sires, 750 dams, and over a population that spans up to four genetically linked generations. Disease severity has been measured by assessing gill lesions in field challenges and repeated measures have been made on individual animals at subsequent AGD infections. The data are suggestive of an innate resistance, with low heritability (h^2=0.16) and an acquired resistance with moderate heritability (h^2=0.38).

The genetic trend for resistance to AGD will be presented, together with the genetic trend for other selection traits (total weight, maturation, and carcass quality). Empirical data will also be presented on change in treatment interval observed in the annual progeny tests, and gains realised in commercial grow-out. The experiences with operational breeding over 10 years and the implications and opportunities for ongoing breeding are discussed.

Keywords: Atlantic salmon, disease resistance, amoebic gill disease, heritability

[§] Corresponding author. Tel.: +61 3 62325459
E-mail address: brad.evans@tassal.com.au

GENETICS OF THERMOTOLERANCE IN RAINBOW TROUT, *Oncorhynchus mykiss*

Dupont-Nivet M.[a, §], Crusot M.[a], Rigaudeau D.[b], Labbé L.[c], Quillet E.[d]

[a] *UMR 1313 GABI, INRA, Jouy en Josas, 78350, France*
[b] *UE 907 IERP, INRA, Jouy en Josas, 78350, France*
[c] *UE 937 PEIMA, INRA, Sizun, 29000, France*

In the context of climate change, adaptation to high or fluctuating temperature is a key issue for cold water species since it will be costly to regulate temperature in farms. Selection is a way to help transition towards a new climate and we need first to investigate genetic architecture of thermotolerance.

Ten isogenic lines (within each line, all fish have the same genotype) of Rainbow trout were used to study acclimatization to chronic temperatures and resistance to acute stress temperature. Three chronic temperature conditions were applied to the lines (aged ~6 months): 12°C (L), 20 °C (H) and a fluctuating one (F): 12°-20°C-12°C every day. Survival and growth were individually monitored during 7 weeks to assess acclimatization to chronic stress. Acute temperature stress was applied at the end of the period: quick and high increase of temperature in a few hours until fish lose equilibrium. Upper Thermal Tolerance (UTT) was calculated as cumulative thermal exposure in degree minutes for each fish.

Survival was high in all groups while growth was significantly lower in F and H groups. Significant genetic * temperature interactions were evidenced for growth which showed genetic variability for acclimatization to different temperatures regimes. Significant genetic variability was found for resistance to acute stress with significant genetic-rearing temperature interactions. However some lines were found resistant or sensible whatever the rearing temperature. Thus it should be possible to select for better acclimatization to chronic high or fluctuating temperatures and for resistance to acute stress. However, no significant correlation was found between responses to chronic stress and acute stress which will complicate introduction of such traits in breeding programs.

Keywords: temperature, thermal stress, genetic variability,

§ Corresponding author. Tel.: + 33 1 34 65 23 49 ; Fax: + 33 1 34 65 23 90
E-mail address: + mathilde.dupont-nivet@jouy.inra.fr

GENETIC VARIATION IN DEVELOPING ATLANTIC SALMON *Salmo salar* TO HYPOXIA TOLERANCE

Andrewartha S.J.[a,*], Hamilton M.[a,*], Elliott N.G.[a,*,§], Frappell P.B.[b,*]

[a] *Agriculture Flagship, CSIRO, Hobart, 7001, Australia*
[b] *IMAS, University of Tasmania, Hobart, 7001, Australia*

The farming of Atlantic salmon is Australia's leading seafood sector. The salmon production cycle is hatching and nursery in freshwater hatcheries and on-going rearing in large seawater cages. While every effort is made in the hatchery to provide ideal, stable growing conditions, little can be done to control the seawater environment where potential growth-limiting factors, such as oxygen concentration, can be highly variable. Animals with high hypoxia tolerance are likely to be more robust and productive in such variable environments. This project examined genetic variation in hypoxia tolerance in developing salmon. Metabolic rate was measured in normoxia and across a range of hypoxic oxygen concentrations at 8° C for thirty-seven families at the eyed-egg stage of development. Twenty-six of the families were measured again once the alevins were newly hatched. Statistically significant (P<0.05) differences in metabolic response to hypoxia were identified among families, indicating that some families have a more robust metabolic physiology for hypoxia exposure than others. The implications of these genetic differences in the context of the Australian selective breeding program, which primarily targets improved growth and disease resistance, are explored.

Keywords: Atlantic salmon, hypoxia, selective breeding

[*]These authors have contributed equally to this work.
[§] Corresponding author. Tel.: +61 3 6232 5263
E-mail address: nick.elliott@csiro.au

HERITABILITY OF COPING STYLES IN FARMED EUROPEAN SEABASS

**Allal F.[1§], Ferrari S.[2], Horri K.[1], Vidal M.-O.[1], Ruelle F.[1],
Vandeputte M.[1, 3], Chatain B.[1], Bégout M.-L.[2]**

[1] *Ifremer, UMR 9190 MARBEC, chemin de Maguelone, 34250 Palavas-les-Flots, France*
[2] *Ifremer, Fisheries laboratory, Place Gaby Coll, 17137 L'Houmeau, France*
[3] *INRA, UMR1313 Génétique animale et Biologie intégrative, 78350 Jouy-en-Josas, France*

The characterization of the behavioural and physiological response strategies, defined as coping style, is expected to bring new keys for the sustainable development of aquaculture, in enhancing animal welfare, reducing disease susceptibility, and more directly improving production performances. To understand the genetic basis of personality traits and reactions to a stressful or challenging situation in European sea bass (*Dicentrarchus labrax*), families from a full factorial mating (10 females x 50 males) were reared in common garden and individually tagged at an early age (95 days post hatching, dph) using microtags. Parentage assignment was performed with VITASSIGN using 12 microsatellite markers, resulting in 1308 uniquely assigned fish. The coping style of the animals was characterized through behaviour based tests at four different ages, categorizing fish into proactive or reactive: a hypoxia avoidance test (at 255 dph) and 3 risk-taking tests (at 276, 286 and 304 dph). We observed significant heritability of the coping style, higher for the average of risk-taking scores (h^2 = 0.42±0.12) than for the hypoxia test (h^2 = 0.23±0.10). The genetic correlation between the three risk-taking scores was very high (r_A = 0.99 – 1) showing that although their repeatability was moderately high (r_P = 0.66-0.73), successive risk-taking tests evaluated the same genetic variation. A mild genetic correlation between hypoxia avoidance and the average risk-taking score (0.43±0.21) suggested that hypoxia and risk-taking tests do not address exactly the same behavioural and physiological responses. In addition, significant genetic correlations were observed between coping styles and phenotypic traits, particularly between hypoxia avoidance and thermal growth coefficients (r_A = -0.45 – -0.55), showing that reactive fish have a higher growth than proactive fish under our experimental conditions. To a lesser extent, higher growth performances of reactive fish were also observed suggested by the genetic correlation between thermal growth coefficient and risk taking score (r_A = -0.12 – -0.23). This study, part of the EU project COPEWELL (FP7), suggests that the use of coping style characterization could represent an additional tool to improve the domestication process, selecting individuals better adapted to farming conditions, but also showing higher growth performances.

Keywords: coping style, behavior, heritability, growth

[§] Corresponding author. Tel.: + 33 4 67 13 04 04; Fax: + 33 4 67 13 04 58
E-mail address: + fallal@ifremer.fr

GENOTYPE-ENVIRONMENT INTERACTIONS OF CHANNEL CATFISH, *Ictalurus punctatus,* ♀ X BLUE CATFISH, *Ictalurus furctatus,* ♂ HYBRIDS-CHANGING CULTURE ENVIRONMENTS-CHANGING CLIMATE

Dunham R.A.[a]*, Alsaqufi A.[a], Youssef N.[a], Makhubu N.P.[a], Su B.[a], Peatman E.[a]

[a]School of Fisheries, Aquaculture and Aquatic Sciences, Auburn University, Alabama 36849 USA

The channel catfish, *Ictalurus punctatus,* ♀ x blue catfish, *Ictalurus furctatus,* ♂ hybrid catfish is the best genotype for pond culture in the USA. The catfish industry is evolving, and currently several culture systems are used including conventional ponds, intensively aerated high density ponds, split ponds, modular systems and in-pond raceways. Performance of hybrid catfish can be further improved through strain selection and likely through reciprocal recurrent selection. Will a single genetic improvement program serve to improve hybrid catfish for these different environments or are multiple breeding programs needed to address the needs of all farms? Several genetic types of hybrids were evaluated in conventional ponds, high density ponds, split ponds and in-pond raceways. Moderate but significant ($P<0.05$) genotype-environment interactions occurred for growth, survival, production, seinability and sexually dimorphic growth. In general, the best genetic types were the same for each environment, however, change in genetic rank was sufficient that some error could occur if choosing the best genetic type for all environments was based on performance in a single environment.

Global climate change could result in water temperature extremes and alterations in salinity in aquaculture environments. Different genetic types of hybrid catfish varied ($P<0.05$) for tolerance of high temperature. Genotype-environment interactions occurred among blue catfish, channel catfish and hybrid catfish for growth and survival at varying salinities and cold temperatures. The heterosis exhibited by hybrids disappeared at high salinity and sub-zero temperatures.

Keywords: catfish, hybrid, genotype-environment interaction

*Corresponding author. Tel.: 1-334-707-7491; Fax: 1-334-844-9208
Email address: dunhara@auburn.edu

GENOTYPE-BY-ENVIRONMENT INTERACTION FOR UNIFORMITY OF GROWTH IN RAINBOW TROUT (Oncorhynchus mykiss)

Sae-Lim P.[a,b,§], Kause A.[b], Janhunen M.[b], Vehviläinen H.[b], Koskinen H.[c], Gjerde B.[a], Lillehammer M.[a], Mulder H.A.[d]

[a] Breeding and Genetics, Nofima AS, Ås, NO-1431, Norway
[b]Biometrical Genetics, Natural Resources Institute Finland, Jokioinen, FI-31600, Finland
[c]Aquaculture Unit, Natural Resources Institute Finland, Tervo, FI-72210, Finland
[d]Animal Breeding and Genomics Centre, Wageningen University, Wageningen,6700 AH, the Netherlands

When a rainbow trout stock from a single breeding program is reared in diverse production environments, genotype-by-environment interaction (GxE) may present itself. Growth and its uniformity are considered as two of the most important traits by trout producers world-wide. However, GxE for uniformity of growth has not been studied. Using a double hierarchical generalized linear model and data from the Finnish breeding program, we quantified the genetic variance and correlation of body weight (BW) and its uniformity, as well as the degree of GxE for uniformity of BW in a breeding (BE) and a production (PE) environment. To investigate whether scale effect (high variance related to high mean) affected the estimated parameter, the data were also log-transformed. Although heritability for uniformity () in the BE (0.014) and in the PE (0.012) was low and of similar magnitude, the genetic coefficient of variation for uniformity was 19 and 21%, respectively, revealing high potential for response to selection. The low heritability for uniformity implies that a large number of relatives are needed to obtain moderate accuracy of selection. Genotype re-ranking of uniformity was moderate (r_g = 0.56) but became strong after log-transformation (r_g = -0.08), indicating independent ranking of genotypes in uniformity across the two environments when the scale effect was accounted for. Due to the strong GxE, especially after log-transformation, the use of sib-testing in the PE is recommended when uniformity is required to be improved across environments. The genetic correlation between BW and uniformity was 0.30 in the BE and 0.79 in the PE, but for the log-transformed BW, the genetic correlations were switched to -0.83 in the BE and -0.62 in the PE. The opposite sign of genetic correlations between BW and uniformity from the raw and log-transformed BW data, respectively, indicate that increased BW is genetically related to increased variance of BW, but to decreased variance of BW after accounting for the scale effect. Hence, the scale effect substantially influences the genetic parameters of uniformity, especially the sign and magnitude of its genetic correlations.

Keywords: genetic heterogeneity of environmental variance, genotype-by-environment interaction, microenvironmental sensitivity, scale effects, uniformity

§ Corresponding author. Tel.: +47 64970390
E-mail address: panya.sae-lim@nofima.no

META-ANALYSIS METHODOLOGY FOR SUMMARIZING GENOTYPE-BY-ENVIRONMENT INTERACTIONS ACROSS AQUACULTURE SPECIES

Kause A.[a],[§]

[a] *Biometrical Genetics, Natural Resources Institute Finland, Jokioinen, FI-31600, Finland*

Aquaculture species are typically farmed in multiple production environments with diverse environmental conditions. This may induce genotype-by-environment interaction (GxE) which may lead to lower-than-expected genetic responses to selection in multiple environments. The genetic responses in multiple environments are influenced by the two forms of GxE, namely, genotype re-ranking and heterogeneity of genetic variation across environments. There is a bulk of studies on genetic parameters of GxE but we lack understanding of the way the two forms of GxE are related, the factors causing GxE, and the impact of GxE on breeding programmes. A literature synthesis of GxE benefits from a standardized uniting of diverse GxE studies conducted with different species, traits and environmental conditions. This study introduces a meta-analysis methodology that enables to unite different GxE studies to quantify the degree to which GxE occurs along environmental gradients. The meta-analysis of 66 estimates of GxE for body weight and environment quality in nine aquaculture species demonstrated that, in general, genotype re-ranking was modest, the 95% confidence limit for the genetic correlation between two environments (r_G) ranging between 0.88-0.94. The extreme r_G estimates ranged from -0.270 to 1.0. As expected, genotype re-ranking was marginally significantly increased with increasing quality difference between two production environments. Moreover, genotype re-ranking increased with increasing heterogeneity of genetic variation, implying that the two forms of GxE are related responses to environment and they share some common genetic and physiological determination. That the two forms of GxE are coupled intensifies the impact of GxE on the genetic responses to be obtained in multiple production environments by a breeding program. Thirdly, the degree of heterogeneity of genetic variation changed in a non-linear fashion when the quality difference between two environments increased. This confirms that compared to re-ranking, the changes in trait variation across environments are less predictable. The results highlight that breeding programs which distribute improved animal material across environments with large environmental differences should account for GxE in breeding value evaluations and in the structure of the breeding program.

[§] Corresponding author. Tel.: +358 29 5326222
E-mail address: antti.kause@luke.fi

GENETIC PARAMETERS AND GENOTYPE x ENVIRONMENT INTERACTIONS FOR GROWTH IN THE RED ABALONE *(Haliotis rufescens)*

Winkler F.[a, c, §], Farías W.[b], Brokordt K.[c, a], Herbinger C.[d]

[a] *Dept. Biología Marina, Universidad Católica del Norte, Coquimbo, 1781421, Chile*
[b] *Programa de Magíster en Ciencias del Mar mención Recursos Costeros, Facultad de Ciencias del Mar, Universidad Católica del Norte, Coquimbo, 1781421, Chile*
[c] *Centro de Estudios Avanzados en Zonas Aridas (CEAZA), Coquimbo, 1781421, Chile*
[d] *Dept of Biology, Dalhousie University, Halifax NS Canada B3T 2B7*

The red abalone (*Haliotis rufescens*) was introduced in Chile with aquaculture purposes in 1977, and its production rose from 1 MT in 1998 to 1,110 MT in 2013. The grow-out of this species takes place in sea based facilities in southern Chile (Chiloé; 42°-43° S) and in land based farms in the north (Atacama to Los Ríos regions 26°5'-38°9' S). The juvenile production is mostly concentrated between Caldera (27°0'S) and Los Molles (32°2'S). Genotype by environment interactions (GEI) can lead to serious problems in the planning of a selective breeding program when individual are cultured in different environments because it can affect the estimations of genetic parameters and of the expected response to selection. In the present work we study the effect of different environments on the estimation of genetic parameters and the existence of GEI for production traits.

Two cohorts of red abalone were created in 2007 and 2009 using a nested design to produce full (FS) and half (HS) sib families. Each FS family was grown separately until approximately 14 month old. After that, each individual was measured (total weight and shell length and width), individually marked, and transferred to baskets in raceway tanks. Samples of each FS family were transferred to commercial farms and cultured at last for one year. Cohort 2007 was tested in two land based farms, in Coquimbo and Caldera and Cohort 2009 in a land based farm in Coquimbo and in a sea based farm in Chiloé. Animals were measured one year after transfer in each cohort. Genetic parameter and GEI were estimated using the packages ASReml 3.0.

Heritability estimates varied between cohorts and traits (0.16 to 0.6). Genetic correlations among traits within farms were high and positives ($r_G \geq 0.82$). No GEI was observed in land based farms in northern Chile (Coquimbo/Caldera). However, GEI was observed for shell length and width between Coquimbo and Chiloé, but not for weight. The appropriateness of one or more selective breeding programs to improve abalone production in Chile is discussed.

*Financed: FONDEF D05I10013, FONDECYT 1110890 and INNOVA 12IDL2-13682 grants.

[§] Corresponding author. Tel.: 56-502209921; Fax: 56-512209812
E-mail address: fwinkler@ucn.cl

ESTIMATES OF HERITABILITY AND GENOTYPE BY ENVIRONMENT INTERACTIONS IN THE PURPLE FRESHWATER PEARL MUSSEL *Hyriopsis cumingii*

Li Q.[a†], Bai Z.[a,c†], Han X.[a], Luo H.[a], Dong S.[a], Li J.[a, b,c§]

[a]*Key laboratory of Freshwater Aquatic Genetic Resources, Shanghai Ocean University, Ministry of Agriculture, Shanghai 201306, China*
[b]*Aquaculture Division, E-Institute of Shanghai Universities, Shanghai Ocean University, Shanghai 201306, China*
[c]*Shanghai Engineering Research Center of Aquaculture, Shanghai Ocean University, Shanghai 201306, China*

Hyriopsis cumingii is the most important freshwater pearl mussel in China. Genetic parameters and genotype by environment interactions were estimated from fifth generation purple *H. cumingii* reared at two sites (Chongming and Jinhua). Six shell growth traits and four inner shell color parameters were recorded in 1142 12-month-old individuals. Microsatellite-based DNA parentage analysis was used to assign the mussels to 13 paternal half-sib families and 37 full-sib families. Heritability estimates for shell length (0.20 ± 0.03), shell height (0.16 ± 0.06), shell width (0.17 ± 0.02), body weight (0.23 ± 0.01), and mantle weight (0.15 ± 0.06) were moderate, while that of shell weight (0.31 ± 0.03) was high; inner shell col.or parameters $L*$, $a*$, $b*$, and dE were 0.33 ± 0.19, 0.17 ± 0.10, 0.69 ± 0.1, and 0.33 ± 0.19, respectively. Purple selective lines of fifth generation *H. cumingii* had high genetic diversity and expected heterozygosity was 0.791. Moderate or high heritability and high genetic diversity suggests that the fifth generation had good selective breeding potential. All growth traits and color parameters, except for color parameter $b*$, exhibited significant genotype by environment interactions at both sites. Considering growth and purple inner shell, the F09 and F10 families are more suitable for culturing in Chongming; the F20, F23, and F34 families are more suitable for culturing in Jinhua; and the F21 and F22 families are suitable for both sites. Attention to the likely genotype by environment interaction affects in future purple *H. cumingii* selective breeding programs and culture practices.

Keywords: Hyriopsis cumingii; growth traits; inner shell color; heritability; genotype by environment interaction

[†]These authors have contributed equally to this work
[§]Corresponding author. Phone: 86-021-61900401; Fax: 86-021-61900401
E-mail: jlli@shou.edu.cn

HERITABILITY AND GxE INTERACTIONS OF DISEASE RESISTANCE TO SUMMER SPAT MORTALITIES IN THE PACIFIC OYSTER *Crassostrea gigas* USING BAYESIAN MODEL

Enez F.[a, §], Puyo S.[a], Boudry P.[b], Lapègue S.[c], González-Araya R.[d], Guémené D.[a], Chapuis H.[a], Haffray P.[a]

[a] *SYSAAF, LPGP/INRA, 35000 Rennes, France*
[b] *Unité Physiologie Fonctionnelle des Organismes Marins, Ifremer, 29280 Plouzané, France*
[c] *SG2M-LGPMM, Ifremer, 17390 La Tremblade, France*
[d] *CNC, 75015 Paris, France*

The summer mortality syndrome (POMS) is a major cause of spat mortality in Pacific oyster world-wide. Since 2008, the increase of mortality (70-95% *vs* 30% before) is associated with a new virus variant (OsHV-1 µvar). This work reports first estimates of genetic parameters and GxE interaction for genetic resistance to the new OsHV-1 µvar in France.

3 cohorts (C4, C5, C6) were produced from wild parents with 20, 16 and 20 sires and 20, 21 and 16 dams. Each sire was crossed with 3 to 5 dams to produce 80, 75 and 80 full-sib families. 170 families (67, 54, 49) were challenged under normal rearing conditions (300 spats per bag or lantern) in summer 2013 in 7 sites in C4 and C5 and in 2 sites in C6 cohorts with, in most cases, 3 replicates per family and per site. Surviving oysters were counted individually at the autumn.

Data analyses were performed by mixed-models. Individual mean weight at the challenge transfer was considered as fixed factor and parental effects (sire, dam, family) and environment effects (site, challenge) as random factors. Heritability and genetic correlation were estimated using Bayesian methods and family rankings were also tested by Wilcoxon signed-rank test for paired data.

Mean bags survivals were 12.8% ± 10.7 (C4), 3.7% ± 4.6 (C5) and 70.7% ± 26.7 (C6). Heritabilities estimated in each cohort were different between sites within cohorts (0.01-0.74). Genetic correlations between sites within cohort were high (0.85-0.99), except in a few cases depending on the cohort. Family rankings were not different according to environments. All data were then considered in the same treatment for which heritability was estimated to 0.26 [0.17;0.36].

Our result provides first estimation of genetic parameters to the resistance to the new OsHV-1 virus variant. Heritabilities of survival were different between cohorts and intermediate when all cohorts were considered. The high mean mortality rate is suggested to limit expression of genetic differences when compared to previous studies. The potential improvement of resistance to summer mortality syndrome by selection is confirmed. The limited GxE interaction is favorable to large valorization of genetic progress in France.

Keywords: Crassostrea gigas, mortality, OsHV-1, heritability, genetic correlations

[§] Corresponding author. Tel.: (+33)2 23 48 50 23
E-mail address: florian.enez@rennes.inra.fr

EVALUATION OF THE ERROR RATE AND SOLUTIONS ASSOCIATED TO DOUBLE DIGESTION RAD GENOTYPING BY SEQUENCING IN THREE EUROPEAN MARINE AQUACULTURE SPECIES

Maroso F.[a][§]**, Hermida M.**[b]**, Pardo B.G.**[b]**, Martínez P.**[b]**, Bargelloni L.**[a]

[a] *BCA, Università degli Studi di Padova, Legnaro, 35020, ITALY*
[b] *Departemento de Genética, Universitade de Santiago de Compostela, Campus de Lugo, SPAIN*

Recent improvements in sequencing technologies (higher throughput and lower prices) have allowed SNP genotyping in a faster, cheaper and more flexible way on a large numbers of samples, exploiting recently developed Genotyping By Sequencing (GBS) techniques. GBS applications cover a wide range of fields, from ecological studies to advance selection practices in farming and breeding context, potentially offering great advantages to studies and applications on these fields. Moreover, analysis can be carried out even when reference genomes are not available. Nevertheless, despite these techniques are straightforward and promising, when real data are managed several biases can seriously affect final results. In our work, we addressed the issue of genotyping errors by analyzing data from double digest, Restriction enzyme Associated DNA genotyping (ddRAD) of sea bream (*Sparus aurata*), sea bass (*Dicentrarchus labrax*) and turbot (*Scopthalmus rhombus*) samples in the framework of a European project aimed to evaluate aquaculture impact on wild populations. Multiplexed 144x sampling libraries were constructed and sequenced in Illumina HiSeq and output data analyzed with the software Stacks, the most commonly used to analyze RAD genotyping data. Parsing the dataset, we detected weaknesses and potential biases of this approach, and searched for possible source of errors in the wet lab protocol (from DNA quality to library preparation), the sequencing step, and the bioinformatic pipeline. Comparing the results from different sequenced libraries, we quantified the loss of information due to quality filtering and normalization issues. In addition, using replicated samples in different libraries, we checked 1500-2000 SNPs/species for systematic biases on genotyping. Several RAD-tag identification and genotyping approaches were tested (e.g. de-novo vs reference genome based), resulting in error rates from 0,5% to 8% of total genotypes depending on the strategy and the species. Finally, we suggest possible ways to decrease biased genotypes, pointing out critical points in both the laboratory practices and the bioinformatic approach. We think that the same procedures may be effective for other different techniques and species, in order to reduce the "noise" caused by wrong genotypes and gain more accurate results from RAD genotyping.

Keywords: ddRAD, GBS, bias, stacks

[§] Corresponding author. Tel.: +34 982822427
E-mail address: francesco.maroso@gmail.com

IDENTIFYING PARALLEL AND NON-PARALLEL GENOMIC CHANGES BETWEEN INDEPENDENT PAIRS OF WILD/DOMESTIC ATLANTIC SALMON POPULATIONS USING A HIGH DENSITY SNP ARRAY

López M.E. [a,d]., Correa K. [b,d], Di Genova A. [c], Moore J-S. [e], Perrier C. [e], Bernatchez L. [e], Gilbey J. [f], Soto C. [h], Bassini L. [a,d], Maass A. [c], Neira R. [a], Figueroa R. [d], Lhorente J.P. [d], Yáñez J.M. [b,d §]

[a] *Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago, 8820808, Chile*
[b] *Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, 8820808, Chile*
[c] *Laboratory of Bioinformatics and Mathematics of the Genome, Center for Mathematical Modeling (UMI 2807 CNRS), Universidad de Chile, Santiago, 8370459, Chile*
[d] *Aquainnovo, Puerto Montt, 5503032, Chile*
[e] *IBIS, Institut de Biologie Intégrative et des Systèmes, Université Laval, Québec, G1V0A6, Canada*
[f] *Marine Scotland Science, PH16 5LB, UK*
[h] *Camanchaca, Puerto Montt, 5502277,Chile*

Artificial selection to captivity can drive genetic changes on a short time scale in domesticated animals. Novel genomic approaches can be used to identify regions of the genome involved, repeatedly or not, in adaptation to captivity. Atlantic salmon (*Salmo salar*) represents an advantageous model for studying the genomic response to artificial selection because some farmed populations have experienced intense artificial selection in a short period of time, and because independent strains originating from different wild stocks are available. In this study, we used the highest density SNP chip currently available to screen 151,510 SNPs seek for selection signatures in two independent domesticated strains (Canada: n = 37 and Scotland: n = 43) and their respective wild ancestor populations (n= 44 and 41). The genetic differentiation between domestic and wild was F_{ST} = 0.16 for Canadian populations and F_{ST} = 0.08 for Scottish populations. To identify genomic responses to selection, we performed genome scans separately on each wild/domestic pair using two approaches (genome scans and latent factor mixed models). A subset of 160 and 93 SNPs was identified showing evidence of selection between wild and domestic populations for Canadian and Scottish populations respectively. None of these SNPs were shared between the two independent comparisons, suggesting most genetic changes leading to adaptation to captivity are non-parallel despite similar selection pressures. Furthermore, we performed a genome wide association analysis combining both pairs and considering "domestic" and "wild" as traits. We found four markers that are associated with domestication, again suggesting few parallel genomic changes. The outlier loci identified here illustrate the usefulness of a high density chip and provide a basis for further studies aiming at detecting genes involved in biological processes related to important traits subjected to domestication and selection in Atlantic salmon.

Keywords: Salmo salar; selective sweeps, domestication, single nucleotide polymorphisms

[§] Corresponding author. Tel.: + 56-2-29785533
E-mail address: + jmayanez@uchile.cl

IDENTIFICATION OF SPECIES-SPECIFIC SNP MARKERS IN TILAPIAS USING DOUBLE-DIGEST RAD SEQUENCING (ddRADseq)

Syaifudin M.[a, b *]**, Bekaert M.B.**[a *]**, Taggart J.B.** [a]**, Hulata G.** [c]**, D'Cotta H.** [d]**, Baroiller J.F.** [d]**, Penman D.J.** [a, §]**, McAndrew B.J.** [a]

[a] *Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK.*
[b] *Program Study of Aquaculture, Agriculture Faculty, University of Sriwijaya, Indonesia.*
[c] *Institute of Animal Science, Agricultural Research Organization, The Volcani Center, Israel*
[d] *Cirad, Persyst, UMR Intrepid, Campus International de Baillarguet, Montpellier, France*

We tested the potential of double digest restriction site associated DNA sequencing (ddRAD-seq) for discovering SNP markers to distinguish between 10 tilapia species (including more than one population and/or subspecies of the most important aquaculture species). Analysis of ddRADseq data detected 635 SNP markers that were homozygous within each species but showed variation among species. A phylogenetic tree based on these SNP markers indicated a similar pattern to most prior phylogenies based on other molecular markers. Further analysis ascertained species-diagnostic SNP markers (i.e. with an allele unique to that single species) as follows: *Oreochromis aureus* (18), *Oreochromis karongae* (10), *Oreochromis mossambicus* (4), *Oreochromis niloticus* (12), *Oreochromis urolepis hornorum* (5), *Oreochromis andersonii* (3), *Oreochromis macrochir* (6), *Sarotherodon galilaeus* (25), *Sarotherodon melanotheron* (40) and *Tilapia zillii* (32). These species-diagnostic SNP markers (155 across 10 tilapia species) were distributed across the genome, ranging from 0.10 SNPs/Mb in linkage group 4 to 0.44 SNPs/Mb in linkage groups 8-24, with the exception of LG3, where no diagnostic SNP markers were found. These SNPs would be of value to investigate hybridization and introgression in a range of captive and wild stocks of tilapias.

Keywords: Tilapia, Species-diagnostic markers, Population, ddRAD-seq, SNP

*These authors have contributed equally to this work.
§ Corresponding author. Tel.: + 44 (0)1786 467901; Fax: + 44 (0)1786 472133
E-mail address: d.j.penman@stir.ac.uk

A GENOMIC APPROACH TO THE GENETIC MANAGEMENT OF AQUACULTURE-BASED STOCK ENHANCEMENT IN A MARINE REEF FISH, THE RED SNAPPER *Lutjanus campechanus*

Norrell A.E.[a], Jones K.L.[b], Saillant E.A.[a][§]

[a] *Gulf Coast Research Laboratory, University of Southern Mississippi, Ocean Springs, 39564, USA*
[b] *Biochemistry and Molecular Genetics, University of Colorado School of Medicine, Denver, 80045, USA*

The red snapper (*Lutjanus campechanus*) is a reef fish of major economic importance in the Southeast United States. Aquaculture of this species is being investigated for captive propagation as a means to provide additional fishing opportunities to anglers in a stock enhancement program, and also to supplement the food market through commercial production. Genetic management is needed to assess and mitigate the potential negative impacts of stocking on the fitness of wild populations. The degree of demographic dependence and local adaptation of geographic populations first need to be determined in order to design appropriate management units for stock enhancement. The objective of this work was to develop and implement high density genome scans to accurately assess genetic variation and local adaptation in wild red snapper and assist with the spatial management of releases. Using P-454 and Illumina sequencing, a draft genome sequence with an average coverage of 25X was generated for red snapper. The obtained assembly was applied as a reference to map Restriction Site Associated DNA (RAD) Tags and characterize associated Single Nucleotide Polymorphisms (SNPs). A 1324 cM sex-averaged linkage map consisting of 5856 markers identified from 4 outbred single pair families was constructed and applied to ordering and orienting genome contigs. The map contains 24 linkage groups, consistent with the haploid number of chromosomes reported in other lutjanids. RAD-Tag sequencing was then implemented in red snapper samples from 3 regions (eastern and western Gulf of Mexico and U.S. east coast off South Carolina) encompassing the exploited range of the species in U.S. waters. Consistent with previous genetic studies, pairwise F_{ST} values between regions were low ($F_{ST} < 0.0004$). However, an outlier analysis revealed 58 candidate SNPs departing significantly from expectations derived from a simulated neutral distribution of F_{ST}. Twenty-four outlier SNPs located on the map showed a non-random distribution across linkage groups. Groups 1 and 13 accounted for 4 and 5 outliers, respectively, potentially signalling genomic regions under spatially divergent selection. Work in progress focuses on refining spatial patterns of population structure and developing a reduced panel of SNPs for fine scale monitoring of populations during enhancement.

Keywords: Lutjanus campechanus, linkage mapping, population genomics, stock enhancement, genetic impacts

§ Corresponding author. Tel.: + 1-228-818-8007; Fax: + 1-228-872-4204
E-mail address: + eric.saillant@usm.edu

ECONOMIC AND ENVIRONMENTAL IMPACTS OF GENETIC IMPROVEMENT IN FISH FARMING DEPEND ON LIMITING FACTORS

Besson M.[a, b, §], M. Vandeputte[b, c], Aubin J.[e], van Arendonk J.A.M.[a], de Boer I.J.M.[e], Quillet E.[b] , H. Komen[a]

[a] Animal Breeding and Genomics Centre, Wageningen University, Wageningen, 6700 AH, Netherlands
[b] INRA, UMR1313 Génétique animale et biologie intégrative, Jouy-en-Josas, 78350, France
[c] IFREMER, Palavas-les-Flots, 34250, France
[d] INRA, UMR1069 Sol Agronomie Spatialisation, Rennes, 35042, France
[e] Animal Production Systems group, Wageningen University, Wageningen, 6700 AH, Netherlands

The target of fish breeding is to increase economic profit by producing faster growing fish with lower feed intake. Genetic improvement, therefore, is also expected to reduce environmental impacts of fish farming. However, little is known about the economic and environmental consequences of improving growth rate and feed efficiency in fish farming.

At farm level, genetic improvement of a trait affects feeding strategy and/or management practices according to the limiting production factor. Evaluating the impact of a genetic improvement requires therefore, (1) to model the whole farm operations via a bioeconomic model and (2) to calculate the environmental impacts along the production chain using Life Cycle Assessment. We calculated environmental and economic values (ENV and EV) of thermal growth coefficient (TGC) and feed conversion ratio (FCR) in two different farming systems: recirculating aquaculture system (RAS) of catfish and sea cages (SC) of sea bass. In RAS, the limiting factors are nitrogen treatment capacity of the bio-filter and fish density. In SC, the limiting factors are fish production quota and oxygen availability. ENV and EV were calculated using the difference in profit and in environmental impacts per kg of fish produced between the current population mean for both traits (μ_t) and the next generation of selective breeding ($\mu_t + \Delta_t$).

The results show that ENV and EV are both dependent on limiting factors. Improving TGC is economically and environmentally beneficial in RAS and SC, only when the limiting factors are respectively rearing density (RAS) and oxygen availability (SC). There is no benefit in improving growth rate when nitrogen treatment capacity or production quota are the limiting factors. On the other hand, improving FCR always increases profit and decreases environmental impacts in RAS and SC irrespective of the limiting factor.

These results emphasize the importance of calculating environmental and economic values in the right farming context in order to develop efficient future breeding programs in aquaculture. FCR is the most important trait increasing profitability and decreasing environmental impacts in fish farming. This result emphasizes, the need for further studies aiming at better characterising the genetic bases of feed efficiency.

[§] Corresponding author. Tel.: + 31 317482335; Fax: + 31 317483929
E-mail address: mathieu.besson@wur.nl

MICROSATELLITE ASSESSMENT OF GENETIC DIVERSITY OF WILD AND CAPTIVE POPULATIONS FOR RESTOCKING THE MIGRATORY CURIMBA *(Prochilodus argenteus)* IN THE SÃO FRANCISCO RIVER (BRAZIL)

Ana P.S.[1], Karine K.C.[1], Maria R.M.[1]

[1] Departamento de Pesca e Aquicultura, Universidade Federal Rural de Pernambuco, Rua Dom Manuel de Medeiros, s/n– Dois Irmãos, Recife-PE, Brazil, 52171-900, email: raquel@depaq.ufrpe.br.

The curimba (*Prochilodus argenteus*) is a migratory freshwater fish endemic of the São Francisco River basin, with a great aquaculture potential. As hydropower development and invasive fish species has altered and degraded many habitats and posed serious risk to the populations of this species, an enhancement program with the release of hatchery-reared fingerlings into the wild has been carried out. A comprehensive genetic baseline evaluation of seven wild populations and a captive broodstock was assessed. Average observed and expected heterozygosities ranged from 0.609 to 0.883 and from 0.730 to 0.827, respectively. Deviations from HWE were significant for all loci in the captive broodstock and for some loci in the wild populations of the middle stretch. Average value of F_{IS} in the captive broodstock was 0.123, contrasting with negative values obtained from the majority of the wild populations. On the other hand, mean pairwise relatedness coefficient across all populations, including the captive one, showed that more than 65% of the dyads had estimates below zero. In the absence of simulations data with known relationship categories to test the precision of this estimator, we speculate that a matrix of relatedness could be constructed to avoid mating close relatives in the captive broodstock. Pairwise relatedness correlates with effective population size and a critical situation was found in the captive broodstock, which showed a value around 50. The wild populations do not seem to be structured as overall F_{ST} was low, although the Bayesian clustering method suggested some level of structuring between the upper/middle versus submiddle/lower and also some structuring among inter-annual arrival waves in the middle stretch of the river. The pairwise F_{ST} between wild populations and captive broodstock showed a moderate divergence with values near 0.10. The total number of alleles of the captive broodstock was 61, while in wild populations this number varied from 62 to 78. The total number of private alleles was 24, varying from one to seven in the wild populations, while in the captive broodstock five were registered. The use of this broodstock for restocking is discussed.

CAN SELECTIVE BREEDING FOR GROWTH OR FILLET YIELD DECREASE ENVIRONMENTAL IMPACT OF FISH FARMING? A GILTHEAD SEA BREAM *(Sparus aurata)* CASE STUDY

Haffray P.[1][§], Acosta Alba I.[2], Cariou S.[3], Bruant J.S.[3], Bugeon J.[4], Vandeputte M.[5,6], Aubin J.[2]

[1] SYSAAF, Campus de Beaulieu, F-35000 Rennes, France
[2] INRA, UMR1069 Sol Agro et Hydrosystème Spatialisation, F-35000 Rennes, France
[3] FMD, F-17840 La Brée les Bains, France
[4] INRA, LPGP, UR1037, Campus de Beaulieu, F-35000 Rennes, France
[5] INRA, UMR1313 GABI, F-78352, Jouy-en-Josas, France
[6] Ifremer, UMR 9190 MARBEC, F-34250 Palavas-les-Flots, France

Life Cycle Assessment (LCA) was applied to assess the environmental impact of genetic selection of growth or fillet yield from input production up to consumer's plate in the gilthead seabream *Sparus aurata* as a case study for farmed fish species.

In a first step, genetic parameters of growth and quality traits (gutted yield, head yield, headed and gutted carcass yield, filet yield, fat muscle content, morphological traits) were estimated in 241 DNA-pedigreed mixed families from 25 dams and 86 sires reared in 2 environments, in a land-based sanitary protected breeding site (Oléron, France) and a growing site in sea cage (Corsica, France). As GxE interactions between the two environments were limited for processing traits (r_A = 0.89 to 0.94), LCA impact categories were assessed using only genetic parameters estimated from the breeding site. Functional unit for the LCA evaluation was 1 kg of fillet consumed.

LCA results show that the farming first step contributed to 79-100 % of the impacts depending on categories (acidification, eutrophication, climate change, land occupation, energy use and primary production use). Commercialization, consumption and recycling represented less than 5 % of the impacts except for energy use and climate change. The cooking step represented close to 18 % of the energy and waste incineration accounted for more than 12 % of climate change.

The impact of selective breeding was estimated after simulating 5 generations of selection with a pressure of 10 % or 3 % on growth or 20 % or 10 % on fillet yield. Genetic improvement of growth had limited impacts on environmental performances, mainly associated with the decrease of the fixed environmental costs (facility). In the other hand, genetic improvement of fillet yield induced a 19 % to 24 % decrease in environmental impacts, mainly associated with a more efficient use of artificial feed per kg of flesh produced, which directly influenced all impact categories.

These results illustrate large indirect effect of selection on fillet yield, on environmental impact and provide original evidence to link genetic selection and the enhancement of environmental performance of aquaculture and livestock, the main goal of aquaculture being to produce flesh for human consumption.

Keywords: aquaculture, genetic parameters, fillet yield, Sparus aurata, life cycle analysis

[§] Corresponding author: Tel: (33) 2 23 48 53 78; Fax (33) 2 23 48 50 20
E-mail address: haffray@rennes.inra.fr

GENETIC PARAMETERS FOR THE OPERCULUM AND JAWS DEFORMITIES IN LARVAE OF GITLHEAD SEABREAM, *Sparus aurata*

Batargias C.[a,*,§]**, Fragoulis S.**[b,*]**, Loukovitis D.**[a,*]**, Tzokas K.**[c,*]**, Koumoundouros G.**[b,*]

[a] *Department of Fisheries and Aquaculture Technology, T.E.I. of Western Greece, Messolonghi, 30200, Greece*
[b] *Biology Department, University of Crete, Heraklion, 71409, Greece*
[c] *ANDROMEDA SA, Patras, 26500, Greece*

Skeletal deformities are a major problem of product quality in finfish aquaculture. Their development is often attributed to the environmental conditions. Existing studies on the genetic basis of skeletal deformities usually record these traits at advanced developmental stages, ignoring the fact that the majority of the skeletal deformities originate during the first ontogenetic period. In the present study, we focused on this period and recorded deformities of the operculum and the jaws. A broodstock consisted of 64 males and 63 females were mass spawned for 4 consecutive days. At the age of 39 days post hatching (dph), a random sample was taken from each tank for phenotyping and genotyping. A subsample of 1,400 larvae was stained for bone and cartilage and phenotyped following a novel approach of staining which did not inhibited the subsequent genotyping process. Gill-cover deformities mainly concerned alterations of the operculum bone in the form of inside folding, atrophy or/and over-ossification. Macroscopically, these alterations resulted to a 10-50% size reduction of the gill cover. Gill-cover deformities were classified into five intensity levels by means of a semi-quantitative scale. Jaw deformities were quantified semi-quantitatively, either with a macro-scale (external morphology) or with a micro-scale (individual anatomical elements) (four levels of intensity for both scales). All deformities were recorded bilaterally. Genotyping was done with a multiplex of nine microsatellites resulting in 95.8% of unambiguously assigned offspring using the exclusion method. Heritability and correlations estimates on the observed scale were estimated by REML using a multi-trait animal model including tanks as fixed effects. The heritability estimates for the operculum traits were very low and insignificant (0.02±0.02). The heritability estimates for the jaw deformities traits was 0.12±0.04 for the macro-scale and ranged from 0.05±0.05 to 0.22±0.06 for the micro-scale. The heritabilities on the liability scale were higher ranging from 0.10 to 0.78. The phenotypic and genetic correlations of the jaw deformity traits with total length were negative, low to moderate and of similar magnitude. The phenotypic and genetic correlations among the jaw traits were in most cases positive, moderate to high and of similar magnitude.

Keywords: heritability, skull deformities, larvae, finfish

[*]These authors have contributed equally to this work.
[§] Corresponding author. Tel.: +302631058424; Fax: +302631058287
E-mail address: batc@teimes.gr

EFFECTS OF STRAIN ON GROWTH PERFORMANCES OF TRIPLOID THAI WALKING CATFISH, CLARIAS MACROCEPHALUS GÜNTHER, 1864

Chatchaiphan S.[a], Srisapoome P.[b] and Na-Nakorn U.[c§]

[a]Graduate Program in Aquaculture, The Graduate School, Kasetsart University, Chatuchak, Bangkok, 10900, Thailand
[b§]Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Chatuchak, Bangkok, 10900, Thailand

The induction of triploidy is expected to obtain animals with superior growth over that of the diploids. Triploidy has been induced in Thai walking catfish (*Clarias macrocephalus*) but the results varied regarding relative growth of diploids and triploids which might be due to strain differences. Therefore, the present study was conducted to evaluate impacts of strains on growth performance of the triploid fish. Cold shock (7°C, commenced at 0 min after fertilization, with shock duration of 25 minutes) was applied to eggs obtained from all possible crosses between two catfish stains. The fingerlings of each group were randomly reared in tanks in triplicates at a stocking density of 75 fish per 1,000 l-tank until they were 240 days old. The results showed that the cold shock induced more than 90% triploidy, but hatching rate was low. At 60 days old, the triploid fish had lower specific growth rate (SGR), absolute growth rate (AGR), body length, and body weight than that of the diploid counter-parts. However, when the fish grew older, the differences between diploid and triploid were not statistically significant except for SGR during 180-240 days old where it was higher for triploids than that of diploid. Interaction between paternal strain×shock was significant for the following traits, body length at 90 days old, body weight at 90 and 120 days old, AGR during 60-90 days, and SGR during 61-90 and 91-120 days old. The interaction between pa-ternal-×maternal strain×shock was significant for body length, body weight and AGR at 180 days old. Sex ratio of triploids was 1:2.7 (female:male) which may be because some females were identified as un-identified sex. Gonadosomatic indices of male and female diploids were significantly higher than those of triploid groups.

Keywords: strain effect, triploid, Clarias macrocephalus, cold shock, sterility, growth

*Corresponding author. Tel.: +66 2 5792924; Fax: + 66 2 5613984
E-mail address: ffisurn@ku.ac.th

GENETIC PARAMETERS FOR UNIFORMITY OF HARVEST WEIGHT IN THE GIFT STRAIN OF NILE TILAPIA ESTIMATED USING DOUBLE HIERARCHICAL GENERALIZED LINEAR MODELS

Marjanovic J.[a, b, §], Mulder H.A.[a], Khaw H.L.[c] and Bijma P.[a]

*[a] Animal Breeding and Genomics Centre, Wageningen
University, Wageningen, 6700AH, The Netherlands
[b] Department of Animal Breeding and Genetics, Swedish University
of Agricultural Sciences, Uppsala, 75007, Sweden
[c] WorldFish, Bayan Lepas,11960, Penang, Malaysia*

Through twelve generations of selection, the GIFT breeding program proved to be very successful in increasing the mean level of harvest weight in tilapia. However, it is often desirable not only to improve the mean level of traits, but also to change their variability. In farmed tilapia, individuals display large size differences within a population, but the genetic background of this variability is almost entirely unknown. Selection for more uniform individuals is possible only in the presence of genetic variation in trait variability. This phenomenon is known as genetic heterogeneity of environmental (residual) variance. The aim of this study was to investigate the potential for genetic improvement in variability of harvest weigh in the GIFT strain of Nile tilapia, by applying double hierarchical generalized linear models. This approach offers simultaneous estimation of genetic parameters for the mean and for the residual variance, which is modelled on the exponential scale. Both untransformed and Box-Cox transformed harvest weight were analysed to study the impact of non-normality on estimates of variance components. Analysis involved 6090 fish with records on harvest weight. The estimated heritability of harvest weight had moderate value of 0.44, which increased to 0.46 after the transformation. We found substantial genetic variation in the residual variance of 0.41 which reduced to 0.27 after the Box-Cox transformation. The given estimates are on a log scale. The genetic coefficients of variation for residual variance were 64% and 52% on untransformed and transformed scale respectfully. These values describe the potential decrease in residual variance when achieving one genetic standard deviation by selection. The genetic correlation between mean and variance was 0.67 for untransformed harvest weight, which points to a strong positive relationship between both traits. The genetic correlation for Box-Cox transformed harvest weight was 0.12. The results obtained in this study indicate a good opportunity to improve variability of harvest weight. The high genetic correlation of 0.67 between harvest weight and residual variance suggests necessity for index selection in order to increase harvest weight and reduce its variation at the same time.

Keywords: uniformity, harvest weight, Nile tilapia, DHGLM, Box-Cox

[§] Corresponding author. Tel.: + 31(0)317 48 31 98; Fax: +31(0)317 48 39 62
E-mail address: jovana.marjanovic@wur.nl

RESPONSE TO SELECTION FOR HARVEST WEIGHT IN A FAMILY BASED SELECTION PROGRAM OF GILTHEAD SEABREAM *(Sparus aurata)*

Thorland I.[a, §], Kottaras L.[b], Refstie T.[a], Dimitroglou A.[b],Papaharisis L.[b], Rye M.[a]

[a] *Akvaforsk Genetics Center AS (AFGC), Sjølseng, 6600 Sunndalsøra, Norway*
[b] *Nireus Aquaculture SA, 19400 Koropi, Greece*

Gilthead seabream (*Sparus aurata*) is an economically important species in European aquaculture. For sustainable improvement of production traits in farmed populations, genetic improvements combined with restricted inbreeding are key factors. While genetic parameters for production traits in the seabream have been frequently reported, documentation of responses to selection for Gilthead seabream is still scarce.

The Nireus' family based breeding program of Gilthead seabream in Greece was initiated by the company Kego SA with production of the first batch of nucleus families by artificial stripping in 2002. Since its establishment the program has been designed and supervised by Akvaforsk Genetics Center (AFGC).The seabream is a protandrous hermaphrodite with males sexual maturing from 2 years of age and females maturing from 3-4 years of age, and the Nireus breeding program is run with overlapping generations.

Performance data recorded at commercial harvest size from ~ 65000 animals representing 623 full- and halfsib families produced in 11 batches are analysed. Growth recorded as body weight at harvest shows medium high heritability (~0.35), while survival during cage culture, subjective scores for external appearance (pigmentation spots and brightness of skin) and jaw deformity show low to medium low heritabilities (0.11 -0.16).

Selection has been based on selection indices primarily focusing on improved growth, but with some relative weights placed on additional traits mainly to prevent undesired correlated responses. The 2013 year class represents generation ~F(2.8). A genetic trend analysis of combined data till date (~F2.6) demonstrates an accumulated genetic gain for body weight at harvest of 100g, which expressed relative to the performance of the base population (F0) amounts to an average selection response of 13% per generation for this trait. The average accumulated inbreeding in the present population is 2.4%.

Keywords: Selection response, Gilthead seabream, breeding program, heritability, harvest weight

[§]Corresponding author. Tel.: +47 975 69 563
E-mail address: ingunn.thorland@afgc.no

THE EFFECT OF PARENTAL RELATEDNESS ON THE FITNESS IN NEXT GENERATION OF THE GUPPY *Poecilia reticulata*

Ismail M.S.[a,b,*], Nakajima T.[a,*], Nakajima M.[a,*,§]

[a] *Graduate School of Agricultural Science, Tohoku University, Sendai 981-8555, Japan*
[b] *Aquaculture Department, University Putra Malaysia, Selangor, 43360, Malaysia*

Reproduction from small number of parents compared with natural population is inevitable in cultured stock. Because of an inbreeding coefficient depend upon the number of parents, rapid increase of inbreeding coefficient is expected in cultured stock. If the increase of coefficient of inbreeding controls less than 10% in 30 generations, more than 100 individuals, 50 pairs, are necessary. It is essential to consider the method to prevent the increase of coefficient of inbreeding without the increase of parental individuals.

Recent progress of a genetic marker leads the correct estimation of genetic variability and genetic relationship between parents. If the effect of inbreeding could estimate from the parental genetic relationship, the inbreeding depression which will occur in their offspring would be able to prevent. In this study, the correlation among genetic variability, relatedness in parental generation, and the fitness in next generation were examined in the three generations of ful-sib mating guppy families. For the estimation of genetic variability and parental relatedness Ritland and Lynchi, 1999), 9 microsatellite DNA markers were used. As the fitness, thermal tolerance and survival rate at 180 days were used. The thermal tolerance were estimated from the disappearance time of normal swimming in 37º water temperature. Genetic variation was estimated as individual heterozygosity, standardized heterozygosity and internal relatedness. Parental relatedness also used for the evaluation of genetic similarity between parents. Multiple regression analysis was used for the analysis of correlation between the fitness and other variants, parental heterozygosity (Individual and standardized) and relatedness (internal and parental).

Significant negative standardized partial regression coefficient was observed between thermal tolerance and parental relatedness, which indicates the dissimilar mates, would be able to prevent the inbreeding depression.

Keywords: Guppy, inbreeding, parental relatedness, standardized heterozygosity, thermal tolerance

*These authors have contributed equally to this work
§Corresponding author

GENETIC PARAMETERS IN ATLANTIC SALMON FOR GROWTH RATE AND CARCASS QUALITY TRAITS RECORDED AT THE SAME BODY WEIGHT OR THE SAME AGE

Kristjánsson Ó.[a, c, §], Gjerde B.[b c], Lillehammer M.[b], Jónasson J.[a]

[a] Stofnfiskur, Hafnarfjörður, 221, Iceland
[b] Nofima, Ås, 1430 ,Norway
[c] Department of Animal and Aquacultural Sciences, Norwegian
University of Life Sciences, 1430 Ås, Norway

In farmed Atlantic salmon *Salmo salar* filet colour and fat are the most important carcass quality traits. Selection has been practised for increased growth and colour and reduced fat, all measured on fish slaughtered at the same age and thus at different weights. Positive genetic correlations between growth and fat, low positive correlations between growth and colour and zero genetic correlations between fat and colour have been found. If selection for faster growth is utilized to harvest the fish at an earlier age, it may be argued that genetic parameters for carcass quality traits should be based on records obtained from fish of the same body weight, close to the average harvested body weight in the industry. The aim of this study was to obtain genetic parameters for growth (gram/day), and filet fat and colour predicted using backscatter of light in the near infrared (NIR) and the visual (VIS) spectra, respectively, measured in fish at same body weight (SW). Similar sized fish was obtained by slaughtering the largest fish at six different times over 5-6 months. Traits records were obtained on a total of 2693 offspring of 103 sires and 206 dams from two year-classes. For comparison, a group of 2437 of their sibs were measured for the same traits, but slaughtered at the same age (SA). Estimates of genetic parameters for the three traits were obtained from an animal model, also including a random effect common to full sibs, analysing data from both groups simultaneously. For each trait estimated heritabilities were similar in the SA and SW groups. The genetic correlation between growth in the two groups was high (0.92±0.04), while it was lower between filet fat (0.59±0.17) and filet colour (0.44±0.26). Genetic correlation between growth and fat in the SA group was 0.63±0.13, while it in the SW group was estimated to -0.21±0.23. Genetic correlations of colour with growth and fat were not significantly different from zero both within and between groups. These results strongly indicate that selection for increased growth will not increase filet fat in Atlantic salmon if the increased growth potential is utilized through earlier slaughter.

Keywords: Genetic parameters, Atlantic salmon, NIR, quality parameters

§ Corresponding author. Tel.: + 354 693 6315 Fax: + 354 564 6301
E-mail address: olafur@stofnfiskur.is

NEGATIVE GENETIC CORRELATION BETWEEN RESISTANCE AGAINST PISCIRICKETTSIA SALMONIS AND HARVEST WEIGHT IN COHO SALMON *(Oncorhynchus kisutch)*

Yáñez J.M.[a, b, §], Barría A.[c], Dufflocq P.[a], Oyarzún M.[b], Neira R.[c], Newman S.[d], Lhorente J.P.[b]

[a] *Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, 8820808, Chile*
[b] *Aquainnovo S.A., Puerto Montt, 5503032, Chile*
[c] *Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago, 8820808, Chile*
[d] *Genus, plc, Hendersonville, TN 37075 USA*

Piscirickettsia salmonis is the main infectious disease agent affecting Chilean coho salmon (*Oncorhynchus kisutch*) aquaculture. In this study, we used data from experimental challenges against *P. salmonis* in full- and half-sibs from 107 families (~2,600 fish) belonging to a coho salmon breeding nucleus. The challenge spanned 48 days and total cumulative mortality across all families reached 40% by the end of the test period. Cumulative mortality rates ranged from 5% to 82% between families, indicating substantial phenotypic variation to susceptibility for *P. salmonis* in this species. Resistance to *P. salmonis* (SRS) was defined as the day of death of each fish. We also measured harvest weight (HW) in 42,657 genetically related individuals of the challenged fish from the same breeding population. We used a bi-variate animal model to estimate (co)variance components and to calculate genetic parameters. For HW we included contemporary group (sex:tank:year) as a fixed effect and age at harvest as a covariate. In addition, tank was included as a fixed effect and weight at end of test as a covariate for SRS. Estimated heritabilities for HW and SRS were 0.38 (± 0.03) and 0.14 (± 0.03), respectively. The genetic correlation between HW and SRS was -0.43 (± 0.13). The levels of genetic variation detected in the present study indicate that selective breeding for these traits is fesaible. However, the magnitude and direction of the genetic correlation between HW and SRS must be taken into account when selecing both traits simultaneously.

Keywords: Coho salmon, genetic correlation, heritability, disease resistance, SRS

[§] Corresponding author. Tel.: + 56-99-810-1584
E-mail address: jmayanez@uchile.cl

PARENTAGE ASSIGNMENT IN SALMON USING HIGH DENSITY SNP PANELS: A SIMULATION STUDY

Grashei K.E.[a, b, *], Kjøglum S.[b, a], Moen T.[a, b], Ødegård J.[a, b, *]

[a] *AquaGen AS, Ås, 1432, Norway*
[b] *AquaGen AS, Trondheim, 7462, Norway*

Salmon farming is one of the largest industries in Norway. In 2013, the turnover from Atlantic salmon farming in Norway was over 1.1 billion tons (~€4.4 billion). The same year, official numbers indicated 198,000 escaped salmon, and the real number may be considerably larger. Due to the large number of escaped salmon relative to wild salmon, there has been great concern regarding introduction of farmed salmon genetic material into the wild salmon populations. Due to this, economic penalties has been introduced for escape incidents. However, one of the main problems is to identify the correct source of the escaped fish. Genomic tracing tools have been suggested as a possible solution to this problem, i.e., by controlling family background of each fish farm, the source of each escaped fish can be indirectly identified through parentage assignment of the escapees. This has also the added effect of removing suspicion raised against other fish farmers in the area. An earlier study (Kjøglum et al., 2012) has indicated a potential for large-scale parentage assignment using SNP data. As a consequence of this, the salmon breeding company AquaGen now offers fully traceable eggs ("TRACK"-eggs) on demand. At the time of writing, 45 million TRACK eggs have been ordered by fish farmers. However, as the number of candidate parents increases, so does the need for additional markers. The aim of the current study was thus to use dense genomic markers for proper parentage assignment in large data sets (thousands of offspring and potential parents). One of the challenges of such data is to develop rapid methods that utilize information from all the available markers. In AquaGen, a ~56k Affymetrix SNP-chip is being used, thus producing data that can also be applied in other types of analyses such as genomic selection, GWAS and population genetic studies. The parentage assignment method used for TRACK eggs was validated in a simulation study. Here, genomes of a large population were simulated over numerous generations. Parentages were assigned using 10k SNP markers, based on a novel method utilizing genomic relationships for parentage assignment. For each scenario, parentages were assigned for ~7000 animals, having between 420 and 6120 possible parental candidates. Preliminary results show that the correct parents are identified in 100% of the offspring, even when assuming up to 5% genotyping errors.

Keywords: Salmon, parentage, SNPs, simulation

*These authors have contributed equally to this work.

§ Corresponding author. Tel.: +47 72 450 500 ; Fax: +47 72 450 525
E-mail address: + kim.erik.grashei@aquagen.no; + jorgen.odegard@aquagen.no

SHRIMP BROODSTOCK MANAGEMENT FOR THE CONTROL OF GENETIC DIVERSITY AND INBREEDING

Pérez-Enríquez R.[a, §], Robles-Cota C.[b], Peiro-López J.[c], Haffray P.[d]

[a] *Aquaculture Program, Centro de Investigaciones Biológicas del Noroeste S.C., La Paz, 23096, Mexico*
[b] *Blue Genetics Mexico, La Paz, 23000, Mexico*
[c] *Acuacultura Mahr, La Paz, 23000, Mexico*
[d] *Syndicat des Selectioneurs Avicoles et Aquacoles Français, Rennes, 35042, France*

Genetics is one of the management alternatives for production improvement. Independently of which feature we want to improve (growth, response to pathogens, feed efficiency) the selection programs carried out by commercial hatcheries have to have a well-designed management scheme that decreases the risk of inbreeding depression; this is achieved by the maintenance of the genetic diversity and the control of the mating structure. The Number of effective breeders (Ne) is a concept closely related to the inbreeding coefficient, and thus it should be maintained at high levels, particularly in closed populations subject to long-term management. The present work shows the evolution of the genetic diversity and inbreeding in a commercial breeding selection program for *Litopenaeus vannamei* in Mexico. Genetic diversity was evaluated through 6 generations using microsatellites as genetic markers. Allele frequencies were calculated and used for the estimation of genetic diversity (number of alleles per locus and heterozygosity) and inbreeding with GenAlEx software. Inbreeding was also estimated by the effective number of breeders (Ne) in each generation. Genetic gain was estimated at G_3 and G_6 by comparison of growth within and between generations, respectively. Following the first generation of selection the genetic diversity has been maintained on similar levels through generations, with 6.6 alleles per locus at generation G_6 and 0.545 of heterozygosity. The management of the broodstock has resulted in low inbreeding at G_6 of 3.2% (estimated by genetic markers) and at 0.16% (estimated by Ne). Genetic gain in growth rate between G_1 and G_3 was 14%, and 38% between the average of G_1-G_3, and the average of G_5-G_6. We discuss the strategies for selection and broodstock management in terms of these results.

Keywords: *Litopenaeus vannamei, hatchery management, genetic improvement, genetic gain, effective population size*

§Corresponding author. Tel.: + 52-612-1238504
E-mail address: rperez@cibnor.mx

ESTIMATES OF GENETIC VARIABILITY AND INBREEDING IN SELECTED POPULATIONS OF EUROPEAN SEA BASS

Hillen J.[a, §], Carr A.[b], Hellemans B.[a], Ogden R.[c], Taggart J.B.[d], Vandeputte M.[e, f], Vergnet A.[e], Volckaert F.A.M.[a], AquaTrace Consortium, Coscia I.[a]

[a] *Laboratory of Biodiversity and Evolutionary Genomics, University of Leuven, Leuven, B-3000, Belgium*
[b] *Fios Genomics, Edinburgh, EH16 4UX, United Kingdom*
[c] *TRACE Wildlife Forensics Network, Royal Zoological Society of Scotland, Edinburgh, EH12 6TS, United Kingdom*
[d] *Institute of Aquaculture, University of Stirling, Stirling, FK9 4LA, United Kingdom*
[e] *Ifremer UMR9190 MARBEC, Chemin de Maguelone, 34250 Palavas-les-Flots, France*
[f] *INRA, UMR1313 Génétique animale et Biologie intégrative, 78350 Jouy-en-Josas, France*

In recent years, the aquaculture industry has increasingly aimed at improving economically important traits, like growth, feed efficiency or resistance to infections, through artificial selection. It represents a great window of opportunity to significantly improve stocks. However, the pitfall of these procedures is that it may decrease genetic diversity and increase inbreeding. Therefore, it is important to monitor the level of inbreeding within a selected strain in order to ensure that genetic diversity remains acceptable throughout the selection process.

We have assessed the level of genetic variability over three generations of two populations of European sea bass (*Dicentrarchus labrax*) from an experimental farm located in France. The first strain originates from Atlantic wild broodstock, and was selected for growth over three generations. The second and third strain originate from Mediterranean wild broodstock and were selected for either high or low weight loss under a starvation regime (as an indirect predictor of feed efficiency) over two generations. We used a genomic approach (ddRADseq – double digest Restriction site Associated DNA sequencing) to screen 159 individuals. Using a suite of hundreds of genomic markers (single nucleotide polymorphisms - SNPs), we reliably estimate the inbreeding levels and genetic variability across generations for both strains. We also assess the relatedness of the individuals within each group and the number of effective breeders, in order to further determine the dynamics of reproduction. Finally, we calculate the genetic diversity within and between groups and generations. Our study is among the first using genomics to quantify inbreeding over generations of farmed sea bass selected for commercially relevant traits. The results provide a first insight on how such selection procedures affect inbreeding and genetic variability even within the short span of a few generations.

Keywords: inbreeding, selection, SNP, genetic

§ Corresponding author. Tel.: +3216 37 37 67
E-mail address: jasmien.hillen@bio.kuleuven.be

GENETIC SIGNATURES OF SELECTION AND ASSOCIATION ANALYSIS OF THE DOMESTICATION EVENT IN SOUTH AFRICAN ABALONE, *Haliotis midae*

Rhode C.[§], Dale-Kuys R., Vervalle J., Bester- Van der Merve A., Roodt-Wilding R.

Department of Genetics, Stellenbosch University, Stellenbosch, 7600, Republic of South Africa

The abalone, *Haliotis midae*, is an important aquaculture species in South Africa and the largest generator of revenue for the mariculture sector. Despite domestication of this species still being in the initial stages, significant differentiation has been observed between wild and cultured populations. The genetic consequences of founder effects, including population bottlenecks, inbreeding, and differential parental contributions, have been well documented in many aquaculture species. The effects of selection, however, remain under-investigated. This study therefore provides a preliminary assessment of the role of selection during a domestication event of a first generation cohort of cultured South African abalone. In order to identify signatures of selection, F_{st}-outlier analyses were conducted using 150 microsatellite markers across wild and cultured populations. Approximately 9% of the genome-wide microsatellite markers were subject to directional selection, whilst 6% to 18% of the loci are thought to be influenced by balancing selection. Genetic diversity estimates for candidate loci under directional selection was significantly reduced in comparison to candidate neutral loci, whilst candidate balancing selection loci demonstrated significantly higher levels of genetic diversity (Kruskal-Wallis test, $P < 0.05$). Pairwise F_{st} estimates based on candidate directional selection loci also demonstrated increased levels of differentiation between study populations. Various candidate loci under selection showed significant inter-chromosomal LD, suggesting possible gene-networks underling adaptive phenotypes. To further investigate the causative effects of selection, and association analysis was done, to evaluate the correlation of allelic content at loci under directional selection with growth in a culture cohort. Only two loci demonstrated significant evidence for association with size, with both loci possessing alleles that correlated significantly with either increased or decreased size. As size is currently the only trait actively selected for in terms of production, the current results suggest that natural selection for adaptation to the novel aquaculture environment is the predominant selective force shaping genetic variation during the initial stages of domestication in abalone. Furthermore, whilst it is currently unclear as to whether these loci represent causative variants for size traits, they may be useful in future molecular-assisted breeding programmes for *H. midae*.

Keywords: abalone; domestication, growth, outlier test, selection

[§] Corresponding author. Tel.: +27 21 808 5834; Fax: +27 808 5833
E-mail address: clintr@sun.ac.za

GENOME-WIDE SNPS PROVIDE INSIGHTS INTO FINE-SCALE POPULATION STRUCTURE AND VARIABILITY IN THE FIJIAN BLACK-LIP PEARL OYSTER *Pinctada margaritifera*

Lal M.M.[a, b, * §]**, Southgate P.C.**[a, b, c *]**, Jerry D.R.**[a, b *] **& Zenger K.R.**[a, b, *]

[a] *Centre for Sustainable Tropical Fisheries and Aquaculture,*
James Cook University, Townsville, QLD 4811, Australia.
[b] *College of Marine and Environmental Sciences,*
James Cook University, Townsville, QLD 4811, Australia.
[c] *Faculty of Science, Health, Education and Engineering,*
University of the Sunshine Coast, Maroochydore DC, QLD 4558, Australia.

Pearl and shell production from the Black-lip Pearl Oyster *Pinctada margaritifera* is a valuable industry and substantial source of livelihood in the Fiji Islands. The industry is almost exclusively dependant on wild oysters for which there are currently no comprehensive management guidelines, as a country-wide genetic stock assessment has not been undertaken. Therefore no information is available on the number of discrete populations present, their levels of genetic fitness, or if domestic translocation of animals is suitable for the establishment of new farms. Consequently, there is a compelling need for genetic resources to resolve these knowledge gaps and inform policy for the sustainable development of the industry.

SNPs are a powerful and highly versatile genetic tool for species management, and were selected here as their genome-wide presence and availability in large numbers provide the detection power required to discover fine-scale levels of population stratification, make assessments of locally adaptive variation and permit association analyses for future selective breeding efforts. The utility and power of 6,753 genome-wide SNP loci were investigated to describe the population structure, diversity and connectivity in four Fijian oyster populations discovered using double-digest restriction-site associated DNA sequencing (ddRADseq). The global F_{st} value was 0.0425, while population pairwise values ranged between 0.0015-0.0825 ($p<0.05$), indicating very little differentiation that is similar to patterns identified for other marine species which experience high levels of gene flow over large spatial scales. The markers described here will also be useful for independent F_{st} outlier detection for investigations of directional and balancing selection, to evaluate any population-specific local adaptations.

Overall, these SNPs provide for the first time the ability to examine fine-scale patterns of genetic structure, adaptive variation and genome-wide association in this commercially important marine mollusc, all of which are requisite for effective and comprehensive stock management. They are also excellent candidates for future research, and highly useful for further population assignment, translocation management and genetic monitoring of the Black-lip Pearl Oyster resource in the Fiji Islands.

Keywords: SNP, genetic diversity, stock management, marker utility, Fiji Islands

[*]These authors have contributed equally to this work.
[§] Corresponding author. Tel.: +61 0414 525 914 or +61 4781 5387 ; Fax: +61 7 4781 5511
E-mail address: monal.lal@my.jcu.edu.au

GENETIC STRUCTURE BASED ON MICROSATELLITES AND COLOR VARIANCE ANALYSIS FOR A BRIGHT RED CLAM, *Paphia amabilis* (PHILIPPI, 1847)

Yu F.[a, *], Li Q.Z.[b, *], Wu X.X.[a, *], Luo B.[b], Zhu J.[a], Wang Y.[a, §]

[a] *Key Laboratory of Tropical Biological Resources of Ministry of Education, Ocean College, Hainan University, Haikou 570228, China*
[b] *Guangxi Academy of Fishery Science, Nanning, 530021, China*

Paphia amabilis is an important fishery shellfish widely distributing along Southeast Asia coast. In recent years, its natural populations have been declining significantly due to over-fishing, which highlights the urgency of its aquaculture. The muscle of *P. amabilis*'s foot and of siphons has a bright red color due to the presence of a new C37 skeletal carotenoids. However, 10.5% to 18.6% individuals were found with yellowish muscle in the wild populations from Vietnam and China. Chi-Square tests revealed that no significant difference of the sex ratio (near 1:1) and the percentage of individuals with yellow muscle among those four populations ($P<0.05$); And although male rate were higher than female rate with yellow muscle, but not significant in winter samples, whereas significant ($P<0.05$) in autumn specimens (BHA, from Beihai), which indicated that the female individuals may enhance the accumulation of carotenoid in breeding season. Histological sections revealed that carotenoids may be mainly concentrated in the subcutaneous fat cells of the clams' foot.

Furthermore, we developed 20 genomic microsatellite markers, and analyzed the population structure of three *P. amabilis* natural populations (CT, from Co To island, Vietnam, n=32; BH, Beihai, Guangxi province, China, n=32; ZJ, Zhanjiang, Guangdong province, China, n=32) in the north of Beibu Bay, South China Sea. The results showed that high levels of polymorphism were observed in all three populations with the number of alleles ranging 1-21 for CT, 1-19 for BH and 1-18 for ZJ. The average observed/expected heterozygosity were 0.58/0.66 for CT, 0.51/0.71 for BH, and 0.47/0.68 for ZJ, respectively. Four (*Pam14,20,36,38*) loci deviated from Hardy–Weinberg equilibrium ($P<0.05$ after Bonferroni correction) in at least 2 populations in the direction of heterozygote deficiency, probably because of the presence of null alleles and substructuring of population samples. Significant genetic differentiation was revealed by globe FST (0.050) and pairwise FST, especially between CT and BH (0.093). No microsatellite marker was found associated with foot color of *P. amabilis*.

*These authors have contributed equally to this work.
§ Corresponding author. Tel.: + 86 (0)13707543005; Fax: + 86 898 66279215
E-mail address: wy2005@163.com

SECOND-GENERATION LINKAGE MAPS REVEAL ERRORS IN THE ASSEMBLY OF THE PACIFIC OYSTER *(Crassostrea gigas)* GENOME AND FACTORS AFFECTING MAP LENGTHS AND MARKER ORDERS

Hedgecock D.[a, §]

[a] *Department of Biological Sciences, University of Southern California, Los Angeles, 90089-0371, U.S.A.*

Linkage maps are needed for identifying quantitative trait loci (QTL) that influence production traits (e.g. survival, growth, sex-determination, and reproductive maturation) of the Pacific oyster. A published genome (Zhang et al. 2012 Nature), moreover, should enable dissection of QTL and identification of candidate genes underlying variation in production traits. First-generation linkage maps for the oyster were based on ~100 microsatellites markers. In some cases, microsatellites were supplemented with hundreds of dominant, largely non-transferrable AFLP markers and, more recently, with dozens of single-nucleotide polymorphisms (SNPs). Altogether, these efforts yielded maps with 10 linkage groups, equal to haploid chromosome number, but with total lengths of ~600-1000 cM and average marker spacing of ~10 cM. To improve coverage and candidate gene-discovery, we developed 1536 SNPs, from protein-coding sequences, and typed these in five, full-sib families of oysters, using an Illumina Golden Gate bead array. We mapped 1095 of these SNPs in one or more families, decreasing marker spacing 10-fold, to 1 cM, and yielding an estimated genome length of ~620 cM, as expected from cytological, flow-cytometric, and genome sequence data. However, marker order, spacing, and fit are variable across families and mapping methods, owing to (1) an abundance of markers segregating from only one parent, (2) widespread distortions of segregation ratios caused by early mortality, as previously observed for oysters, and (3) genotyping errors. Selection has idiosyncratic effects that depend on the number, dominance, and phase of viability loci. A high-density, consensus linkage map of 660 framework markers provides a starting map for future work. We assign 1087 SNPs to 623 genome scaffolds; all but two map to the same linkage group, across families, providing confidence in assignment of SNPs to linkage groups. However, of 261 genome scaffolds containing two or more mapped markers, 101 (39%) have SNPs that map to two or more different linkage groups. These observations suggest that the genome, which has good coverage of gene-sequences, comprises a large number of miss-assembled scaffolds, which could hinder progress in QTL mapping. Information from even denser, third-generation, linkage maps will be useful in improving the assembly of the oyster genome.

Keywords: SNPs, map length, marker order, segregation distortion, genotyping errors

[§] Corresponding author. Tel.: +1 (213) 821-2091; Fax: +1 (213) 740-8123
E-mail address: + dhedge@usc.edu

IDENTIFICATION OF SINGLE NUCLEOTIDE POLYMORPHISM MARKERS ASSOCIATED WITH BACTERIAL COLD WATER DISEASE RESISTANCE AND SPLEEN SIZE IN RAINBOW TROUT

Liu S.*, Vallejo L.R., Palti Y., Gao G., Marancik D.P. and Wiens G.D.

*USDA/ARS National Center for Cool and Cold Water Aquaculture,
11861 Leetown Rd, Kearneysville, WV 25430, USA*

Bacterial cold water disease (BCWD) is one of the frequent causes of elevated mortality in salmonid aquaculture. Previously, we identified and validated microsatellite markers associated with QTL (quantitative trait loci) for BCWD resistance and spleen size in rainbow trout. The objective of this study was to identify single nucleotide polymorphism (SNP) markers associated with BCWD resistance and spleen size using both genome-wide association studies (GWAS) and QTL mapping approaches. A total of 298 offspring from the two half-sib families used in our previous study to validate the significant BCWD QTL on chromosome Omy19 were genotyped with RAD-seq (restriction-site-associated DNA sequencing), and 7,849 informative SNPs were identified. Based on GWAS, 18 SNPs associated with BCWD resistance and 20 SNPs associated with spleen size were identified. QTL mapping revealed three significant QTL for BCWD resistance. In addition to the previously validated dam-derived QTL on chromosome Omy19, two significant BCWD QTL derived from the sire were identified on chromosomes Omy8 and Omy25. Consistent with previous study, there was no spleen size QTL on chromosome Omy19 in these two families. However, one significant QTL for spleen size on chromosome Omy2 was identified. The SNP markers reported in this study would facilitate fine mapping to identify positional candidate genes for BCWD resistance in rainbow trout.

Keywords: Bacterial cold water disease, Rainbow trout, Flavobacterium psychrophilum, QTL, SNP

*Corresponding author. Tel: 203 724 8340; Fax: 304 725 0351
E-mail address: sixin.liu@ars.usda.gov

A HIGH DENSITY GENETIC LINKAGE MAP FOR RAINBOW TROUT *(Oncorhynchus mykiss)* CONTAINING 47,839 SNPS

Baranski M.[a, §], Palti Y.[b], Moen T.[c], Grove H.[d], Gao G.[b], Lien S.[d], Liu S.[b], Rexroad C.[b]

[a] *Nofima, Ås, 1430, Norway*
[b] *USDA-ARS-NCCCWA, Kearneysville, 25430, USA*
[c] *AquaGen, Trondheim, 7462, Norway*
[d] *NMBU-IHA (CIGENE), Ås, 1430, Norway*

High-density SNP arrays have become the tool of choice for QTL mapping, genome-wide association studies and genomic selection. More recently, high-density linkage maps generated by SNP array data have proven to be crucial for the accurate assembly of scaffolds and contigs in whole-genome sequencing efforts. Rainbow trout is an important aquaculture species world-wide, and a first draft of the genome sequence of this species is now available. Earlier mapping studies have identified QTL for important commercial traits including disease resistance, and combining the resources of a high density genetic map with genome sequence data will facilitate the fine mapping of these loci and the identification of candidate genes and mutations. In this study, a 57K Affymetrix SNP array was used to genotype 2,464 samples collected across 46 full-sib families from a commercial Norwegian population and 10 from an experimental USDA breeding population. Following quality control of raw genotype data, linkage mapping was performed with Lep-MAP software. First, SNPs were assigned to linkage groups with the 'SeparateChromosomes' command using increasing LOD thresholds until the observed number of linkage groups corresponded with the haploid chromosome number in this species. Additional SNPs were subsequently added to the groups with the 'JoinSingles' command at a more relaxed LOD threshold, and finally SNPs were ordered in each linkage group with the 'OrderMarkers' command. Numerous iterations were performed to optimise error and recombination parameters. A total of 47,839 SNPs were mapped to 29 linkage groups, with an average of 1,650 SNPs per group. The number of SNPs assigned to each group ranged from 754 to 2,934. The total distances covered by the male and female maps were 2,214 cM and 4,248 cM, respectively. In all 13 chromosomes known to have homeologous pairing with at least one other chromosome arm, the female/male recombination ratios were >2.0, while in non-duplicated chromosomes the female/male recombination ratio ranged from 1.0 to 2.0, with the exception of chromosomes Omy15 and Omy21. This map is currently being used to map QTL for a number of commercially important traits, and will be used to improve the assembly of the rainbow trout genome.

Keywords: SNP, linkage map, Rainbow trout, Affymetrix, genome

§ Corresponding author. Tel.: +47 6497 0396
E-mail address: matthew.baranski@nofima.no

GENE MAPPING IN THE SENEGALESE SOLE *(Solea senegalensis)*

Rodríguez M.E., Portela-Bens S., Merlo M.A., Cross I., Rebordinos L.

*Área de Genética. Facultad de Ciencias del Mar y Ambientales,
Universidad de Cádiz. 11510, Puerto Real, Cádiz, Spain.*

In aquaculture, an industry of vital importance in Spain, the application of genetic tools to improve the cultivation of crop species of interest is critical for future development. Flatfish is one of the most interesting groups of fish in this regard, since these fish are increasingly being cultivated as an alternative to the more traditional bream and sea bass. Specifically, sole (Solea senegalensis) is a species of great economic value, mainly in the south and western Spanish coasts, due largely to its rapid development, fast rate of growth and high-quality meat. Therefore, the development of genetic tools and applications in this species would be of significant value for the development and improvement of sole cultivation, which currently suffers several serious limitations related mainly to reproduction, metamorphosis and pathologies.

The sole has a karyotype with 42 chromosomes and 48 chromosome arms, comprising 3 metacentric pairs, 2 submetacentric pairs, 4 subtelocentric pairs and 12 acrocentric chromosome pairs. Distinguishing pairs within each of the above categories is a difficult task because of their similarity in size and morphology.

Integrated genetic and physical maps bring together information from linkage analysis with sequencing of genes and their chromosomal location. The quality of a map depends on its degree of resolution, i.e. on the ability to measure the distance between elements that are situated very close together. Integrated genetic maps are extremely valuable resources and allow comparative studies of synteny to be performed and applied advantages, such as positional cloning, to be obtained. A physically-anchored genetic map also allows an approach oriented to sequencing of the complete genome or of individual chromosomes. Physical mapping of genes is particularly important in fish due to the high degree of genetic interference in small chromosomes and the difficulties in identifying individual chromosomes.

Therefore, we have sequenced BAC clones by NGS technology and used them as probes in FISH simple, double and multiple. In order to to obtain a genetic integrated map.

The results presented are a major advance in the understanding of the molecular and genomic organization of Senegalese sole, and they represent a starting point for further studies applied to aquaculture, especially at the level of expression. Also, the preliminary study genetic map is a source of information that can be used in a complementary manner with physical maps and / or linkage.

Keywords: Senegalese sole, BACs, Solea senegalensis, genetic map, genomics

GENETIC LINKAGE MAP AND QTL ANALYSIS OF GROWTH RELATED TRAIT IN PACIFIC BLUEFIN TUNA

Uchino T.[a], Hosoda E.[a], Nakamura Y.[b], Sekino M.[b], Fujiwara A.[b], Yasuike M.[b], Sugaya T.[c], Tanaka Y.[d], Kumon K.[d], Sano M.[a], Sakamoto T.[a, §]

[a] *Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, Tokyo, 108-8477, Japan*
[b] *Aquatic Genomics Research Center, National Research Institute of Fisheries Science, Fisheries Research Agency, Kanagawa, 236-8648, Japan*
[c] *Marine Invertebrate Animals Research Center, National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, Hiroshima, 722-0061, Japan*
[d] *Research Center for Tuna Aquaculture, Seikai National Fisheries Research Institute, Fisheries Research Agency, Kagoshima, 894-2414, Japan*

Pacific bluefin tuna, *Thunnus orientalis*, has a high market value. However, the wild population of bluefin tuna has decreased in recent years. Recently, the complete aquaculture technology was established for this specie and artificial seeds are beginning to be used for production. Therefore, there is much interest in creating broodstock with commercially valuable traits, such as rapid growth. To genetically improve the broodstock, a genetic linkage map located many DNA markers is needed to efficiently find markers associated with a Quantitative trait loci (QTL) that can be used in marker-assisted selection (MAS) breeding programs. In this study, we have developed sex-specific microsatellite (MS) linkage maps using the tuna draft genome sequence and attempted to identify growth related QTL.
In order to collect sibling individuals, a parentage test was conducted using 500 individuals produced from random mating, and 197 individuals were identified as a full-sib population. Standard length of these sibling individuals was measured for QTL analysis, and an average was 8.3 mm. We selected the longest 1,000 scaffolds that showed sequence homology to a part of the medaka genome sequence. 398 pairs of PCR primers were designed adjacent to Microsatellite (MS) regions in the selected scaffolds. Construction of linkage maps was performed with a LOD threshold of 4.0. An examination of the segregation pattern of the 398 MS markers revealed that 344 of the markers each on a different scaffold had linkage relationships with each other. Both the female and male maps consisted of 24 linkage groups, corresponding to the number of tuna chromosome pairs. The marker positions of each linkage group enabled us to identify scaffolds that account for 15.6 % (115.5 Mb) of the draft genome. We performed QTL analysis of growth-related trait (standard length) with composite interval mapping (CIM) method. Permutation tests were performed (1,000 replicates) to determine the threshold for LOD at 5 % level. QTL analysis revealed one significant QTL on LG10 of female map. This QTL will be useful for MAS programs in tuna aquaculture.

Keywords: Pacific bluefin tuna, genetic linkage map, growth related QTL

*These authors have contributed equally to this work.
§ Corresponding author. Tel.: + 080-6561-8435
E-mail address: + wingin.side88@gmail.com

MARKERS FOR RESISTANCE TO WHITE SPOT SYNDROME VIRUS IN BLACK TIGER SHRIMP *(Penaeus monodon)* AND CORRESPONDANCE TO CSNPS IN WHITE SHRIMP *(Litopenaeus vannamei)*

Robinson N.[ac§], Baranski M.[a], Gopikrishna G.[b], Vinaya Kumar K.[b], Shekhar M.S.[b], Gopal C.[b], Panigrahi A.,[b], Balasubramanian C.P.[b], Ashok Kumar J.[b], Rajendran V.[b], Moghadam H.[a], Aranguren F.[d], Salazar M.[d], Gitterle T.[e] and Vijayan K.K. [b]

[a]Nofima, PO Box 5010, 1432 Ås, Norway.
[b]Central Institute of Brackishwater Aquaculture, Raja Annamalai Puram, Chennai, Tamil Nadu, India.
[c]Flinders University, Sturt Road, Bedford Park, SA 5042, Australia.
[d]Ceniacua – Centre for Aquaculture Research Columbia, CARRERA 70F N° 78 A 84, Bogota, Columbia.
[e]SyAqua, Unit 1902-4, 140 One Pacific Place, Sukhumvit Road, Klongtoey 10110, Bangkok, Thailand.

White spot syndrome virus (family Nimaviridae, genus *Whispovirus*, WSSV) is a lethal pathogen that can cause up to 100% mortality in shrimp within 7-10 days after the onset of infection. Dead or infected shrimp are the main vector for horizontal transmission of the disease, and the disease can be vertically transmitted from brood shrimp to post larvae. As yet, there are no treatments with proven efficacy against WSSV. One of the best options for reducing the incidence of WSSV disease would be to produce shrimp with a heightened resistance to the disease. Family-based selection using controlled challenge tests is one means of increasing resistance, however this trait has been shown to be lowly heritable in *L. vannamei* and similarly, limited evidence has been found for genetic variation for resistance to WSSV in *P. monodon*. There is a need for tests that can be used to directly select individuals that have gene variants promoting resistance to WSSV.

Despite the low heritability of WSSV resistance, breeding survivors from artificial infections under high selection pressure resulted in an increased resistance of the selected population after several rounds of selection, suggesting that this trait is an excellent candidate for marker-assisted selection (MAS). To find markers in *P. monodon*, a powerful design comprising through marker coverage of the genome and a mapping population with reliable phenotypic recordings was used. Here we checked for correspondence between the quantitative trait loci (QTL) found to affect WSSV resistance in *P. monodon* and cSNPs identified in *L. vannamei* using next generation sequencing technologies.

Several regions with suggestive QTL for WSSV resistance (measured as hours survival post-challenge) were detected in *P. monodon*. Genes mapping to these QTL regions, and corresponding SNPs found in the *L. vannamei* genome, are discussed. The potential use of these SNPs as QTL markers for *L. vannamei* WSSV resistance will be tested experimentally in our current project.

Keywords: White spot syndrome virus; disease resistance; quantitative trait loci; Penaeus monodon; Litopenaeus vannamei

§ Corresponding author. Tel.: +61 448984002
E-mail address: nick.robinson@nofima.no

EXTENT OF GENOME-WIDE LINKAGE DISEQUILIBRIUM IN FARMED ATLANTIC SALMON *(Salmo salar L.)* USING HIGH-DENSITY GENOTYPES

**Yáñez, J.M.[a, §] , Correa K.[a,b], López M.E.[b,c], Lhorente J.P.[b],
Figueroa R.[b], Bassini L. [b,c], Di Genova A.[d], Maass A.[d]**

[a] *Facultad de Ciencias Veterinarias y Pecuarias, Universidad
de Chile, Santiago, 8820808, Chile*
[b] *Aquainnovo, Puerto Montt, 5503032, Chile*
[c] *Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago, 8820808, Chile*
[d] *Laboratory of Bioinformatics and Mathematics of the Genome, Center for Mathematical
Modeling, Universidad de Chile, Beauchef 851, Santiago, 8370456, Chile*

The extent and decay of linkage disequilibrium (LD) among single nucleotide polymorphism (SNP) impacts genome-wide association studies and implementation of genomic selection. The availability of a high-density SNP genotyping platform for Atlantic salmon makes it possible to investigate LD at higher resolution. In this study, we characterized LD extent and decay in a commercial population of Atlantic salmon. Fin clip samples were taken from 86 fish belonging to a breeding population from Chile. Samples were genotyped using a 159K SNP Axiom® myDesign™ Genotyping Array. To estimate LD we used r^2 coefficient calculated across all pairs of syntenic markers. A non-linear model was fitted to determine the effects of marker distance and chromosome on the decay of LD. LD decayed rapidly and r^2 was found to be less than 0.2 for SNP pairs separated by 25 kb. At short distances between markers (< 10 kb), this breeding population showed high levels of LD (r^2 = 0.37). Furthermore, the decay of LD was found to be significantly different among chromosomes. Longer ranges of LD extent were found for chromosomes *Ssa15*, *Ssa16* and *Ssa19*, for which the expected LD between SNP markers separated by 10 kb were r^2 = 0.45, 0.46 and 0.52, respectively. On the other hand, LD decayed rapidly in chromosomes *Ssa7* and *Ssa27*, for which the expected LD between SNP markers separated by 10 kb were r^2 = 0.23 and 0.24, respectively. Higher levels of LD in *Ssa15*, *Ssa16* and *Ssa19* may reflect selection for traits that are strongly influenced by QTL of large effect on these chromosomes in this breeding population. The pattern of genome-wide LD showed a rapid decay with increasing physical distance between SNP markers. The SNP panel used in this study, which on average has one SNP every 14 kb, can be used to detect association between markers and traits of interests and also capture high-resolution information for genome-enabled predictions, since LD dropped below 0.2 only at distances higher than 25 kb. Further studies aimed at detecting selection signatures in chromosomes *Ssa15*, *Ssa16* and *Ssa19* may help to identify genomic regions involved in traits subjected to selection on this commercial population.

Keywords: Atlantic salmon, linkage disequilibrium, SNP, genomic selection

§ Corresponding author. Tel.: + 56-2-29785533
E-mail address: jmayanez@uchile.cl

USE OF NEXT-GENERATION SEQUENCING IN THE PACIFIC OYSTER TO DISCOVER AND GENOTYPE SNP MARKERS FOR BUILDING THIRD-GENERATION LINKAGE MAPS

Arias-Pérez A.[a, b, §], Hedgecock D.[b]

[a] *Departamento de Bioloxía Celular e Molecular, Universidade da Coruña, A Coruña, 15071, Spain*
[b] *Department of Biological Sciences, University of Southern California, Los Angeles, 90089-0371, U.S.A.*

The use of protocols based on advances in DNA sequencing technologies can facilitate the development of genomic tools for the aquaculture industry. Such tools include linkage maps that are fundamental to QTL mapping of performance and production traits, to positional cloning, or to improve the assembly of reference genomes. For the Pacific oyster *Crassostrea gigas*, as for most aquaculture species, the density of available maps has limited their utility. Different technologies and types of markers have been employed to build linkage maps with tens to hundreds of markers. However, these approaches are labor intensive, expensive and yield a limited number of markers. To build a third-generation, high-density linkage map for one, full-sib, Pacific oyster family (two F_1 parents and 129 F_2 offspring), we employed a next-generation sequencing based protocol that permits simultaneous discovery and genotyping of thousands to tens of thousands of SNP markers. A restriction enzyme was used to target a subset of the genome, and barcoded adapters allowed pooling of multiple individuals in the same library and sequencing of that library in a single lane of an Illumina flow cell. A preliminary analysis of the sequence data revealed 1,922,129 potential variants, which mapped to 5,303 of the 11,969 scaffolds (>100 bp) that comprise the reference genome (Zhang et al 2012 Nature 490:49). After removing potential variants with low coverage, non-segregating variants, variants with unexpected offspring genotypes, and individuals with a low number of sequences, a preliminary analysis of the data identified 3,664 segregating SNP markers. When these SNPs and 37 microsatellite markers were used to build a linkage map, we obtained ten main linkage groups, comprising 1,442 markers and spanning 874.5 cM, with an average spacing of 0.8 cM. In some cases, more than one of these markers mapped to the same scaffold, but unexpectedly, 100 of these 237 scaffolds (42%) have markers that map to different linkage groups. This suggests that a large number of scaffolds are miss-assembled. The protocol employed in this work will help to map QTL for production traits in the Pacific oyster and to refine the assembly of the genome.

Keywords: next-generation sequencing, linkage map, SNPs, Pacific oyster

[§] Corresponding author. Tel.: + 34 981 167 000 ext. 2030; Fax: + 34 981 167 065
E-mail address: aarias@udc.es

BLUNT SNOUT BREAM, MEGALOBRAMA AMBLYCEPHALA, GENOME REVEALS THE EVOLUTION AND ADAPTATION TO HERBIVOROUS DIET

Liu H. and Wang W.*

Fisheries College of Huazhong Agricultural University, Wuhan, China

Blunt snout bream, *Megalobrama amblycephala*, is an important herbivorous cyprinid freshwater fish and mainly distribute in the Yangtze River of China. It has become the most economically important freshwater aquaculture specie due to its desirable qualities for aquaculture such as herbivorous feeding habit, resistance to disease, and reproductive performance. A high-quality draft genome of *M. amblycephala* sequencing at 100 × coverage using Illumina short-read sequencing technology was studied in order to elucidate the molecular mechanisms underlying its feeding habits and other traits. The generated genome of *M. amblycephala* was around 1.1 G in size, with the contigN50 and scaffold N50 values of 49 kb and 839 kb, respectively. Using the annotation pipeline and RNA-seq transcript evidence, we annotated a total of 23,696 protein-coding genes. Phylogenetic analysis found that the *M. amblycephala* lineage has 624 gene families expanded and 7,084 contracted compared with other fish species. *M. amblycephala* had a developed digestive s.ystem with all kinds of protease, and amylase genes compared with other carnivorous and omnivorous fish species. It was also found two predicted digestive cellulose genes, beta-glucosidase. Unexpectedly, however, the two genes may have no functions due to the mutations of catalytic active site and putative proton donor site. Meta-analysis found that the gut microbial communities of *M. amblycephala* dominated by *Fusobacteria*, *Bacteroidetes*, and *Firmicutes* were similar to other herbivorous animal, underlying that its unique traits of herbivorous diet might not depend on its own genetic composition and is more related with its gut microbiome. Comparative analysis showed that the olfactory receptor (OR) genes in *M. amblycephala* genome were extensive expansion, especially, the beta type of ORs. Our analysis also identified frame shift mutations in T1R1 gene, suggesting that it lost the taste senses of umami. Collectively, study on the whole genome sequence of *M. amblycephala* provides new insights in understanding the evolution and adaptation to its unique herbivorous behavior and a valuable resource on genetic improvement.

Keywords: Megalobrama amblycephala, genome, evolution and adaptation, herbivorous diet, olfactory receptor (OR) genes

GENOME SEQUENCING OF THE TURBOT *(Scophthalmus maximus;* PLEURONECTIFORMES) A FLATFISH OF HIGH AQUACULTURE VALUE

Figueras A. [a], Corvelo A.[b], Robledo D.[c], Hermida M.[d], Pereiro P.[a], Gómez J.[b], Carreté L.[e], Bello X.[f], Marcet-Houben M.[e], Forn-Cuní G.[a], Abal-Fabeiro J.L. [f], Pardo B.G. [d], Taboada X.[c], Fernández C.[d], Rubiolo J.A.[d], Álvarez-Dios J.A.[g], Gómez-Tato A.[h], Viñas A.[c], Maside X. [f], Gabaldón T.[e], Novoa B.[a], Bouza C.[d], Alioto T.[b], Martínez P.[d] [§]

[a] Instituto de Investigaciones Marinas (IIM) (CSIC), Vigo, 36208, Spain
[b] Centre Nacional d'Anàlisi Genòmica (CNAG), Parc Científic de Barcelona, 08028, Spain
[c] Dep. de Xenética, Fac. de Bioloxía (CIBUS) da USC, Santiago de Compostela, 15782, Spain
[d] Dep. de Xenética, Fac. de Veterinaria da USC, Lugo, 27002, Spain
[e] Bioinformatics and Genomics Programme, Centre for Genomic Regulation (CRG), Barcelona, 08003, Spain
[f] Departamento de Bioloxía Celular e Molecular, Centro de Investigaciones Avanzadas (CICA), Universidade da Coruña, A Coruña, 15071, Spain
[g] Departamento de Matemática Aplicada, Facultad de Matemáticas. Universidad de Santiago de Compostela. 15782 Santiago de Compostela, Spain
[h] Departamento de Geometría y Topología, Facultad de Matemáticas. Universidad de Santiago de Compostela. 15782 Santiago de Compostela, Spain

Turbot is a marine flatfish (*Pleuronectiformes)* with an increasing aquaculture value in Europe and China. Here, we report the turbot genome assembly integrated with all previous transcriptomic and mapping resources both for boosting breeding programs and for identifying specific evolutionary signatures related to its particular lifestyle. Whole genome paired-end (PE) sequencing was performed using Illumina GAIIx from different libraries to facilitate assemblage: i) PE libraries with insert sizes of 200bp and 500bp rendering >85Gb (120x coverage); ii) mate-pair (MP) libraries of 3 and 5kb producing ~6Gb (9x) and 12Gb (17x), respectively; and iii) a fosmid library of 140,000 clones rendering ~12Gb (8x). The assembly resulted in 544 Mb (~85% genome size), with a contig-N50 of 31.2kb and scaffold-N50 of 4.2Mb (95% assembly including 287 scaffolds). Turbot genes were identified using empirical transcriptome data and bioinformatic predictions, 22,803 protein-coding genes and 7,521 RNA genes being identified. The turbot genome was compared with our last version of the genetic map (~600 markers), the scaffolds being anchored to specific linkage groups covering >80% of the genome. This information enabled us to refine genome assembling, establish relationships between physical and genetic distances, and investigate teleost chromosome evolution by comparative mapping. Gene family trees were analyzed regarding other sequenced vertebrates to predict orthology and paralogy relationships, and to characterize duplication events. Evolutionary relationships based on 327 genes rendered a highly supported topology mostly in accordance with previous data, identifying stickleback as the closest model fish to turbot. Transposable elements (TE)-derived DNA represented nearly 7% of the genome (~38 Mbp), a fraction slightly higher than that found in other Neoteleostei (~3%), but much lower than in *Danio rerio* (~40%; Ostariophysi). All major TE orders were represented, and MITE DNA-transposons were the most abundant superfamily (27% TE). Finally, the genetic architecture of the most important aquaculture traits, growth, reproduction and immune response, was analyzed, and QTL regions previously identified were used as reference for gene mining and functional enrichment. An important amount of candidate genes and functionally enriched-related genomic regions for relevant productive traits were identified, representing an essential starting-point for future marker assisted selection programs.

Keywords: turbot genome, transcriptome, phylome, transposable elements, genetic architecture

[§] Corresponding author. Tel.: +34982822425; E-mail address: paulino.martinez@usc.es

GENOME SEQUENCING OF 12 PUFFERFISHES

Hosoya S.[a, §], Tasumi S.[a], Kobayashi H.[b], Kikuchi K.[a, §]

[a] *Fisheries Laboratory, University of Tokyo, Shizuoka431-0214, Japan*
[b] *NODAI Genome Research Center, Tokyo University of Agriculture, Tokyo156-8502, Japan*

In contrast to terrestrial domestic animals, aquaculture fishes often have a number of closely-related species. Furthermore, these closely-related species also tend to be diverse in many traits, and thus have the potential for genetic improvement through selective breeding.

Fugu, *Takifugu rubripes* (also known as tiger pufferfish), is one of the most important aquaculture species in Japan. Besides, the genome of fugu, due to its compact size, has been used as a model genome for identifying genes and other functional elements in vertebrate genomes. The *Takifugu* genus has undergone extensive radiation in the last 2-5 million years, resulting in about 20 species, including fugu, that exhibit an impressive diversity in morphology, physiology, life history and behavior. Thus, *Takifugu* species form an attractive model system for the studying the genetic basis of phenotypic diversity among closely-species, and the utilization of closely-related species for genetic improvement.

In this study, were sequenced whole genomes of 11 *Takifugu* species and a close relative, *Torquigener hypselogeneion*, using the Illumina sequencing technology. Overall, we were able to map around 97% of the reads of those *Takifugu* species to the reference genome sequence of fugu (Fugu ver.5 made by Fisheries Lab, University of Tokyo and IMCB), suggesting that genetic distances among the closely related species of *Takifugu* are very close. Indeed, the DNA sequence of the 11 *Takifugu* species showed approximately 99% identity to the reference sequence of fugu. Phylogenetic analysis revealed discrepancies between the whole genome-based taxonomy and the previously reported mitogenome-based one. Analysis of the intra- and interspecific diversity in the sequenced genomes suggests extensive gene flow between many of the closely-related species. These interspecific hybridizations may have been important in facilitating subsequent diversification of *Takifugu* species. We believe that the data from the re-sequenced genomes in this study will be a valuable resource for linking genotype and phenotype among *Takifugu* species and will facilitate the genetic improvement of fugu and other aquaculture fish.

Keywords: fugu, Takifugu, closely-related species, whole genome sequencing, breeding

[§]Corresponding author. Tel.: +81-53-592-2821; Fax: +81-53-592-2822
E-mail address: ahosoya@mail.ecc.u-tokyo.ac.jp, akikuchi@mail.ecc.u-tokyo.ac.jp

DE NOVO GENOME ASSEMBLY OF THE AFRICAN CATFISH *(Clarias gariepinus)*

Kovács B.[a,§], Barta E.[c], Pongor S.L.[b], Uri Cs.[a], Patócs A.[b], Orbán L. [d], Müller T.[a], Urbányi B.[a]

[a] *Department of Aquaculture, Szent István University,
Páter Károly u. 1., H-2100,Gödöllő, Hungary*
[b] *Molecular Medicine Research Group, Hungarian Academy of Sciences and
Semmelweis University, Szentkirályi u.46, 1088 Budapest, Hungary.*
[c] *Agricultural Genomics and Bioinformatics Group, National Agricultural Research and
Innovation Center (NARIC), Szent-Györgyi Albert u. 4., H-2100 Gödöllő Hungary.*
[d] *Reproductive Genomics Group, Temasek Life Sciences Laboratory, 1 Research
Link National University of Singapore, Singapore 117604, Singapore*

Following the carp, the African catfish (*Clarias gariepinus*) is produced in the second largest volume in Hungary and farmed in many other countries. In addition, it is one of the models for reproductive fish endocrinology (hormonal regulation, puberty) and sex determination. During our previous work two sex-specific DNA markers were identified, demonstrating the presence of strong chromosomal sex determination and male heterogamety in this species. Among catfish species, only the genome of channel catfish (*Ictalurus punctatus)* is available currently. However, *I. punctatus* is not a close relative of *Cl. gariepinus* and therefore its genome is unlikely to be a good reference for a positional cloning approach. Moreover, in the *Clariidae* family both female and male heterogamety have been described, like in many other families or orders among fishes.

In order to learn more about the genome architecture of African catfish and improve our understanding of the differences between the male and female genomes, we have started a *de novo* genome sequencing and assembly project. For the genomic DNA library preparation we used a single male individual. Paired-end and mate-pair libraries were generated and sequenced by Illumina HiSeq 2000. The data processing involved the alignment of about 711 million unique reeds (71 billion bases) corresponding to ~60x coverage of the African catfish genome. Sequence assembly was performed with SOAPdenovo, Minia and Allpaths-lg. The best result was generated by Allpaths-lg (1,276 scaffolds, 940 Mb, N50:600219, N75:191375), ~400 Mb of unmapped nucleotide. Gene model prediction is underway using as extinct evidence protein and EST data from phylogenetically related taxa.

This represents the first report of a draft genome sequence for African catfish and the second for all the >2,200 catfish species. Sex-specific markers are good starting points for identification of genetic regions and genes involved in sex determination. Moreover the draft genome will be a valuable source of information for marker detection/selection, genetic improvement, conservation and basic biology studies, however, additional genome re-sequencing and exome sequencing from different lineages must be carried out to reach these future aims.

Keywords: Clarias gariepinus, Next generation sequencing, De Novo, Sex-specific marker

§ Corresponding author. Tel.: + 36 28 522 000/1610; Fax: + 06 28 522-927
E-mail address: Kovacs.Balazs@mkk.szie.hu

ACCURACY OF POPULATION-WIDE AND WITHIN-FAMILY GENOMIC SELECTION IN ATLANTIC SALMON

Sonesson A.[a, §], Meuwissen T.H.E.[b], Baranski M. [a], Moghadam H.[a], Lillehammer M.[a], Norris A.[c], Bakke, H.[d], Lund V.[e],

[a] *Nofima, Ås, 1430, Norway*
[b] *NMBU, Ås, 1430, Norway*
[c] *Marine Harvest, Sandnes, 4319, Norway*
[d] *SalmoBreed, Bergen, 5003, Norway*
[e] *Salmar, Kverva, 7266, Norway*

In aquaculture, the focus of genomic selection is to improve traits that can only be measured on the siblings of selection candidates, and not the candidates themselves. Population-wide linkage disequilibrium (LD) or within-family linkage analysis (LA) genomic information can be used to calculate the relationship between individuals. The aim of this study is to compare the accuracy of selection with traditional BLUP, GBLUP_LD and GBLUP_LA. Using cross-validation, accuracy of selection was calculated as the correlation between phenotype and breeding value scaled by the heritability of the trait, i.e. the accuracies are comparable over traits. Approximately 1,500 fish were genotyped with a 37K Affymetrix SNP array for each trait, resulting in around 31,000 informative SNPs. For resistance to pancreas disease, the accuracy of selection was 0.31 with BLUP. With GBLUP_LD, accuracy was 0.70 with 20 selection candidates per family, and with 10 fish per family, the accuracy decreased to 0.55. When the number of SNPs decreased to 10,000 and 3,000, accuracy decreased to 0.66 and 0.65, respectively. The GBLUP_LA had higher accuracy than GBLUP_LD and was less sensitive to the number of candidates per family than GBLUP_LD, accuracy reduced from 0.78 to 0.76 when the number of selection candidates was reduced from 20 to 10 per family. For resistance to salmon lice, the accuracy of selection for BLUP was 0.53. GBLUP_LD had higher accuracy of 0.59 than GBLUP_LA of 0.48 with a family size of around 20. In conclusion, accuracy was always higher with GBLUP than BLUP, and the efficiency of the methods differed between traits, which have different heritability and genetic architecture.

Keywords: genomic selection, accuracy of selection

§ Corresponding author. Tel.: +47 93098047
E-mail address: anna.sonesson@nofima.no

GENOMIC SELECTION FOR BCWD RESISTANCE IN RAINBOW TROUT USING RADSNP AND SNP GENOTYPING PLATFORMS, SINGLE-STEP GBLUP AND BAYESIAN VARIABLE SELECTION MODELS

Vallejo R.L.[a, *, §], Leeds T.D.[a, *], Liu S.[a], Gao G.[a], Welch T.J.[a], Wiens G.D.[a], Palti Y.[a, *]

[a] National Center for Cool and Cold Water Aquaculture, USDA-ARS, Kearneysville, West Virginia 25430, USA

Bacterial cold water disease (BCWD) causes significant economic losses in salmonid aquaculture. At the National Center for Cool and Cold Water Aquaculture (NCCCWA), we have pursued selective breeding to increase rainbow trout genetic resistance against BCWD and found that post-challenge survival is moderately heritable and responds to selection. Genomic selection (GS) is a recently developed methodology that is revolutionizing animal breeding and we plan to use GS in our breeding scheme to increase accuracy of selecting best genetic value animals. In this study, we aimed to 1) predict genomic breeding value (GEBV) for BCWD resistance in NCCCWA population; 2) compare the reliability of pedigree-based model (PED) with three GS models including BayesB and C and single-step GBLUP (ssGBLUP); and 3) compare the reliability of genomic predictions from two genotyping platforms (RADSNP and 50K SNP chip) when using GS models. The BCWD phenotypes survival days (DAYS) and survival status (STATUS) were recorded in training animals (n=583). The reliability of genomic predictions () was assessed using validation animals (n=53) that had PED estimated breeding value (EBV) for DAYS and STATUS. The reliability was assessed through predictive ability () defined as the correlation between EBVs and GEBVs, Then reliability was estimated as where is reliability of EBVs. For STATUS, all the GS models (BayesB and C and ssGBLUP; 59 – 130% increase) outperformed the PED model in reliability of genomic predictions. For DAYS, BayesB and C (69 – 109%) outperformed the PED model; however, the PED model outperformed ssGBLUP (-19 – -6% decrease). Overall, BayesB () estimated higher reliability GEBVs than BayesC () and ssGBLUP (). The number of polymorphic markers after quality control were ~42K and ~10K for the chip and RAD platforms, respectively. Hence, it is somewhat surprising that the RADSNP platform () was as efficient as the SNP chip (). However, we find that the SNP chip is higher throughput and more practical for large scale studies and currently is as cost-effective as RAD sequencing. The accuracy of GS for BCWD resistance in rainbow trout is expected to increase using larger training and validation-testing samples.

Keywords: Genomic selection, disease resistance, rainbow trout, single-step GBLUP, Bayesian methods

[*] These authors have contributed equally to this work.
[§] Corresponding author. Tel.: 304-724-8340; Fax: 304-725-0351
E-mail address: roger.vallejo@ars.usda.gov

WITHIN-FAMILY GENOMIC SELECTION IN AQUACULTURE BREEDING PROGRAMMES

Saura M.ª, Villanueva B.ª, Fernández J.ª, Toro M.A.[b, §]

ª *Departamento de Mejora Genética Animal, INIA, Madrid, 28040, Spain*
[b] *Departamento de Producción Agraria, ETS Ingenieros Agrónomos, Madrid, 28040, Spain*

A recent proposal for implementing cost effective genomic selection in fish breeding programmes has been to combine within-family genomic breeding values, based on low-density genotyping, with conventional family breeding values. In this study, we have investigated genetic gain and inbreeding when applying within-family genomic selection in the simplest scenario of starting with base populations in global linkage equilibrium. Populations composed of 50 families were simulated. The genome consisted of 10 chromosomes, with 100 markers and 1 or 100 QTLs per chromosome. The chromosome length (L) was 0.1, 1 or 100 Morgans. Two heritabilities (h^2 = 0.1 and 0.4) and two selection scenarios were considered. In scenario 1, all the individuals within each family (100 full-sibs) were genotyped and phenotyped. In scenario 2, 100 sibs were genotyped and phenotyped (training set) and 100 sibs were only genotyped (testing set) within each family. We also considered three mating systems: random, assortative and dissasortative.Ten generations of selection based on genomic breeding values computed from within-family GBLUP were carried out. One male and one female per family were selected to be the parents for the next generation. All results presented refer to generation 10. The inbreeding level achieved (<0.04) was similar across all simulated cases. Genetic gain was lower under scenario 2 than under scenario 1, as expected. For scenario 1, genetic gain increased from low (L = 100M) to moderate (L = 1 M) linkage by 24% and 57% for h^2= 0.4 and h^2= 0.1, respectively, when 100 QTLs controlled the trait. For h^2= 0.4, increasing linkage further (i.e., to L = 0.1) did not lead to higher gains. However, for h^2= 0.1, decreasing L from 1 to 0.1 M, increased gain by up to 16%. For scenario 2, increasing linkage from low to moderate values led to increases in gain of 278% and 243% for h^2= 0.4 and h^2= 0.1, respectively. Differences in gain from both scenarios when a single QTL was simulated were much smaller. In all cases, the effect of the mating system on both genetic gain and inbreeding was minimal.

Keywords: within-family genomic selection, genetic gain, inbreeding, mating systems

§Corresponding author. Tel.: +34 914524900 Ext.1645
E-mail address: miguel.toro@upm.es

DEVELOPMENT OF GENOMIC RESOURCES AND WHOLE GENOME PREDICTION IN PACIFIC WHITE-LEG SHRIMP *(Litopenaeus vannamei)*

**Jerry D.R.[a], Raadsma H.W.[b], Khatkar M.S[b], Prochaska J.[c],
van der Steen H.[c], Jones D.B.[a], Zenger K.R.[a] [§]**

*[a] Centre for Sustainable Tropical Fisheries & Aquaculture, and College of Marine
and Environmental Sciences, James Cook University, Townsville QLD, Australia.
[b] Reprogen - Animal Bioscience, Faculty of Veterinary Science,
University of Sydney, Camden NSW, Australia.
[c] Global Gen, Jalan Raya Narogong Km 14, Desa Cikiwul
Bantar Gebang Bekasi, 17310 Indonesia.*

The Pacific white-leg shrimp (*Litopenaeus vannamei)* is an important tropical aquaculture species that has benefited from selective breeding. Traditional breeding programs for *L. vannamei* rely on phenotypic selection of families to produce shrimp with improved growth and disease tolerance traits. However, recent advances in quantitative animal breeding and genomics can now rapidly advance genetic progress particularly for complex or difficult to measure traits.

Here we used high-throughput genotyping technologies to develop and test genome-wide genomic resources for *L. vannamei* incorporating 8,967 SNPs (8,616 novel and 351 from the public domain). A total of 1,934 samples were genotyped, including 1,134 female and 123 male parents of 416 families, along with 677 nauplii pools. Following SNP quality control, the average minor allele frequency of 7,150 SNPs in the breeding population was 37%. Missing parent genotypes using pooled SNP frequency data was also used to impute missing parental genotypes and found to be 99.3% accurate. The average linkage disequilibrium (LD) of the population (r^2) was 0.1. A comprehensive linkage map incorporating 4,390 SNPs mapped to 45 linkage groups covering 97.9% of the genome was also generated. There was no evidence of inbreeding or a reduction in genetic diversity over time within this farmed population.

Genome-wide prediction analyses were conducted using mean family allele frequencies of 5,893 SNPs post filtering, and the family mean growth trait (g/d). Genomic selection was investigated by dividing the data on 416 families into a training set and a validation/test set. Direct Genetic Values (DGV) was estimated using best linear unbiased prediction (BLUP) utilizing genomic relationships to estimate the genetic merit of an individual. The accuracy of DGV in mirror prediction (randomly dividing families in training and test set) for growth was "high" (0.63-0.69). Other traits such as disease resistance were also investigated. This project demonstrates that genomic selection has potential application with moderate number of SNPs, family average phenotypic records, and based on family DNA pool frequency data.

Keywords: Litopenaeus vannamei, growth traits, SNP discovery, Genomic selection, aquaculture.

[§] Corresponding author. Tel.: + 61-7-4781 6532; Fax: + 61-7-4781 4585
E-mail address: kyall.zenger@jcu.edu.au

QTL-SELECTION CONTRIBUTES TO INCREASED RESISTANCE TO CARDIOMYOPATHY SYNDROME (CMS) IN ATLANTIC SALMON *(Salmo salar L.)*

Kjøglum S.[a, §], Moen T.[a], Korsvoll S.A.[a], Ødegård J.[a], Santi N.[a]

[a]*AquaGen AS, Trondheim, 7462, Norway*

In Norwegian fish farming industry there are annually around 100 outbreaks of Cardiomyopathy syndrome (CMS). CMS is a cardiovascular disease caused by the *Piscine myocarditis* virus (PMCV). Disease outbreaks often occur late in the seawater phase and the loss of fish that are approaching harvest weight represents a high economic value.

Based on data from two field outbreaks and a challenge test for CMS, AquaGen found a relative strong QTL for increased CMS resistance. In the first CMS outbreak high correlations were found between level of PMCV in the heart, histopathology changes in heart muscle and mortality related to CMS. Level of PMCV was measured in 1083 fish, that also were genotyped for 6000 SNP-markers. A QTL was found, explaining 12% of the total variation of level of PMCV in the heart and 28% of the genetic variation.

In the next generation a challenge test for CMS were performed, with high and low performing groups based on data from the first field outbreak. In addition, a new field outbreak also provided data for CMS related mortality. The QTL was comfirmed in both these CMS-outbreaks, and individuals with two copies of the good allele had better performance than individuals with no copies of the good allele, regarding level of PMCV virus, histopathology score and mortality.

The use of salmon eggs that have been selected for resistance to CMS is currently the only available measure that has a documented effect against CMS.

Keywords: Atlantic salmon, QTL, CMS

[§] Corresponding author. Tel.: +47 72 450 500; Fax: +47 72 450 525
E-mail address: sissel.kjoglum@aquagen.no

GENOTYPING-BY-SEQUENCING USING CUSTOM ION AMPLISEQ™ TECHNOLOGY AS A TOOL FOR GENOMIC SELECTION IN ATLANTIC SALMON (Salmo salar)

Baranski M.[a, §], Jowdy C.[b], Moghadam H.[a], Norris A.[c], Bakke H.[d], Sonesson A.[a], Meuwissen T.[e], Lillehammer M. [a], Lund V.[f]

[a] Nofima, Ås, 1430, Norway
[b] Cofactor Genomics, St. Louis, 63110, USA
[c] Marine Harvest, Sandnes, 4319, Norway
[d] SalmoBreed, Bergen, 5003, Norway
[e] NMBU, Ås, 1430, Norway
[f] Salmar, Kverva, 7266, Norway

Traditional genomic selection relies on high density genotyping that captures sufficient linkage disequilibrium between markers and QTL across families. High-density SNP arrays are normally used for this purpose in live stock but can be prohibitively expensive for aquaculture species where economy of scale is typically more limited. Within-family genomic selection (WFGS) based on linkage with few SNPs per chromosome has been shown to be a powerful alternative genomic selection strategy for aquaculture species. However, highly cost efficient genotyping methods for such 'moderate' SNP numbers are lacking, meaning that the theoretical cost saving of implementing WFGS cannot currently be realised. Genotyping-by-sequencing (GBS) is a promising approach to target this niche, given that genotyping efficiency can directly scale with rapid developments in sequencing technology. The most common GBS approaches are derivatives of RAD sequencing, which randomly targets loci across the genome based on the distribution of restriction enzyme cut sites. A limitation of RAD-GBS is that it cannot target *a priori* selected SNPs, and there is typically incomplete overlap of sequenced sites across samples. Targeted amplicon sequencing is an emerging alternative to RAD-based GBS, enabling genotyping of selected SNPs. Ion AmpliSeq™ is an amplicon approach based on ultra-high multiplex PCR that requires low amounts of input DNA and can produce genotypes through a single day workflow. Here, we validate a custom Ion AmpliSeq™protocol for SNP genotyping in Atlantic salmon.Initially, a primer pool was developed from around 1000 SNPs selected from the published Atlantic salmon SNP map. Following PCR, we sequenced 29 high quality libraries comprising 19 samples and replicates on an Illumina NextSeq 500 (PE 150bp reads). Reads were mapped to the SNP sequences using Bowtie, and genotypes called with Freebayes and custom scripts. We obtained sequence for 941 targeted SNPs with a minimum coverage of 100x in all samples and an average coverage per locus ranging from 551x to 8925x. Genotype concordance between replicate samples was 100%. Further optimisation will focus on increasing robustness of the workflow to varying template yields and quantities, and optimising the sample multiplexing level to reach a per sample pricepoint significantly lower than SNP arrays.

Keywords: Genotyping, sequencing, Atlantic salmon, AmpliSeq™, SNP

§ Corresponding author. Tel.: +47 6497 0396
E-mail address: matthew.baranski@nofima.no

EFFECT OF IMPUTED MARKER GENOTYPES ON ACCURACY OF GENOMIC SELECTION IN AQUACULTURE POPULATIONS

Vela-Avitúa S.[abc], Ødegård J.[ad]

[a] *Institute of Animal and Aquacultural Sciences, Norwegian University of Life Sciences, Ås 1432, Norway*
[b] *Nofima, Ås, 1431, Norway*
[c] *AFGC, Sunndalsøra, 7462 Norway*
[d] *AquaGen AS, Trondheim, 7462, Norway*

Implementation of genomic selection in breeding programs requires the use of genome-wide dense markers, and the availability of such information has been the main limitation for its implementation in many species. As genotyping technology advance, genotyping for denser markers at affordable costs had increased. However, in many cases, a large number of animals are genotyped at sub-optimal densities, due to economic constraints or changes in technology over time. In such cases, imputation has been proposed as a tool to "fill in" missing genotypes of individuals genotyped at lower densities. Imputation methodologies use either information on population-wide parameters or family relationships. The main aim of this study was to compare different imputation methodologies with respect to accuracy of genomic selection at different scenarios with respect to target densities and structure of the densely vs. sparsely genotyped animals. Accuracy of GEBV after imputation was studied using simulated data of a typical aquaculture population. Eight different scenarios were studied, varying target densities (960 or 2900 SNPs/Morgan), number of markers in the sparsely genotyped animals (10 or 100 SNPs/Morgan) as well as imputation strategy (down or up the pedigree). Three different public softwares were used: Beagle (Bg), AlphaImpute (AI) and FImpute (FI). After imputation, genomic relationship matrices were constructed and EBVs estimated by GBLUP and traditional pedigree based BLUP. Predictive ability was estimated as Pearson's correlation coefficient between EBVs and true breeding values (BV). G-EBV's accuracies outperformed pedigree-based methods in almost every case, but to a varying degree for the different software packages. Imputing down the pedigree improved reliability of selection for AI and FI compared with a one-step approach only using non-imputed target genotypes in parents. Imputing up the pedigree marginally increased accuracies for FI and AI, but the opposite was observed for Bg where it was in some cases even outperformed by a classical pedigree-based model. Reduction in sparse density had a substantial effect, with 100 SNPs/Morgan being consistently better than 10 SNPs/Morgan (4.5-8%). The use of imputation down the pedigree was always substantially better than not including genotypes (4-5%) which produced accuracies at the level of pedigree-based methods.

Keywords: Genomic selection, Imputation, Marker Density.

[§] Corresponding author. Tel.: +42 94436282
E-mail address: sergio.vela@afgc.no

CANDIDATE GROWTH GENES IDENTIFIED BY QTL FINE MAPPING IN BIGHEAD CARP *Aristichthys nobilis*

Sun Y.H.[a, b, *], Liu H.Y.[a, c, *], Feng X.[a, c], Yu X.[a], Fu B.D.[a], Tong J.[a, §]

[a] *State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, Chinese Academy of Sciences, Wuhan, 430072, China*
[b] *Wuhan Aquaculture Science Research Institute, Wuhan, 430207, China*
[c] *University of Chinese Academy of Sciences, Beijing, 100039, China*

QTL fine mapping provides a powerful tool to identify genomic regions with potential genes involving traits of interest. Bighead carp (2n=48) is one of the most important aquacultured fish in China and some Asian countries, and molecular breeding technologies have been desirable for this large cyprinid species with sex maturation of about five years. In this study, QTL fine mapping were performed to identify candidate genes for five growth traits (TL: total length, BL: body length, BH: body height, HL: head length, BW: body weight) in two full-sib families of bighead carp. In a 7-months-old family, a genetic linkage map with 905 SSRs was constructed for genome scan, and three genome-level QTLs at the same genomic region of the linkage group 9 (LG9) for BL, BH, BW, and 11 LG-level QTLs at the LG17 for TL, BL, BH, HL, BW, were identified, with phenotype variance explained (PVE) of 18.4-25.5%. In an another 42-months-old family, LG-based fine mapping for the LG16 was carried out after initial genome scan for potential QTLs, then a total of 11 genome-level QTLs for BL, BH, BW, and a total of 10 LG-level QTLs for TL, BL, BH, HL, were identified, respectively, with the PVE of 17.4-32.0%. Homology searches against public databases and unpublished silver carp and bighead carp genomes by using sequences of the SSRs tightly-linked to the QTLs demonstrated such genes as *GH*, *TP53BP2*, *mxil*, *fat3a* in early growth stage (7 months) and *THR*β*2*, *NKIRAS1*, and *CSPG5* in adult growth stage (42 months) of bighead carp. Homologous of these candidate genes in model fish and/or mammals are functionally related to either hormonal regulation of body growth or cell growth/proliferation. Some of these genes were subject to association analysis between their genic SNPs and growth traits, and positive results have been confirmed. In summary, by QTL fine mapping and comparative genomics analyses we have revealed several candidate genes that may have genetic effects on growth in bighead carp, and those homologous identified in this study are choice of growth genes for marker (gene)-assisted selection and other molecular breeding programs of the species.

[*]These authors have contributed equally to this work.
[§] Corresponding author. Tel.: + 86 27 6878 0751; Fax: + 86 27 6878 0123
E-mail address: jgtong@ihb.ac.cn

ESTIMATES OF HERITABILITY FOR DISEASE RESISTANCE TO SRS USING GENOMIC RELATIONSHIPS PREDICTED USING HIGH DENSITY SNP DATA IN ATLANTIC SALMON AND RAINBOW TROUT

Martínez V.[a, §], Santi N.[b], Ødegård J.[b], Moen T.[b]

[a]FAVET-INBIOGEN. FONDECYT 1120608. Faculty of Veterinary Sciences, University of Chile, 8820808, Chile.
[b]BLUE GENOMICS SPA. Puerto Varas, 5550513. Chile.

Estimates of heritability are important in order to understand to what extent the variation in breeding values explained the observed variability for complex traits. This parameter is not only important in developing breeding programs, but also in conservation and evolutionary studies and on the genetics of disease resistance. One of the main bacterial diseases in the Chilean salmon aquaculture is the rickettsial syndrome (SRS) due to *P. salmonis*. Although estimates of heritability were obtained previously based on the resemblance of collateral sibs, there are no estimates based on genomic relationships. In this study we estimate the heritability for several traits explaining both resistance and robustness using information obtained from a large-scale challenge using cohabitation. The genomic matrix was constructed using 220 K SNPs evenly covering the Atlantic salmon genome, and genotyped in 1,700 individuals sampled from 144 families of the commercial breeding program. Survival was measured as a binary trait, but also in terms of endurance (number of days to die). Robustness was measured as the weight and length gain of individual fish before and after the challenge test. Several univariate and multivariate models were used that properly account for the genetic covariance between traits involved in disease resistance. Using the complete set of SNPs, the prediction of genomic inbreeding was negligible. The mean genomic relatedness in the population was consistent with the depth of the pedigreed data and the structure of matings in the population. For the complete multivariate model, which includes endurance and survival, corrected for body weight, the heritability estimates were 0.44 (0.05) for endurance and 0.49 (0.05) for the binary trait. These estimates are very consistent with estimates obtained in earlier challenge tests for the same disease in other populations. Increasing the body weight increases the probability of survival. The heritability estimates for robustness as obtained for increase in body weight was 0.38 (0.04). The heritability estimates were inflated when body weight was not included as a covariate in the model. Estimates of the heritability in rainbow trout for the same disease were consistent. These are the first estimates of heritability of SRS resistance, for both species using genomic relationships based of a number of SNPs with high coverage, which efficiently captured the genetic variability at the whole genome level. FONDECYT 1120608.

§ Corresponding author. Tel.: + 56 9 63060306 ; Fax: + 56 22 9785641
E-mail address: vmartine@u.uchile.cl.

A GENOME-WIDE ASSOCIATION STUDY FOR SEX DETERMINATION IN ATLANTIC SALMON

Covelo-Soto L.[a,§*], Morán P.[a], Kent M.P.[b], Saura M.[c]

[a] *Departamento de Bioquímica, Genética e Inmunología,*
Universidad de Vigo, Vigo, 36310, Spain
[b] *CIGENE, Norwegian University of Life Sciences, Aas, 1432, Norway*
[c] *Departamento de Mejora Genética Animal, INIA, Madrid, 28040, Spain*

Sex-determination (SD) of salmonids has remained an important challenge for the study of wild populations and aquaculture, as controlling the sex ratio is essential to develop appropriate breeding schemes. While birds and mammals show highly conserved master genes responsible for gonad differentiation, high genetic variation has been described in fish species regarding the genes responsible for SD, the number of genes involved in such decision and the relationships between them. Recently, a highly conserved sex-determining gene, sdY (sexually dimorphic on the Y-chromosome) has been described in many salmonids species, however observations strongly suggest that, in addition to sdY gene, SD comprises a complex trait in this group. The recent availability of high density SNP arrays has provided the opportunity to address this issue from a genome-wide perspective. Here, we have performed a genome-wide association analysis (GWAS) to identify genomic regions associated with SD in wild Atlantic salmon. We used data from a 220,000 SNP panel for A. salmon (Affymetrix) in 96 individuals from 6 rivers covering the complete distribution range of the species in Spain, which represents the South distribution limit of the species in Europe. Quality control filters were applied on both samples (dish quality control > 0.82, call rate > 0.97) and SNPs (R package SNPolisher, Affymetrix). Data were also filtered for HWE ($P < 1x10^{-5}$) and MAF (< 0.01). After filtering, 95 individuals and 165,136 SNPs remained for the analysis. GWAS was performed with the GenABEL package in R, using mixed model and regression by including the factor river as a fixed effect. Preliminary results revealed association between SNPs located at Ssa2 and SD, as reported by recent studies. Potential association with other chromosomes was also found according to the QQ-plot, thus suggesting that sex can be regarded as a complex trait in this species, although further research is required. Phase 2 of the Salmon Sequencing Project employing next-generation sequencing technologies has already started, which will provide information about the genomic regions involving the trait. Our results provide preliminary information for the development of new molecular sexing approaches in aquaculture breeding programs.

Keywords: Atlantic salmon, GWAS, molecular sexing, sex-determination

[§] Corresponding author. Tel.: +34 986 813899; Fax: +34 986 812556
E-mail address: laracovelo@uvigo.es

THE EFFECTS OF EARLY LIFE STRESS ON THE EPIGENOME AND TRANSCRIPTOME OF ATLANTIC SALMON *(Salmo salar)*

Moghadam H.[a, §], Tveiten H.[b], Robinson N.[a], Andersen Ø.[a], Burgerhout E.[a], Johnsen H.[b]

[a] *Nofima AS,Osloveien 1, N-1432 Ås, Norway*
[b] *Nofima AS, Muninbakken 9-13, N-9291 Tromsø, Norway*

Exposure to environmental stress during early life stages can alter the rate and timing of various developmental processes, including muscle development, growth, stress resilience and survivorship. In particular, environmental stimuli can result in epigenetic marks that affect the genomic machinery of a cell and the regulation patterns of gene transcription. To assess how stress affects the methylome and genome-wide transcriptome in Atlantic salmon during early life stages, fish were treated using cold shock and air-exposure once a week from the eye stage until start feeding. The fish were either stressed prior to hatching, post-hatching, pre- and post-hatching or not stressed at all. Genomic DNA was extracted from six randomly chosen replicates of each treatment and the control group and DNA libraries were constructed and sequenced on an Illumina HiSeq system following digestion with *MspI* restriction enzyme and bisulfite conversion. To assess the profiles of transcript expression, whole mRNA was extracted from the same individuals and sequenced using the Illumina platform. In order to accurately estimate the levels of expression and investigate the patterns of methylation, particularly at the promoter regions, and since there is not yet any publicly available annotation of the Atlantic salmon genome, previously sequenced whole transcriptome data from approximately 400 individuals, representing both sexes, different tissues and various life stages, where integrated, assembled and annotated at the genome level. Comparative analysis of the transcriptome expression between the control and stressed individuals revealed a core set of genes that have consistently increased or decreased their expression levels in response to stress, irrespective of the developmental stage. Similarly, a core group of CpG sites with consistent hyper- or hypo-methylation profiles across all stressed individuals were detected. On the other hand, enrichment of functional gene categories and methylation patterns that are specific to each developmental stage were also detected. The signatures of these genomic modifications seem to have persisted (throughout the length of the experiment) even for those individuals that only received stress prior to their hatching. These findings highlight how environmental programming during early development can lead to DNA structural modification and regulation of gene expression with potential long-lasting effects on an organism's health, metabolism and capacity to adapt to environmental perturbations.

Keywords: methylation; epigenetics; transcriptomics; high throughput sequencing; bisulfite sequencing; Atlantic salmon; environmental stress

[§] Corresponding author: Tel.: +47 405-10-494
E-mail address: hooman.moghadam@nofima.no

GENOME-WIDE ANALYSIS OF DNA METHYLATION OF ATLANTIC SALMON IN RESPONSE TO STRESS

Covelo L.[1], Reyes D.[2], González R.[2], Pérez-Figueroa A.[1], Morán P.[1], Vidal R.[2]

[1] *Departamento de Bioquímica, Xenética e Inmunoloxía, Campus Universitario de Vigo, Universidade de Vigo, Vigo 36310 Spain.*
[2] *Laboratory of Molecular Ecology, Genomics, and Evolutionary Studies, Department of Biology, University of Santiago de Chile, Santiago, Chile*

As with other aquaculture species, intensive aquaculture of Atlantic salmon often faces various stressors which have negative impacts on several productive parameters. Therefore to improve production efficiency it is crucial to understand stress response at the physiological and molecular level. Although a great number of studies have been realized in the transcriptomic and physiological level, little is known about the role of the epigenetic process involved in the response to stress in fishes. In this study we conducted handling/crowding stressor experiments on two different strains of Atlantic salmon considering 4 tissues. In silico analysis of CpGo/e ratios using publicly available sequence of data revealed that DNA methylation is a common feature of the Atlantic salmon genome, and that specific functional categories of genes have significantly different levels of methylation. Then, we used F-MSAP method to assess the extent and pattern of methylation. The methylation levels ranged from 46,70 to 35,90% with full methylation state being predominant. AMOVA analysis of methylation differentiation determined that only muscle showed a significant differentiation among unmethylated and methylated states. Additionally, spleen showed a significant pattern of methylation among strains. Our study present the first look at the tissue-specific methylation pattern of Atlantic salmon under stress. Our long term goal is to identify and characterize the role of epigenomics in the process de stress and infection of salmonids.

Keywords: Salmon, stress methylation, epigenetics

GENE EXPRESSION PROFILES DEFINING HOST RESISTANCE TO INFECTIOUS PANCREATIC NECROSIS VIRUS IN ATLANTIC SALMON FRY

Houston R.D.[a]§, Taggart J.B.[b], Bishop S.C.[a], Bron J.E.[b], Bekaert M.B.[b]

[a] *The Roslin Institute, University of Edinburgh, Midlothian, EH25 9RG, Scotland, UK*
[b] *Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK*

Background: Infectious Pancreatic Necrosis (IPN) is a highly contagious birnavirus disease of salmonid fish which often results in high levels of morbidity and significant mortalities in aquaculture. In some Atlantic salmon stocks, there is segregation for a major locus (QTL) affecting host resistance to IPNV. However, the biological mechanisms underlying the genetic resistance conferred by the favourable QTL allele are not well known. Earlier microarray comparisons between resistant and susceptible families suggested that a marked innate immune response was observed in susceptible fish by day seven post infection that was apparently absent in resistant fish, suggesting that key mechanisms defining genetic resistance operate early in the infection process. Herein we report on a series of experiments aimed at elucidating the genes and pathways that are differentially expressed between resistant and susceptible fish prior to and immediately following experimental infection with the IPN virus.
Methods: The offspring of three commercial breeding families with both parents heterozygous (R/S) for the QTL were given a successful immersion challenge of IPNV. Each family was split into six tanks (50 fry per tank; two tanks per sampling timepoint; three timepoints, 1, 7, & 20 days post challenge). An additional sample of 100 fry (50 from duplicate tanks) from each family was taken immediately prior to challenge (timepoint 0). Parents and fry were genotyped at IPN QTL-linked markers to determine the fry QTL genotype. For timepoints 0 and 1, from each of the three families, Illumina-based high depth RNA-seq analysis was performed on RNA from six fry carrying the "RR" genotype and six fry carrying the "SS" genotype (total n = 72).
Results: Approximately 1.6 billion 100 bp PE sequence reads from all fry were aligned to the Atlantic salmon reference genome sequence and a comprehensive reference transcriptome was constructed, containing ~80 K different transcripts. The transcripts were annotated and approximately 88 % of all publicly-available EST sequences showed significant alignment to at least one transcript. Analyses of the differential expression of transcripts between RR and SS fry, within and across families, revealed enrichment of differential expression in pathways relevant to the host response to the IPN virus. The potential role of these genes and pathways in mediating the effect of the QTL resistance allele is discussed.

Keywords: disease resistance, RNASeq, QTL, virus, immune response

§ Corresponding author. Tel.: +44 131 651 9224
E-mail address: ross.houston@roslin.ed.ac.uk

GENE EXPRESSION PROFILE ANALYSIS OF MANILA CLAM (*Ruditapes philippinarum*) HEMOCYTES AFTER A *Vibrio alginolyticus* OR *Perkinsus olseni* CHALLENGE USING AN IMMUNE-ENRICHED OLIGO-MICROARRAY

Moreira R.[1], Romero A.[1], Milan M.[2], Bargelloni L.[2], Novoa B.[1], Figueras A.[1§]

[1]Instituto de Investigaciones Marinas (IIM-CSIC), Vigo, Spain
[2]Department of Comparative Biomedicine and Food Science (BCA)
University of Padova. Viale dell'Università 16, 35020 Legnaro, Italy.

The Manila clam (*Ruditapes philippinarum*) is a cultured bivalve with worldwide commercial importance, and diseases in this species can cause high economic losses. For this reason, interest in the immune genes in this species has recently increased. The present work describes the construction of the first *R. philippinarum* microarray containing immune-related hemocyte sequences and the application of this microarray to study the gene transcription profiles of hemocytes from clams that were challenged with the bacteria *Vibrio alginolyticus* or the protistan parasite *Perkinsus olseni* through a time course. Our results highlight the importance of a fast response in bivalves and the effectiveness of their innate immune system. With regard to the *V. alginolyticus* challenge, genes related to signaling, transcription and apoptosis were typically expressed as early as 3 hours post-challenge (hpc), while characteristic immune genes appeared later at 8 hpc. This immune-triggering response could have affected a high number of processes that seemed to be activated 24 hpc to overcome the *Vibrio* challenge, including the expression of many cytoskeleton molecules, which is indicative of the active movement of hemocytes. In fact, functional studies showed an increment in apoptosis, necrosis or cell migration after the infection. Finally, 72 hpc, activity returned to normal levels. The results show that the key point to overcome the challenge seemed to be 8 hpc, when we detected immune functions that could lead to the destruction of the pathogen and the activation of a wide variety of processes related to homeostasis and defense.
The analysis of the transcriptome profile after *P. olseni* infection revealed an early phase of infection (day 5) that was characterized by no mortality and by the increased expression of genes associated with pathogen recognition, production of nitrogen radicals and antimicrobial activity. This phase was followed by an intermediate stage (days 10 and 14), when the pathogen was most likely multiplying and infecting new areas of the body, and animals began to die. In this stage, many genes related to cell movement were over-expressed. Thirty days after infection metabolic pathway genes were the most affected. Apoptosis appears to be important during pathogenesis.

Keywords: Manila clam, hemocytes, Vibrio alginolyticus, Perkinsus olseni, microarray, immune response, Gene Ontology, Blast2GO

[§]Corresponding author. Tel.: +34 986 231 930; Fax: +34 986 292 762
E-mail address: antoniofigueras@iim.csic.es

COMPARATIVE ANALYSIS OF MICRORNAs TRANSCRIPTOME EXPRESSION IN CHITRALADA, RED STIRLING AND IN CROSSBRED NILE TILAPIA *(Oreochromis niloticus)* USING HIGH THROUGHPUT SEQUENCING

Herkenhoff M.E.[a, §], Bovolenta L.A.[b], Dias M.A.D.[c], Hilsdorf A.W.[d], Pinhal D.[a]

[a] *Department of Genetics, São Paulo State University, Botucatu - SP, 18618-970, Brazil*
[b] *Department of Physics and Biophysics, São Paulo State University, Botucatu - SP, 18618-970, Brazil*
[c] *Department of Animal Sciences, Federal University of Lavras, Lavras, 37200-000, Brazil*
[d] *Unit of Biotechnology, University of Mogi das Cruzes, Mogi das Cruzes,08780-911, Brazil*

MicroRNAs (miRNAs) are a class of small RNAs that inhibit gene expression post-transcriptionally by pairing in their target mRNA. Since miRNAs are key elements in gene regulation, they have also become targets of investigations directed to animal breeding of farming animals. MiRNAs participate actively in growth, metabolism, muscle development and fat deposition. Nile tilapia is the most important farmed fish species in Brazil. Recently, crossbreeding has been carried out between two strains of Nile tilapia, Chitralada and Red-Stirling, producing individuals with hybrid vigour, which display the superior growth performance of Chitralada and the red coloration of Red-Stirling preferred by consumers. Previous studies have shown that some miRNAs may help to promote hybrid vigour. Based on this assumption, the present work aims to assess a potential role of miRNAs in fostering the hybrid vigour in crossbred animals. For this purpose, the miRNA transcriptome of parental and crossbred animals was identified and characterized by high throughput sequencing and bioinformatics analysis. Skeletal muscle samples (n=4) of five and six month old animals were chosen. This analysis showed a suite of miRNAs differentially expressed between control groups and the crossbred. Notably the miRNAs (let-7; mir-16; mir-24; mir-122; mir-124; mir-135a; mir-153; mir-192; mir-194; mir-216b; mir-219; mir-301; mir-455; mir-458 families) were more expressed in Red-Stirling than in the crossbred and Chitralada. Also several miRNAs-3p (mir-124; mir-196; mir-301; mir-7641 families) were low expressed in Chitralada, in comparison to crossbred and Red-Stirling. Among these miRNAs let-7 and miR-122 have been previously enrolled in fish growth. Let-7 plays a role in the GH-2 expression and interacts with myostatin whereas miR-122 might be associated with fat deposition. Further investigation to determine candidate target genes of these differentially expressed miRNAs in skeletal muscle is under development. Although preliminary, these results are promising to improve our understanding about the correlative effects between miRNA expression and hybrid vigour.

Keywords: miRNA; Nile tilapia; skeletal muscle; aquaculture; growth

§Corresponding author. Tel.: + 55 (14) 3880-0363; Fax: + 55 (14) 3815-3131
E-mail address: herkenhoff@ibb.unesp.br

FROM TILAPIA'S COMPARATIVE TRANSCRIPTOME ANALYSIS TO CHARACTERIZATION OF NUTRIENT TRANSPORTERS

Rozenberg P., Ronkin D., Nitzan T., Seroussi E., Doron-Faigenboim A., Cnaani A.*

Institute of Animal Science, Agricultural Research Organization, Bet Dagan 50250, Israel

Tilapias are a group of freshwater fishes, which vary in their euryhaline capabilities. Comparative analysis of closely related species that differ in their salinity tolerance can be a powerful tool to study the genetic and physiological basis of this trait. The anterior- and posterior-intestine transcriptome of the highly salinity-tolerant *Oreochromis mossambicus* and the more sensitive *O. niloticus*, acclimated to seawater and freshwater, was sequenced using the Illumina Hi-Seq. The results of gene expression and gene-ontology (GO) analyses indicate a species-specific salinity-dependent gene expression patterns in the anterior intestine. Overall, between 182 and 404 genes were significantly up- or down-regulated in seawater in the two species and two intestinal sections, including 70 genes with inversed salinity response, up-regulated in one species and down-regulated in the other. From these genes we have focused on peptide and amino acid transporters. Seawater- and freshwater-acclimated fish were sampled at three time points after feeding (6, 24, 72 h), representing different digestive stages. Genes encoding to the PepT1a, PepT1b, PepT2, $B^{0,+}AT1$, and B^0AT1 were analyzed for their expression, protein localization, and biochemical activity along the intestine. We found salinity-dependent differential expression and localization of these nutrients transporters along the intestine, correlated them with the different digestive stages, and characterized factors affecting their activity.

Keywords: Next Generation Sequencing, Transcriptome, Peptide transporters, Amino acid transporters, Salinity

* Corresponding author. Tel.: +972-3-968-3566; Fax: +972-3-960-5667
E-mail address: avnerc@agri.gov.il

TRANSCRIPTOME AND MICRO-RNA ANALYSIS REVEALS NOVEL INSIGHTS INTO DEVELOPMENT OF INTERMUSCULAR BONE IN TELEOSTS

Gao Z.X.[a, §], Wang W.M.[a], Yi S.K.[a], Wan S.M.[a], Chen B.X.[a, b]

[a] *College of Fisheries, Huazhong Agricultural University, Wuhan, Hubei 430070, China*
[b] *Animal Husbandry and Fisheries Research Center of Haid Group Co., Ltd, Guangzhou 511400, China*

Intermuscular bone, which occurs only in the myosepta of the lower teleosts amongst vertebrates, is attracting increasing more attention of researchers due to its particular development, as well as the unclear gene regulations. Blunt snout bream (*Megalobrama amblycephala*), which is widely cultured in the Chinese freshwater polyculture system with high economic value, belongs to the economically important cyprinid species. Due to its high production and consumption, selecting a less or even no intermuscular bone strain becomes another focus of *M. amblycephala* molecular selective breeding.

In the present study, we investigated the transcriptome and microRNA features of three key tissues (white muscle, connective tissue and intermuscular bone) in *M. amblycephala*. A total of 117,644,718 trimmed reads were generated and assembled into 49,477 non-redundant unigenes with an average length of 649 bp. Totally 13,231 differentially expressed unigenes (DEGs) were identified based on pairwise comparison of three tissues, among which, 394 DEGs were differentially expressed in the three libraries, with 193 (48.98%) and 173 (43.91%) DEGs exhibited higher expression level in intermuscular bone and connective tissue. These DEGs were associated predominantly with metabolic pathways, MAPK signaling pathway, PI3K-Akt signaling pathway, TGF-beta signaling pathway, and osteoclast differentiation. In addition, 740, 706 and 134 specifically expressed unigenes were identified from connective tissue, intermuscular bone and white muscle, respectively.The results from microRNA anlalysis revealed the sequences and expression levels of 218 known (belonging to 103 families) and 19 novel miRNA genes. Of these microRNAs, 117 known and 11 novel microRNA sequences exhibited significant expression differences between the two libraries, with 66 and 62 differentially expressed microRNAs exhibiting higher expression in the connective tissue and intermuscular bones libraries, respectively. The expressions of 11 differentially expressed miRNAs were selected to validate in 9 tissues. Among the high-ranked predicted gene targets, differentiation, cell cycle, metabolism, signal transduction and transcriptional regulation were implicated.This study provided the first comprehensive transcriptome and microRNA resources of intermuscular bone for teleosts and the obtained findings offered the first insights into the mechanism that may lead to the cell differentiation of connective tissue and contributes to a better understanding of the regulatory network involved in the development of intermuscular bone in cyprinid species.

Keywords: Megalobrama amblycephala; teleosts; microRNA; transcriptome; intermuscular bone

§ Corresponding author. Tel.: +86-27-87282113; Fax: +86-27-87282114
E-mail address: +gaozexia@hotmail.com

STRESS SPECIFIC GENE EXPRESSION PATTERNS IN RELATION TO EARLY LIFE STRESS IN THE GILTHEAD SEA BREAM (SPARUS AURATA)

Sarropoulou E.[a, §], Tsalafouta A.[b], Sundaram A.Y.M.[c], Papandroulakis N.[a], Oulas A.[a], Leithaug M.[c], Gilfillan G.D.[c], Kotoula G.[a], Pavlidis M.[b]

[a] *Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, P.O. Box 2214, 710 03 Heraklion, Crete, Greece*
[b] *Department of Biology, University of Crete, 71409 Heraklion, Greece*
[c] *Norwegian Sequencing Centre (NSC), Oslo University Hospital, Oslo, Norway*

The gilthead sea bream (*Sparus aurata*) is, along with the European sea bass (*Dicentrarchus labrax*), one of the commercially important European aquaculture species. For healthy development, larval stages are the most critical period and ensure high performance and superior quality of the subsequent developmental phases of the life cycle under captivity. However, the effect of early life stress on fish performance has not yet thoroughly investigated in exothermic species. In the present study the effect of mild husbandry stressors applied at two different early developmental phases i.e. first feeding onwards flexion and flexion onwards the development of all fins, on the transcriptome of control and stressed larvae has been investigated. For each sampling point, three biological replicates were collected and their transcriptome was sequenced using Illumina paired-end technology. In total 202,754 transcripts were obtained after de-novo assembly and filtering with Trinity package. Differential expression analysis (DEG) revealed that larvae exposed to stress during the period between first feeding and flexion are more sensitive than in larvae exposed to stress after the flexion stage. Interestingly more transcripts were found to be down and not up regulated due to stress. The present study reveals differences at the transcriptome level in relation to early life stress for the first time. In addition, this is to our best knowledge first report of a DEG transcriptome study in the gilthead sea bream investigating the effect of stress during development.

Keywords: Development, stress, Sparus aurata, RNAseq, differential expression

§ Corresponding author. Tel.: +30-2810-337753; Fax: + 30-2810-337753
E-mail address: sarris@hcmr.gr

INCIPIENT TRANSITION OF A SEX DETERMINING GENE IN *Takifugu* PUFFERFISH

Ieda R.[a], Hosoya S.[a], Tasumi S.[a], Suzuki S.[b], Kikuchi K.[a]*

[a]Fisheries Laboratory, University of Tokyo, Hamamatsu, Bentenjim 2971-4 Japan
[b]Minami-Izu Laboratory, National Research Institute of
Aquaculture, FRA, Shizuoka, Minami-izu 415-0156 Japan

It is interesting to note that although genetic sex determination is widespread among vertebrates, the molecular pathways underlying this process do not appear to be conserved across major taxa. In teleosts in particular, the different master sex-determining (SD) genes in different lineages appear to have evolved independently and have been frequently replaced by new genes. The evolutionary transition between SD systems is obviously best studied using closely-related species with distinct master SD genes.

Takifugu genus has undergone an adaptive radiation in the last 2-5 million years, resulting in about 20 extant species including fugu (tiger pufferfish). Fugu is one of the most economically important food fish in Japan and also is the first fish with a fully sequenced genome. Previously, we have shown that sex in fugu appears to be determined by a missense single nucleotide polymorphism (SNP) in the *Amhr2* gene.

In this study, we have taken advantage of this finding and the rich genomic resources of fugu to explore the genetic basis of sex determination in closely-related species of fugu. We found that while sex in the majority of *Takifugu* species is likely determined by the SNP in the *Amhr2* gene, it is clearly not the case in a few species. To confirm this, we performed genome-wide linkage analysis and identified new SD loci distinct from the *Amhr2* locus in these species. Interestingly, the transition of the SD system appears to be in progress at least in one species, as a small percentage of males still retains the "sex-determining SNP" on the *Amhr2* gene. These results indicate that fugu and its closely-related species can be an excellent model group for investigating the transitions between alternative master SD genes.

Keywords: closely-related species, fugu, sex-determining gene, transition of the SD system

*e-mail: akikuchi@mail.ecc.u-tokyo.ac.jp
Tel: +81-53-592-2821, Fax: +81-53-592-2822

SEX CHROMOSOME EVOLUTION AND MECHANISM FOR SEX DETERMINATION AND REVERSAL REVEALED BY WHOLE-GENOME SEQUENCING AND METHYLATION SEQUENCING IN HALF-SMOOTH TONGUE SOLE *Cynoglossus semilaevis*

Chen S.L.[a,§], Zhang G.J.[b], Shao C.W.[a], Huang Q.F.[b], Liu G.[b] , Song W.T.[a], Sha Z.X.[a], Xie M.S.[a], Liu Y.[a], Wang N.[a], Yang C.G.[a], Hu Q.M.[a], Schartl M.[c], Tang Q.S.[a], Wang J.[b]

[a] Yellow Sea Fisheries Research Institute, CAFS, Key Laboratory for Sustainable Development of Marine Fisheries, Ministry of Agriculture, Qingdao 266071, China.
[b] BGI-Shenzhen, Shenzhen 518000, China.
[c]Physiologische Chemie I, University of Würzburg, D-97074 Würzburg, Germany.

We sequenced whole genomes of a male (ZZ) and a female (ZW) half-smooth tongue sole (*Cynoglossus semilaevis*). 21,516 genes were annotated. A high density SNP genetic map was developed with 12,142 SNPs using RAD-seq technique. The genetic map contains 22 linkage groups (20 autosomes and 2 sex chromosomes) with an average interval of 0.1562 cM. Based on the difference of sequencing depth of Z/W linked-scaffolds between female and male, together with the high-resolution genetic map, we assembled the Z and W chromosome of tongue sole. We found that the sex chromosomes of tongue sole are derived from the same ancester as chicken, but not as any known fishes. Notably, the sex chromosome of tongue sole and chicken showed convergent evolution, and Z chromosome of tongue sole exhibited partial dosage compensation in female as that of chicken.

In order to identify sex determining gene, four genes involved in sexual development in other vertebrates were identified on the Z chromosome of tongue sole. For sf-1, patched2 or follistatin, expression patterns at the sex determination stage and gene methylation in normal males and females, were incompatible with a role as a male sex determining gene. Only Csedmrt1 displayed many features that make it an outstanding candidate for a master sex-determining gene. Only the Z contains a functional copy of dmrt1. It is male specifically highly expressed in germ cells and presomatic cells at the sex determination stage and persists at high levels during testis development. This is paralleled by demethylation of the dmrt1 promoter region. Its expression in sex-reversed ZW males was up-regulated to a level as in normal ZZ males. All these are defining features for a dosage-dependent male sex-determining gene.

By comparing female/male genome, some sex-linked SSR markers were found and used to develop molecular technique for genetic sex identification of ZZ, ZW and WW fish. We found that phenotypic female accounts for only 10-30% in cultured populations, and more than 90% of pseudo-male offspring sex-reverted into a pseudo-male. Whole-genome methylation sequencing revealed that all second-generation pseudomales had inherited the Z chromosome from their sex-reversed fathers and retained the paternal methylation pattern, implying that trans-generational inheritance of DNA methylation status is particularly important for the inheritance of sex reversal.

Keywords: half-smooth tongue sole, Cynoglossus semilaevis, whole genome sequencing, ZW chromosome evolution, sex determination mechanism.

§Corresponding author: Tel: 008653285844606; Fax: 008653285811514
E-mail address: chensl@ysfri.ac.cn

EPIGENETIC CHANGES OF SEX GENES INDUCE SEX REVERSAL IN BARRAMUNDI *Lates calcarifer*

Domingos J.A.[§], Budd A.M., Banh Q.Q., Zenger K.R., Jerry D.R.

*Centre for Sustainable Tropical Fisheries and Aquaculture,
College of Marine and Environmental Sciences
James Cook University, 4811 Townsville, QLD, Australia*

Barramundi (*Lates calcarifer*) is an emergent aquaculture fish species with excellent farming attributes. However, barramundi breeding programs to boost productivity are hindered by a lack of reproductive control. Barramundi, being a large protandrous hermaphrodite, change sex from male to female at 3-5 years of age. As a result, hatcheries need to maintain male broodstock for several years before they can be bred as females. This increases size and scale of infrastructure and decreases the rate of genetic improvement because crosses are performed on fish from over-lapping generations. The genetic mechanisms leading to this sex-change have not been elucidated and there is currently no intervention option available for hatchery managers to control or speed up the sex transition process.

Recent research strongly suggests that epigenetics plays a critical role in determining go-nadal fate in some fishes. In sequential hermaphrodites like barramundi where male and female genomes are identical, it is possible that lifetime cumulative changes in gonadal *CpG* methylation profiles beyond a threshold level may trigger male to female sex reversal. To test this hypothesis, DNA methylation levels of 162 *CpG* sites within the promoters and first exons of six sex-related genes from broodstock and wild barramundi testis and ovaries (n = 40) were investigated by bisulphite amplicon NGS.

Results showed that regardless of origin, barramundi *dmrt1* and *nr5a1* 5'-methylcytosine (C^m) content was significantly lower in testis than in ovaries; whereas *Cyp19a* and *amh* C^m content was significantly higher in testis than in ovaries ($P<0.001$). *Foxl2* and *Sox8* were unmethylated ($C^m <10\%$) in both sexes. In what has been hypothesized as the key testis stabilising gene *dmrt1*, for instance, average methylation levels were 2.6 times greater in ovaries (24.3±3.5 %) than in testis (9.5±4.0 %) in 22 *CpG* sites, with the greatest difference (42.7 *vs.* 3.8%) occurring at -350bp of TSS, adjacent to putative transcription factors binding sites. This work demon-strates that gene-specific methylation/demethylation changes during barramundi adult-hood are key biological processes underlying sex reversal. Further investigation on factors which may accelerate epigenetic changes will lead to better broodstock sex-control and contribute to efficient breeding programs for hermaphrodite fishes like barramundi.

Keywords: Protandrous hermaphrodite, methylation, dmrt1, cyp19a

§ Corresponding author. Tel.: + 61- 7 4781 4154 ; Fax: + 61- 7 4781 5511
E-mail address: jose.domingos1@ jcu.edu.au

FAST TURNOVER OF SEX DETERMINATION AND GENOMIC INCOMPATIBILITIES IN HYBRIDIZING SCULPINS *(Cottus)*

Cheng J.[§], Nolte A.W.

*Evolutionary Genetics of Fishes, Max-Planck Institute for
Evolutionary Biology, Plön, 24306, Germany*

Sex determination in fish has been documented as a very flexible process with respect to evolutionary patterns observed among phylogenetically diverse species. This is a major barrier to evolutionary or applied studies with lacking of information on sex determination mechanisms among different species. Here, two fish species *Cottus rhenanus* and *Cottus perifretum* are known to be involved in the formation of hybrid lineages in the river Rhine. Understanding the sex determination mechanism and developing sex-associated markers will help us to learn about the processes that affect raised fitness of the hybridizing *Cottus*. We have identified genomic regions in the parental species that cause hybrid incompatibilities and tests whether these manifest in a sex specific manner. Interspecific F_2 crosses were analyzed for 255 evenly dispersed markers for genetic mapping and to detect transmission distortion as sign for genetic incompatibilities. QTL mapping using the F_2 crosses has found sex determination region on different linkage group in the two parental species. The segregation of microsatellites from the two regions shows that both species have a male heterogametic (XY) system. Genetic incompatibilities were incomplete, varied among individuals and populations and were not associated with the heterogametic sex. Next-generation sequences of pooled DNA from 50 males and 50 females of hybrid *Cottus* have been mapped against 6 parental reference genomes to identify SNP markers. We estimated allele frequency differences between the male and female hybrid *Cottus* DNA pool and ordered them along the stickleback genome according to BLAST searches to identify sex associated regions. Three candidate regions show signs of association with sex, and verification required. Homology relationships of candidate genomic regions in *Cottus* indicate that sex determination is not based on the same genomic regions found in other fish species. This suggests a fast evolutionary turnover of the genetic basis of sex determination in *Cottus* which, together with the small size of the heterogametic regions, may contribute to the absence of fitness effects related to Haldane's rule.

Keywords: sex determination, scorpaeniformes, QTL mapping, pool-seq, hybrid incompatibility

[§]Jie Cheng: Tel.: + 49 4522 763-375; Fax: + 49 4522 763-281
E-mail address: jiecheng@evolbio.mpg.de

INVESTIGATING THE GENETICS OF SEX DETERMINATION IN EUROPEAN SEA BASS (Dicentrarchus labrax) USING RAD-SEQ

Palaiokostas C.[1], Bekaert M.B.[1], Taggart J.B.[1], Gharbi K.[2], McAndrew B.J.[1], Chatain B.[3], Penman D.J.[1#], Vandeputte M.[3,4]

[1] Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland, United Kingdom
[2] Edinburgh Genomics, Ashworth Laboratories, King's Buildings, University of Edinburgh, Edinburgh EH9 3JT, Scotland, United Kingdom
[3] Ifremer, Chemin de Maguelone, 34250 Palavas-les-Flots, France
[4] INRA, UMR1313 Génétique animale et Biologie intégrative, 78350 Jouy-en-Josas, France

European sea bass (*Dicentrarchus labrax*) is one of the most important farmed species for Mediterranean aquaculture. The observed sexual growth and maturity dimorphism in favour of females adds additional value to deciphering its sex-determining system. Current knowledge indicates the existence of a polygenic sex-determining system interacting with temperature. Restriction-site Associated DNA (RAD) sequencing was used in a test panel of 175 offspring originating from a F2 factorial cross between two dams and four sires from one F1 family between parents with contrasted sex tendencies. In total, 1,156,659,542 raw reads (100 bases long) were produced (578,329,771 paired-end reads). After removing low quality sequences (quality score under 30), ambiguous barcodes and orphaned paired-end reads, 76.7% of the raw reads were retained (886,927,866 reads) from which 56,696 unique RAD-tags were retrieved. In order to maximise the number of informative markers and minimise the amount of missing or erroneous data, we used RAD-tags retrieved in at least 75% of the samples, and carrying one or two single nucleotide polymorphisms (SNPs). The family structure was identified *a posteriori* using R/hsphase and Vitassign with the SNP markers from the offspring and parents. A consensus SNP-based linkage map was constructed, consisting of 5,097 SNPs grouped into 24 linkage groups. Indications for putative sex-determining QTL are provided in linkage groups 13, 19 and 21. A preliminary study was conducted testing whether breeding values, estimated by including the additive SNP effects, could be used as a predictive measure of phenotypic sex. Prediction of phenotypic sex within each of the eight full-sib families, using genomic models (MCMC-BLUP, BayesCPi), consistently outperformed pedigree BLUP. The advantage of using genomic models was particularly evident in dam half-sib family 2, where one of the sex-determining QTLs had been detected. Altogether, our results further support the polygenic hypothesis for sex determination in sea bass, while they also highlight the potential use of within-family genomic selection to rank individuals within families for their estimated breeding value for sex tendency.

Keywords: RAD-seq, Dicentrarchus labrax, QTL, Genomic Selection

§ Corresponding author. Tel.: + 44 (0)1786 467901; Fax: + 44 (0)1786 472133
E-mail address: d.j.penman@stir.ac.uk

DOES EARLY GROWTH PLAY A ROLE IN THE SEX DETERMINATION OF EUROPEAN SEABASS *Dicentrarchus labrax?*

Vandeputte M.[1,2§], Horri K.[1], Allal F.[1], Ferrari S.[3], Vidal M.-O.[1], Ruelle F.[1], Bégout M.-L.[3], Chatain B.[1]

[1] Ifremer UMR9190 MARBEC, Chemin de Maguelone, 34250 Palavas-les-Flots, France
[2] INRA, UMR1313 Génétique animale et Biologie intégrative, 78350 Jouy-en-Josas, France
[3] Ifremer, Ressources Halieutiques de La Rochelle, Place Gaby Coll, 17137 L'Houmeau, France

European sea bass has a polygenic sex determinism with temperature influences, which remains hard to predict and manipulate. To investigate the interactions between early growth and sex determination, we studied West Mediterranean domesticated sea bass from a 50♂x10♀ full factorial cross, reared as a single batch at 15°C to promote female sex determination. Temperature was increased to 25°C from 70 to 119 days post-hatching (dph) to increase growth. Fish were individually tagged at 95 dph (575 mg) with microtags and were individually weighed at 95, 115, 136, 157, 200, 256 and 325 dph, and then sexed. Parentage was recovered with 12 microsatellites; 1134 fish had appropriate pedigree, sex and growth data. Multivariate sire models were run to investigate the links between growth and sex (considered a threshold trait with an underlying sex tendency). Heritability of sex tendency was 0.39±0.12 on the underlying scale. The sex dimorphism of body weight in favour of females rose from +27% at 95 dph (BW_{95}) to +40-45% between 115 and 325 dph, and the sex dimorphism of DGC was maximal for DGC_{95-115}. If differential growth is seen as a consequence of phenotypic sex, growth should be corrected by a fixed sex effect. In this case, there was a positive, although not significant (r_A=0.18±0.12) genetic correlation of sex tendency with DGC_{95-115}, while it never exceeded 0.05 later than 136 dph. The genetic correlation of BW with sex tendency regularly decreased from 0.45±0.18 for BW_{95} to -0.20±0.17 for BW_{325}. If differential growth is seen as a cause of phenotypic sex, no fixed sex effect should be modelled, which then led to a r_A of sex tendency with DGC decreasing from 0.60±0.11 for DGC_{95-115} to 0.21±0.15 for $DGC_{256-325}$, while the r_A of BW with sex tendency also decreased from 0.72±0.10 for BW_{115} to 0.48±0.14 for BW_{325}. In all cases, the genetic and environmental correlation of growth and sex tendency was maximal at the earlier stages, especially before the first reported signs of sex differentiation. This leads us to think that post-larval growth is, at least partially, a cause and not a consequence of sex determination in sea bass.

Keywords: Sex determination, quantitative genetics, growth

§ Corresponding author. Tel.: + 33 4 67 13 04 07; Fax: + 33 4 67 13 04 58
E-mail address: + marc.vandeputte@jouy.inra.fr

GENE EXPRESSION ANALYSIS AT THE ONSET OF SEX DIFFERENTIATION IN TURBOT *(Scophthalmus maximus)* AT DIFFERENT REARING TEMPERATURES

Robledo D.[a, §], Ribas L.[b], Cal R.[c], Sánchez L.[d], Piferrer F.[b], Martínez P.[d], Viñas A.[a]

[a] *Departamento de Genética, Facultad de Biología (CIBUS), Universidad de Santiago de Compostela, Santiago de Compostela, 15782, Spain*
[b] *Institut de Ciències del Mar, Consejo Superior de Investigaciones Científicas (CSIC), Barcelona, 08003, Spain*
[c] *Instituto Español de Oceanografía, Centro Oceanográfico de Vigo, Vigo, 36390, Spain*
[d] *Departamento de Genética, Facultad de Veterinaria, Universidad de Santiago de Compostela, Lugo, 27002, Spain*

Controlling sex ratio is essential for the aquaculture industry, especially in those species with sex dimorphism for relevant productive traits. The turbot (*Scophthalmus maximus*), a very important fish for aquaculture industry in Europe, shows one of the largest sexual growth dimorphisms amongst marine cultured species, being the production of all female populations a desirable goal for industry. Although an important knowledge has been achieved on the genetic basis of sex determination (SD) in this species, the master SD gene remains unknown and precise information on key genes at the critical stage of sex differentiation is lacking. In the present work, we tackled the analysis of the expression profiles of 29 relevant genes related to sex differentiation from the first larval stages (60 days post fertilization, dpf) up to 135 dpf, when male and female gonads are differentiating. Also we considered the influence of three temperature regimes on the sex differentiation process. Three genes were the first to show differential expression between sexes and allowed us to sex turbot accurately at 90 dpf, considered as the time of the onset of sex differentiation: *cyp19a1a*, *amh* and *vasa*. Genes related to primordial germ cell (PGC) and PGC proliferation (*vasa*, *gsdf*, *tdrd1*) increased their expression between 75–90 dpf, and, specifically *vasa* and *tdrd1*, presented higher expression in females at more advanced stages (105 dpf). Furthermore, two genes present on the sex determining region of turbot were studied, *sox2* and *fxr1*. Our results suggest that *sox2* could be discarded as sex determining gene, while *fxr1* presented an expression pattern which could be consistent with a function as sex determinant, being up-regulated in females at 105 dpf. We also detected significative changes in the expression level of several genes (for example *cyp11a*, *dmrt2* or *sox6*) depending on culture temperature and our data suggest that the β-catenin could be involved in male-to-female sex reversal at cold temperatures, since the expression of *ctnnb1* is higher at lower temperatures in males.

Keywords: turbot, sex, gonad differentiation, temperature, qPCR

§ Corresponding author. Tel.: +34981516965
E-mail address: diego.robledo@usc.es

FEMALE SPECIFIC MARKERS AND ATTEMPTS OF ALL-FEMALE PRODUCTION IN HALF-SMOOTH TONGUE SOLE CYNOGLOSSUS SEMILAEVIS

Zhang Q., Wang X., Yu H., Wang Z., Qi J., He Y.

Key Laboratory of Marine Genetics and Breeding, Ministry of Education, College of Marine Life Science, Ocean University of China, Qingdao, 266003, China

Half-smooth tongue sole (*Cynoglossus semilaevis*) is an important flatfish species in both marine aquaculture and fisheries. It has female heterogametic (ZZ/ZW) sex determination system and shows a significant sexual dimorphic growth difference, with females have fast growth rate and final body size than males do. Thus, production of all-female progeny has attracted great attention for its great commercial significance. Several attempts were made to obtain WW super females for all-female production.

Meiotic and mitotic gynogenesis were successfully induced by hydrostatic pressure shocks. Several hundreds of mitotic gynogens and several thousands of meiotic gynogens were obtained. Chromosome observations showed that all the survivors from both groups were ZZ males.

A W chromosome-specific DNA library was constructed through chromosome microdissection. Then W-specific FISH probes were developed and numbers of female-specific markers were screened. Thus FISH based cytogenetic sexing and PCR based molecular sexing methods were established.

Using the W-specific markers, large number of sex reversed (ZW) neo-males were identified from commercially cultured fish. Crosses between these neo-males and normal females (ZW×ZW) yielded thousands of individuals. Molecular detections showed that 63.6% of these individuals possessed W-specific markers. However, all of these genetic female individuals were proved to have ZW chromosome constitution by chromosome observation. The expected WW super female was not identified.

Although ZW×ZW crosses produced higher percentage of genetic females than normal ZZ×ZW crosses, growth performance of this population was not as good as control population.

Triploids were induced by suppression of the second polar body release of normal ZZ×ZW crosses. The triploidy rate in juvenile progeny was about 73%. PCR assessment with W-specific markers showed that 46% triploids were females. Chromosomes of 36 genetic females were observed, all of them showed ZWW chromosome constitution. ZZW chromosome constitution was not observed. Again, growth performance of this triploid population was not as good as control diploid population.

Our results showed that production of all-female population in half-smooth tongue sole through gynogenesis and sex reversed ZW neo-males are not possible at present stage, because the WW super female is inviable.

Keywords: Cynoglossus semilaevis, sex-specific marker, all-female, gynogenesis

§ Corresponding author. Tel.: +86-532-82031806; Fax: +86-532-82031806
E-mail address: qzhang@ouc.edu.cn

INVENTING TETRAPLOID BREEDING FOR ANIMALS USING THE EASTERN OYSTER *C. virginica* AS THE MODEL

Allen Jr. S.K.[a, §], Kube P.[b], Small J.[a]

[a] *Aquaculture Genetics and Breeding Technology Center, VIMS, Gloucester Point, Virginia, USA*
[b] *Department, CSIRO Food Futures Flagship, Hobart, Tasmania, Australia*

Tetraploids are a regular feature of plant breeding but not for animals. The sole exception, at least as judged by the extent of their use, is oysters of the genus *Crassostrea*. For three species, the utility of tetraploids – to make triploid progeny – have been clearly demonstrated. Tetraploid *C. gigas* have been in use commercially since about 2000 and *C. virginica* – since 2003. Polyploid development with other *Crassostrea* spp., such as *C. ariakensis*, has been intermittent but shows equal utility. Despite the enormous production of triploid oysters for commercial purposes (approaching 3B seed annually), little in the way of domestication of tetraploids has been accomplished. Rather, the attributes of tetraploid "lines" is generally derived from the starting material used to produce them in the F_0. How should tetraploids be domesticated after the F_0? Models for quantitative genetics of polyploidy species (plants) exist, so this approach requires a family based program. Yet, tetraploids can originate through three different protocols and their karyotypes may vary accordingly in subtle ways. Cytogenetically, evidence suggests the karyotype of tetraploids is unstable and may vary among successive generations. In addition, chromosome loss may have deleterious effects. How does this chromosome instability translate in quantitative genetic analysis? Probably, *de novo* tetraploids require a period of domestication, the artificial selection equivalent of diploidization in evolution. Furthermore, tetraploids *per se* are not the commercial end product, triploids are. Does genetic progress in tetraploids (presumably a parallel selection program to diploids) obtain in the tetraploid x diploid cross or do other, non-additive (heterosis), or epigenetic (chromosome loss) factors prevail? None of these questions have been answered. At ABC, we have begun a collaboration with CSIRO to integrate diploid and tetraploid breeding, which is warranted in our region due to the exceptional reliance of the industry on triploids (90%). The considerations above have been incorporated into the design of this breeding program and will be discussed with evidence from earlier studies on tetraploids that also inform our strategy.

Keywords: oyster, breeding, polyploidy, chromosomes, tetraploid

§ Corresponding author. Tel.: + 1-804-684-7710 ; Fax: + 1-804-684-7717
E-mail address: + ska@vims.edu

ENVIRONMENTAL DNA (eDNA): A NEW FORENSIC TECHNIQUE TO DETECT PATHOGENS IN FARMED FISH

Gomes G.B.[§], Miller T.L., Hutson K.S., Jerry D.R.

*Centre for Sustainable Tropical Fisheries and Aquaculture,
College of Marine and Environmental Sciences
James Cook University, 4811 Townsville, QLD, Australia*

Disease compromises the sustainability of global aquaculture. Among many pathogens affecting farmed fishes, ciliate protozoans are a particular concern as mortalities occur with little or no warning. Parasitic ciliates are one of the largest causes of production loss in farmed fish. Currently there are no efficient sentinel methods to detect the presence or the abundance of these aquaculture pathogens. A novel and innovative detection method is urgently needed to overcome the difficulty in correctly identifying ciliate parasites.

Environmental DNA (eDNA) is a new molecular detection approach which can screen genetic material released in the environment (water or sediment) by both macro and microorganisms. Environmental DNA detection methodologies have been gaining popularity in conservation and forensic studies, and more recently, have been applied to perform parasite surveys in natural water bodies. Using the globally distributed freshwater ciliate parasite *Chilodonella* spp. as a model we show that the eDNA approach also has merit in detecting the presence of these parasites in fish aquaculture.

Water samples from an Australian freshwater barramundi or Asian seabass (*Lates calcarifer*) farm were collected in three different points within a pond over 10 months. Using qPCR and TaqMan probe technology based on the 18S small subunit ribosomal gene we were able to detect and quantify the presence of *Chilodonella* spp. in water samples before, during and after epizootic events. The ability to detect the presence and abundance of ciliate pathogens prior to disease outbreaks has the potential to revolutionise the way economically important parasites are detected prior to potential outbreaks. When combined with data on local environmental parameters (e.g. low oxygen levels, high or low temperatures, nitrate and nitrite parameters), the use of eDNA can lead to development of better predictive models to pre-empt actual disease outbreaks and aid management of these pathogens.

Keywords: Parasites, pathogen monitoring, disease, detection, Chilodonella spp.

[§]Corresponding author. Tel.: +61- 7 4781 5397; Fax: +61- 7 4781 5511
E-mail address: giana.bastosgomes@my.jcu.edu.au

A BAC TRANSGENIC ANALYSIS OF THE asip1 LOCUS REVEALS DEVELOPMENTAL MECHANISMS OF DORSO-VENTRAL PIGMENTATION IN FISH

Cal L.[a]§; Gómez-Marín C.[b]; Gómez-Skarmeta J.L.[b]; Cerdá-Reverter J.M.[c], Kelsh R.N.[d], Rotllant J.[a]

[a] *Aquatic Molecular Pathobiology Group, Institute of Marine Research (IIM-CSIC), Vigo, 36208, Spain*
[b] *Gene Regulation and Morphogenesis Group, Andalucian Center of Developmental Biology (CABD-CSIC/Pablo de Olavide University), Seville, 41013, Spain*
[c] *Department of Fish Physiology and Biotechnology, Institute of Aquaculture Torre de la Sal (IATS-CSIC), Castellón, 12595, Spain*
[d] *Department of Biology and Biochemistry, Centre for Regenerative Medicine and Developmental Biology Programme, Bath, BA2 7AY, United Kindom*

Pigment abnormalities are one of the leading causes in performance loss in flatfish aquaculture. Nevertheless, and in spite of the importance of the problem, there is scanty information about the molecular regulation of fish pigmentation which makes the control of this problem difficult. To date, the most important regulator of skin and hair pigmentation in vertebrates is the Agouti signaling protein (ASIP)- melanocortin 1 receptor (MC1R) system: the *agouti* locus encodes the Agouti-signaling protein (ASIP), which antagonizes the effects of α-Melanocyte-Stimulating hormone (α-MSH) binding to the MC1R on the follicular melanocyte, resulting in a decrease in the production of eumelanins (dark pigments) and an increase in pheomelanins (red/yellow pigments). A key aspect of mammalian pigment pattern, that is widely conserved, is the pale *ventrum* and darker *dorsum*; the underlying mechanism is regulation of ASIP expression, with high ventral *Agouti* driving pale belly color. Likewise, fish often show a pale *ventrum*, but here the pigmentation mechanism is very different with independent cell-types, each expressing chemically distinct pigments (not just melanins), being differentially distributed. Recently, we have shown that not only the *agouti* gene (*asip1*), but also even the elevated ventral expression levels, are conserved in fish. It is of interest, in order to know the molecular regulation of fish pigmentation, to establish how Asip1 functions to regulate fish pigment pattern formation. In order to explore the mechanism of Asip1 in zebrafish pigment pattern formation, we used a BAC transgenic approach to allow characterization of *asip1* cis-regulation regions. We have generated a BAC transgenic zebrafish line in which 5' and 3' *agouti* locus regulatory regions direct *eGFP* overexpression. Our initial observations showed a graded dorso-ventral expression of *eGFP*. Therefore, *eGFP* expression in the *asip1-iTol2-eGFP-BAC* transgenic fish faithfully recapitulated the endogenous expression pattern of *asip1* mRNA. This opens the way to characterizing the mechanisms generating this crucial graded distribution of *asip1.*

§ Corresponding author. Tel.: +34 986231930 ext 289; Fax: + 34 986292762
E-mail address: lauracal@iim.csic.es

VERIFICATION OF ISOGENIC NATURE OF CLONAL LINES IN THE ATLANTIC SALMON *(Salmo salar)* THROUGH ddRADseq

Oral M.[1§], Taggart J.B.[1], McAndrew B.J.[1], Penman D.J.[1], Fjelldal P.G.[2] and Tom Hansen[2]

[1] Institute of Aquaculture, School of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland, UK
[2] Institute of Marine Research (IMR), Matre Research Station, NO-5984 Matredal, Norway

Isogenic clonal lines of fish have similar potential as a tool in aquaculture related research as inbred lines of mice have shown in the other areas of biological research, although to date they are available in only a few species. These can be produced in two generations through androgenesis or mitotic gynogenesis. Next generation sequencing allows large numbers of SNP markers to be used to verify the production of such lines, in particular the efficiency of gamete inactivation and the elimination of spontaneous meiotic gynogenetics. In this study, we used double-digest restriction site associated DNA sequencing (ddRADseq) to analyse the development of isogenic clonal lines in the Atlantic salmon (*Salmo salar*) from the out-bred founder generation, via reproductively mature mitotic gynogenetic (double haploid) clone founders to five putative clonal lines (produced by meiotic gynogenesis from double haploid clone founders). Haploid gynogenetic embryos were analysed in parallel as a control for duplicated loci.

Keywords: Isogenic clonal lines, ddRADseq, Atlantic salmon, aquaculture

§ Corresponding author. Tel.: +44 (0)1786 466593; Fax: +44 (0)1786 472133
E-mail address: mo19@stir.ac.uk

TISSUE-SPECIFIC TRANSCRIPTOMES OF *Mytilus galloprovincialis* REVEAL NEW FUNCTIONS

Moreira R.[1], Canchaya C.[2], Novoa B.[1], Posada D.[2], Figueras A.[1§]

[1]Instituto de Investigaciones Marinas, IIM – CSIC, Vigo, Spain
[2]Universidade de Vigo, Vigo, Spain

The Mediterranean mussel (*Mytilus galloprovincialis*) is a cosmopolitan, cultured bivalve with worldwide commercial and ecological importance. There is a need to increase our knowledge of the molecular mechanisms involved in *Mytilus* physiology from a qualitative and quantitative point of view. In order to start filling this gap we have used RNA-Seq to study the transcriptome of mantle, muscle and gills from naïve mussels and hemocytes exposed to distinct stimuli.

After the analysis of the complete mussel transcriptome, we confirmed that we have achieved a completeness of the transcriptome of 95.16%. As mussel and oyster are model species for bivalves, we compared them and found that the shared proteins with a sequence identity over 80% represent less than 10% of their transcriptomes. In the whole mussel transcriptome it is important to highlight that the response to infectious diseases and cancer were pathways highly represented. However, only 55% of the transcripts were shared across all tissues. Hemocyte and gill transcriptomes were most different, with 60% shared transcripts, while mantle and muscle transcriptomes were most similar, with 77% shared transcripts. The transcriptomes showed characteristic expression profiles in agreement with their structures and functions: stimulated hemocytes confirmed their immune function showing a high representation of defense and immune-related expressed genes; the gills presented many transcripts assigned to both structure and recognition of non-self patterns; the mantle showed an abundance of transcripts related to reproduction and shell formation and, finally, the muscle expressed many myofibril and calcium-related proteins.

However, we could find other complex and specialized functions of each tissue, not previously reported: gills and its probable osmotic and homeostatic function; muscle revealed unexpected defense functions; and in mantle additional and interesting antifungal and sensorial functions were discovered, but also hematopoiesis transcripts were exclusively expressed in mantle, confirming its possible role as the hematopoietic tissue in bivalves. This information will provide new insight to bivalves, and specially mussel, physiology.

Keywords: Mytilus galloprovincialis, transcriptome, NGS, RNA-Seq, NOISeq, KEGG, Gene Ontology, Blast2GO.

§Corresponding author. Tel.: +34 986 231 930; Fax: +34 986 292 762
E-mail address: antoniofigueras@iim.csic.es

APPLICATION OF RNA-SEQ IN INVESTIGATING A MAJOR PARASITIC DISEASE OF TURBOT (Scophthalmus maximus), ENTEROMYXOSIS

Ronza P.[a, §*], Robledo D.[b, *], Losada A.P.[a], Bermúdez R.[c],
Pardo B.G.[d], Martínez P.[d], Quiroga M.I.[a,]

[a] *Departamento de Ciencias Clínicas Veterinarias, Facultad de Veterinaria,*
Universidad de Santiago de Compostela, Lugo, 27002, Spain
[b] *Departamento de Genética, Facultad de Biología (CIBUS), Universidad de*
Santiago de Compostela, Santiago de Compostela, 15782, Spain
[c] *Departmento de Anatomía y Producción Animal, Facultad de Veterinaria,*
Universidad de Santiago de Compostela, Lugo, 27002, Spain
[d] *Departmento de Genética, Facultad de Veterinaria, Universidad*
de Santiago de Compostela, Lugo, 27002, Spain

Enteromyxum scophthalmi is an intestinal myxozoan parasite which poses a serious threat for turbot (*Scophthalmus maximus*) aquaculture, an important industry with a production over 10,000 tons/year in Europe and over 60,000 tons/year in China. Currently there are not any therapeutic options available for enteromyxosis. To develop effective prevention and treatment strategies, knowledge on host-parasite interaction and host immune response must be gathered. In this sense, we previously performed an RNA-seq analysis on turbots at 42 days post infection (dpi), histopathologically classified as severely infected. This allowed us to depict a general view of the pathogenetic mechanisms involved in severe enteromyxosis. Aiming to further understanding the pathogenesis of this disease, a similar analysis was performed on slight *E. scophthalmi*-infected turbot (24 dpi) uncovering the early transcriptomic changes induced by the parasite. We carried out RNA-seq on kidney, spleen and pyloric caeca; pooled samples by organ from 3 specimens were used as control and compared with individual samples from 3 infected individuals. We found a total of 469, 215 and 189 differentially expressed (DE) genes for kidney, spleen and pyloric caeca, respectively, lower figures than those previously found at 42 dpi: 1316, 1377 and 3022 respectively. Nevertheless, several genes were shared between the 24 and 42 dpi responses in each tissue. At 24 dpi, DE immune related genes were mainly found in kidney, which appeared to first response to the infection in comparison with spleen, and pyloric caeca, the primary site of infection. Regarding immune response, a remarkable result was the opposite expression pattern of interferon-related genes observed between the two time points, showing up-regulation at early infection. On the other hand, in pyloric caeca, genes related with B cells and C-type lectin CD209, a marker of antigen-presenting cells, were found up-regulated both at 24 and 42 dpi. The combined approach of RNA-seq analyses and pathologic observations is greatly helping to elucidate the host-parasite interaction and the pathogenesis of this threatening disease. This constitutes an important step in the identification of candidate genes for incoming effective strategies to control enteromyxosis.

Keywords: turbot, enteromyxosis, RNA-seq, pathogenesis, host-parasite interaction.

[*]These authors have contributed equally to this work.
[§] Corresponding author. Tel.: + 34982822306
E-mail address: paolo.ronza@usc.es

EXPLORING THE GENETIC BASIS OF RESISTANCE TO PANCREAS DISEASE IN ATLANTIC SALMON *(Salmo salar)*

Gonen S.[a, §], Baranski M.[b], Thorland I.[c], Norris A.[d], Grove H.[e], Arnesen P.[d], Bakke H.[f], Lien S.[e], Bishop S.C.[a] and Houston R.D.[a]

[a] *The Roslin Institute and Royal (Dick) School of Veterinary Studies, University of Edinburgh, Midlothian, EH25 9RG, UK*
[b] *Nofima, PO Box 210, 1431 Ås, Norway*
[c] *Akvaforsk Genetics Center AS, Sjølseng, 6600 Sunndalsøra, Norway*
[d] *Marine Harvest, Sandviksboder 78a, 5035 Bergen, Norway*
[e] *Department of Animal and Aquacultural Sciences and Centre for Integrative Genetics, Norwegian University of Life Sciences, 1431, Ås, Norway*
[f] *SalmoBreed AS, Bontelabo 2, 5003, Bergen, Norway*

Pancreas disease (PD) is currently one of the major viral diseases affecting Atlantic salmon aquaculture, resulting in high levels of morbidity and mortality, reductions in production efficiency and economic losses on affected farms. Natural field outbreaks of PD have only been reported in the post-smolt stage of the salmon lifecycle, although laboratory viral challenge studies have demonstrated that infection at the juvenile fry stage is possible. Heritability of resistance to PD has been estimated at 0.21 (± 0.005) in a natural field outbreak in a population of post-smolts. However, the underlying genetic architecture of this host genetic variation in resistance has not been explored. The aim of this study was to confirm the presence of heritable variation for resistance, and to identify and validate QTL influencing resistance to PD in two large, independent populations of farmed Atlantic salmon. The first population (POP 1) consisted of ~5,500 Atlantic salmon fry, and the second population (POP 2) consisted of ~5,000 post-smolts. Both populations were challenged with the same strain of the PD causing salmonid alphavirus (SAV). In both populations, a high heritability of resistance to PD was estimated, and estimates were relatively concordant across both datasets (~0.5 in POP 1 and ~0.4 in POP 2). QTL mapping studies conducted independently within in the two populations identified a total of six putative QTL affecting resistance. A single QTL, located on the distal end of chromosome 3, was repeatedly identified across all analyses and in both populations. This suggests similar biological mechanisms underlying a proportion of the genetic resistance across both lifecycle stages, with positive implications for this QTL for use in marker-assisted breeding on Atlantic salmon farms. SNP markers showing significant population-level association with this QTL have been identified. Application of these markers in selective breeding programs may contribute towards improving resistance amongst fish stock, and further characterisation of the QTL may enhance the understanding of the biological basis of resistance to this disease.

Keywords: Pancreas disease, Salmonid alphavirus, Atlantic salmon, Heritability, QTL mapping

[§] Corresponding author. Tel.: +44 131 6519100
E-mail address: gonenserap@gmail.com / serap.gonen@roslin.ed.ac.uk

ANNOTATION OF *Seriola lalandi* REFERENCE TRANSCRIPTOME OF LARVAE AND DIFERENTIAL GENE EXPRESSION BETWEEN NORMAL AND SKELETAL DEFORMED INDIVIDUALS

Patel A.[a], Hernandez E.[a], Barra V., Martínez V.[a, §]

[a] *FAVET-INBIOGEN. Dpt of Animal Science, Faculty of Veterinary Sciences, University of Chile, Stgo, 8820808, Chile.*
[b] *ACUINOR, Caldera, Chile*

The presence of skeletal deformities in farmed fish is an important problem in aquaculture causing huge economic loss and raising animal welfare issues. *S. lalandi*, a carangidae, have been reported to possess anomalies of various skeletal tissues in culture environment mainly during the larval phase. The deformities observed are fused vertebrae, bended lower jaw, shorter jaw, operculum shorter or missing and compacted body and tail. Identification of the genes and its networks that are involved in skeletal development will help to understand the molecular mechanisms behind these phenotypes. We deep sequenced normal and skeletal deformed individuals on 23 days after post hatch to generate a *de novo* assembly, and subsequently annotated and compared the gene expression between normal and skeletal deformed individuals. The full assembled transcriptome predicts 40,066 genes, 15,744 were associated gene ontology (GO) with a mean GO graph level of 4.38. We could construct 337 KEGG pathways maps comprise of several functional categories: amino acid metabolism (292), lipid metabolism (276) and carbohydrate metabolism (263). We identified 2,309 differentially expressed genes that were significant at 0.01 FDR and at least a 2-fold change between two groups. Of these, 1,262 genes (54.65%) were down-regulated and 1,047 genes (45.34%) were up-regulated in the deformed group compared to that of phenotypically normal individuals. GO enrichment analysis of genes found to be down regulated in deformed group were mostly from the proteinaceous extracellular matrix term (unigenes (58). We found a substantial decrease in counts of COL1A1 and Bmp2 transcripts in skeletal deformed groups. Some of the most prominent ECM proteins secreted by chondrocytes are the collagens, a single molecule that associate into chains to form a triple helical structure. Mutations in genes that encode the collagens have been reported to be associated to skeletal abnormality in human, zebrafish and gilthhead sea bream. Therefore, enrichment of GO term 'extracellular matrix' in our global differential transcriptome analysis highlights the importance of these cellular components in skeletal development process of *S. lalandi*.

Keywords: Seriola lalandi, RNA-seq, larval stage, skeletal deformities, digital gene expression

§ Corresponding author. Tel.: +56 96 306 0306
E-mail address: vmartine@u.uchile.cl

ALLELE SPECIFIC EXPRESSION ON LIVER AND HEAD KIDNEY OF Salmo salar WITH DIFFERENTIAL SUSCEPTIBILITY TO THE CHALLENGE WITH *Piscirickettsia salmonis.*

Dettleff P. and Martínez V.

FAVET-INBIOGEN, Faculty of Veterinary Science, University of Chile, Santiago, Post code 8820808, Chile.

The allele specific expression (ASE) determines the existence of imbalances in the expression of one allele relative to the other. This analysis is possible using deep sequencing of mRNA obtained from different tissues and susceptibility background. The aim of this study is to identify SNPs that present ASE in Atlantic salmon challenged with *Piscirickettsia salmonis,* based on large scale challenge test for this disease. A *de novo* assembly was obtained using a sample of individuals of different families, representing the population under analysis. In this study, samples of liver and head kidney of susceptible and resistant individuals to the disease were used for RNA-seq analysis, as obtained throughout the challenge test. SNPs present in the transcripts of the two groups were identified, determining heterozygotes in the population and the number of reads for each allele. To identify ASE, the deviation of the expected ratio 50:50 of the expression of each allele was evaluated using a Chi-square test (FDR <0.01). Subsequently, the SNPs with ASE in each sample were compared, identifying those SNPs with ASE in both groups and tissues. Additionally the SNPs that present ASE exclusively on susceptible or in resistance group were determined. A total of 4063 heterozygotes SNPs were identified. Of these, 170 SNPs present ASE in both tissues and in both groups, present on 59 different transcripts. To evaluate the molecular and biological function of these genes a Gene Ontology analysis was conducted. The results show that genes with ASE in all individuals are associated with metabolic processes of organic substances; nitrogen compounds metabolic processes and biosynthetic processes. Additionally 174 SNPs (in 62 transcripts) were identified with ASE within the susceptible group. In the case of resistant group, a total of 102 SNPs (in 43 transcripts) with ASE exclusively in this group were obtained. The SNPs with ASE of these transcripts could be of importance in disease resistance against this bacterium, due to the fact that they could be used in marker-assisted selection for tolerance against this pathogen. Still, further studies are required in order to understand how these *cis* acting factors explain disease resistance in practice. Funding: FONDECYT 1120608, CONICYT scholarship for PhD.

Keywords: *salmon, allele specific expression, SRS, RNA-seq*

POPULATION GENETICS AND TRANSCRIPTOMICS OF MANILA CLAM *(Ruditapes philippinarum)* AND CARPET-SHELL CLAM *(R. decussatus):* IMPLICATIONS FOR AQUACULTURE.

Saavedra C.[a,*], Milan M.[b], Cordero D.[a], Leite R.[c], Peña J.B.[a], Delgado M.[d], Liu B.[e], Ruesink J.[f], Cancela L.[c,g], Bargelloni L.[b] and Patarnello T.[b].

[a] Instituto de Acuicultura Torre de la Sal, CSIC, 12595 Ribera de Cabanes (Castellón), Spain
[b] Department of Comparative Biomedicine and Food Science, University of Padova, Viale dell'Università 16, Legnaro (Padova), Italy
[c] CCMAR/University of Algarve, Campus de Gambelas, Faro, Portugal.
[d] Centre d'Aqüicultura, Institut de Recerca i Tecnologies Agroalimentaries, Sant Carles de la Ràpita (Tarragona)
[e] Institute of Oceanology, Chinese Academy of Sciences, Qindao, China
[f] Department of Biology, University of Washington, Seattle, EE.UU.
[g] Department of Biomedical Sciences and Medicine, University of Algarve, Faro, Portugal

Knowledge of the genetics of populations is essential to understand performance differences among races and local stocks of shellfish species. We have studied the population genetics of the native Asian -but now globally cultured- Manila clam, and the native European carpet-shell clam, which are exploited in Europe by a combination of wild bed harvesting and supplementation with hatchery spat. Using mtDNA and nuclear markers we found that both species show clear phylogeographic breaks at the Mediterranean Sea (carpet-shell clam) and at the East China Sea (Manila clam). Moreover, we confirmed a Japanese origin for American and European Manila clam populations, which in spite of the short time interval passed since the introduction, have experienced a considerable genetic differentiation, and some loss of genetic variability in Europe. To study the potential functional significance of phylogeographic differences we used a transcriptomic approach in *R. decussatus*. We studied the gene expression in the gill tissue of clams from four populations sampled along the north-south thermal gradients of Atlantic and west Mediterranean European coasts with a microarray containing 14003 oligonucleotide probes. Clams were acclimated for three months in a common environment before the experiment. Using a multiple regression approach we found that a model based on the latitude and longitude of the populations, an index of phylogeographic differentiation (based on 3 polymorphic introns), and their interactions, explained a considerable fraction of the interindividual variation in gene expression for 1706 genes (FDR = 0.22). Functional characterization uncovered an abundance of stress-related genes in the group of differentially expressed genes. These results imply that the choice of the geographic origin of clam spat or breeders could have important consequences on the results of population supplementation or breeding programs.

Keywords: microsatellites, mitochondrial DNA, introns, microarray, stress

* Corresponding author. Tel.: + 34964319500; Fax: + 34964319509
E-mail address: saavedra@iats.csic.es

IDENTIFICATION OF BACTERIAL COMMUNITY COMPOSITION IN TILAPIA BIOFLOC SYSTEM UNDER DIFFERENT ENVIRONMENTAL CONDITIONS USING PCR-DGGE TECHNIQUE

Suloma A., Mabroke R.S., Tahoun A.M., Zidan A.N., El-Menofy W., Adam M. and El-Shafiey M.H.M.

[1]Fish Nutrition Laboratory (FNL), Animal Production Department, Faculty of Agriculture, Cairo University, 12613, Giza, Egypt

This work designed to study the impact of different parameters namely water dissolved oxygen, alkalinity, pH, ammonia, organic carbon source and water temperature on the water quality and biofloc formation and to Monitor the distribution of microbial populations inhabiting different culture using PCR-DGGE. Five experiments were conducted to study the effect of pH / alkalinity (Exp. 1), dissolved oxygen (Exp. 2), ammonia (Exp 3), organic carbon source (Exp.4.) and water temperature (Exp 5) on water quality and the microbial community structure under BFT system. All environmental factors in water in the rearing units were fixed among different experiments except for the factor under study. All treatments (four levels with three replicates) were randomly assigned to 70 L plastic tanks. Each treatment was consist of three replicates, and tilapia were stocked per tank according to their size. The treatment systems were provided daily with tilapia feeds and carbon source. Diffusive stone aerators were installed in each tank to provide adequate mixing and dissolved oxygen concentration (DO) above 4 mg /L. *Bio-floc samples for DNA extraction were taken from all experimental treatments after 15 and 30 days. Bio-flocs were harvested from 50 mL of tank water and biomass were collected by centrifugation (10 min, 8000 X g). To obtain DNA for further analysis of the total bacterial community by DGGE, a single round of PCR reaction will be performed using oligonucleotides primers designed to anneal to conserved positions regions of the bacterial 16S rRNA gene, such as V3 region, as described by Muyzer et al. (1993).* The chemical properties of water samples were determined according to APHA, 1998.

Changes in environmental condition caused differences in water quality parameters among treatments. Low oxygen level led to decreased biofloc volume, zooplankton abundance, pH and nitrate. Glucose was the best carbon source during the first week while cellulose improved water quality parameters after the system start-up indicating that BFT has the ability to utilize lignocellulosic agricultural residues as carbon sources for pond feeding. Sequencing of the fractions generated from PCR-DGGE analyses are still in progress..

Keywords: bacterial community composition, tilapia, biofloc system, PCR-DGGE

*Corresponding author: (Tel.): + 201006176195
E-mail address: suloma2001@agr.cu.edu.eg

GENETIC VARIATION IN PACIFIC OYSTERS (*Crassostrea gigas*) FOR RESISTANCE TO OSTREID HERPESVIRUS-1

Kube P.D.[a][§], Dove M.C.[b], Cunningham M.[c], Kirkland P.D.[d], O'Connor W.A.[b], Elliott N.G.[a]

[a] *CSIRO Agriculture Flagship, Hobart, 7000, Australia*
[b] *New South Wales Department of Primary Industries, Taylors Beach, 2316, Australia*
[c] *Australian Seafood Industries, Hobart, 7010, Australia*
[d] *New South Wales Department of Primary Industries, Menangle, 2567, Australia*

Ostreid herpesvirus-1 (OsHV-1) is the causative agent of mass mortalities of cultured Pacific oysters (*Crassostrea gigas*) in different regions worldwide. This virus has devastated farmed stocks in France, New Zealand and parts of Australia. Genetic selection for disease resistance is one potential management tool and research in Australia has aimed to understand the genetic architecture of disease resistance and develop methods to include selection for resistance into an existing selective breeding program.

Data has been collected from a series of field trials in Botany Bay, NSW, where animals were exposed to a natural challenge. These trials have tested both spat (6 months old) and juveniles (12 months old) and have been done for three genetically linked year classes with 43, 54 and 78 families per year class (175 families in total). Genetic variation has been present in all trials and heritabilities (on the observed scale) ranged between 0.18 and 0.60. The genetic correlation between a spat and juvenile challenge was moderately high (r_g=0.76) and in a combined analysis the heritabilities of spat and juvenile survival were 0.39 and 0.27 respectively.

However, expression in field trials has sometimes been problematic and the implementation is logistically difficult due to the unpredictable nature of the disease and the variability inherent in a natural challenge. Consequently, a laboratory challenge model has been adapted for routine genetic evaluation. The laboratory challenge is based on an immersion challenge and involves challenging with "low" and "high" doses of virus to achieve discrimination of mortality across all families. Early results for the laboratory challenge are indicating a moderate heritability (h^2=0.2) and a moderate genetic correlation with field challenges (r_g=0.7).

Selection for OsHV-1 resistance has become a primary trait in the Australian Pacific oyster selective breeding program and two generations of resistance selection have now been successfully completed. Progeny test data can be reliably obtained at 6 months of age when using the laboratory challenge system, which provides good opportunities to make rapid genetic progress for this trait.

Keywords: Pacific oysters, disease resistance, Ostreid herpesvirus-1, heritability

[§] Corresponding author. Tel.: +61 3 62325241
E-mail address: peter.kube@csiro.au

A FIRST STEP FOR SUSTAINABLE BREEDING PROGRAMMES IN PIKEPERCH *(Sander lucioperca)* THROUGH THE EVALUATION OF THE GENETIC VARIATION IN DOMESTICATED BROODSTOCKS AND NATURAL POPULATIONS

Tsaparis D.[a], Kyriakis D.[b], Ekonomaki K.[a], Darivianakis S.[a], Fontaine P.[c], Tsigenopoulos C.S.[a, §]

[a] *Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Centre for Marine Research (HCMR), Heraklion, 71003, Greece*
[b] *Department of Biology, Aristotle University of Thessaloniki, Thessaloniki, 54124, Greece*
[c] *Animal and Functionality of Animal Products (UR AFPA), University of Lorraine - INRA, Vandoeuvre-lès-Nancy, 54505, France*

The pikeperch is a temperate Eurasian freshwater/brackish water fish species with growing interest for the European aquaculture. Wild populations of pikeperch show signs of decline in many areas of its natural range of distribution (from Finland to the Aegean Sea, and East to the Aral and Caspian basins) due to human activities, such as the destruction of natural habitats and/or overfishing. The species has been introduced in northern Russia, Italy, Spain, Turkey, the North African countries (from Morocco to Tunisia) and many other regions. There are only a few commercial hatcheries that produce pikeperch in Europe. In principle, each farm uses its own stock, captured either from the wild or supplied by another farmer. There-fore, pikeperch populations differ from one farm to another depending upon the geographi-cal origin of the captured wild populations, which were at the base of the captive stocks. The main objective of the present study was to develop and use highly informative and efficient for the species, multiplex panels of microsatellite loci, in order to assess the genetic variabil-ity of thirteen domesticated populations from commercial farms and of eight wild popula-tions. In total, DNA from 971 fish samples (fin clips) was extracted using standard protocols. Two multiplexes (4-plex & 7-plex) were developed, optimized and finally used for genotyping of samples while all 21 populations were analyzed for basic population genetics parameters (allelic richness, heterozygosity indices, inbreeding coefficients). The genetic differentiation among locations was quantified by F_{ST} values. Genotyping of wild and domesticated popu-lations and the comparative evaluation of their genetic status are extremely useful for the future establishment of genetic breeding programmes for sustainable optimal performance through domestication of pikeperch. Results indicate that most of the populations show medium to low levels of genetic diversity and some of them may be inbred. Differentiation between broodstocks was high in most cases, while lowest values were estimated for pairs of known common origin or geographic proximity.

Keywords: microsatellites, differentiation, freshwater aquaculture

§ Corresponding author. Tel.: +30 2810337854; Fax: +30 2810337870
E-mail address: tsigeno@hcmr.gr

RECORD OF LACK OF INTERMUSCULAR BONES IN SPECIMENS OF *Colossoma macropomum* (CHARACIFORMES): UNUSUAL PHENOTYPE TO BE INCORPORATED INTO GENETIC IMPROVEMENT PROGRAMS

Perazza C.A.[a], Menezes J.T.B.[b], Silva L.A.[c], Pinaffi F.V.[c], Ferraz J.B.S.[c], Hilsdorf A.W.S.[a]

[a]*Unit of Biotechnology, University of Mogi das Cruzes, Mogi das Cruzes,08780-911, Brazil*
[b]*Biofish Aquaculture Consulting, Porto Velho, 76801-504, Brazil*
[c]*Department of Veterinary Sciences, University of São Paulo, Pirassununga, 05508-270, Brazil*

Intermuscular bones are small spicule-like bones inserted in the myosepta, extending horizontally backward and marginally downward located the both sides of the muscle fillet of teleost fishes. These bones appeared to be a response to strains undergone by the muscles. Other studies pointed out other functions, such as transmission of force between musculature segments, overall growth of body firmness, and restrain myomere deformation during contraction. Intermuscular bones are present in important farming fish species such as carps (*Cyprinus carpio*) and Milk fish (*Chanos chanos*). Intermuscular bones are also a common characteristics found in fishes of order Characiformes. Representatives of this order are typically freshwater fish mostly diverse in the Neotropical region throughout Central and South America. Numerous of Characiformes species are important fish food production either by farming or fishing. Tambaqui, *Colossoma macropomum*, is the largest Characiforme species native to the Amazon and Orinoco river basins of South America. Tambaqui is a fast-growing, omnivorous and tolerates poor water quality making it an outstanding species for aquaculture. Tambaqui farming is a growing industry being the largest native fish cultivated in Brazil. Intermuscular boneless tambaqui was found out in a fish farming located in in the north-western part of Brazil. In 2012, one fingerling batch was sold for growth-out operation farming enterprise that reported that after fillet processing the individuals of that batch had no intermuscular bones. Having received such information, the tambaqui hatchery manager started searching for the parents of the intermuscular bones tambaqui among hatchery broodstock used during previous breeding season. We used x-ray and ultra-sound images to assess the intermuscular bones. We found out 50 intermuscular-boneless tambaquis among 120 tested broodstock. The potential of this phenotype for tambaqui farming is enormous. The presence of intermuscular bones in the loin eye area of tambaqui is an undesirable farming characteristic because it poses problems in filleting. To disclosure the genetic mechanism behind this phenotype is an important step to incorporate it into a breeding program will improve the tambaqui cuts in processing plants which remove manually intermuscular bones making the processing more labor-intensive work.

Keywords: tambaqui; intermuscular bones; pin bones; spicule-like bones; processing

*These authors have contributed equally to this work.
§Corresponding author. Tel.: + 55 11 4798-7210; Fax: +55 11 4798-7106
E-mail address: wagner@umc.br

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DISCOVERY AND VALIDATION OF SNPS BETWEEN TWO COMMERCIALLY IMPORTANT CRASSOSTREA SPECIES: *C. gigas* (PACIFIC OYSTER) AND *C. angulata* (PORTUGUESE OYSTER)

Cross I., García A., Merlo M.A., Rodríguez M.E., Portela-Bens S., Rebordinos L.

Área *de Genética. Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz. 11510, Puerto Real, Cádiz, Spain*

The Portuguese oyster, *Crassostrea angulata*, is an edible cupped oyster of major commercial importance, playing an important role as biosensor of coastal water pollution. It is assumed to be native to the Northwest Pacific region and introduced into many countries around the world. The conservation of *C. angulata* in southern Europe is endangered by the current expansion of the Pacific oyster *C. gigas* aquaculture and by pollution of its natural habitat. We have sequenced the genome of *C. angulata* and identified SNPs based on the *C. gigas* reference genome; functional annotation for SNPs of *angulata* has also been carried out. Comparisons of the sequences with oyster.v.9 reference assembly yielded 1,29 million SNPs. From the total number of SNPs, 151,620 were located in 20,908 genes from the *C. gigas* database. Out of the SNPs annotated in the oyster sequence assembly, 77,534 were found in protein-coding regions containing 32,994 non-synonymous SNPs. The analysis of Gene Ontology (GO) terms associated with gene regions containing SNPs revealed that significant GO terms showing differences between the two oyster species were related to the binding and catalytic activities. These activities are related to response to stress caused both by drying and by metal salt contamination. In the Biological Process domain, the GO terms ion transport, phosphorylation and proteolysis processes, among other, showed many polymorphic genes in *C. angulata*. These results reveal that most of the gene polymorphisms observed in *C. angulata* are associated with processes related to genome adaptation to abiotic stress in estuarine regions and support that genetic polymorphisms may be the base to the observed ability of *C. angulata* to retain the phenomenally high concentrations of toxic heavy metals. Our results also provide the framework for future investigations to establish the molecular basis of phenotypic variation of adaptive traits and should contribute to the management of the species' genetic resources.

To validate the observed variation, various methods can be used for SNP genotyping. High resolution melting (HRM) is a method for mutation screening and genotyping that does not require fluorescently labelled probes. In this study, several SNP markers were obtained using an improved small-amplicon HRM method, which could be potentially useful for genetic, genomic and ecologic studies of the oyster.

Keywords: oysters, Crassotrea angulata, Crassostrea gigas, genome adaptations, SNPs

DEVELOPMENT AND IMPLEMENTATION OF CUSTOM-MADE SYSTEMS FOR CONTROL AND PREVENTION OF PATHOGENS IN AQUACULTURE

Espiñeira M.[a§]**, Alonso M.**[a]**, Lago F.C.**[a]**, Vieites J.M.**[a]

[a] *Department of Molecular Biology, ANFACO-CECOPESCA, Vigo, 36310 Spain.*

The global aquaculture production has increased rapidly in the last decade, positioning itself as a quality product in international markets. Currently, one of the major problems of aquaculture farms is the high mortalities that infections by pathogens, parasites, bacteria and viruses cause. These pathogens do not only reduce culture performance due to they cause death of the fish, but also produce serious losses in aquaculture, reduce the growth rate in the fish, and can make the fish are not marketed due to skeletal muscle abnormalities, in cases with high intensity of infection.

The best method to avoid losses associated with the occurrence of diseases in aquaculture is the implementation of routine control and prevention systems in farms. These are designed specifically for the needs of each production system. These systems have to be fast, allowing early detection of diseases and accelerating the adjustment of the control measures to prevent the spread of infection systematically.

In order to facilitate routine control and prevention of these infections, ANFACO-CECOPESCA has developed molecular tools for early detection of pathogens. These tools had been improved by adopting non-invasive sampling techniques, allowing to carry out routine checks on farms growing without altering the welfare of farmed fish.

The molecular methodologies which are developed in ANFACO-CECOPESCA for pathogen detection can be applied regardless of the stage of the life cycle of the pathogen or the intensity of infection, and are easily implemented in hatcheries and farms. These tools had also been improved by adopting non-invasive sampling techniques, allowing to carry out routine checks on farms growing without altering the welfare of farmed fish. These methodologies are fast, robust, specific, effective and adapted to the needs of each production system.

Keywords: Aquaculture, Pathogens, Real Time PCR, Prevention, Control.

§ Corresponding author. Tel.: +34 986 469 301; Fax: +34 986 469 269 ;
E-mail address: montse@anfaco.es ;

TRIPLOIDIZATION: A METHOD TO ALLEVIATE MATURATION OF LARGE-SIZE FISH

Felip A.[§]

Department of Fish Physiology and Biotechnology, Institute of Aquaculture of Torre de la Sal (IATS-CSIC), Ribera de Cabanes,12595, Castellón, Spain

The most important feature of triploids is their sterility. Normal gametogenesis does not occur in triploids and thus, energy that they do not divert to reproduction is available for somatic growth. However, it is known that performance of triploids is species specific. Nevertheless, since sexual maturation is often considered as a problem in fish, due to it perturbs growth and product quality, induced sterility could be an effective method to solve these problems. In this context, triploidization might satisfy the demand for large-size fish as there is an increasing interest and willingness to pay for value added product forms. It can be the case of the European sea bass (*Dicentrarchus labrax* L.), a highly-prized marine teleost in Mediterranean areas that attains puberty at 2 years of age in males and around 3–4 years in females, after which time it reproduces once a year during the winter (December-March). Although sea bass is still almost sold as whole fish (350-400 g fish), the production of big-sized fish (more than 24 months of age and 2 Kg) is starting to make an appearance. Induction of triploidy in the sea bass using cold shocks (0ºC), 5 min after fertilization and duration of 10 min has demonstrated that meiosis is completely altered in both sexes and they do not produce mature gamete, thus conferring functional sterility in the fish. Of note, while older triploids retain impaired reproductive endocrinology, a sexually related dimorphic growth is observed with triploid females attaining the largest sizes. These observations indicate that triploidy could be an interesting option for fish aquaculture, especially for the production of large-size sea bass including Royal sea bass (> 1 Kg) and Imperial sea bass (> 3 Kg). It would be better valuated and not affected by complications related to sexual maturity, thus following consumer and market requirements. Together, triploidization might be considered an approach for the reproductive and biological containment while it alleviates sexual maturation and satisfies the demand for larger market size production.

Keywords: sterilization, finfish production, market demands, biological confinement, sea bass

[§] Corresponding author. Tel.: + 34-964319500 ; Fax: + 34-964319509
E-mail address: afelip@iats.csic.es

TRIPLOID ORNAMENTAL (KOI) COMMON CARP *Cyprinus carpio* FEMALES DEVELOP LARGE OVARIES AND PRODUCE MASS ANEUPLOID PROGENY WHEN CROSSED WITH DIPLOID MALES

Gomelsky B.[§], Delomas T.A., Schneider K.J., Anil A., Warner J.L.

Aquaculture Division, Kentucky State University, Frankfort, 40601, USA

It is generally accepted that induced triploidy in fish has a stronger impact on the reproductive system of females than males. Usually, triploid females develop very small ovaries that do not contain advanced vitellogenic oocytes. In contrast, in the present study the investigation of gonad development in 4-year-old triploid koi from a heat-shocked progeny has revealed unexpectedly well-developed ovaries in females. Gonadosomatic indexes of triploid females varied from 4.2% to 30.1% with a mean value of 17.0% (vs. 21.3% in diploid females from a control progeny). Ovaries of triploid females were filled with fully grown oocytes. The functional reproductive ability of 5- and 6-year-old triploid females was investigated in two consecutive years. Six triploid females released from 350 to 780 g (9.8-23.6% of female body weights) of ovulated eggs (224,000 to 402,000 eggs per female) after hormonal injection. Eggs obtained from triploid females were fertilized with sperm from normal diploid koi males. The percentages of live embryos next day after fertilization varied from 26 to 87% with a mean value of 56%. Embryo development and hatching of larvae proceeded normally but mass mortality of hatched larvae occurred at the swim-up stage. Nevertheless, about 88,000 swim-up larvae were obtained and stocked in outdoor tanks for further rearing; a total of 280 juveniles (or less than 0.5% of stocked larvae) were collected. Flow cytometric analysis of nuclear DNA content showed that most larvae and juveniles were aneuploid with ploidy ranging from 2.24n to 2.88n and a mean value of 2.56n; three out of 142 analyzed juveniles were diploid. This shows that triploid koi females produced aneuploid eggs with a ploidy range from haploid to diploid and a modal ploidy around 1.5n, similar to the production of aneuploid spermatozoa observed earlier for triploid males in fish. Some larvae and juveniles obtained from triploid females had ploidy ranging from 3.8n to 4.0n; apparently these fish resulted from spontaneous suppression of the second meiotic division in aneuploid (1.4n -1.5n) eggs. This study demonstrated a rare case when ovaries of triploid females developed large quantities of fully grown aneuploid oocytes.

Keywords: common carp, koi, triploidy, reproduction, aneuploidy

[§] Corresponding author. Tel.: +1 502 597 8114; Fax: + 1 502 597 8118
E-mail address: boris.gomelsky@kysu.edu

DEVELOPMENT AND CHARACTERIZATION OF NOVEL TETRANUCLEOTIDE MICROSATELLITE MARKERS IN THE NOBLE CRAYFISH (*Astacus astacus* L.) SUITABLE FOR MULTIPLEX PCR

Gross R.[a, §, *], Kõiv K.[a, *], Pukk L.[a], Kaldre K.[a]

[a] *Department of Aquaculture, Estonian University of Life Sciences, Tartu, EE-51006, Estonia*

Noble crayfish is the most highly appreciated freshwater crayfish species in Europe, which is threatened due to a long-term population decline. The genetic background has rarely been considered, however, when selecting material for re-introductions, supplemental stocking or captive breeding, the main reason being a rather limited knowledge of intra-species genetic diversity. The application of microsatellites as genetic markers in studies of genetic diversity in the noble crayfish has been limited because of the low number of available loci that can be reliably genotyped. Therefore, the aim of the current study was to develop a set of new polymorphic tetranucleotide repeat microsatellite markers that are easily scorable and suitable for multiplexing. We used Illumina MiSeq next generation sequencing technology. *Hin*fI restriction enzyme digested and purified genomic DNA from three specimens of crayfish was prepared for sequencing using ClaSeek Library Preparation Kit and Illumina TruSeq DNA LT Sample Prep kit. The libraries were validated by qPCR and sequenced on MiSeq flowcell with 2 x 300 bp paired-end reads using MiSeq Reagent Kit v3. QDD version 3.1 software was used for tetranucleotide repeat motif detection, sequence selection and PCR primer design. A total of 48 primer pairs were initially tested for amplification success and polymorphism among eight individuals of the noble crayfish. This resulted in 24 polymorphic markers from which 19 were further selected for multiplexing in a single PCR reaction using Type-it kit (QIAGEN) and fluorescently labeled primers. This 19-plex marker panel was genotyped in a single run of AB3500 Genetic Analyzer and the loci were tested for variability in four populations of the noble crayfish from Estonia and Czech Republic. All 19 loci conformed to the linkage and Hardy-Weinberg equilibria. The number of alleles per locus ranged from 2 to 7 and the observed heterozygosity varied from 0.03 to 0.52.

[*]These authors have contributed equally to this work.
[§] Corresponding author. Tel.: + 372 7313489; Fax: + 372 7313489
E-mail address: riho.gross@emu.ee

INTERSPECIFIC GYNOGENESIS WITH CRYOPRESERVED SPERM IN ZEBRAFISH (*Danio rerio*)

Kovács B.[a],[§], Bercsényi M.[b], Csenki Zs.[a], Bakos K.[a], Kovács R.[a], Csepeli A.[a], Németh S.[b], Uri Cs.[a], Bernáth G.[a], Horváth Á.[a], Bokor Z.[a], Urbányi B.[a], Orbán L.[c]

[a] *Department of Aquaculture, Szent István University,*
Páter Károly u. 1., H-2100,Gödöllő, Hungary
[b] *Department of Animal Sciences and Breeding, University of*
Pannonia, Deák Ferenc u. 16, H-8230 Keszthely, Hungary
[c] *Reproductive Genomics Group, Temasek Life Sciences Laboratory,*
1 Research Link, The NUS, Singapore 117604

Contrary to other model animals, highly homogeneous/inbred strains of zebrafish (Danio rerio) are not available. Only two gynogenetic lines (C32 and another line from Shinya and Sakai) and some partially inbred strains (SJD, SJC, SJA and IM) were established. However, their genetic analysis revealed that 7% (C32), 11% (SJD) 15% (SJA) and 5% (IM) of tested loci were polymorphic in them, respectively. However, it is not clear whether these types of strains have such a "high" genetic diversity in zebrafish or will become extinct.

Gynogenesis is one of the fastest ways to produce highly inbred strains. However, the production of gynogenetic zebrafish lines has some disadvantages (i.e., logistical difficulties related to sperm, high mortality of embryos and larvae, biased sex ratio, low quantity and quality sperm, etc.). In this study, an outbred AB-type strain was used to generate genetically homogeneous strains of zebrafish. Eggs were obtained by stripping anesthetized spawners. Gamma ray-irradiated carp *(Cyprinus carpio)* or goldfish *(Carassius auratus auratus)* sperm samples were cryopreserved and later used for in vitro egg activation. To obtain homozygous fish, the activated eggs were subjected to a heat shock according to the appropriate protocol. Only 184 larvae hatched from more than 18,000 eggs. Most of them died due to different developmental disturbances. Finally, 14 individuals reached adulthood, but only 7 males and 2 females were fertile. These individuals were used to produce the F1 generation. Until now in the F1 generation consists of 46 individuals produced by one pair of brooders and only a single individual displays the male phenotype.

Our results show that irradiated and cryopreserved sperm of carp and goldfish can be used for gynogenesis in zebrafish. The survival rate was lower than 1% as it was expected. We observed a sex-biased nature of the highly homogenous lines both in the gynogenetic (male biased) and the F1 generation (female biased; ~98%). Presumably this is a result of the polygenic sex determination observed in domesticated strains of zebrafish.

To the best of our knowledge, this is the first experiment that produced gynogenetic zebrafish individuals with interspecific, cryopreserved sperm.

[§] Corresponding author. Tel.: + 36 28 522 000/1610 Fax: + 06 28 522-927
E-mail address: Kovacs.Balazs@mkk.szie.hu

TETRAPLOIDIZATION AND INDUCTION OF MITOTIC GYNOGENESIS IN STURGEON

Lebeda I.[§], Flajšhans M.

University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, 389 25 Vodňany, Czech Republic

In the present study, artificial tetraploidization in two model sturgeon species, *Acipenser baerii* and *Acipenser ruthenus* was performed for the first time. A relatively high efficiency of tetraploidization treatment was achieved by applying a heat shock at 37°C for 2 min timed at the end of female pronuclei formation and the beginning of pronuclei migration 0.8–1.0 τ_0 (duration of one mitotic division). Fertilized and shocked eggs developed in up to 31% of tetraploid larvae in sterlet (a functionally diploid species), and up to 34% of octaploid larvae in Siberian sturgeon (a functionally tetraploid species). In both cases, doubled DNA content in these progenies was confirmed by flow-cytometry. Most of the larvae with doubled DNA content exhibited body malformations; thus, the possibility of producing adults with this technique is questionable. Subsequently, this technique was applied for mitotic rediploidization of gynogenetic progeny of sterlet induced with UV-irradiated sperm at doses of 200 and 300 J/m². The maximum efficiency of mitotic gynogenotes production (2.3%) was obtained in group of eggs activated by spermatozoa irradiated with 300 J/m2 and treated with heat shock at 37 C° for 2 min on $0.79\tau_0$ after activation. This group of progeny contained 33.3% of diploid larvae, while all progeny of gynogenetic control group that haven't been subjected to heat shock were haploid. Samples for microsatellite analysis of putative mitotic gynogenotes were collected on 6th day after hatching, when flow-cytometry assay showed absence of survived haploid larvae. Solely maternal heredity and diploidy of mitotic gynogenotes was confirmed with analysis of 6 polymorphic microsatellite loci (Spl 163; Spl 101; Spl 173; AfuG 135; Aox 45; AciG 35). The study was financially supported by the Ministry of Education, Youth and Sports of the Czech Republic - projects „CENAKVA" (No. CZ.1.05/2.1.00/01.0024), "CENAKVA II" (No. LO1205 under the NPU I program) and by the Czech Science Foundation (No. 14-02940S).

Keywords: sturgeon; gynogenesis; microsatellites; mitotic shock; polyploidy.

[§] Corresponding author. Tel.: +420 387774612
E-mail address: ilebeda@frov.jcu.cz

GENOME-WIDE MARKER SEGREGATION DISTORTION IN MUSSELS Mytilus spp.

Peñaloza C.[§], Bishop S.C., Houston R.D.

Division of Genetics and Genomics, Roslin Institute, Edinburgh, EH25 9RG, UK

Selective breeding of mussels is at a formative stage. Consequently, the development and use of genomic markers may significantly assist mussel aquaculture breeding and production. However, molecular genetic markers typically exhibit an unusual pattern of segregation in mussel species, which could potentially limit their use in parental assignment and linkage mapping. Despite the importance of understanding the phenomenon of segregation distortion in bivalve molluscs, the reason why it occurs remains unknown.

To gain further insight into this phenomenon we studied the genome-wide inheritance pattern of SNP markers in two mussel families using Restriction-site Associated DNA (RAD) sequencing. We discovered a total of 4,537 putative SNPs. RAD sequence analysis showed that the mussel genome is extremely polymorphic, with and average frequency of 1 SNP each 25bp. The majority of these markers (72%) deviated from Mendelian segregation ratios within the families, showing a trend towards a deficiency of heterozygotes genotypes in the offspring. In addition, many SNP alleles and haplotypes were observed in the offspring without being present in their parents. We confirmed the data obtained by RAD sequencing through Sanger sequencing of PCR-amplified RAD loci. Therefore, these unusual features of marker segregation in mussels appear to be of biological – rather than technical – origin.

We are currently undertaking additional experiments in unrelated populations to investigate possible causes of these unorthodox segregation patterns. Specifically, controlled biparental crosses were carried out and the genome of mussel gametes and larvae at different ages is being sequenced using RAD-seq. The aim is to confirm our previous observations and assess the potential importance of natural selection in shaping the genotype frequencies of mussels.

In conclusion, the high density SNP markers developed in this study may provide a useful genetic resource for selective breeding purposes. Nevertheless, the high frequency of distorted markers and the frequent presence of non-parental alleles in the offspring indicate that several aspects of marker inheritance in mussels have yet to be fully understood before they can be applied in selective breeding.

Keywords: Mussel, RAD sequencing, SNP, segregation distortion

§ Corresponding author. Tel.: +44 (0)131 651 9228
E-mail address: v1cpeal@exseed.ed.ac.uk

APPLICATION FOR MICROINJECTION OF YELLOWTAIL *Seriola quinqueradiata.* PROLONGED ONE-CELL STAGE IN YELLOWTAIL EGGS TO APPLY MICROINJECTION.

Okamoto H.[a,§], Shimada Y.[a], Yamaguchi T.[a], Satoh J.[a], Yoshida K.[b], Hotta T.[b], Noda T.[b], Mizuochi H.[b], Chujo T.[b], Shima Y.[b], Inoue N.[a], Nagoya H.[a], Araki K.[c], Usuki H.[a]

[a]*National Research Institute of Aquaculture, FRA, Kamiura, Saiki, Oita 879-2602, Japan*
[b]*Seikai National Fisheries Research Institute, FRA, Nunoura, Goto, Nagasaki 853-0508, Japan*
[c]*National Research Institute of Aquaculture, FRA, Tamaki, Watarai, Mie 879-2602, Japan*

Yellowtail (*Seriola quinqueradiata*) production is Japan's largest aquaculture sector. Seeds are obtained from natural resources and reared to commercial size. However, in the future, the natural seed will have to be replaced with artificial seed to conserve natural resources and meet market demand year round. To be a viable replacement for natural seed, artificial seed must be commercially beneficial. To improve breeding, genetic traits to improve or acquire beneficial characteristics in target commercial species are required. This paper details a method for the prolonged retention of one-cell stage eggs for microinjection to promote genome editing in yellowtail breeding. RNA from artificial nucleases is injected into either blastodiscs or one-cell blastomeres in fertilized eggs prior to first cleavage. The one-cell stage duration is almost 30–40min at 20°C. Two methods of storing one-cell stage eggs for prolonged periods were investigated. The first is keeping unfertilized eggs following spawning. The second is slowing the pace of development, under cool conditions, following fertilization. Keeping unfertilized eggs for a time makes it possible to delay the timing of fertilization. When we tested the stability of unfertilized eggs, they maintained the capacity to make embryo bodies for 2–4 hours at 16–18°C. Additionally, when the temperature was reduced from 20°C to 18°C first cleavage was delayed by 30 minutes. These two methods make it possible to prolong the period until microinjection in artificial seeds from thirty minutes to four hours. This study provides useful information on microinjection and genome editing in yellowtail breeding.

INTERGENERIC ANDROGENESIS THROUGH HYBRIDIZATION BETWEEN RELATED SPECIES OF FAMILY *CLARIIDAE*

Olaniyi W.A.[a,§], Omitogun O.G.[b,*]

[a] *Department of Environmental Biology and Fisheries, Adekunle Ajasin University, PMB 001, Akungba-Akoko, 342111, Ondo State, Nigeria*
[b] *Department of Animal Sciences, Obafemi Awolowo University, Ile-Ife, 220005, Osun State, Nigeria*

The present study registered successful production of androgeneic populations through intergeneric androgenesis between two closely related species of the family Clariidae. Ultra-violet (UV) irradiated eggs of both Clarias gariepinus and Heterobranchus bidorsalis species were fertilized with heterologous sperm of the alternate species, and consequently subjected the haploid embryo to cold shock treatment to restore viable diploidy. The characteristic morphological traits such as presence/absence of adipose fin, and genome analysis using sex-specific DNA markers, CgaY1 and CgaY2 were both employed for the androgeneic population evaluation. The adipose fin length and adipose fin depth [% standard length (cm)] respectively for the androgeneic C. gariepinus population revealed no (0; 0) and maximum of 13.58; 1.63; and androgeneic H. bidorsalis with 12.91±1.26; 3.96±0.57. CgaY1 and CgaY2 proved the androgeneic progenies sex to be true by showing extra bands of ~2 kb and ~350 bp respectively in the male genome pools, thereby confirming the male sexual phenotypic features. However, the presence of adipose spatial properties or its negligible contribution renders the progenies to be clonal androgeneic hybrids. Thus, the resistance of the species eggs cytoplasm or mtDNA to UV treatment generated none 100% total homozygous cloned progenies. According our knowledge, this study, for the first time presents successful intergeneric androgenesis between two different species of family Clariidae.

Keywords: Intergeneric androgenesis, Clarias gariepinus, Heterobranchus bidorsalis, heterologous sperm, sex-specific DNA markers

[*]These authors have contributed equally to this work.
[§] Corresponding author. Tel.: +2348033799789; +2348070867687
E-mail address: waolaniyi@gmail.com

STEM CELL PROLIFERATION AND MUSCLE HYPERPLASIA CONTINUE DURING NUTRIENT STARVATION IN THE ATHERINID FISH *Odontesthes bonariensis*

Simó I.[a], Sciara A.A.[a], Arranz S.E.[a§]

[a] Instituto de Biología Molecular y Celular de Rosario (CONICET-UNR) y Área Biología, Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Rosario, 2000 Argentina.

Growth rate is the most important trait in aquaculture. Postembryonic muscle growth depends on proliferative myogenic precursor cells (MPCs). Refeeding after nutrient starvation is followed by a phase of accelerated growth known as compensatory growth. Although fish show a particularly robust capacity for catch up growth, the biological mechanisms and the role of MPCs in this process remain elusive.

To give insight into the basis of this process, pools of 200 mg fishes were set: a control group was continuously fed and a treated group was starved for 2 weeks. At 7 and 10 days after the start of the experiment fishes received an intra peritoneal injection of 5-ethynyl-2'-deoxyuridine in order to track and label MPCs. Both weight and length of starved fishes were significantly lower than controls (p<0.01). After starvation, a significant increase in the percentage of fibers with a diameter lower than 20 μm was observed compared to control group (p<0.01). Interestingly, the number of fibers did not change between groups showing that hyperplasia but not hypertrophy continues during the fasting period. By quantifying the MPCs we demonstrated that proliferation continued during starved periods.

In order to gain insight into the relationship between body mass and muscle growth during refeeding, a new experiment was performed. Two different pools of 550 mg fishes were set. A control group was continuously fed, while the second pool was starved for 2 weeks and then refed for another 2 weeks. Both weight and length of starved fishes were significantly lower than control group (p<0.01) but at the end of the experiment no significant differences were observed. Like in previous experiment, the percentage of fibers with diameter lower than 20 μm was significantly higher in starved fishes than in controls (p<0.01). The number of muscle fibers increased along the experimental period in both groups and did not differ between them. At the end of the experiment no significant difference were observed on fiber muscle sizes of control and reefed fishes. The results show that the muscle gain during compensatory growth is mainly due to a compensatory hypertrophy of small fibers generated during starvation.

DNA BARCODING FOR IDENTIFICATION OF COMMERCIAL PERUVIAN CLAM *Transennella pannosa* USING MITOCHONDRIAL AND NUCLEAR MARKERS

Sotil G.[a,b§], Francia J.C.[b]

[a] *Laboratory of Genetics, Area of Aquaculture Research,
Instituto del Mar del Perú (IMARPE), Callao 01. Peru*
[b] *School of Marine Biology, Scientific University of the South (UCSUR), Lima 18, Perú*

T. pannosa is a direct consumption and an exported hydrobiological resource, basically exploited from natural banks of Sechura Bay, Piura, in the north of Peru. This clam is distributed in Peru and Chile coasts, and is considered with aquaculture potential due to its high commercial demand, principally exported to Spain and United States markets. We evaluated the utility of three molecular markers for specie identification with application in authentication and efficient food traceability of the bivalve. Organisms were morphologically identified based on shells shapes and two different groups, with concentric lines and concentric rhomboids figures, were distinguished. Mitochondrial (COI) and nuclear (18S and ITS2) DNA regions from 10 specimens collected in Sechura were amplied by PCR. Sequences with 681 base pairs (bp) from the COI mtDNA gene, 1381 bp from rDNA 18S, and 283 bp from the rDNA internal transcribed spacer ITS2 regions were analyzed. Higher nucleotide diversity was observed in COI (0.0022) and ITS2 (0.0028), compared with the diversity of 18S (0.0007). COI sequences showed 4 variable sites and 31% of GC content, while ribosomal markers 18S and ITS2 showed 52.4% and 62.3% GC content, respectively. Sequences were incorporated in iBOL and GenBank databases. No previous nucleotide register was found for the specie. The 18S sequences showed match similarities with other genera from the family Veneridae (99% with genera Nutricola and 98% with genera Gemma), while COI and ITS2 showed low match similarities with *Gemma gemma* (88%) and with *Protothaca staminea* (74%), respectively. The specie was well differentiated by using COI and ITS markers, providing a good phylogenetic resolution, and unambigous species-level identification for samples analysed. They can be considered as molecular tools for authentication and reliable tracebility of the resource, in order to protect products and market niches.

Keywords: Veneridae, COI, ITS2, food traceability, species identification

[§] Corresponding author. Tel.: + 511 6106400
E-mail address: gsotil@imarpe.gob.pe / gsotil@yahoo.com / jfrancia@cientifica.edu.pe

DNA TYPING ACROSS TEN TILAPIA SPECIES USING CYTOCHROME C OXIDASE SUBUNIT I (COI)

Syaifudin M.[a,b] [*], Bekaërt M.[a*], Taggart J.B.[a], Hulata G.[c], D'Cotta H.[d], Baroiller J.F.[d], Penman D.J.[a,§], McAndrew B.J.[a]

[a] *Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland, UK*
[b] *Program Study of Aquaculture, Agriculture Faculty, University of Sriwijaya, Indonesia*
[c] *Institute of Animal Science, Agricultural Research Organization, The Volcani Center, Israel*
[d] *Cirad, Persyst, UMR ISEM, Campus International de Baillarguet, Montpellier, France*

The tilapias are a group of cichlid fish that are widely cultured in both developed and developing countries. With many different species and sub-species of tilapia, and extensive use of interspecies hybrids, identification of tilapia species is of importance in aquaculture and in wild populations where introductions have occurred. Mitochondrial DNA cytochrome c oxidase subunit I (COI) sequence is widely used as a "barcode of life" for species identification. This study involved ten species of tilapia from all three common genera, and included more than one population and/or subspecies from the major commercial species. It was undertaken in parallel to research to develop species-specific markers from nuclear DNA using double-digest restriction site associated DNA sequencing (ddRADseq). Most species could be discriminated using the COI sequence, with some exceptions. *O. andersonii - O. macrochir* and *S. melanotheron - S. galilaeus* could not be separated. West African *O. niloticus* exhibited COI haplotypes typical of *O. aureus*, as previously reported in the literature, although nuclear markers clearly indicated the differences between these two species. Both *O. niloticus* and *O. mossambicus* haplotypes were detected in Indonesian stocks that were nominally *O. niloticus*. The *O. mossambicus* haplotypes are likely to have been derived from feral stocks present in Asia. As a marker for species discrimination, the COI DNA barcode has limitations: in addition to those highlighted above, it is a single, maternally inherited marker, and thus of limited use in analysing cases of hybridization/introgression. However, it is still likely to be useful in combination with multiple nuclear DNA markers.

Keywords: Tilapia, DNA barcode, COI gene, population.

[*]These authors have contributed equally to this work
[§] Corresponding author. Tel.: + 44 (0)1786 467901; Fax: + 44 (0)1786 472133
E-mail address: d.j.penman@stir.ac.uk

QUANTITATIVE GENETICS OF MORPHOLOGICAL ABNORMALITIES IN FARMED GILTHEAD SEABREAM (*Sparus aurata L.*)

Afonso J.M.[*§], **Negrín-Báez D.**[*], **Lee-Montero I., Navarro A.**[*], **Astorga N., Montero D., Ginés R., Robaina L., Izquierdo M., García-Cortés L.A.**[ç], **Toro M.A.**[§], **Zamorano M.J.**[*]

* Grupo de Investigación en Acuicultura, Universidad de Las
Palmas de Gran Canaria (ULPGC), Arucas, 35413, Spain
ç Dpto. de Mejora Genética Animal – INIA, Carretera La Coruna nº 7, Madrid, 28040, Spain
\$ Universidad Politécnica de Madrid (UPM), Paseo Juan XXIII, nº 11, Madrid, 28040, Spain

Morphological abnormalities in farmed gilthead seabream (*Sparus aurata* L.) is a major problem, affecting to the viability of the companies due to their economic influence on fish sale price and production level. Environmental and genetic causes have been described on gilthead seabream like are; abiotic, xenobiotic, biotic, nutritional, associated to the culturing system or polygenic. Genetic causes are revised and discussed. Several studies have contributed to the understanding of genetic determination of morphological abnormalities in gilthead seabream: family associations, inheritance, consanguinity effect, heritabilities and QTL. In family associatons, Afonso *et al.* (2000) reported an association for LSK skeletal deformity (Lordosis-Scoliosis-Kyphosis) to only one family from 31 full sib families, on experimental population. Negrín-Báez *et al.* (2015a) confirmed the LSK association to five families (2 males and 4 females implied) from 89 full sib families, on industrial population. In inheritance, Negrín-Báez *et al.* (2015a) were carried out directed matings between breeders with normal morphology and skeletal deformities (operculum, lordosis or vertebral fusion), and reported significant prevalence of the same deformity of breeders, on descendants. In consanguinity, Astorga (1999) found a higher incidence of deformed larvae from crosses of full sibs than half sibs families and control crosses. In heritability, Astorga *et al.* (2004) estimated a high heritability for presence/absence of deformities, considering as deformity class thirty-eight kinds of morphological abnormalities. Lee-Montero *et al.* (2015) estimated medium values of heritabilities for whole deformities trait (any kind of skeletal deformities). In QTL, the identification of Quantitative Trait Loci was studied in the context of PROGENSA breeding program. A set of 13 multiplex PCR reactions containing 106 microsatellite markers of gilthead seabream genetic map were used (Negrín-Báez *et al.* 2014). The prevalence of severe or important skeletal deformities (LSK complex, lordosis, vertebral fusion and lack of operculum) was analyzed. A significant relationship between the prevalence of these deformities and specific families and breeders was detected. On these families, LSK-related significant QTLs were found and four of them showed an extremely large effect. A strong association between the genotype of closely located markers of these QTLs and the phenotype was observed. For lack of operculum, two QTLs significant were detected. One significant QTL was detected for vertebral fusion, lordosis and jaw deformity, as well. These results confirm genetic components of skeletal deformities in gilthead seabream, giving extensible and accumulable tools to improve fish quality at industrial scale, and represent a major step towards the location of genes that determine their presence in this species and to reduce their prevalence at industry.

Keywords: Morphological anomalies, Genetic Improvement, Heritability, QTL

*These authors have contributed equally to this work
§ Corresponding author. Tel.: +928459735; Fax: +928451182
E-mail address: juanmanuel.afonso@ulpgc.es

DEVELOPMENT OF THE SYSTEM TO DETERMINE PARENTAGE AND SIBSHIP IN HATCHERY POPULATION FOR STOCK ENHANCEMENT PROGRAM OF JAPANESE FLOUNDER *Paralichthys olivaceus*

Ando D.[a,§], Ikeda M.[a], Sugaya T.[b], Katamachi D.[b], Fujii T.[b], Kijima A.[a]

[a] *Laboratory of Integrative Aquatic Biology, Graduate School of Agricultural Science, Tohoku University, Konori, Onagawa, Oshika, Miyagi 981-8555, Japan*
[b] *National Research Institute of Fisheries and Environment of Inland Sea, Fisheries Research Agency, Maruishi, Hatsukaichi, Hiroshima 739-0452, Japan*

Parentage assignment using DNA markers has been introduced as an efficient tool in selective breeding and monitoring of reproductive success of hatchery broodstock for stock enhancement program, especially in mass spawning species. However, sibship determination in the offspring without the prior parentage assignment is difficult and the success depends on genetic individual identification power of the DNA markers. We prepared a highly polymorphic DNA marker set of 12 microsatellites DNA and 1,873 nucleotides in mitochondrial DNA (concatenating the first half of control region and entire of ND2 gene sequences) of Japanese flounder *Paralichthys olivaceus*, and tested the determination power for sibship among the artificial seeds without their parental genotype data through comparing the true sibship revealed by parentage assignment and the likelihood sibship estimated by COLONY program (Jones & Wang, 2010). At first, we screened 120 wild captive broodstock without tags for their sex and the 192 offspring in a hatchery using both of the microsatellite and mitochondrial DNA markers. All of the offspring could be completely assigned to 27 sires and 37 dams, and the number of full- and half-sib dyads was 357 and 1,960, respectively. While the results of likelihood estimation using only offspring's microsatellite data showed 357 full-sib and 1,956 half-sib dyads. The value of type I and II errors, precision, recall, and F-measure on full-sib was 0.000% and 0.283%, 1.000, 0.997, and 0.998, respectively. On half-sib, those were 0.283% and 0.835%, 0.935, 0.829, and 0.879. These results indicate that a combination of the DNA marker set we prepared and COLONY program can determine sibship with high accuracy, and would contribute to breeding program for indirect prediction of breeding values and genetic management of hatchery populations for stock enhancement program of this species.

Keywords: parentage assignment, sibship, Japanese flounder, microsatellite DNA, mitochondrial DNA

§ Corresponding author. Tel.: + 81-225-53-2436; Fax: + 81-225-53-2436
E-mail address: daiki870530@yahoo.co.jp

QUANTITATIVE AND QUALITATIVE ANALYSIS OF TRAIT PERFORMANCE IN F1 HYBRID OF *Amphiprion ocellaris × A. percula*

Balamurugan J.[a], Ajith Kumar T.T.[a,b]

[a]Centre of Advanced Study in Marine Biology, Annamalai University
Parangipettai - 608502, Tamil Nadu, India
[b]ICAR-National Bureau of Fish Genetic Resources, Lucknow - 226002, India
[b]ttajith87@gmail.com

The present study was attempted to evaluate the quantitative and qualitative trait performance of F1 hybrid produced by inter-crossing of *Amphiprion percula* (sire) and *A. ocellaris* (dam). Captive developed cross broodstock, *Amphiprion percula* and *A. ocellaris* displayed successful pair formation, after 5 months of rearing. Results showed that there is significant variation in the length of newly hatched hybrid larvae (3.26 mm ± 0.22) against the larvae of purebred, *A. ocellaris* and *A. percula*. Hybrids also exhibited significant variation in feed conversion efficiency (0.036 ± 0.06) and specific growth rate (3.63% ± 0.44) compared to the purebred, *A. ocellaris* (0.031 ± 0.004; 3.02% ± 0.19) and *A. percula* (0.031 ± 0.004; 2.80% ± 0.42). The species were confirmed using proportional measurements and molecular methods (COI and 16S gene sequencing). Identified key characters of *A. percula*, *A. ocellaris*and hybrids are dorsal spine (11-12), anal spine (2), dorsal rays (15-16), anal rays (12-13) and gill rakers (10-12). COI gene sequence showed minimum genetic distance of less than 1% within genus (0.078) and much lesser within species (0.044). The K2P distance of hybrid showed maximum likelihood to the *A. ocellaris* (female) as compared to *A. percula* (male). Although clownfish are protandrous hermaphrodite, the heredity of mtDNA was maternal. Likewise, 16S genesequence showed very less genetic variability between *A. ocellaris*, *A. percula* and F1 hybrid. Further, principal component analysis using Bray Curtis similarity cluster revealed that 65.20% of hybrids were inherited with parental traits (26.10% maternal, 4.35% paternal and 4.35% distinct from both parents). Hence, it was recommended that the selection of both the sexor preferably female, with superior traits helps to produce high number of positive trait values in F1 generations, which helps in enhancing the trade value.

Keywords: A. ocellaris, A. percula, crossbreeding, PCA, COI gene, 16S gene

SIZE-SPECIFIC GROWTH RATES OF FIVE GENETICALLY IMPROVED NILE TILAPIA STRAINS REARED IN NET ENCLOSURES SET IN EARTHEN PONDS IN THE PHILIPPINES

Basiao Z.U.[a,§], Quilang J.P.[a,*], Bandong C.M.S.[a,*], Mateo R.[b,*]

[a] *Institute of Biology, University of the Philippines Diliman, Quezon City 1103, Philippines*
[b] *Purok 2 Barangay Villa Cuizon, Science City of Muñoz, Nueva Ecija, Philippines*

In the late 1980s, several genetic improvement programs selecting for fast growth were undertaken in the Philippines in order to improve the genetic quality of the tilapia stocks grown in Asia as these stocks were reported to be introgressed with the Mozambique tilapia genes. In our study, the growth rates of five improved strains of Nile tilapia namely GIFT PHL (Genetically Improved Farm Tilapia) Philippines, GIFT MA (Genetically Improved Farm Tilapia) introduced by the WORLD FISH CENTER, Malaysia in 2013, FaST (Freshwater Aquaculture Center Selected Tilapia), BFAR GET-EXCEL, and the SEAFDEC-ST (SEAFDEC Selected Tilapia) were compared in net enclosures set in earthen ponds. In summary, the performance of the strains, ranked best to worst, was: FaST, GIFT MA, GIFT PHL, BFAR GET-EXCEL, and SEAFDEC-ST strain. Commercial feed was given during the experimental period. Early growth (1 – 30 days post-swimup) was evaluated in replicated fine-mesh net cages (1 x 1 x 1.2m) set in earthen ponds. The five strains showed significantly different early size-specific growth rates based on standard length and body weight. The GIFT MA and the FaST strains showed superior growth in length (2.207%/ day and 2.146 %/day, respectively) while the GIFT MA and GET-EXCEL showed significantly high size-specific growth based on body weight (5.630 %/day and 5.509 %/day; respectively) whereas the FaST and the GIFT PHL had similar size-specific growth rates (5.000 %/day and 4.902 %/day; respectively). The SEAFDEC-ST strain consistently showed the lowest size-specific growth rate (1.540%/day; 4.891 %/day). After 120 days of on-growing in bigger size (2.4x 6 x 1.2m) net cages, the FaST strain consistently showed superior size-specific growth both in standard length (0.465 %/day) and body weight (0.989 %/day). However, based on body weight, the FaST strain was not significantly different from the GIFT MA (1.023 %/day), and the GIFT PHL (0.997 %/day), nonetheless the FaST strain was significantly heavier than the GET-EXCEL strain (0.904 %/day). The SEAFDEC strain was consistently inferior in growth (0.250 % mm/day; 0.711% g/day) compared to the other four strains after 120 days of growing in net-cages set in an earthen pond. We discuss implications of these results, as well as future directions of selective breeding in developing countries.

Keywords: growth, selective breeding, Nile tilapia, GIFT

*These authors have contributed equally to this work
§Corresponding author. +639209140876
E-mail address: zbasiao08@gmail.com

GENETIC PARAMETERS FOR RESISTANCE TO *Caligus rogercresseyi, Piscirickettsia salmonis* AND BODY WEIGHT IN RAINBOW TROUT (*Oncorhynchus mykiss*)

Bassini N.L.[a,b], Neira R.[b], Yáñez J.M.[a,c], Newman S.[d], Oyarzún M.[a], Erranz F.[a], Figueroa R.[a], Lhorente J.P.[a,§]

[a] *Aquainnovo, Pto.Montt, 5503032, Chile*
[b] *Programa de Doctorado en Acuicultura, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago, 8820808, Chile*
[c] *Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, 8820808, Chile.*
[d] *Genus,plc, Hendersonville, TN 37075 USA.*

Genetic improvement of disease resistance represents a primary strategy for controlling infectious diseases in farmed fish. Piscirickettsiosis, caused by the intracellular bacteria *Piscirickettsia salmonis* and caligidosis, produced by the ectoparasite *Caligus rogercresseyi* are important diseases generating large economic losses in salmonid farming systems. The objectives of this study were to estimate genetic parameters for resistance to piscirickettsiosis (SRS) and caligidosis (CAL), and body weight at harvest (HW) in a population of rainbow trout (*Oncorhynchus mykiss*). The data for resistance to SRS and CAL were obtained from 2,421 and 2,589 rainbow trout smolts, respectively, belonging to 232 full- and half-sib families. Resistance to SRS was defined as the day of death of each fish post- intraperitoneal injection of 0.2 ml of a previously determined LD50. *C. rogercresseyi* resistance was measured as the parasite load after experimental infestation. We also measured HW in related individuals of the challenged fish. We used a multi-trait animal model to estimate (co)variance components and to calculate genetic parameters. For HW we included contemporary group (sex:tank:year) as a fixed effect and age at harvest as a covariate. For SRS and CAL we included tank as an additional factor and weight at end of test as a covariate. Heritabilities for HW, SRS and CAL were 0.57 (±0.03), 0.44 (±0.06) and 0.08 (±0.02), respectively. Genetic correlations between HW and SRS, HW and CAL and SRS and CAL were 0.00 (±0.11), −0.07 (±0.14) and −0.38 (±0.14), respectively. There is a favorable genetic correlation between SRS and CAL, because selecting for fish with greater survival implicitly assumes a lower parasite count. There is no evidence in the present study of genetic associations between HW and SRS and CAL.

Keywords: disease resistance, Piscirickettsia salmonis, Caligus rogercresseyi, heritabilities, genetic correlations

[§] Corresponding author. Tel.: + 56 813 92520
E-mail address: + jean.lhorente @aquainnovo.com

SEVAN TROUT *(Salmo ischchan* KESSLER) PRODUCTION IN ARMENIA AND PRIMARY GENETIC ANALYSIS OF SELECTED BROODSTOCK

Bogdanowicz W.[a*], Mkrtchyan J.[b§*], Asatryan A.[b*], Rutkowski R.[a*]

[a]Institute of Museum and Institute of Zoology Polish Academy of Sciences, Wilcza 64, 00-679 Warszawa, Poland
[b]Biological Department, Sevani ishkhan CJSC, Yerevan, 0014, Armenia

Lake Sevan is the largest lake in the Caucasus. Since the 1930s, unsustainable utilization of lake water for irrigation and hydropower generation has disrupted its ecological balance. Between 1933 and 2000, the water level has dropped by almost 20 meters and the water volume has declined by nearly 45%.

These drastic changes of the lake's hydrological system severely have impacted its biodiversity, particularly the endemic Sevan trout (*Salmo ischchan* Kessler), which is listed in the Red Book of Armenia. As a consequence of the desiccation of spawning grounds and uncontrolled fishing, two out of four Sevan trout subspecies – the winter ishkhan (*S. i. ischchan*) and the bojak (*S. i. danilewskii*) have disappeared completely and are now considered extinct, and the summer ishkhan (*S. i. aestivalis*) and the gegharkuni (*S. i. gegarkuni*) are classified as critically endangered.

The government project "Restoration of Sevan trout stocks and development of aquaculture" was launched in 2013, aiming to resolve the main problems of Lake Sevan, namely to restore trout stocks in the lake and to provide bases for further development of the Sevan trout production in Armenia. For this purpose the main objective is to collect viable broodstock, which will provide optimal production and restocking.

The main aim of the present study was to characterize basic genetic parameters in gegharkuni ishkhan broodstock in the Gegharkunik region, Armenia. 116 muscle tissue samples were collected and used as a source of DNA. Genetic variability in broodstock under study was estimated using 11 microsatellite markers. Analysis indicated a moderate level of genetic diversity; the mean number of alleles per locus was 7.45 and observed heterozygosity was 0.506. The inbreeding coefficient (F_{IS}), estimated for the whole broodstock was low and non-significant. Similarly, individual estimates of inbreeding (internal relatedness and homozygosity by loci) were rather low, suggesting outbreeding in this population.

Thus primary genetic studies of gegharkuni ishkhan broodstock have revealed moderate genetic diversity and no indication of inbreeding, indicating that this broodstock could be used for intensive production and restocking. The same studies should also be performed for the broodstock of summer ishkhan.

Keywords: Sevan trout, genetic variability, inbreeding

[*]All authors have contributed equally to this work.
[§] Corresponding author. Tel.: + 37477328777.
E-mail address: jon.mkrtchyan@sevantrout.com

A LOW DENSITY SINGLE NUCLEOTIDE POLYMORPHISM PANEL FOR ASSIGNMENT OF PARENTAGE, GENETIC SEX, AND CONTINENT OF ORIGIN IN ATLANTIC SALMON *Salmo salar*

Buchanan J.T.[a], Fujimoto A.A.[a], Hori T.[b], Plouffe D.A.[b], Stannard J.A.[a]

[a] Center for Aquaculture Technologies, San Diego, CA, 92121, USA
[b] Center for Aquaculture Technologies Canada, Souris, PE, COA 2BO, Canada

Single nucleotide polymorphisms (SNPs) are the marker of choice for selective breeding in as they are a highly abundant marker and ideally suited for automation and high-throughput technologies. The objective of this study was to design a low density, 95-SNP panel to enable identification of genetic sex and assignment of parentage at a low cost. SNP loci previously identified for Atlantic salmon were selected for the array including two SNPs intended to detect the presence of the male-specific gene conserved amongst salmonids. Preliminary genotyping results suggested that 69 of the 95 SNP assays, including the sex-specific SNPs, could be auto-scored reliably. The array was subsequently tested with a population of 2124 juvenile Atlantic salmon (1564 diploid and 560 triploids) from 57 families comprised of full and half siblings from a single year class. The test population were pooled in equal numbers per family at first feeding and reared communally using standard hatchery procedures. Physical tagging (passive integrated transponder) and blood sample collection occurred at approximately 10 g. Genomic DNA was extracted from blood stored in ethanol using a low-cost method, subjected to pre-amplification, and prepared for SNP analysis. Results indicate that this low-density SNP panel is an effective tool for assigning parentage with 99.4% accuracy for diploid animals and 95.5% accuracy for triploid animals using 65 SNP loci. Genetic sex of brood stock animals was correctly assigned 100% of the time. Additional SNPs can be added to this array to maximize the information obtained from a single SNP-genotyping run. For example, traceability has been achieved by adding SNPs which can be used to predict continent of origin i.e. Atlantic salmon of North American or European descent. The array can be further customized with the addition of SNPs associated with traits of interest. The use of low density SNP arrays allows rapid, automated genotyping of commercial Atlantic salmon brood stock to support and accelerate selective breeding programs in a cost-effective manner.

Keywords: SNP, low-density array, parentage assignment, Atlantic salmon, genotyping

MATE SELECTION IN AQUACULTURE BREEDING USING DIFFERENTIAL EVOLUTION

Carvalheiro R.[a][§], Yoshida G.M.[a], Queiroz S.A.[a]

[a]*Department of Animal Science, Universidade Paulista Júlio Mesquita Filho (FCAV), Jaboticabal-SP, 14884-900, Brazil.*

Mate selection refers to selection and mating decisions being performed simultaneously. A differential evolution algorithm was developed for defining mate selection in aquaculture breeding. The selected mates were defined based on the optimization of an objective function considering the average inbreeding and expected merit of the future progeny, the coancestry of selected parents and constrains related to the maximum number of mates for each candidate. Functions of expected progeny variability can also be accommodated in the objective function aiming, for example, to prioritize positive assortative mating for the top dams and corrective mating for the remaining. Different weights can be assigned for each component of the objective function to explore the multi-dimension response surface of the components and determine the weights which provide the best compromise in maximizing de genetic merit and controlling inbreeding in the short and long term. The objective function can also be maximized constraining one of the components as, for example, imposing a maximum allowed value for coancestry. To increase computational efficiency, an indirect approach was adopted to calculate coancestry, and linked lists were used for the storage and calculations involving sparse matrices. The algorithm was written in FORTRAN 90. Its efficiency was tested using different simulated and real datasets. For a Nile tilapia dataset containing roughly 7.000 pedigree information and mate selection candidates from 30 different families, the program provided a solution for the 145 mates to be performed in few minutes (~10 min), in a 4GB RAM desktop. The convergence from objective functions accounting for average inbreeding of the future progeny was slower due to the complexity of the function to be optimized and the multimodality of its response surface. The expected consequence of using the algorithm, in contrast with empirical procedures for controlling inbreeding, is to promote higher genetic progress and to control inbreeding more effectively.

Keywords: differential evolution, evolutionary algorithms, mate selection

[§] Corresponding author. Tel.: +55 (16) 3209-7450
E-mail address: rcar@fcav.unesp.br

COMBINING VITASSIGN AND COLONY: AN EFFICIENT PRACTICAL PROCEDURE FOR PARENTAL ASSIGNMENT WITH MISSING PARENTS

Doan Q.K.[a], Vandeputte M.[a,b], Chatain B.[a], Vergnet A.[a], Allal F.[a,§]

[a] *Ifremer, UMR 9190 MARBEC, chemin de Maguelone, 34250 Palavas-les-Flots, France*
[b] *INRA, UMR1313 Génétique animale et Biologie intégrative, 78350 Jouy-en-Josas, France*

The contribution of parentage assignment in selective breeding of aquaculture species is undeniable. However, breeding programs often face practical management problems and it is not uncommon that some broodstock genotypes miss because of premature death, traceability problems or sample quality problems. This may lead to unexpectedly low parentage assignment and decrease markedly the potential of genetic improvement. In this study, we explored the potential of combining two softwares, VITASSIGN and COLONY, for obtaining parentage assignment in the case of a few missing parental genotypes in a full factorial mating design. Nine dams were crossed to 60 sires in a full factorial mating scheme and 2000 offspring were reared in a single batch. Biological samples of offspring and parents (caudal fin or sperm) were genotyped for 12 microsatellite markers at Labogena (Jouy-en-Josas, France). Due to low sample quality, 2 dams, 2 sires and 9 offspring could not be genotyped. First pedigree assignment trials were run with this partial dataset (1991 offspring, 7 dams and 58 sires). Using VITASSIGN, an exclusion-based parentage assignment software, 40.8% of offspring were assigned to single parent pair (55.8% allowing up to 2 mismatches). Using Colony, a maximum likelihood parentage software, highly probable pedigree was obtained for 52.6% of the offspring. The average posterior probabilities of 259 additional potential dams genotypes generated by Colony were collected over 7 plausible configurations, and 2 dams showing posterior probabilities higher than 0.95 were identified. The next pedigree assignment included those two inferred dam genotypes, and resulted in 78.0% perfect match in VITASSIGN (92.4% allowing up to 2 mismatches) and in 77.1% assignment in Colony. Finally, candidate sires and dams with missing loci or genotyping errors were corrected based on the genotypes inferred by Colony (12 more parent genotypes were corrected or completed, for a total of 29 corrected alleles). In the end, using VITASSIGN, 96.4% of the offspring were uniquely assigned (86.1% with perfect match and 96.4% with up to 2 mismatches allowed), and only 3.4% of the offspring could not be assigned.

Keywords: pedigree assignment, VITASSIGN, Colony, missing genotypes

§ Corresponding author. Tel.: + 33 4 67 13 04 04; Fax: +33 4 67 13 04 58
E-mail address: fallal@ifremer.fr

EVALUATION OF GENETIC DIVERSITY AND DIFFERENTIATION IN THREE IBERIAN LOCALITIES OF *Donax trunculus* USING MITOCHONDRIAL AND NUCLEAR MARKERS

Fernández-Pérez J.[a,§], Nantón A.[a], Arias-Pérez A.[a], Méndez J.[a]

[a] *Departamento de Bioloxía Celular e Molecular, Facultade de Ciencias, Universidade da Coruña, A Coruña 15071, Spain*

The wedge clam *Donax trunculus* is an Atlantic-Mediterranean warm-temperate species found from Senegal to the northern coast of France, including the Mediterranean and Black Sea. It is commercially exploited in several countries and constitutes an important fishing resource due to its high economical value. In Galicia (northwest coast of Spain) this species has been subjected to intense harvesting and, in some natural beds, became non-existent. The production of this clam could be increased by effective management of natural and hatchery stocks, and transplant of individuals from other regions. However, it is known that restocking techniques may have deleterious effects on the genetic composition of natural populations. Consequently, this should be assisted by genetic studies in order to evaluate the state of the resource and to contribute to a sustainable management. Nevertheless, genetic studies are practically inexistent in *D. trunculus.* In this work we used mitochondrial and nuclear markers to study the genetic diversity and population differentiation of three localities of *D. trunculus* in the Iberian Peninsula. A fragment of the mitochondrial cytochrome c oxidase subunit I (COI) and seven microsatellite markers, arranged into a multiplex PCR (Multiplex 1) previously optimized, were amplified. The COI fragment (430 bp) displayed an elevated number of haplotypes (H= 40), a high haplotypic diversity (h = 0.925) and a low nucleotide diversity (ϖ = 0.008). Furthermore, all microsatellites were polymorphic in the studied localities, mean number of alleles per locus between 3.333 and 21.333, with an expected mean heterozygosity per marker between 0.245 and 0.882. F_{ST} values obtained with the COI fragment (0.06) and with microsatellites (0.02) showed significant genetic differentiation among the three localities, suggesting the presence of barriers to gene flow. This genetic differentiation should be taken into account in relation to restocking strategies to avoid putting at risk the genetic diversity of the species.

§ Corresponding author. Tel.: + (34) 981167000 ext: 2030; Fax: + (34) 981167065
E-mail address: jenyfer.fernandez.perez@udc.es

PERFORMANCE OF THE NATIVE CICLHID (*Cichlasoma urophthalmus*) AFTER TWO GENERATIONS OF FAMILY SELECTION

Fernández-Pérez M.[a], Contreras-Sánchez W.[b], Gallegos-Bravata F.[a], Contreras-Rodríguez G.[a]

[a]*Laboratorio de Acuacultura. División Académica de Ciencias Agropecuarias. Universidad Juárez Autónoma de Tabasco. Villahermosa, Tabasco C.P. 86000. México.*
[b]*Laboratorio de Acuacultura Tropical. División Académica de Ciencias Biológicas. Universidad Juárez Autónoma de Tabasco. Villahermosa, Tabasco C.P. 86000. México.*

Selection constitutes one of the means by which a breeder can select genetic traits of a population by electing the organisms that will be utilized in the reproductive processes. We stablished a family selection program for the native cichlid *Cichlasoma urophthalmus,* with the objective of obtaining a genetically improved population. Twenty-two families were used, starting from wild fish obtained from three different localities (Comalcalco, Centro y Centla) in the State of Tabasco, Mexico. The descendants from each family and a corresponding control group were stocked in three floating cages (2x1x1.2 m). In the first phase, fish were stocked at a density of 300 fish per m^{-3} and grew-up to first selection. Afterwards, fish were stocked at a density of 20 fish per m^{-3} until second selection. In every selection the best performing fish from a cage (10%) were selected. The best results were obtained from the Comalcalco population locality averaging 32.7 ± 0.12 g in the first selection and 231.0 ± 0.10 g in the second. The gain obtained was 11.61% and 46.12% in the 1st and 2nd selection respectively. For the second generation a rotational breeding plan was used with the best performing 24 families from the Comalcalco population. To evaluate growth, we used the same methods and criteria applied for the first generation. Results from the second generation indicate that the fish averaged 54.0 ± 0.13 g in the first selection and 285.3 ± 0.08 g in the second. Average gain was 8.4% and 18%, respectively. Genetic selection in this native species constitutes a step stone for regional aquaculture.

Keywords: Castarrica; family selection; genetic improvement, performance

§ Corresponding author. Tel.: + (52) 993995201
E-mail address: mafepez@hotmail.com

COMBINED DAM ROTATIONAL MATING AND DNA-PARENTAGE ASSIGNMENT TO ESTIMATE GENETIC PARAMETERS OF GROWTH AND QUALITY TRAITS IN A MASS SPAWNING FISH SPECIES, THE ASIAN SEA BASS *Lates calcarifer* REARED IN THE JAVA SEA

Fitrianis Y.[a], Wahjudi B.[a], Muhyi S.[a], Quittet B.[b], Bestin A.[b], Michel A.[a], Haffray P.[b,§]

[a] *FEGA MARIKULTURA, Femina Group, Jakarta 12910, Indonesia*
[b] *SYSAAF, Station LPGP/INRA, 35042 Rennes, France*

Mass spawning prevents to optimize mating and inflates the number of progeny to DNA-assign due to high genetic variance in eggs number spawned between dams.

We report the first application of dam rotational mating in a mass spawning aquaculture species, the Asian sea bass *Lates calcarifer* reared in the Java Sea, to estimate genetic parameters of growth and processing traits and initiate domestication. The female capacity to spawn during 2 to 4 successive days was used to create genetic ties between parents and cohorts and to standardize the number of progenies pooled by dams by spawning day. Each day, 10 dams (1 per tank) were rotated between breeding tanks (n = 10) in 2 to 3 independent runs per cohort in two cohorts (CO2 and CO3). The number of larvae was equalized at hatching between dams before their pooling by day of spawn. Fish were graded to limit cannibalism until their transfer in sea cage at 148 or 189 days of age.

At 634 or 419 days (CO2; 1260.3 g; CO3; 1683.6 g), 500 fish per cohort were processed. 64 and 48 families from 20 dams and 31 sires (CO2) or 15 dams and 54 sires (CO3) were represented and considered for the BLUP genetic data treatment. In the CO2 cohort, heritability of body weight (0.34 ± 0.14), non-trimmed fillet yield (0.28 ± 0.12) and headed and gutted carcass yield (0.35 ± 0.16) were intermediate, the two later being highly correlated (0.79 ± 0.15). This avoided to filet the fishes in the CO3 cohort, heritability of headed and gutted carcass yield being higher than for fillet yield. Heritability of dorsal or belly trimming yields were limited (0.11 ± 0.08 and 0.08 ± 0.06). On the whole data set of the two cohorts, heritability of body weight was intermediate (0.25 ± 0.14) as for gutted yield (0.20 ± 0.05) or headed and gutted carcass yield (0.37 ± 0.08).

These results demonstrated the usefulness of dam rotational mating to create genetic ties and to initiate domestication and genetic selection for growth and processing traits in a mass spawning aquaculture species, the Asian sea bass.

Keywords: aquaculture, genetic parameters, fillet yield, Lates calcarifer, rotational mating

§ Corresponding author: E-mail: yfa_vet38@yahoo.com

ANALYSIS OF KINSHIP DEGREE AND GENETIC VARIABILITY OF PACU (*Piaractus mesopotamicus*) THROUGH MICROSATELLITE MARKERS

Hata M.E.[a], Pazetto B.R.[a], Sato L.S.[a], Jorge P.H.[a], Mastrochirico-Filho V.A.[a], Martínez P.[b], Foresti F.[c], Porto-Foresti F.[d], Hashimoto D.T.[a,§]

[a] *Center of Aquaculture, University of São Paulo State, Jaboticabal, 14884-900, Brazil*
[b] *Department of Genetics, University of Santiago de Compostela, Lugo, 27002, Spain*
[c] *Department of Morphology, University of São Paulo State, Botucatu, 18618-970, Brazil*
[d] *Department of Biology, University of São Paulo State, Bauru, 17033-360, Brazil*

With the increasing number of overfished species, as well as drastic changes in their natural environments, the development of a correct exploitation of aquaculture resources could support the demand of animal protein. Breeding programs can ensure maximization of productive performance in farming systems, through optimizing the yield of the target species. Thus, studies of genetic variability using molecular markers is essential, as a resource to begin a breeding program with a wide variety of genotypes, as well as to avoid the genetic bottleneck and inbreeding depression during the generations. The present study was performed to analyze the genetic variability of one broodstock of pacu *Piaractus mesopotamicus* (collected in the Center of Aquaculture, UNESP, Brazil) by microsatellite markers, in order to know the genetic profile of these animals and improve the genetic management in production systems. The analysis of 31 individuals (seven microsatellites) showed a variation of alleles from two (*loci* Pme20 and Pme28) to thirteen (*locus* Pme32), with an average of 7.57 alleles per locus. The observed heterozygosity (Ho) levels ranged from zero (*locus* Pme28) to 0.25 (*locus* Pme32), with an average of 0.11. The expected heterozygosity (He) ranged from 0.019 (*loci* Pme20 and Pme28) to 0.26 (*locus* Pme32), with an average of 0.17. In relation to the inbreeding coefficient (F_{is}), except for only one *locus* (Pme20), all had positive values, which means heterozygote deficiency. Analysis of kinship degree demonstrated that most of the broodstock was considered genetically unrelated (57.7%). However, 42.3% of the individuals had kinship relation, with 21.6% considered as half-sib and 20.7% as full-sib. Genetic studies focusing on better understanding of the pacu genomes in farming stocks are considered essential for aquaculture, especially to provide resources for the development of management and breeding programs of this species.

Keywords: inbreeding, molecular markers, Brazilian aquaculture, Neotropical fish

GENETIC RELATIONSHIPS AMONG MEMBERS OF THE PACIFIC ABALONE SPECIES COMPLEX INFERRED FROM POPULATION MITOGENOMIC DATA

Ikeda M.[a][§][*], Nishisako M.[a][*], Sekino M.[b][*], Hara M.[c][*], Kijima A.[a][*]

[a] *Laboratory of Integrative Aquatic Biology, Graduate School of Agricultural Sciences, Tohoku University, Onagawa, Miyagi 986-2242, Japan*
[b] *Research Center for Aquatic Genomics, National Research Institute of Fisheries Science, Fisheries Research Agency of Japan, Yokohama, Kanagawa 236-8648, Japan*
[c] *Tohoku Ecosystem-Associated Marine Sciences, Tohoku University, Sendai, Miyagi 981-8555, Japan*

A species complex of Pacific abalone (genus *Haliotis*) is classified into three putative species including two subspecies: *H. discus discuss*, *H. d. hannnai*, *H. madaka*, and *H. gigantia*. Taxonomic status of Pacific abalone consistent with morphological, biological, and ecological characteristics suggests that sympatric *H. d. discus*, *H. madaka*, and *H. gigantia* each should be categorized as species and *H. d. hannai* represents an allopatric variety of *H. d. discus*. Authentic identification of these abalone species is required for the aquaculture and stock enhancement programs, however, subtly morphological differences together with the variable nature of their external characteristics on which environmental conditions have a significant influence. Recent study using nuclear microsatellite DNA markers and the individual assignment tests revealed a solid genetic boundary between *H. gigantia* and *H. d. hannai*, *H. d. discus*, and *H. madaka*. Meanwhile, there has been no information for the species identification and genetic relationships among them based on intensive mitochondrial DNA analysis. Hence, we studied to search genetic evidence to distinguish the members of Pacific abalone species complex and to examine genetic relationships among species based on population mitogenomic data, that is, 8,189 nucleotides concatenated complete or partial sequences of each 15 genes in the mitochondrial genomes of 10 to 36 individuals collected from 2 to 4 localities along the coast of Japanese Archipelago in each species. Small but significant genetic differentiation between/among sample populations was detected in all species, suggesting that gene flow is restricted within the distribution ranges and existence of management units which should be conserved in all species. The neighbor-joining tree for all sample populations showed that the populations of *H. gigantia* consisted of a clustered group distant from other species but the populations of *H. d. discus*, *H. d. hannnai*, and *H. madaka* formed an another cluster group without unity of species. The phylogenetic tree of all haplotypes demonstrated two major clades separated with high bootstrap probability. The haplotypes of *H. discus* and *H. gigantia* completely sorted into each clade, respectively. But almost all haplotypes (95%) and the remains (5%) of *H. madaka* belonged to the clades of *H. discuss* and *H. gigantia*, respectively. These results mean that species identification of Pacific abalone is difficult using only mitogenomic data. This may be caused by recent speciation of *H. madaka* and existence of introgression of mitogenome between *H. madaka* and other two species.

Keywords: Pacific abalone, species identification, population mitogenome, genetic relationship

[*]These authors have contributed equally to this work.
[§] Corresponding author. Tel.: +81-225-53-2436; Fax: + 81-225-53-2436
E-mail address: + ikusyu@bios.tohoku.ac.jp

THE GENETIC STRUCTURE OF NATURAL AND ARTIFICIAL SEA TROUT (*Salmo trutta*) POPULATION IN RIVERS SALACA AND GAUJA, LATVIA

Karkliņš A.[1], Škute N.[1]

Life science and technology institute Daugavpils University, Parades1A, Daugavpils Latvia

Sea trout (*Salmo trutta* L.), with other representatives of the *Salmonidae* fish, is among the most valuable biological resources in Latvia. It was shown that microsatellite loci are useful markers to study genetic structuring within species. Therefore microsatellites could be of great help in an accurate characterization of sea trout natural and artificial populations. The study of genetic differentiation of sea trout population from Salaca and Gauja river was based on microsatellite DNA analysis. Eight different microsatellite primers were used*: Str15, Str73, Str85, Str543, Str79, Str60, Strutta58, Strutta 12.* The length of the microsatellite alleles was determined by ABI 310 DNA analyzer. The obtained data were statistically analyzed by Gen Alex program and population genetic parameters were determined. It was shown, that the level of heterozigosity decreases upstream the river Salaca, but the number of alleles increases, the greater the level of heterozygosity, the higher the number of average alleles and the number of private alleles. In the natural population the higher upstream the river the fish live, the genetically more distant to each other they are. The artificially reproduced population, however, is genetically more distant within itself, as the naturally reproduced population is. 37% of molecular diferentiation has been observed between natural and artificial reproduced populations, they are considered as two different populations.

Corresponding author email address: *natalja.skute@du.lv*

GENETIC IMPROVEMENT OF FEED CONVERSION EFFICIENCY VIA INDIRECT SELECTION IN RAINBOW TROUT

Kause A.[a,§], Kiessling A.[b], Martin S.A.M.[c], Houlihan D.F.[c], Ruohonen K.[d]

[a] *Natural Resources Institute Finland, Jokioinen, FI-31600, Finland*
[b] *Swedish University of Agricultural Sciences, Umeå, SE-90183, Sweden*
[c] *University of Aberdeen, Aberdeen, AB24 2TZ, The United Kingdom*
[d] *EWOS Innovation AS, Dirdal, N-4335, Norway*

Feed is one of the largest costs of aquaculture production, making the improvement of feed conversion efficiency (FCR) of great importance. To directly select for FCR (=daily feed intake/daily gain), feed intake needs to be recorded but its recording is very difficult in fish. An indirect alternative is to select for traits that are genetically correlated with FCR. Lipid deposition is a potential indicator trait because in fish, and in farmed animals, lean animals are more efficient than fat animals. Using data on 1001 individual rainbow trout of 2.2kg body weight, we quantified the benefit of replacing feed intake in a selection index by three alternative lipid traits: whole body lipid%, muscle lipid%, and percentage of viscera weight of total body weight (reflecting visceral lipid). Individual feed intake was recorded using the method in which fish are fed with feed pellets with glass ballotini, an x-ray revealing the amount of feed consumed. The genetic correlation between daily gain and FCR was favourable -0.63 (±0.30), and given the simulated population structure, index theory calculations for EBV selection showed that selection for daily gain improved daily gain by 7.0% and FCR by 4.1%. Simultaneous selection for daily gain and against feed intake (direct selection to improve FCR) increased genetic gain in FCR by 1.57 fold compared to sole selection for daily gain. Replacing feed intake in the selection index by whole body lipid%, muscle lipid%, or viscera% increased genetic gain in FCR by 1.23, 1.60, and 1.01 fold, respectively, compared to sole selection for daily gain. Consequently, indirect selection for daily gain and against muscle% was as effective as direct selection for FCR. The reason for the efficiency of muscle lipid% is its high genetic variation relative to feed intake, and the strong genetic correlation between muscle lipid% and feed intake. The results highlight the benefit of controlling muscle lipid deposition on the genetic improvement of FCR and product quality. Because lipid in muscle and viscera are genetically distinct traits, care must be taken to simultaneously select for multiple lipid traits to effectively control for whole body lipid deposition.

Keywords: Breeding programme; Feed intake, Index selection; Production efficiency; Quantitative genetics

§ Corresponding author. Tel.: + 358 29 5326222
E-mail address: antti.kause@luke.fi

THE EFFECT OF INTERSTRAIN HYBRIDIZATION ON THE PRODUCTION PERFORMANCE IN THE PACIFIC OYSTER *Crassostrea gigas*

Kong L., Song S., Li Q.[§]

Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China

Intra-specific hybridization was carried out using three strains (C, J and K) of *Crassosrrea gigas*, which were successively mass selected for two generations from three culture stocks. Detailed comparison on larval growth development, the growth and survival of the spats and adult were carried out among six hybrids and three parental groups. By way of the serial growth traits measurements (shell height, shell length and the whole body weight), the obtained results revealed that six hybrid crosses were inferior to those of parental crosses to different extend at larval stage, while at spat and adult stages some differences were observe. It was noted that the cross CJ outperformed all the other groups in all growth traits and at harvest, the shell height, shell length and the whole body weight reached to 75.64(± 11.68) mm, 46.35(± 9.06) mm and 42.11(± 10.53) g, respectively. For spats and adults survival rate, the hybrid crosses CJ and CK were larger than three parental crosses and that of the reciprocal hybrids crosses CK and KC were significantly ($P< 0.05$) larger than the parental crosses CC and KK. Considering the growth and survival conditions, the results concluded that the crosses CJ, CK and KC in this work were deemed to be the better hybridization combinations by genetic improvement, which could have significant implications for the development of oyster aquaculture.

Keywords: Crassostrea gigas; growth; survival; heterosis

[§] Corresponding author. Tel.: +86 0532 82031622;Fax: +86 0532 82032773
E-mail address: qili66@ouc.edu.cn

INTROGRESSIVE CROSSBREEDING OF TWO *O. niloticus* STRAINS TO DEVELOP IMPROVED STRAINS OF RED TILAPIA

Lago A.A.[a*], Rezende T.T.[b*], Freitas R.T.F.[a*], Hilsdorf A.W.S.[c,*§]

[a]*Department of Animal Science, Federal University of Lavras, Lavras, MG, 37200-000, Brazil*
[b]*Department of Agriculture, , Federal University of Lavras, Lavras, MG, 37200-000, Brazil*
[c]*Unit of Biotechnology, University of Mogi das Cruzes, Mogi das Cruzes, SP, 08701-970, Brazil*

Aiming to suggest strategies to develop an crossbred of *Oreochromis niloticus* with high performance and red colouration were performed introgressive crossbreeding program using Red Stirling and Chitralada strains, having 50%, 75% and 87.5% of genic proportion of Chitralada. Experiments were performed in the Brazilian Industry of Fish Ltd., São Paulo, Brazil. Seven genetic groups were obtained namely, Chitralada (C) and Red Stirling (R); the hybrids F1, fish-female Red Stirling × fish-male Chitralada (RC) and its reciprocal (CR); the backcrossing (RC1), fish-female CR × fish-male Chitralada (CR×C) and its reciprocal (C×CR); and the recurrent backcrossing RC2 [C(C×CR)]. Progenies were distributed in 28 hapa 1×1×1m containing 100 fish each, for 90 days. After this period, 15 fish-females and 15 fish-males weighing 15 g were collected from each parcel. Fish were identified by using electronic chips classified according to their weight and allocated in 8 net cages, four for female fingerling and four for female, each cage having all weight class. *Biometric* data were collected each 21 days up to 190 ongrowing period. The R software was used for statistical analysis. For growth, were analysed the exponential, logistic and Gompertz models. Values of heterosis, maternal heterosis, paternal heterosis and maternal effects for weight and length were evaluated by least squares procedures. The genetic groups were all ranked according to breeding value for growth (recorded as body weight and length). The growth was different between the groups for fish-males and fish-females, on which the adjusted model was the exponential. For the fish-males, the crossbreeding with the best performance in the groups with red colour was found in the RC2, showing that gene introgression was effective. While for fish-female was found in the RC1 (C×CR), which was observed the paternal heterosis of 21.58%. Concluding, because of differences in crossings using female and male as well as the increase in performance of Red fish, we recommend a breeding program to achieve a red female and male lines exploring the paternal *heterosis on females.*

Keywords: *Backcrossing; aquaculture; growth; breeding program; heterosis.*

*These authors have contributed equally to this work.
§ Corresponding author. Tel.: + 55 11 99912-4250; Fax: + 55 11 47987106;
E-mail address: wagner@umc.br

CORRELATED RESPONSE IN COLOR FLESH AFTER SIX GENERATION OF SELECTION FOR HARVEST WEIGHT IN COHO SALMON, (*Oncorhynchus kisutch*)

Lhorente J.P.[a], Newman S.[b], Neira R.[c], Filp M.[a], Figueroa R.[a], Yáñez J.M.[a,d,§]

[a] *Aquainnovo, Pto.Montt, 5503032, Chile*
[b] *Genus, plc,Hendersonville, TN 37075 USA*
[c] *Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago, 8820808, Chile*
[d] *Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, 8820808, Chile*

A commercial breeding program of coho salmon was established in Chile in 1997. The nucleus consists of two independent populations (EVEN and ODD lines, depending on the spawning year). Each population is comprised of 120 full-sib families per generation, which were generated using a hierarchical design (3-4 female per male). We used data from eight generations of selection, incorporating 42,657 and 42,157 records for harvest weight (HW), and 6,140 and 6,741 records for fillet color (COL) measured as salmoFan score for EVEN and ODD population, respectively. Pedigree size was 79,345 and 75,322 fish for EVEN and ODD populations, respectively. Genetic parameters, breeding values (EBVs) and predicted genetic trends were estimated with a bi-variate animal model including HW and COL as dependent variables. The model included contemporary group (Year:Sex:Cage) for both traits and age at harvest and plant weight as covariates for HW and COL, respectively. Estimated heritabilities for HW and COL were 0.38 (±0.03) and 0.10 (±0.02) for EVEN and 0.40 (±0.03) and 0.18 (±0.03) for the ODD population, respectively. Genetic correlations between HW and COL were 0.45 (±0.13) and 0.32 (±0.14) for EVEN and ODD populations, respectively. Genetic trend was estimated as the regression of average EBV on generation. After eight generations of selection for HW, genetic gain was between 230 and 177 gr per generation in EVEN and ODD lines, respectively. Moreover, we found a genetic response in COL (+0.04 salmoFan color card score, ~ 0,5% per generation) after selecting for HW in both populations, likely due to the positive genetic correlation between HW and COL.

Keywords: Coho salmon, harvest weight, fillet color, genetic correlation, genetic gain

§ Corresponding author. Tel.: + 56-9-98101584
E-mail address: + jmayanez@uchile.cl

GENETIC PARAMETERS OF AMOEBIC GILL DISEASE RESISTANCE IN ATLANTIC SALMON *Salmo salar*

Lillehammer M.[a,§], Norris A.[b], Løvoll M.[c], Gjerde B.[a]

[a] *Nofima, Ås, Norway*
[b] *Marine Harvest ASA, Dublin, Ireland*
[c] *VESOVikan, Namsos, Norway*

Amoebic gill disease (AGD) is caused by the amoeba *Paramoeba perurans*, which colonises gill tissue of several fish species. In salmon farming, AGD has been a major problem in Tasmania for decades, and is currently an emerging issue in Northern Europe. The treatment consist of repeated baths of freshwater or H_2O_2, which is expensive, logistically challenging and invasive. In Tasmania, selective breeding for lower gill-score in filed test has successfully increased the interval between the treatments over generations. The aim of this study was to estimate genetic parameters for AGD resistance of Atlantic salmon (*Salmo salar*) under controlled and field conditions in Northern Europe. Smolt from 150 families from the Marine Harvest nucleus was infected with *P. perurans* in a controlled challenge test (n=20 fish per family) while another sample of fish from the same families (n=40 fish per family) were placed at a high-risk location in Ireland where AGD-infections occurred naturally. Gill scores were obtained after two subsequent infections both in the controlled test and in the field, and mortality was recorded after the second treatment in the field. Heritability of gill-score ranged from 0.10 to 0.20. Genetic correlation between first and second gill-score was close to zero and non-significant in the challenge test, and 0.70±0.10 in the field test. Genetic correlations between gill-scores from challenge test and field-test ranged from -0.07 and 0.38. The fish from the field test suffered >20 % mortality shortly after the second freshwater treatment which was associated to poor gill health. This mortality had heritability of 0.06±0.01, and was positively genetically correlated to first (but not second) gill score in the challenge test. Genetic correlations between mortality and gill score in field was close to zero and non-significant. The challenge test hence had low power to predict field gill score, but some power to predict field mortality. The first gill score in the field was a good predictor for second gill score in the field.

Keywords: AGD, genetic parameters, Atlantic salmon, challenge test

§ Corresponding author. Tel.: + 476497 0405
E-mail address: marie.lillehammer@nofima.no

HERITABILITY ESTIMATIONS FOR TOXIN ACCUMULATION, COLOR AND GROWTH RELATED TRAITS IN MEDITERRANEAN MUSSEL

Martínez P.[1], Pino-Querido A.[1], Guerra-Varela J.[1,2], Pardo B.G.[1], Vera M.[3], Álvarez-Castro J.M.[1], Toro M.A.[4], Fuentes J.M.[5], Blanco J.[5]

[1] Department of Genetics, University of Santiago de Compostela, Spain
[2] Geneaqua SL, Lugo, Spain
[3] Department of Biology, University of Girona, Spain
[4] Department of Animal Production, University Politécnica de Madrid, Spain
[5] Center of Marine Research (CIMA), Vilagarcía de Arousa, Spain

Mediterranean mussel (*Mytilus galloprovincialis*) is one of the main species of the European aquaculture, Galicia being (NW Spain) the main producer (~200,000 t/year). The principal problem for mussel production is accumulation of toxins coming from microalgae blooms, which even increased in recent years determining the banning of commercialization for long periods at specific areas. Considering the natural occurrence of microalgae blooms, the most efficient way for reduction toxin accumulation in mussels is through selective breeding programs provided a significant heritability for the trait. The best way to estimate heritabilities is to manage a large set of families reared under a common environment using a randomized design. Families were obtained in hatchery by crossing 10 females x 19 males stimulated with temperature changes, and when progenies reached the appropriate size were transported to an area where a toxic episode was occurring. Progeny allocation was done with a microsatellite tool constituted by 9 markers. A total amount of 170 full-sib and above 600 (via father and via mother) half-sib families were finally obtained. Heritabilities and correlations (genetic and phenotypic) for okadaic acid, the main diarrheic toxin, concentration after a toxic episode and after a depuration period at indoor facilities were estimated using around 2200 offspring per experimental condition. We took advantage of available families to estimate the same parameters for other relevant traits for production like growth-related traits and color. Heritabilities were moderate and significant after both the accumulation and detoxification periods (h2 ~ 0.35), suggesting the possibility of reducing toxin concentration through breeding programs in mussels. Growth-related traits also showed moderate heritabilities (~0.30), while color showed a very high heritability (~0.90), making them suitable for selection depending on producer and consumer demands. Interestingly, growth-related traits showed negative significant genetic and phenotypic correlations with toxin concentration, while color showed positive ones, strongly suggesting that bigger and whitish mussels may accumulate less toxin. The results of our study support the viability of breeding programs to face the main problems of mussel industry, but this approach will determine a change in seed production, the wild seed being replaced or complemented with hatchery-produced seed.

Keywords: *Mytilus galloprovincialis, heritability, mussel production, algal toxins*

LESSONS FROM STUDIES ON DISEASE RESISTANCE IN ATLANTIC SALMON (*Salmo salar*) POPULATIONS: THE *Piscirickettsia salmonis* CASE IN THE CHILEAN AQUACULTURE

Martínez V.*, Detleff P., Bravo C., Machuca A., Barra V., Patel A.

FAVET-INBIOGEN. University of Chile, 8820808, Chile

Understanding the genetic variability of disease resistance of salmon populations is a very important issue in practice in order to design efficient breeding programs. Still, most of the studies use phenotypes for assessing disease resistance, the mortality after the challenge of individuals coming from a given pedigreed population. Although, empirical studies show that the genetic correlations between the challenge test and disease outbreaks are generally high, there is little information regarding the use of indicator traits that can more appropriately ascertained, for increasing the repeatability of challenge tests. This is of much importance in *Piscirickettsia salmonis* challenge testing due to the unpredictable behavior of the bacteria, and hence the replication of the trials is challenging. In this study, we use different molecular indicators obtained from the host-pathogen interaction in order to measure disease resistance and tolerance more effectively. A number of families were challenge tested using a virulent strain of *P. salmonis* in order to obtain mortality curves and disease resistance between families. The heritability for the binary trait dead or alive was significant (h^2=0.4). We characterize the transcriptome of individuals using Illumina sequencing at high depth of a set of families that differ significantly in terms of the family mean. The results show that the transcription patterns are quite sensitive to the phenotypes used, i.e. when the pedigree was not considered for gene expression, it yields a number of genes that significantly differ between groups, but they lack biological significance in terms of disease response. Using extreme families we obtained a number of genes with biological significance that differs significantly. These genes are related to immune and bacterial response and lipid metabolism. In general terms, gene expression is exacerbated in susceptible individuals, which is a classical finding in several studies of disease response. Also bacterial load is negligible in resistant families. Moreover, the transcriptional response (RNAseq) of the bacteria clearly is dependent on the culturing environment, which may explain why the bacterial load differs between the different tissues and organs examined. This study highlights the importance of developing a judicious strategy that includes not only the mortality, but also other indicators explaining more effectively disease resistance in *Salmo salar*. FONDECYT 1120608.

Keywords: Disease resistance, Quantitative genetics, breeding programs, SNP-chip

* Corresponding author. Tel.: + 56 9 63060306 ; Fax: + + 56 22 9785641
E-mail address: vmartine@u.uchile.cl

DEVELOPING THE NATIONAL BREEDING PROGRAM OF *Seriola lalandi* FOR THE DIVERSIFICATION OF THE CHILEAN AQUACULTURE

Martínez V. [a,*], Hernández E. [a], Lacamara J.[b], Barra V., Patel A.[a]

[a] *FAVET-INBIOGEN. Dpt of Animal Science, Faculty of Veterinary Sciences, University of Chile, Stgo, 8820808, Chile.*
[b] *ACUINOR, SPA., Caldera, Chile.*

Developing breeding programs from wild species, invoke interesting questions regarding the development of breeding programs for the Chilean industry, based on long-term objectives at the "*Programa de la Diversification de la Acuicultura Chilena (PDACH)*". This is a private public consortium whose objective is to develop the Seriola lalandi aquaculture in the north of Chile. However, this species lack sufficient genomic resources required for assessing broodstock relationships, genetic contributions and population assessment. This is a very important issue in marine species, where, it is not possible to keep family records during the first stages of development due to communal spawning. Thus inbreeding and relatedness can increase dramatically if algorithms for maintaining the rate of inbreeding when selecting broodstock are not used. We are aiming at developing the required genomic tools in order to design sound breeding programs, that can provide selected larvae based on judicious breeding goals within the program. This will be done using the Affymetrix platform. We are developing a whole genome draft (10 to 15x), sequencing different libraries constructed from a single individual) using the Illumina platform. This sequence will be used for reduced representation, whole genome re-sequence and transcriptome mapping of larvae and adults in order to characterize complex traits such as growth, skeletal deformities and nutrigenomics. We have discovered more than 100K SNPs in coding regions using deep sequencing of normal and deformed larvae (in excess of 60 millions reads per individual), some of this markers has been validated in a larger sample of an independent population. A total of 10K indels and 3k microsatellites were predicted using the transcriptome data. All the data is consistent when using the *de novo* transcripts assembly. The SNPs characterized at the genome level, will be available for use in genomic selection, calculation of genomic inbreeding and relatedness and for characterize the effects of selection at the genomic level, while developing a SNP chip platform specifically for this species.

Keywords: Heritability, Genetic variation, breeding programs, deformies, SNP-chip

* Corresponding author. Tel.: + 56 9 63060306 ; Fax: + + 56 22 9785641
E-mail address: vmartine@u.uchile.cl

COMBINING BREEDING PRACTICE AND QUANTITATIVE GENETIC THEORY: EXAMPLES FROM A FISH BREEDING NETWORK IN DENMARK

Meier K.[a][§], Norberg E.[a], Thomsen B.[b], Sørensen A.C.[a]

[a]*Center for Quantitative Genetics and Genomics, Department of Molecular Biology and Genetics, Aarhus University, Tjele, 8830, Denmark*
[b]*Danish Aquaculture Organization, Silkeborg, 8600, Denmark*

In 2012 a breeding network was established in the Danish aquaculture industry to accommodate the farmers' needs to intensify breeding efforts. The breeding effort, breeding goals and breeding practice differs between individual farmers and companies. Hence, a decentralized approach with specific breeding programs for each farmer is needed. To accommodate this need a breeding network, with collaboration between the Danish Aquaculture Organization, the fish farmers, and Aarhus University, was established. The aim of the network is to combine quantitative genetic theory and practical breeding knowledge to optimize the current breeding practice for the individual farmer and obtain and share knowledge between members within the network. Examples of practical aspects in breeding plans that have been optimized and where knowledge have been obtained and shared within the network are given.

Keywords: Fish breeding, Breeding network, Stochastic simulation, Optimal contribution selection

[§] Corresponding author. Tel.: +4529611435; Fax: +4587154995
E-mail address: kristian.meier@mbg.au.dk

GENETIC EVALUATION OF A WILD STOCK OF *Rhamdia quelen* (SILURIFORMES, HEPTAPTERIDAE) AS FOUNDERS OF A STOCK FOR SUPPORTIVE BREEDING BASED ON MICROSATELLITE MARKERS

Mendonça B.B.[a],[*],[§], Fernández-Cebrián R.[b],[*], Martínez P.[c], Batlouni S.R.[d], Porto-Foresti F.[a]

[a] *Biology Department, São Paulo State University (UNESP), Bauru,* 17033-360, *Brazil*
[b] *Geneaqua S. L. C/ Primavera, Nº 40, 1ºB. E-27002 Lugo, Spain*
[c] Genetic *Department, University of Santiago de Compostela, Lugo, 27002, Spain*
[d] *São Paulo State University Aquaculture Center (UNESP), Jaboticabal, 14.884-900, Brazil*

Brazil presents the greatest richness and endemism of freshwater fish species in the world with great potential for aquaculture development in a worldwide scale, although extractive fishing is still responsible for most fishery production. Extractive fishing together with anthropogenic fragmentation of freshwater ecosystems represent the major factors for the wild Brazilian populations decline. Aiming to minimize these impacts, restocking has been implemented in areas with dam constructions, but these actions were done without genetic criteria and have negatively affected wild populations. In view of the freshwater fish commercial importance, the present study was aimed to evaluate the genetic diversity in a wild population of *Rhamdia quelen* and its putative idoneity as founders of a hatchery stock for supportive breeding management to reinforce populations in areas close to Small Hydroelectric Plants. This catfish species is important for aquaculture and extractive fishing. We applied eight microsatellite loci to evaluate genetic diversity in R. quelen populations from three Small Plants Constructions in Sapucaí River, Alto Paraná basin, São Paulo (Brazil).The microsatellites loci displayed a mean number of alleles per locus (N_a) of 9.250 and expected heterozygosity (H_E) of 0.691, these values being similar when compared with previous studies on freshwater fish species. The set of eight microsatellite loci employed in this study was informative to evaluate relatedness between pairs (r) of sampled individuals, and for parentage analysis, displaying combined non exclusion probability for the first parent (NE-1P) of 1.796×10^{-1}, probability for the second parent (NE-2P) of 1.295×10^{-3} and parent pair probability (NE-PP) of 1.126×10^{-5}. Although most pair-wise comparisons were unrelated (76.39%) and only a small part were considered as full-sib (4.15%), an important proportion (19.46%) were classified as half-sib,thus suggesting a careful design of matings to avoid inbreeding in the supportive breeding stock.

Keywords: fresh water species, microsatellite markers, restocking, genetic diversity, kinship, Small Plants.

[*]These authors have contributed equally to this work
[§] Corresponding author. Tel.: +055 14 99152-2189; Fax: +0 55 143103-6092
E-mail address: bruna.bmendonca@hotmail.com

DEBRIS RECYCLING IN GENOME SEQUENCING: REFERENCE DEVELOPMENT AND MICROSATELLITES ISOLATION FOR POPULATION GENETIC ANALYSES OF THE MARBLED FLOUNDER *Pseudopleuronectes yokohamae*

Minegishi Y.[a,§], Ikeda M.[b], Kijima A.[a,b]

[a] *Tohoku Ecosystem-Associated Marine Sciences, Graduate School of Agricultural Science, Tohoku University, Onagawa, Oshika, Miyagi 986-2242, Japan*
[b] *Onagawa Field Center, Graduate School of Agricultural Science, Tohoku University, Onagawa, Oshika, Miyagi 986-2242, Japan*

Marbled flounder *Pseudopleuronectes yokohamae* is a commercially valued fish in Japan. Due to its benthic lifestyle with low dispersal ability, the populations of this species can be easily influenced by human activities including fishing and marine environmental changes like the massive tsunami occurred in Japan in 2011. In order to comprehensively evaluate the genetic consequences of those impacts on this species, the genetic diversity needs to be investigated at a genomic scale. Genome-wide analyses such as variant detection are often performed based on reference genome information. Therefore, species-specific genome sequences are ideal as a reference to maximally utilize the genomic data of the species of interest and interpret them correctly. Here we developed the draft genome sequence of the marbled flounder, specifically aiming at use as a reference for population genetic analyses of this species, by alignment of short reads to determine variants at homologous sites of the genome. For this particular purpose, we designed it to contain most sequences of the genome even though gene/chromosome structures are fragmented. We sampled a single individual of the marbled flounder in Sendai Bay, Miyagi, Japan, and performed sequencing of the genome of this specimen using IonTorrent PGM up to 35.4 × coverage (23.7 gigabases) of the genome of this species. The trimmed reads were *de novo* assembled into 525,502 contigs, and their summed length was 547.8 megabases, corresponding to 81.8% of the genome of this species. Among RAD-tag reads collected from six individuals, 92 – 95% of the reads were successfully mapped to the assembled contigs, and at maximum 75,472 SNPs were determined. This shows that this draft genome sequence developed in this study works as a reference for population genetic analyses of the marbled flounder. Furthermore, from the unassembled reads of *de novo* assembly, we designed a total of 331,368 primer pairs for 86,732 unique microsatellite sequences. Arbitrarily selected 96 pairs were tested and 16 out of them were characterized as novel microsatellite loci of this species. This study presents a strategy of the purpose-specific genome sequencing but also a potential to make use of the debris of genome sequencing.

Keywords: Pseudopleuronectes yokohamae, genome sequence, draft genome, microsatellites

§ Corresponding author. Tel.: +81 225 90 3969; Fax: +81 225 90 3969
E-mail address: y.minegishi@m.tohoku.ac.jp

GENOTYPE BY ENVIRONMENT INTERACTION FOR HARVEST WEIGHT, GROWTH RATE, AND SHAPE BETWEEN MONOSEX AND MIXED SEX NILE TILAPIA (*Oreochromis niloticus*)

Omasaki S.K.[a,b§], Komen H.[a], Kahi A.K.[b], Charo-Karisa H.[c], van Arendonk J.A.M.[a]

*[a]Animal Breeding and Genomics Centre, Wageningen University,
P. O. Box 338, 6700 AH, Wageningen, the Netherlands.
[b]Animal Breeding and Genomics Group, Department of Animal Sciences,
Egerton University, P.O Box 536, Egerton-20115, Kenya.
[c]State Department of Fisheries, P.O Box 58187, Nairobi-00200, Kenya.*

In Kenya, Nile tilapia *(Oreochromis niloticus)* are mostly grown in ponds. To avoid excessive reproduction and stunted growth, fry are treated with methyl-testosterone to make all-male populations (monosex). For a national breeding program that aims to provide fry to small-holder pond farmers, it is important to assess the genetic correlation *(r_A)* for traits between the mixed sex breeding candidates from the nucleus and monosex production fish. The purpose of the study was to estimate the genetic parameters for harvest weight (HW), growth rate, expressed as daily growth coefficient (DGC) and body shape described by ellipse (Ec = (Lenght - Height)/(Lenght + Height)) and investigate the genotype by environment interaction (G x E) between mixed sex and monosex populations for these traits. Data were collected on progeny of 48 sires and 76 dams from the F_2 generation of local *O. niloticus* strains, kept at Sagana Aquaculture Research Station, Kenya. Each 3 days old mixed sex full sib family was divided into two groups of 50 individuals each. One group was fed a diet treated with methyl-testosterone (MT) to induce sex reversal and the other group reared on a control diet (CO). After hapa rearing, tagging and weighing, fish were randomly divided and stocked in 6 earthen ponds, three for CO and 3 for MT fish. After 5.5 months, fish were harvested, photographed and weighed. Genetic parameter estimates for HW, DGC, and shape were obtained on 2105 fish. Heritability estimates for HW ranged between 0.21 – 0.24 for both MT and CO, while for DGC the estimates ranged between 0.26 – 0.32. Heritability estimates for Ec was 0.07 and 0.12 for MT and CO, respectively. Genetic correlations for HW and length between MT and CO were 0.74 and 0.77, respectively, suggesting low G x E. The corresponding r_A for DGC, height and shape were low; 0.59, 0.46 and -0.19, respectively, denoting presence of G x E. It is concluded that G x E between the mixed sex nucleus and monosex production fish is important, and that a breeding program for Nile tilapia needs to include production performance from MT treated siblings.

Keywords: Oreochromis niloticus; harvest weight; monosex; genotype by environment interaction; Kenya

[§] Corresponding author. Tel: +31647598757
E-mail address: simion.omasaki@wur.nl; skomasaki@gmail.com

MOLECULAR MARKER (ALLOZYME) ASSISTED SELECTION OF LATENT PRODUCTION TRAITS AND FINGERPRINTS OF NOVEL STRAINS OF FISH (*Clarias gariepinus*) AND SHELLFISH (*Macrobrachium vollenhovenii*)

Oyebola O.O.[a,§], Jacobs J.M.[b], Omorodion B.N.[a], Akinade S.A.[a], Akinsola Y.Y.[a]

[a] *Department of Aquaculture and Fisheries Management,
University of Ibadan, Ibadan,00000, Nigeria[1]*
[b] *Biotechnology Center, Animal Laboratory, Federal University
of Agriculture, Abeokuta,00000, Nigeria[2]*

The need to develop low cost and simple instruments for identification and marker assisted selection for production traits in novel fish and shellfish necessitated this study.

Male and female brooders of *Clarias gariepinus* strains were selected based on phenotype and expression of reference marker - <14.7kDa band on allozyme profile of12.5% SDS poly-acrylamide gel electrophoresis (SDSPAGE).Individuals possessing anteriorly serrated pec-toral spine and did not show the reference marker were selected for C-variants. Individuals without anteriorly serrated pectoral spine but showed the reference band were selected for S-variant (novel). 18 brooders (1.0-1.2 kg) containing both sexes were selected for both vari-ants. Females were characterized for body morphometric and egg production indices. Males were characterized for body and gonad morphometric and sperm quality. Pooled eggs and milts of the S and C variants were used to produce inbred lines. The inbreds were analyzed for production traits: %fertilization, feeding behaviour, fry survival and weight during 21 days rearing period. Reference marker was confirmed in progenies. Allozyme fingerprints of dis-covered morphotypes of *Macrobrachium vollenhovenii* (individuals possessing equal length of left and right side cheliped-EA, longer cheliped at left side–LL and shorter cheliped at left side-SL) were also investigated on SDSPAGE.

C. gariepinus variants expressed significantly different production traits (P<0.05). Com-pared to C, female S (novel) had shorter dorsal ray count-DRC (65±1) and standard length-SL (44.65±0.64 cm); lower egg weight (136.56±3.24 g) and stripping percentage (13.67±0.32 %). Male S had shorter DRC (65±3), higher SL (50.65±2.19 cm); higher number of gonad teeth (15±1), gonad length (5.7±0.71 cm) and % gonad/body length (5.10±0.13) while gonad weight (6.35±0.67 g) and % gonad/body weight were lower. S had higher % sperm motility (67.78±9.72) and % live/dead sperm (93.78±3.77). Inbred of S had higher % fry swim up during feeding (85.0±10.6 %) and mean weight of fry (0.04±0.02 g) while inbred of C had higher % fertilization (51.03±26.70) and % fry survival (62.45±3.61). All S-variant progeny inherited <14.7kDa band while all C progeny did not. Fingerprints of LL were 18.4 and 50.0 kDa bands while 20 kDa separated SL from EA.

In conclusion, allozyme technique was useful for marker assisted selection for latent pro-duction traits in *C. gariepinus* and for fingerprinting of novel fish/shellfish strains.

Keywords: molecular identification key, novel fish and shellfish, aquaculture potentials

These authors have contributed equally to this work
§ Corresponding author. Tel.: +234-803-3950-321
E-mail address: olusegun.oyebola@yahoo.com

GENETIC VARIATION OF MILKFISH (*Chanos chanos*) BROODSTOCK AND HATCHERY-PRODUCED FINGERLINGS AND JUVENILES IN THE PHILIPPINES

Quilang J.P., Basiao Z.U.

Institute of Biology, College of Science, University of the Philippines 1101 Diliman, Quezon City

The milkfish (*Chanos chanos*) is an important food fish and is widely farmed in the Philippines, Indonesia, and Taiwan. Milkfish fry used to occur in tremendous numbers along shorelines in the Philippines and had been the traditional source of seed supply for the milkfish culture industry. However, because of the unpredictability and seasonality of fry from natural spawning grounds, farmers are relying heavily on hatchery-produced fry from both local and abroad. In this study, fin clips from broodstock and muscle tissues from juvenile fish or fingerlings were collected from four government-run (SEAFDEC-Iloilo, BFAR-Dagupan, BFAR-Bohol, and BFAR-Albay) and two private hatcheries (CDO Aquafarm-Zambales and Oversea Milkfish Hatchery-Cebu). For comparison, milkfish fingerlings grown in a private hatchery from fry imported from Bali, Indonesia were also included. PCR primers were designed to amplify the full-length sequence of the cytochrome b (1,141 bp) and the 3' peripheral domain of the control region (472 bp). The mitochondrial cytochrome b and control regions were sequenced from 50 specimens from each site, except for SEAFDEC-Iloilo wherein successful PCR amplification and DNA sequencing was obtained for only 49 specimens. Milkfish from SEAFDEC-Iloilo had the highest nucleotide diversity (ϖ=1.44%), followed by milkfish from CDO Aquafarm-Zambales (1.23%), Indonesia (1.20%), BFAR-Dagupan (1.16%), BFAR-Albay (1.08%), BFAR-Bohol (1.00%), and Oversea Milkfish Hatchery-Cebu (0.89%). In terms of haplotype diversity (*H*), milkfish from SEAFDEC-Iloilo had the highest (0.996) followed by BFAR-Dagupan (0.989), Indonesia (0.968), CDO Aquafarm-Zambales (0.909), Oversea Milkfish Hatchery-Cebu (0.896), BFAR-Albay (0.873), and BFAR-Bohol (0.820). Although these genetic diversity values can be considered high, those obtained for milkfish from BFAR-Albay, BFAR-Bohol, and Oversea Milkfish Hatchery-Cebu were consistently lower than those from the other hatcheries. These data can be used by the managers of these hatcheries to look into their broodstock management practices to ensure that the genetic quality of their broodstock do not deteriorate further and to consider using stocks with high genetic variability as replacement for old and aging breeders. The data also show that that the genetic diversity values of milkfish produced from hatcheries in the Philippines are comparable if not better than those from a sample imported from Indonesia.

Keywords: broodstock, genetic variation, hatchery, milkfish, population genetics

§ Corresponding author. Tel.: + 63-2-9205471
E-mail address: + jpquilang@gmail.com

GENETIC ASSESSMENT OF PHILIPPINE MILKFISH (*Chanos chanos*) STOCKS BASED ON NOVEL MICROSATELLITES FOR MARKER-AIDED BROODSTOCK MANAGEMENT

Romana-Eguia M.R.R.[a,§,*], Santos B.S.[b,*], Ikeda M.[c,*], Basiao Z.U.[b,*], De Jesus-Ayson E.G.[d], Kijima A.[c]

[a] *Southeast Asian Fisheries Development Center, Aquaculture Department, Binangonan, 1940 Rizal, Philippines*
[b] *Institute of Biology, University of the Philippines, Diliman, 1101 Quezon City, Philippines*
[c] *Laboratory of Integrative Aquatic Biology, Graduate School of Agricultural Sciences, Tohoku University, Onagawa, Miyagi 986-2242, Japan*
[d] *Southeast Asian Fisheries Development Center, Aquaculture Department, Tigbauan, 5021 Iloilo, Philippines*

Reports on genetic diversity within/among milkfish populations using DNA markers are sparse. Earlier work dealt with evolutionary relationships among wild populations to define management units in the Indo-Pacific region where milkfish naturally abound. However, application of milkfish genetic markers in broodstock development and management remains to be investigated. Here, nine novel microsatellites developed by NGS were utilized to (a) describe the genetic structure of Philippine wild and hatchery-bred milkfish; (b) monitor the impact of domestication selection and inbreeding; and (c) formulate marker-aided broodstock management methods, a prerequisite to genetic improvement. Milkfish samples from three wild populations: Claveria (CLA), Currimao (CUR), Camiguin (CAM); eleven local hatchery stocks: SEAFDEC Integrated Hatchery (SIH), SEAFDEC Big Hatchery-Igang batches 1 and 2 (SBH-I1 and SBH-I2), SEAFDEC Big Hatchery-Dumangas (SBH-D), Hautea Hatchery (HH), Sual Pangasinan Hatchery (SPH), BFAR Dagupan Hatchery (BDH), BFAR Bohol Hatchery (BoH), BFAR Palawan Hatchery (PAL), Zambales Hatchery-P_0 (ZH-P_0), Zambales Hatchery-F_1 (ZH-F_1); and a hatchery stock from West Java in Indonesia (WJH), were examined. An Indonesian stock was included since in the Philippines, Indonesian milkfish fingerlings are imported and farmed for having purportedly better production traits. Genetic diversity indices such as expected heterozygosity (*He*) and allele frequency (*A*) ranged from 0.655 to 0.697 and 9.2 to 11.1, respectively. AMOVA showed significant but low genetic differentiation among the milkfish populations (F_{ST} = 0.013; P=0.000,) since much of the variation is attributed to intrapopulation differences (98.6%). The oldest hatchery stock SIH (30-35 years) had relatively moderate genetic variability (*He* = 0.66, *A* = 10.6), which is lower than that of 5-year old SBH-I1 (*He*= 0.687, *A*= 11.5) considering that both stocks originally came from the same source in the wild. A reduction in genetic diversity was seen when a local hatchery stock (ZH-P_0 *He* = 0.66 and *A*= 10.8) was monitored after one generation (ZH-F_1; *He* = 0.65, *A*=9.3). Finally, the Indonesian stock WJH had genetic variability levels (*He*= 0.66; *A*= 10.5) comparable with local stocks. Results of genetic analyses are herein discussed in the context of promoting effective milkfish broodstock management practices for the production of good quality seed stock.

Keywords: *milkfish microsatellites, broodstock management, genetic variability, inbreeding, selective breeding*

*These authors have contributed equally to this work.
§ Corresponding author. Tel.: + (63-2)9277825; Fax: +(63-2)9277825
E-mail address: +mreguia@seafdec.org.ph

SIGNIFICANT GENETIC DIVERGENCES AMONG THAILAND AND CHINESE POPULATIONS OF THE ASIAN MOON SCALLOP *Amusium pleuronectes*: GEOGRAPHIC ISOLATION, SELECTION PRESSURE, OR BOTH?

Su D.J.[*], Cheng L.[*], Fu X.L., Chen X.F., Zhu J.T., Wang Y.[§]

*Key Laboratory of Tropical Biological Resources of Ministry of Education,
Ocean College, Hainan University, Haikou 570228, China*

The Asian moon scallop, *Amusium pleuronectes* (Linnaeus, 1758) belongs to Pectinidae (Mollusca: Bivalvia), characterized by its bicolor circular shells (the right shell is white and the left one is red brown). This species, naturally distributing along the Indo-Pacific Coast, is an important fishery resource. The recent catch statistics, however, showed that its production has visibly reduced. Besides, *A. pleuronectes* is functional hermaphrodite, so its selfing may profoundly influences its hatchery spats production mode and population genetic structure. Here we developed the first set of 52 microsatellite makers for this species, and selected 16 to analyze the population genetic structure of four *A. pleuronectes* natural populations (TH, from the east coast of the Gulf of Siam, Thailand; BH, Beihai, the north of Beibu Bay, China; LG, Lingao, the northwest of Hainan Island, China; QH, Qionghai, the east costal of Hainan Island, China; n=32/population). The results showed that medium levels of polymorphism were observed in all four populations with the average allele number ranging from 10.6±4.1 to 12.5±3.5. The average observed/expected heterozygosities were 0.51/0.79 (BH), 0.67/0.81 (LG), 0.67/0.78 (QH), and 0.61/0.86 (TH), respectively. 4-5 loci deviated from Hardy-Weinberg equilibrium ($P<0.05$ after Bonferroni correction) in each population, with three loci (*Ap29,37,39*) deviated in at least 3 populations in the direction of heterozygote deficiency, probably because of the presence of null alleles and the selfing trait of this species. Significant genetic divergences was detected between Thailand and Chinese populations (pairwise F_{ST} 0.068 of TH:BH, 0.077 for TH:QH, and 0.050 for TH:LG, with globe F_{ST} 0.054), which may due to high levels of regional isolation. Comparative genetic divergence between QH and LG, BH may result from the selection pressure, e.g. the intensity and frequency of typhoon disturbance in Qionghai have been much higher than that in Beihai and Lingao, even in Thailand. The minimal inbreeding coefficient F_{IS} happened at QH (0.078) and the highest F_{IS} at TH (0.205) and BH (0.185) may also due the differences of selective pressure. We argue that both selective pressure and geographic isolation were responsible to varying degrees for generating the significant phylogeographic structure.

*These authors have contributed equally to this work
[§] Corresponding author. Tel.: + 86 (0)13707543005; Fax: +86 898 66279215
E-mail address: wy2005@163.com

RESPONSE TO SELECTION FOR HARVEST WEIGHT IN SEA BASS (*Dicentrarchus labrax*)

Thorland I.[a,§], Kottaras L.[b], Refstie T.[a], Papanna K.[b], Papaharisis L.[b], Rye M.[a]

[a] *Akvaforsk Genetics Center AS (AFGC), 6600 Sunndalsøra, Norway*
[b] *Nireus Aquaculture SA, 19400 Koropi, Greece*

Nireus Aqauculture SA operates a large scale family based breeding program for European seabass (*Dicentrarchus labrax*) in Greece, designed and supervised by Akvaforsk Genetics Center (AFGC). The program was originally established by KEGO SA in 2001 with base material collected broadly from Mediterranean sources, which since 2008 has been supplemented with mass selected material assumed to be largely unrelated to the original base. Till date 807 full- and maternal halfsib families(representing 737 sires and 292 dams) have been produced by single pair matings across12 batches of nucleus families. A nested mating design is applied, for which eggs of individual female breeders are fertilized with semen from~3 (range 1-6) males. Generations are overlapping, and the average generation interval is approx. four years. Families are reared separately until a random sample within family is individually tagged at an average size ~15gr and subsequently pooled and performance tested in commercial grow-out environments. At commercial harvest size individual body weight, jaw deformity score, and body shape are recorded, and mean family survival calculated. Recently work on controlled testing of nucleus families for resistance to vibriosis and pasteurellosis has been initiated, but not yet fully implemented (results not shown).

Quantitative genetic analyses of performance data demonstrate substantial additive genetic variation for harvest weight (h2~.45), and low to medium heritabilities (.14–.26) for the other traits investigated.Genetic correlations between growth and other traits are generally low, and no substantial unfavourably genetic correlations have been revealed. Consistent ranking of families across production environments indicatel ow genotype by environment interaction for growth.

Selection has been based on selection indices primarily focusing on harvest weight, with some relative weight placed on additional traits to prevent undesired changes. The accumulated selection response for harvest weight in the nucleus (~F2.3) is estimated to +138gr, or 23% per generation relative to F0 (data 9 batches). The average level of inbreeding in the present population is below 2%.

Broodstock lines are produced from high ranked nucleus families to meet Nireus' demand for fry. Nireus' commercial production of seabass is expected to fully based on selected broodstock by 2016.

Keywords: European sea bass, breeding program, selection response, harvest weight, heritability

§ Corresponding author. Tel.: +47 975 69 563;
E-mail address: ingunn.thorland@afgc.no;

HAPLOTYPE DIVERSITY OF MITOCHONDRIAL DNA D-LOOP REGION IN THE PERUVIAN ROCK SEABASS *Paralabrax humeralis*

Torres Y.[*], Sotil G.[*,§], Merino J.P., Cueva D.

Laboratory of Genetics. Area of Aquaculture Research. Instituto del Mar del Perú (IMARPE). Callao 01. Peru

Paralabrax humeralis is an important fishery bentho-pelagic resource, well-appreciated food fish and with great aquaculture potencial in Peru and other countries; however its population structure and genetic diversity still remain unknown. We evaluated the haplo-type diversity of the mitochondrial DNA d-Loop region in the native rock seabass from two geographical locations (Piura and Callao, north and central of Peru, respectively). Sequences of 513 pb in thirty individuals were analyzed by PCR product direct sequencing. A total of 27 haplotypes were identified, with 36.1% of G+C content, haplotype diversity of 0.993 and nucleotide diversity of 0.01098 (ranged from 0.0095 to 0.0127, for Callao and Piura respectively) Thirty four mutations (2 polymorphic in Callao and monomorphic in Piura, while 13 polymorphic in Piura and monomorphic in Callao, and 19 shared mutations) were detected. There were 33 nucleotide polymorphic sites, accounting for 6.43% of total sequence, with 14 singleton variable sites and 19 parsimony informative sites. Our results contribute to the knowledge of the Peruvian population variability of *P. humeralis*, showing a very high genetic diversity and preliminary suggesting connectivity between both geographical locations. This study points out the use of the mtDNA d-Loop as a good molecular marker for the genetic diversity studies in this specie, for assessing the fisheries impact on the population status and for the selection of organisms with desirable traits for aquaculture.

Keywords: polymorphism, genetic variation, population, bentho-pelagic, aquaculture.

[*]These authors have contributed equally to this work.
[§] Corresponding author. Tel.: + (051)208-8650 – Anexo 845;
E-mail address: gsotil@imarpe.gob.pe; y.torres.ascurra@gmail.com

CURRENT GENETIC STATUS OF NATURAL AND CULTURED BEDS OF EUROPEAN FLAT OYSTER (*Ostrea edulis*): RESTORATION AND RESISTANCE TO BONAMIOSIS DISEASE

Vera M.[a,b,§], Carlsson J.[c,d], Carlsson J.E.L.[c], Villalba A.[e], Cao A.[e], Pardo B.G.[a], Vilas R.[a], Flannery G.[c], Lynch S.[c], Gómez-Tato A.[f], Álvarez-Dios J.A.[f], Cross T.[c], Culloty S.[c], Martínez P.[a]

[a] Department of Genetics, University of Santiago de Compostela, Lugo, 27002, Spain
[b] Department de Biology (Genetics Area), University of Girona, Girona, 17071, Spain
[c] School of Biological, Earth and Environmental Sciences/Aquaculture and Fisheries Development Centre, University College Cork, Cork, Ireland
[d] School of Biology & Environmental Science, University College Dublin, Dublin, Ireland.
[e] Centro de Investigacións Mariñas (CIMA), Xunta de Galicia, Vilanova de Arousa, 36620, Spain.
[f] Department of Applied Mathematics, University of Santiago de Compostela, Santiago de Compostela, 15782, Spain

The flat oyster (*Ostrea edulis*) is one of the most economically valuable mollusc in Europe, but aquaculture production and harvesting of natural beds have been greatly reduced due to the effects of the parasite *Bonamia ostreae*. Therefore, characterization of current natural and cultured populations is required to develop long term bed restoration programmes, by enhancing genetic diversity and tolerance to bonamiosis. Different locations from Denmark, the Netherlands, United Kingdom, Ireland, France and Spain were sampled in two consecutive years (2010 and 2011). These samples were analyzed using 16 microsatellite loci to study temporal and geographical genetic composition. Temporal variation was low, although sometimes significant probably due to unequal spawning events. Regarding geographical differences, samples from different countries showed genetic differences, and Ireland and France showed differences among locations within country. Clustering analyses grouped the data into three main geographic regions: one group constituted by Holland and Denmark; another by French, Irish and English samples; and a third group exclusively from Spanish locations. Effective population sizes (Ne) were high (> 500) which would reflect population stability and low genetic drift impact which would be insufficient to reduce genetic variability over time. The presence of both local and regional genetic structure shows the potential for local adaptation in flat oysters and suggests caution when transplanting individuals (especially between distant geographical regions). Finally, six wild populations, three naïve (not previous contact with the parasite; Limfjorden, Loch Ryan and Tralee Bay) and three tolerant (reported contact for a long period with *B. ostreae*) (Rossmore, Quiberon and Ortigueira) were analyzed with markers associated with Differentially Expressed (DE) genes identified after comparing control and challenged individuals with the parasite in order to detect signals of divergent selection. Two loci, related to the oyster immune response, showed strong signals of significant divergence and should be further studied and might be valuable for Marker Assisted Selection (MAS) programmes. Moreover, a large list of candidate genes that might be important for Bonamia resistance was produced and is available for future studies.

Keywords: Restoration, population genetics, conservation, Bonamia resistance, Ostrea edulis

§ Corresponding author. Tel.: +34-972-41-81-68; Fax: +34-972-41-82-77
E-mail address: manuel.vera@usc.es, manuel.verar@udg.edu

A MICROSATELLITE MARKER TOOL FOR PARENTAGE ANALYSIS IN THE NEO-TROPICAL FRESHWATER FISH PACÚ (*Piaractus mesopotamicus*, Holmberg, 1891)

**Villanova G.V.[ab*], Posner V.[a*], Sánchez S.[c], Díaz J.[ab],
Sciara A.A.[ab], Martínez P.[d], Arranz S.E.[ab§].**

[a] *Acuario Río Paraná Laboratory, School of Biochemical and Pharmaceutical
Sciences, National University of Rosario, Rosario, (2000), Argentina.*
[b] *CONICET, Rosario, (2000), Argentina.*
[c] *School of Veterinary Science, UNNE. Northeast Institute of Ichthyology,Corrientes, Argentina.*
[d] *Genetics Department, University of Santiago de Compostela, Lugo, Spain.*

The pacú (*Piaractus mesopotamicus*) has become the most important fish in aquaculture in Argentina and is one of the most cultivated fish in Brazil and other South American countries. Although the pacú is the focus of major aquaculture efforts, appropriate parentage tools have not yet been developed for this species and little information is available about genetic diversity and structure of wild and cultured populations.

In this study eighteen marker loci were standardized and tested for utility to perform parentage analysis in *P. mesopotamicus*. Markers were tested in three populations from Paraná and Paraguay Rivers from Argentina and Brazil. In addition families of pacú reared in an isolated environment were used to evaluate the usefulness of analyzed loci (Mendelian proportions, null alleles, genotyping errors) using a a Mendelian exclusion approach. Both probabilities EXCL1 and EXCL2 were obtained for different groups of loci to find the minimum number of loci required for a consistent parentage evaluation. Based on genotyping accuracy from family data, and genetic diversity and frequency of null alleles estimated from wild populations (n=79), a set of eleven markers was initially selected. The theoretical exclusion probabilities for this set of markers were EXCL1=0.9692 and EXCL2=.0.998. A final selected group of eight microsatellites was decided, theoretical exclusion probabilities values got slightly reduced to EXCL1= 0.9658 and EXCL2= 0.997. According to the ML analysis performed with Cervus it was expected to achieved 98% assignments at a single family with these eight markers. The microsatellite tool was evaluated for relatedness estimation using the actual family groups obtained in captivity with known kinship relationships. The estimated classification was contrasted against the true family data.

Results from this work are relevant for the genetic management of *P. mesopotamicus* in captivity and to broodstock organization. The set of loci microsatellites selected prove to be helpful for solving unknown parentage in captive progenies and could be used for exploratory relatedness estimation among broodstock individuals with unknown relationships for stock organization.

Keywords: fish production, relatedness estimation, broodstock organization.

[*]These authors have contributed equally to this work.
[§] Corresponding author. Tel.: + 543414237070 int. 649; Fax: + 543414237070 int. 607
E-mail address: sarranz@fbioyf.unr.edu.ar

COMPARISON OF FERTILIZATION, HATCHING AND GROWTH RATE IN STURGEON HYBRIDS AND PUREBREDS

Vuong D.T.§, Havelka M., Šachlová H., Lebeda I., Flajšhans M.

University of South Bohemia in Ceske Budejovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, Zátiší 728/II, 389 01 Vodňany, Czech Republic

There is growing interest in research of sturgeons as well as their hybrids, not only because of conservation purposes but also for aquaculture. In this study, individually identified mature broodstock of evolutionary octaploid Siberian sturgeon (*Acipenser baerii*) and Russian sturgeon (*A. gueldenstaedtii*) were artificially propagated. Fitness related characteristics i.e. fertilization, hatching and growth rates were analysed in resulting purebreds and hybrid families. The level of heterozygosity and genetic polymorphism among analysed families were investigated at several microsatellite loci. Both fertilization and hatching rates were significantly higher (P<0.05) in hybrids of Russian sturgeon females x Siberian sturgeon males (RxS) (92.8 and 63.4%, respectively) and Siberian sturgeon females x Russian sturgeon males (SxR) (93.6 and 69.4%, respectively) compared to purebreds of Russian sturgeon (RxR) (85.2 and 53.6%, respectively) and Siberian sturgeon (SxS) (90.0% and 55.4%, respectively). Significant growth differences were recorded for the RxS group on day 37 post-hatching with 15.0 ± 1.0 g vs. 11.5 ± 0.5, 12.0 ± 0.0 and 12.0 ± 1.0 g for SxR, SxS and RxR, respectively. The significantly higher growth rates in hybrid groups of RxS and SxR compared to SxS and RxR purebreds were observed on 151, 459, and 569 days after hatching. Similar pattern was recorded for mean survival rates after 569 days post-hatching: 61.07 ± 26.69, 66.07 ± 29.71, 60.85 ± 15.78 and 58.27 ± 22.98 for RxS, SxR, SxS and SxS, respectively. On other hand, there was no significant difference in heterozygosity among analysed families. It was contrary to general consideration that the transfer of genetic material from one species into another via hybridization may serve as a source of adaptive genetic variation. These results suggested that studied sturgeon hybrids had higher survivability and grew faster under hatchery conditions. Therefore they could be potentially useful in sturgeon aquaculture. However, their negative effects to maintain genetic integrity of species should be seriously considered. In addition, their fertility potential may increase threat of backcrossing if these hybrids escape from aquaculture.

Keywords: sturgeon, hybridization, ploidy, aquaculture production.

§Corresponding author. Tel: +420 387774753
E-mail address: vdoi_thi@frov.jcu.cz

GENETIC IMPROVEMENT OF YELLOW PERCH II: PRODUCTION PERFORMANCE AND GENETIC VARIABILITY OF SELECTED POPULATIONS

Wang H-P.[a,§], O'Bryant P.[a], Yao H.[a], Rapp D.[a], Hartleb C.[b], Lynch W.[c]

[a] *Aquaculture Research Center, and Aquaculture Genetics and Breeding Laboratory, The Ohio State University South Centers, Piketon, OH, 45661, USA*
[b] *Department of Biology, University of Wisconsin-Stevens Point, Stevens Point, WI 54481, USA*
[c] *Mill Creek Perch Farms, Marysville, OH 43040, USA*

The development of the yellow perch *Perca flavescens* aquaculture industry has been hindered due to the relatively slow growth of currently cultured populations of this species. As a part of the effort to enhance aquaculture production of yellow perch, we have undertaken a selective breeding program for improving their growth rate using a marker-aided cohort selection with different strategies. On-station and on-farm tests of performance of the third generation of improved lines were conducted at different latitudes using both separate rearing and communal rearing approach, and the same protocol. Results showed that our improved fish exhibited 27.6% - 42.1% higher production, and 25.5% - 37.5% higher growth rates in the condition of having 12.3% - 27.8% higher survival than local strains across the three testing sites. This improvement would make it feasible for yellow perch to reach market size within 14 months in pond culture conditions and in 9 months in recirculating aquaculture systems.

Performance test of OSU improved perch vs. Bell Aquaculture industrial strains was also conducted in recirculating aquaculture systems. Two strains, 500 fish of each, were provided by Bell Aquaculture. OSU provided 500 fish from its genetically improved lines. Each strain was tagged with Visible Implant Elastomer color tags, and stocked to each of two 6' x 6' round recirculating tanks and communally raised in the same environment for an accurate comparison. After 6-month test, OSU genetically improved fish outgrew Bell Aquaculture perch by 43.66% on average. This result shows OSU improved perch not only significantly grow faster in pond conditions, but also in recirculating tank system.

In total, 3,318 broodfish and 600 random fish from two overlapping lines consisting six generations of selected populations and a random population were genotyped using eight microsatellite markers to investigate genetic structure and diversity of selected populations among and within these generations. The results showed that the marker-aided cohort selection breeding strategy could avoid depletion in genetic variability of selected populations and the levels of variation were appropriate to proceed with a long-term selective breeding program in yellow perch.

§ Corresponding author. E-mail address: wang.900@osu.edu

ESTIMATION OF HERITABILITIES FOR BODY WEIGHT AND LENGTH IN ASIAN SEA BASS (*Lates calcarifer*)

Yenmak S.[a], Poompuang S.[a,§], Koonawootrittriron S.[b], Senanan W.[c]

[a]*Department of Aquaculture, Faculty of Fisheries, Kasetsart University, Bangkok, 10900, Thailand*
[b]*Department of Animal Science, Faculty of Agriculture, Kasetsart University, Bangkok, 10900 Thailand*
[c]*Department of Aquatic Science, Faculty of Science, Burapha University, Chon Buri, 20131 Thailand*

The Asian sea bass (*Lates calcarifer*) is an important food fish species in Thailand which can be cultivated in both brackish water and freshwater ponds, as well as in cages in coastal areas. Thailand is a major international supplier for fry and fingerlings, although the genetically improved strains of sea bass are not available. In this study, genetic parameters for body weight and length at fingerling were estimated for sea bass stock from a medium-sized private hatchery to determine the potential for selective breeding. The experiment was conducted under a working condition at the hatchery. Fry were produced by mass spawning 18 males and 48 females which were marked by PIT tags for identification. Pedigrees were reconstructed based on seven microsatellite loci (*LcaM*27, *LcaM*32, *Lca*58, *Lca*98, *Lca*185, *Lca*260 and *Lca*284) using the computer program COLONY 2. Mass spawning produced 97 full-sib and 349 half-sib families. Families with fewer than five progeny were excluded from the datasets. At 12 days post-hatch, fry were graded into six size classes and reared separately until 90 days. They were graded every five to seven days. A total of 807 individuals were measured at 90 days post-hatch with the average of 563.58±220.90 mg for body weight, 32.49±8.71 mm for total length and 8.99±2.30 mm for body depth. Sea bass fingerlings displayed relatively large size differences, likely due to strong competition within the population. Variance components were estimated by multivariate animal model using ASReml. Estimates of heritability S.E. were 0.15 0.09 for body weight, 0.20 0.12 for body length and 0.19± 0.11 for body depth. It is likely that the heritabilities were underestimated due to the effect of size grading. Body length was highly correlated with body depth, with the value S.E. of 0.94 0.01. However, genetic correlation was moderate between body weight and body length (0.41 0.00).The results suggested selection for body length for improvement offingerling body weight in this sea bass stock.

Keywords: Asian sea bass, heritability, genetic correlation, pedigree, size grading

§ Corresponding author. Tel.: +66-2-579-2924; Fax: +66-2-561-3984
E-mail address: supawadee.p@ku.ac.th

COMPARISON BETWEEN OPTIMUM CONTRIBUTION AND MATE SELECTION IN AQUACULTURE BREEDING

Yoshida G.M.[a][§][*], Queiroz S.A.[a][*], Ribeiro R.P.[b][*], Oliveira C.A.L.[b][*], Carvalheiro R.[a][*]

[a]Department of Animal Science, Universidade Estadual Paulista Júlio
Mesquita Filho (FCAV), Jaboticabal-SP, 14884-900, Brazil.
[b] Department of Animal Science, Universidade Estadual de
Maringá, Maringá-PR, 87020-900, Brazil.

The aim of this study was to compare optimum contribution (OC) and mate selection (MS) in aquaculture breeding, regarding parameters associated to genetic progress and inbreeding. The dataset contained pedigree information of 6,722 Nile tilapias from four generations (G4), and standardized expected breeding values (EBV) for a growth trait. Males and females presenting positive EBVs, from the 30 families of the fourth generation, were considered as selection and mate candidates, with a restriction in the maximum number of allowed mates per individual (1 for female and 4 for male). The target objective function (OF) to be optimized was: $OF = w1*x'EBV + w2*x'Ax + w3*$, where w1, w2 and w3 are the weights associated to the expected genetic merit of the future progeny (x'EBV), the coancestry of selected parents (x'Ax) and the predicted average inbreeding of the future progeny (), respectively, and x is the vector of genetic contributions for each candidate, to be optimized as well. In both cases, OC and MS, the OF was optimized using an evolutionary algorithm based on differential evolution. For OC, the OF was optimized in two steps. Firstly, was ignored and x was obtained under different values of w1 and w2. The minimization of was performed in a second step (for OC) after defining the proper set of values for w1 and w2, which allowed reduction of coancestry without substantially compromising the expected genetic merit. The results indicated no superiority of performing selection and mating decisions simultaneously (MS) over the strategy of selecting based on OC followed by mating reducing inbreeding. Under equivalent weights, OC and MS presented similar results, regarding the components considered in the OF. It is not clear in which extend this result is population specific or related to the optimization algorithm implemented. Further investigation is intended to be performed, fine-tuning the algorithm and using different data, to assess the reproductivity of the results.

Keywords: Oreochromis niloticus, objective function, optimization, evolutionary algorithms

[*]These authors have contributed equally to this work.
[§] Corresponding author. Tel.: +55 (16) 98176-0832
E-mail address: grazyoshida@hotmail.com

SPATIAL AND TEMPORAL GENETIC AND REPRODUCTIVE PATTERNS OF THE OYSTER *Crassostrea hongkongensis* IN MAOWEI SEA, CHINA

Yu F.[a], Li Q.Z.[b], Yang Y.C.[a], Luo B.[b], Zhu J.T.[a], Wang Y.[a,§]

[a] *Key Laboratory of Tropical Biological Resources of Ministry of Education, Ocean College, Hainan University, Haikou 570228, China*
[b] *Guangxi Academy of Fishery Science, Nanning, 530021, China*

The oyster *Crassostrea hongkongensis* is the main aquaculture species along south China coast and of significant economic value. It favors high temperature and low salinity so that it is mostly found in estuaries. Maowei Sea, an inland sea in northern Beibu Bay, is not only the "natural pasture" for this oyster but also the largest natural seedling field in China because Qinjiang River joins it, bringing rich food. The natural spat production in Maowei Sea, however, reduced dramatically due to the interception of Qinjiang River, and the low spring rainfall several years ago. Some farmers then introduced the spats from Zhuhai (Guangdong). In order to monitor and evaluate the changes of the population genetic structure of aboriginal oysters in Maowei Sea, we used 16 microsatellite markers to screen Zhuhai (ZH) and Maowei Sea wild populations (MW), three natural spat populations [year 2012 (12L), 2013 (13L) and 2014(14L)]. The results showed that medium levels of polymorphism were observed in all five populations, with similar average allele number (allele richness) 11.9 (11.0) for 14L, 11.3 (10.3) for 13L, 11.2 (10.6) for 12L, and 11.3 (11.0) for ZH, 10.5 (10.2) for MW. The average observed/expected heterozygosities were also closed ranging from 0.54/0.80 (12L) to 0.64/0.77 (MW). Three natural spat populations showed higher genetic divergences from ZH ($F_{ST,}$ 0.031-0.042) than that from MW ($F_{ST,}$ 0.006-0.015), and significantly genetic divergences were found between ZH and MW, suggesting the impact of introduction of Zhuhai oysters might be limited. *Ne* estimates obtained with four methods were variable and generally small (33-256). But no changes in allele richness are evident among adult and spat collections, which may due to the reproductive characters of the oyster. Serial histological sections showed that oyster gonads sexually differentiated from late April and ended in late September, with two spawning peak in May and July-August. Although a batch of survival larvae were only produced by a few adults, resulting in genetic draft, lots of adults' seedlings may survive throughout the six months reproductive season, which overall maintained Maowei Sea oyster population's structure temporally stable. (Research supported by the NSFC #41376174)

Keywords: Crassostrea hongkongensis, microsatellite, population genetic structure, effective population number Ne, reproductive cycle

§ Corresponding author. Tel.: + 86 (0)13707543005; Fax: + 86 898 66279215
E-mail address: wy2005@163.com

PLOIDY LEVEL AND PERFORMANCES IN HYBRIDS BY CROSSING BLUNT SNOUT BREAM (*Megalobrama amblycephala,*♀) WITH TOPMOUTH CULTER (*Erythroculter ilishaeformis,*♂)

Zheng G.D., Chen J., Jiang X.Y., Zou S.M.[§]

*Key Laboratory of Freshwater Aquatic Genetic Resources,
Shanghai Ocean University, Shanghai 201306, China*

In the present study, two groups with distant genetic background were obtained by inter-generic crossing between "*Pujiang No. 1*" good breed of blunt snout bream (*Megalobrama amblycephala,*♀) and topmouth culter (*Erythroculter ilishaeformis,*♂). Our results showed that the fertilizing rates (90.0%) and hatching rates (80.6%) in intergeneric cross group were surprisingly high, which is no different with the those in their self-crossing groups. Both hybrid A (96.2%) and hybrids B (3.8%) were obtained within hybrids of blunt snout bream♀×topmouth culter♂. The morphological characters of hybrids A were between its parents, the hybrids B had the same morphological characters to the female parent of the blunt snout bream. The relative DNA content of hybrids was examined by Flow Cytometery. The results showed that the hybrids A and B were diploid with the same DNA contents as their parents. Two pairs of specific loci (TTF6 and TTF10) were screened by microsatellite (SSR) analysis. The results indicated that hybrids A inhertited the genetic materials from both female and male parents respectively. The hybrids B inherited only the maternal ge-netic materials which show it is gynogenetic progenies. The hybrids A grew faster than both blunt snout bream and topmouth culter, which suggest an obvious growth advantage of intergeneric hybrids in the earth pond. Our studies shine a light on constrution a hybrid strain with improved growth traits by intergeneric cross between blunt snout bream and topmouth culter. In addition, the production of gynogenetic progenies can be applied to establish the pure line during selection breeding of new breed of blunt snout bream.

Keywords: blunt snout bream, topmouth culter, intergeneric crossing, gynogenesis, growth advantage

[§] Corresponding author. Tel.: +86-21-61900345; Fax: +86-21-61900345
E-mail address: +smzou@shou.edu.cn

GENOME-WIDE MICROSATELLITE GENETIC RESOURCES IN AN ICONIC AFRICAN FRESHWATER FISH, THE NILE PERCH, *Lates niloticus*

Basiita R.K.[a,b,§*], Zenger K.R.[a*], Jerry D.R.[a*]

[a] *Centre for Sustainable Fisheries and Aquaculture, College of Marine and environmental Sciences. James Cook University, Townsville QLD, 4811, Australia*
[b] *National Fisheries Resources Research Institute, Kajjansi, National Agricultural Research Organization, Kampala. P. O Box 530, Uganda*

Nile perch is a globally important fresh water finfish species with significant potential for aquaculture, however, to date there are no species specific genetic resources available for population genetic, parentage and commodity traceability studies. Microsatellites are highly polymorphic genetic markers whose development can be achieved *de novo* in species using next generation sequencing. Mining 160,000 reads produced by Roche 454 shotgun sequencing, 31 microsatellite loci (2-4 bp unit length and ≥8 repeats) were developed, synthesized and tested for polymorphism. Of these, 12 markers were selected and characterized by genotyping a population of Nile perch from Lake Victoria. The markers were found to be polymorphic with a mean allelic richness (N_A) of 4.6 (range of 2-10 alleles per locus) and a mean expected heterozygosity (H_E) of 0.47 (range 0.1-0.8). Preliminary findings indicate the robustness of these markers for use in studies of population genetic structure, connectivity, broodstock identification and parentage analysis in this species. This sets precedence for developing genetic resources for other species important to freshwater aquaculture in Africa.

Keywords: Aquaculture, Nile perch, 454 Next Generation sequencing, Microsatellites

*These authors have contributed equally to this work.
§ Corresponding author. Tel.: + 61411331266
E-mail address: Basiita.komugisha@my.jcu.edu.au; Basiitarose@gmail.com

FAST REAL TIME PCR FOR CONTROL OF INTRA-SPECIES RECYCLING IN AQUACULTURE FEED, FOCUSED TO THE MOST RELEVANT FISH SPECIES FARMED IN EUROPE

Espiñeira M.[a§]**, Alonso M.**[a]**, Lago F.C.**[a]**, Vieites J.M.**[a]

[a] *Department of Molecular Biology, ANFACO-CECOPESCA, Vigo, 36310 Spain.*

Recent regulations in animal feed composition prohibit the intra-species recycling in order to avoid potential safety risk to human and animal health. The application of these regulations is effectively checked in case of ruminants and livestock farms by microscopic and molecular techniques. But in aquaculture, the lack of fast and specific tools to check feedstuffs composition, difficult the intra-species recycling control.

To date, the most studies of species identification and detection in feedstuffs were focused to land species, in order to guarantee the feed composition for livestock farms. But scarce studies were focused to fish species composition in aquaculture feed. Recent regulations have generated the need of control of feedstuffs used in aquaculture by effective and specific analytical techniques.

The present work describes four methodologies based in real time PCR for detection of the most relevant fish species farmed in Europe: gilthead sea bream *(Sparus aurata);* sea bass *(Dicentrarchus labrax);* turbot *(Scophthalmus maximus);* and rainbow trout *(Onchorynchus mykiss),* in order to guarantee the intra-species recycling regulation in aquaculture feedstuffs.

Keywords: Real Time PCR, gilthead sea bream (Sparus aurata), sea bass (Dicentrarchus labrax), turbot (Scophthalmus maximus), rainbow trout (Onchorynchus mykiss), aquaculture, feed.

§ Corresponding author. Tel.: +34 986 469 301; Fax: +34 986 469 269
E-mail address: montse@anfaco.es

SIGNATURE OF ARTIFICIAL SELECTION IN A BREED OF COHO SALMON *Oncorhynchus kisutsh*

Hosoya S.[a, §], Kikuchi K.[a], Nagashima H.[b], Onodera J.[b], Sugimoto K.[b], Satoh K.[b], Matsuzaki K.[b], Yasugi K.[c], Nagano A.J.[c, d], Kumagayi A.[b], Ueda K.[b], Kurokawa T.[e]

[a] *Fisheries Laboratory, University of Tokyo, Shizuoka431-0214, Japan*
[b] *Inland Fisheries Experimental Station, Miyagi Prefecture*
Fisheries Technology Center, Miyagi 981-3625, Japan
[c] *Center for Ecological Research, Kyoto University, Shiga 520-2113, Japan*
[d] *JST PRESTO, Saitama 332-0012, Japan*
[e] *Tohoku National Fisheries Research Institute, FRA, Miyagi 985-0001, Japan*

Artificial selection has often left signatures within the genome of the breeding population. Such signatures can be detected by means of a population genomics approach. Identification of these footprints of selection can pave the way to find genes and genetic markers that may facilitate further genetic improvement. In this study, we analyzed two divergent populations of coho salmon (*Oncorhynchus kisutsh*), one of which was obtained by selection for increased growth during the past 15 years and the other with no intentional selection, in order to identify loci affected in the early stages of a breeding program.

We first compared fork length between the two populations reared in a communal pond and found that the average fork length of the selected population was significantly longer than that of the unselected population. We then obtained a genome-wide set of SNPs by ddRAD-seq from the two populations. Close to 430,000 SNP sites were detected, out of which approximately 3,000 were considered reliable, and were used for subsequent analysis.

Phylogenetic analysis based on SNPs showed that the two populations are clearly separated from one another, indicating that two populations have genetically differentiated after several generations of captive breeding. We compared allele frequencies between populations at each locus, and found significant divergence at 150 sites. A subset of these sites are likely to be linked to regions associated with the growth phenotype, and will be available as genetic markers for the improvement of the breed. This work was supported by AFFRC, Japan.

Keywords: coho salmon, artificial selection, body size, ddRAD-seq, marker assisted selection

[§]Corresponding author. Tel.: +81-53-592-2821; Fax: +81-53-592-2822
E-mail address: ahosoya@mail.ecc.u-tokyo.ac.jp

BARRA GONE WILD: INVESTIGATING THE PREVALENCE AND GENETIC RISK OF FARMED BARRAMUNDI *Lates calcarifer* ESCAPEES IN NORTHERN AUSTRALIA

Noble T.H.[a,b*§], Smith-Keune C.[a*], Jerry D.R.[a,b*]

[a] *College of Marine and Environmental Sciences, James Cook University, Townsville, 4811, Australia*
[b] *Centre for Sustainable Tropical Fisheries and Aquaculture, James Cook University, Townsville, 4811, Australia*

Expansion of sea-cage aquaculture has increased incidences of large-scale escapes of farmed fish. Aquaculture populations commonly differ in genetic profile from wild populations due to few broodstock being used to produce progeny and artificial selection pressures imposed by the culture environment. When mixed with wild populations, farmed fish can significantly affect the genetic profile of the natural population.

Escape events have been well-characterised for temperate fishes, but few studies have reported the occurrence and persistence of tropical fish escapees from aquaculture farms. In 2011 a cyclone destroyed a large barramundi, *Lates calcarifer*, sea-cage facility in the Hinchinbrook region, Queensland, releasing ~280 tonne of fish. One year after the escape event genetic samples were collected from 403 barramundi within Hinchinbrook Channel. Fish were assigned as farm escapees or wild by matching multi-locus microsatellite genotypes to the hatchery broodstock used to produce progeny stocked into the sea cages. DNA parentage analyses confirmed escaped barramundi had become established in the Hinchinbrook population, with 31% of fish sampled of farm origin. A single male-female pairing accounted for 32% of these escapees. Lower levels of genetic variation, high relatedness, and deviations from Hardy-Weinberg expectations were evident among escaped fish. These genetic differences raised concerns over escapee fish introgessing into the wild populationand affecting the genetic integrity and level of relatedness of the receiving population long term. Results demonstrate that escaped farmed barramundi can survive and integrate into local wild populations. Given the high prevalence of escapee fish in the Hinchinbrook Channel and the high relatedness among escapee fish, it will be important to monitor any genetic changes in this population into the future so as to detect any deterimental consequences that might arise due to inbreeding and reductions in fitness.

Keywords: escapee, sea-cage aquaculture, parentage, genetic impact, Lates calcarifer

*These authors have contributed equally to this work
§ Corresponding author. Tel.: +6147005969
E-mail address: +tansyn.noble@my.jcu.edu.au

METHODOLOGY FOR ERADICATION OF INVASIVE ALIEN SPECIES. A NEW METHOD OF GENE-INDUCED SUPPRESSION FOR ALIEN POPULATION (GISAP), AND THE FIRST WHOLE-GENOME ANALYSIS OF BLUEGILL (*Lepomis macrochirus*)

Okamoto H.[a*§], Kurita J.[c*§], Nishiki I.[b], Iwasaki Y.[b], Fujiwara A.[b], Yamaguchi Y.[a], Oku H.[c], Masaoka T.[c], Ishikawa T.[a], Kawamura K.[d]

[a]*National Research Institute of Aquaculture, FRA, Kamiura, Saiki, Oita 879-2602, Japan*
[b]*National Research Institute of Fisheries Science, FRA, Yokohama, Kanagawa 236-8648, Japan*
[c]*National Research Institute of Aquaculture, FRA, Tamaki, Watarai, Mie 519-0423, Japan*
[d]*Faculty of BioResources, Mie University, 1577 Kurimamachiya, Mie 514-8507, Japan*

As invasive species, terrestrial or aquatic animals and plants can lead to social and environmental problems all over the world. They can cause the extinction of endemic or endangered species and the destruction of indigenous ecological systems. In particular, damage of inland aquatic ecosystems can severely impact fresh water fisheries sustained by domestic species. In fresh water ecosystems of Japan, some of the most problematic alien species are the bluegill (*Lepomis macrochirus*) and largemouth bass (*Micropterus salmoides*). The bluegill population is estimated to be much greater than that of largemouth bass. Because of the presence of indigenous species, large lakes or ponds cannot be drained for the purposes of exterminating the invasive fish. Therefore, current management involves trying to catch the fish in nets, cages or electric devices, as well as destroying spawning nests, which incurs great physical and economic cost every year. Further, while these current methods make it possible to maintain the alien population at low levels, they do not result in total eradication. We propose here a new method for eradicating invasive alien species that compliments the current strategy of physical capture. It comprises three development steps: 1) production of sex-specific sterile fish, particularly male carriers of female-specific sterile genes, 2) multiplication of sterile-gene carrier fish, 3) tests for planting sex-specific sterile fish in a lake, pond or river, and monitoring the population size of alien species. The most important aim of this method is to reduce the number of fertile females by using male fish to carry female-specific sterile genes into the population, and thus eliminating the capacity for reproduction. The eradication method of "gene-induced suppression for alien population" will suppress fertile genes by replacing them with sterile genes in the population, leading to reduction in the number of fertile females and ultimately suppressing reproduction of the entire population. We report the simulation of gene-induced suppression for alien population and the first genome analysis of bluegill.

*These authors have contributed equally to this work
§ Corresponding author: Tel.: +81-972-32-2125; Fax: +81-972-32-2293
E-mail address: hiokamot@affrc.go.jp, kuritaj@affrc.go.jp

GENETIC EVALUATION OF WILD POPULATIONS OF THE MIGRATORY FISH PIAU (*Leporinus obtusidens*) AND A CAPTIVE BROODSTOCK IN A RESTOCKING PROGRAM IN BRAZIL

Karine K.C.[1], Ana P.S.[1], Maria R.M.[1]

[1] *Departamento de Pesca e Aquicultura,Universidade Federal Rural de Pernambuco, Rua Dom Manuel de Medeiros, s/n– Dois Irmãos, Recife-PE, Brazil, 52171-900, email: raquel@depaq.ufrpe.br.*

The *Leporinus obtusidens* is a large migratory fish, being an important resource that inhabits large South American basins. Due to the river fragmentation, overfishing and the introduction of exotic species, their populations are not considered stable anymore, especially in the sub middle and lower stretches of the São Francisco River basin, which hires the second most important hydroelectric plant complex in Brazil. A restocking program has been developed, however, hatchery supplementation programs consisting in the release of fingerlings into dams and rivers need to be aware of the risks related to the introduction of a large number of unrepresentative genotypes from captive broodstocks. In order to investigate the feasibility of a single restocking program to provide fingerlings to the most critical stretches of this river, ten microsatellite markers were genotyped in six wild populations collected during spawning season and a captive stock was also sampled. Average observed (H_o) and expected heterozygosities (H_e) varied from 0.647 to 0.823 and from 0.718 to 0.840, respectively. Hardy-Weinberg deviations corrected by Bonferroni were significant for the majority of the loci (7/10) in the captive population and for some isolated cases in the wild populations. Inbreeding coefficient was 0.09 in the captive broodstock and effective population size (N_e) was 33, a critical number considering the 50/500 rule. The overall genetic divergence (F_{ST}) among wild populations was low suggesting an intense gene flow, however, the highest value occurred between the populations from the upper and lower São Francisco River basin. On the other hand, pairwise values between wild populations and captive broodstock was higher than 0.10 in all cases. The Bayesian clustering technique confirmed the absence of structure, also suggesting that the most contrasting populations are those from the upper and lower stretches. Most of the private alleles (6/19) were observed in the upper population followed by middle one, while submiddle, lower and captive broodstock had no more than one private allele. We discuss the implications of using this captive broodstock to restocking other stretches rather than submiddle and lower river basin.

IMPROVEMENT OF A MARKER-ASSISTED BREEDING SYSTEM TO INCREASE THE PROPORTION OF NATIVE ALLELES IN A HUNGARIAN BROWN TROUT BROODSTOCK

Ősz Á.[a,*,§], Kovács B.[a,*], Kánainé Sipos D.[a], Hoitsy Gy.[b], Snoj A.[c], Urbányi B.[a], Horváth Á.[a,*]

[a] *Department of Aquaculture, Szent István University, Gödöllő, 2100, Hungary*
[b] *Hoitsy & Rieger Kft., Lillafüred, 3517, Hungary*
[c] *Department of Animal Science, University of Ljubljana, Domzale, 1230, Slovenia*

According to the analyses of the control region of mitochondrial DNA, five evolutionary lineages were identified among European brown trout *(Salmo trutta m. fario)* populations (Atlantic, Danubian, Mediterranean, Adriatic, Marble). Further studies have revealed that most European broodstocks have originated from the Atlantic lineage, additionally because of the stocking of several natural populations hybridization and introgression with Atlantic lineage have occurred.-

Due to the hydrogeography of Hungary, brown trout populations should theoretically belong to the Danubian lineage, however our results have showed that hybrids with a high share of Atlantic alleles have dominated in almost every investigated population, both natural and farmed stocks. Our current study includes two broodstocks in Hungary as well as six natural populations. Eight published molecular markers (three PCR-RFLP and five microsatellite markers) have been used to distinguish the lineages of the brown trout.

After genotyping, a marker assisted breeding system has been developed for the largest Hungarian brown trout broodstock (Lillafüred) in order to increase the proportion of native alleles through generations in case of these loci. The system consists of several components. Fish are tagged with PIT tags and an alias is assigned to each PIT number for easier identification. The second component is a scoring system that was developed based on the share of the Danubian genotype and private alleles in the population by prioritizing these over the Atlantic genotype and more common alleles. Finally, the score is linked to the alias in the PIT tag reader, thus the information is available to the farmer, whenever he scans the PIT tag of the fish. This allows quick selection of individuals for spawning.

After the introduction of our system, the proportion of the Danubian genotype has increased from 20-40% to 50-60% in the F1 generation that originated from selected parents. Thus, an efficient selection system was created in Lillafüred to establish a native brown trout broodstock in case of these loci.

Keywords: Salmo trutta, lineages, mitochondrial DNA, marker assisted breeding system, microsatellite

*These authors have contributed equally to this work.

§ Corresponding author. Tel.: + 36 28 522 000/1611; Fax: + 06 28 522-927
E-mail address: Osz.Agnes@mkk.szie.hu

GENETIC EVALUATION OF HATCHERY-PRODUCED SEED AND CANDIDATE BEDS FOR STOCK ENHANCEMENT PROGRAMMES IN THE CLAM *Venerupis pullastra*

Pereira S.[a], Freire R.[a], Arias A.[a], Cerviño A.[b], Ojea J.[b], Martínez D.[b], Méndez J.[a], Insua A.[a, §]

[a] Bioloxía Celular e Molecular, Universidade da Coruña, A Coruña, 15071, Spain
[b] Centro de Cultivos Mariños, Xunta de Galicia, Ribadeo, 27700, Spain

Commercially important shellfish populations are often supplemented with hatchery-reared seed to increase their production. However, this practice may affect the genetic diversity, effective population size and fitness of wild populations due to the potentially different genetic background of the introduced seed. In this study, microsatellite markers, coupled with DNA parentage analysis, were used to monitor the level of genetic diversity of a hatchery mass spawning event of *Venerupis pullastra*, a clam with high commercial value in the Atlantic coast of the Iberian Peninsula. The level of genetic differentiation between seed, broodstock and wild samples was also assessed to know whether the broodstock was genetically representative of the wild population of origin and to characterize candidate beds for stock enhancement programmes. Samples, were collected in Galicia (northwest Spain) where *V. pullastra* is intensively exploited, and in south Portugal, where its abundance has declined dramatically due to overfishing. A Galician broodstock, with 139 individuals and balanced sex proportions (67 females and 72 males), and 150 hatchery-produced seeds were analysed. The effective population size was 40% of broodstock census size mainly because not all spawners contributed to the seed. Allelic richness and observed and expected heterozigosity of the hatchery-reared seed, although lower, were not significant different from that computed for breeders. No significant genetic differentiation was found between broodstock and one sample collected previously at the same population, but significant F_{ST} values were found when hatchery-produced seed was compared with each tested sample including the broodstock. The genetic differentiation of the seed was low (F_{ST}<5%) with respect to samples from northwest Spain and high (F_{ST}>20%) with respect to south Portugal due in part to the existence of genetic differences among wild populations but also to the genetic composition of the seed The hatchery seed production of *V. pullastra* does not seem to compromise seriously the genetic diversity but improvements in the hatchery practices are still required. Moreover, to minimize the genetic impact of population enhancement programmes in the area studied the spatial genetic structure of this species should not be ignored.

Keywords: Venerupis pullastra, microsatellites, genetic diversity, parentage, genetic differentiation.

[§] Corresponding author. Tel.: 34881012138; Fax: 34981167065
E-mail address: insuax@udc.es

IS THERE A GENETIC TRACE OF SHRIMP AQUACULTURE ON WILD POPULATIONS IN MEXICO?

Pérez-Enríquez R.[a, §], Medina-Espinoza A.J.[a], Millán-Márquez A.M.[a], Cruz-Hernández P.[a], Saucedo-Barrón C.J.[b]

[a] *Aquaculture Program, Centro de Investigaciones Biológicas del Noroeste S.C., La Paz, 23096, Mexico*
[b] *Instituto Sinaloense de Acuacultura y Pesca, Culiacán, 80129, Mexico*

It has been recognized that the release of aquaculture stocks may impact the genetic composition and variability of wild populations, resulting diverse issues that may compromise their long term fitness. Some of these issues are: 1) the reduction of the genetic diversity and increase of inbreeding; 2) the homogenization of the genetic structure; 3) the displacement of genes adapted to local conditions; and 4) the modification of adaptability by domestication. In Mexican shrimp aquaculture, inadvertent release of large amounts of individuals to the wild may occur from farms with earthen ponds during harvesting or natural phenomena such as hurricanes or from hatcheries during the larvae production period. In this study we genetically characterized a wild population of whiteleg shrimp *Litopenaeus vannamei* from the state of Sinaloa. We obtained samples from 2011 from which allele frequencies and genetic diversity parameters (number of alleles per locus, heterozygosity) were calculated using previously reported microsatellites. The analysis on 10 sites of Sinaloa showed a mean genetic diversity of 15.6 alleles per locus and 0.69 observed heterozygosity. Deviations from HWE were observed at most locations. To test whether these deviations are a result of admixture with aquaculture stocks, we will compare the genetic profiles of archive samples from the wild (2001) to recent wild (2011-2012) and hatchery samples (2007). A new set of microsatellite markers (mean number of alleles per locus 11.8 (range 5-20), and 0.836 expected heterozygosity (range 0.69-0.91)) obtained by NGS was developed for this purpose.

Keywords: genetic diversity, genetic impact, inbreeding, release, Litopenaeus vannamei

[§]Corresponding author. Tel.: + 52-612-1238504; E-mail address: rperez@cibnor.mx;

GENETIC DIFFERENTIATION BETWEEN TWO SILURIFORM SPECIES AND HYBRIDIZATION INVESTIGATIONS IN SOUTH AMERICAN RIVERS: CONTRIBUTIONS FOR AQUACULTURE AND CONSERVATION

Prado F.D.[a, §, *], Fernández-Cebrián R.[b, *], Martínez P.[c, *], Senhorini J.A.[d, *], Foresti F.[e, *], Porto-Foresti F.[a, *]

[a] *Biology Department, São Paulo State University (UNESP), Bauru, 17.033-360, Brazil*
[b] *Geneaqua S. L. C/ Primavera, Nº 40, 1ºB. E-27002 Lugo, Spain*
[c] *Genetic Department, University of Santiago de Compostela, Lugo, 27.002, Spain*
[d] *Laboratory of Genetics and Reproduction, (CEPTA/IBAMA), Pirassununga, 13.630-970, Brazil*
[e] *Morfology Department, São Paulo State University (UNESP), Botucatu, 18.600-000, Brazil*

Neotropical catfishes *Pseudoplatystoma reticulatum* and *P. corruscans* are endemic Siluriforms of South American river basins. However, in recent decades, the occurrence of hybrids and genetic introgression in the wild have increased, mainly resulting from escapes and introduction of hybrids produced in aquaculture. This contamination can lead to irreversible ecological and genetic modifications and also affect aquaculture, since pure broodstock samples are usually obtained in the natural environment. This study aimed to identify the occurrence of hybrids and genetic introgresion in sympatric (Paraguai, Cuiabá, Taquari, Negro, Aquidauana, Miranda and Paraná Rivers) and alopatric populations of these species (only *P. corruscans* - Verde, Paranapanema and Mogi-Guaçu) from La Plata basin (Brazil and Argentina) using eight microsatellite markers and 394 individuals. With exception of Aquidauana and Mogi-Guaçu, all populations presented no signals of hybridization. Considering all loci, high allelic differences was verified between species: from 94 allelles observed for *P.reticulatum* and 150 for *P. corruscans*, only 24 was shared; the average Fst between species was of 0.364; genotype assignments clearly identified two groups, corresponding to *P. reticulatum* (average q_1=0.995) and *P. corruscans* (average q_2=0.995). In Aquidauana and Mogi-Guaçu populations,alleles from both parental species occurred in the gene pool, indicating the presence of hybrids. Additionally, Aquidauana presented heterozigote excess for 3 loci (P<0.006), genotype assignment of q_1=0.911 and q_2=0.089 and linkage disequilibrium involving 5 loci, suggesting latter genetic introgresion events with *P. reticulatum*. In Mogi-Guaçu, five loci showed deviations from Hardy-Weinberg equilibrium (P<0.006), linkage disequilibrium was verified among all loci (P<0.006), and the average genotype assignment was of q_1=0.472 and q_2=0.528, indicating the presence of F1 hybrids and more recent hybridization events or escapes. These results demonstrated that the contamination of aquatic environments by hybrids is an alarming reality, even in alopatric populations, confirming that escapes and introductions of hybrids from aquaculture are the most probable hypotesis for the presence of hybrids in the nature. Otherwise, the identification of sites without hybrids may be useful as a source of pure broodstock individuals for aquaculture, the establishment of genetic banks and priority conservation programs.

Keywords: Pseudoplatystoma, Neotropical catfishes, genetic introgression, microsatellites, management, genetic conservation

*These authors have contributed equally to this work.
§ Corresponding author. Tel.: +551431036078; Fax: + 55143103-6092;
E-mail address: + prado.fernandad@gmail.com

FORENSIC IDENTIFICATION BY DNA BARCODE TECHNIQUE OF SHARK SPECIES COMMERCIALLY EXPLOITED BY THE FISHING FLEETS OF THE COAST OF SÃO PAULO, BRAZIL

Ramos M.G.[a][§], Prado F.D.[a], Foresti F.[b], Porto-Foresti F.[a]

[a] *Biology Department, São Paulo State University (UNESP), Bauru, 17.033-360, Brazil*
[b] *Morfology Department, São Paulo State University (UNESP), Botucatu, 18.600-000, Brazil*

Sharks and rays form the group of elasmobranchs and are among the largest vertebrate evolutionary success. They are found in a wide variety of habitats, including inland waters, coastal, estuarine, open sea and even in the deep ocean regions. The fisheries exploitation without control currently represents the biggest threat to these populations, and some species are at serious risk of overfishing, many even threatened with extinction. This study aimed to identify the species caught by fishing fleets of the coast of the state of São Paulo, Brazil, working with the monitoring of artisanal and industrial fishing sharks in the main points of fishing landings. Also to verify the reliability of statistical data, which are not always accurate. This is due to the rather usual practice of removing the head and fins, most of the time even before landing, which prevented the morphological identification of animals, resulting in a global shortage of information about capturing and trading, making it almost impossible to evaluate their effects. Genetically, 271 samples were analyzed, collected in 2008, 2009 and 2014 in the cities of Santos, Cananéia and Ubatuba. These samples were identified using genetic methods, which consisted of amplification of the mitochondrial gene cytochrome c oxidase subunit I (COI) (universal genetic marker for identification of species of animals) and sequencing in an automatic sequencer. It was later made the analysis and editing of sequences in *Geneious 4.8.5* program and compared on the gene bank online BOLD *System*. It was possible to identify 242 individuals, with values greater than 98% similarity to sequences previously deposited in the BOLD. Among these samples, 13 species were identified, the most collected: *Rhizoprionodon lalandii* (86 individuals), *Prionace glauca* (43 individuals), *Sphyrna lewini* (44 individuals), *Squalus cubensis* (28 individuals), *Carcharhinus brevipinna* (14 individuals), *Sphyrna Zygaena* (10 subjects) and 16 individuals of other species. These results demonstrated that there are marketing endangered species by CITES, such as *S. lewini* and *S. zygaena*. Therefore this genetic analysis is essential to propose a management plan and conservation of these species that are fundamental to the maintenance and protection of marine biodiversity.

Keywords: sharks, Bold System, endangered species, extinction, molecular makers.

*These authors have contributed equally to this work.
[§] Corresponding author. Tel.: +55 19 997815190; Fax: + 55 14 31036092
E-mail address: maice_ramos@hotmail.com

THE IMPACTS OF ARTIFICIAL HYBRIDISATION IN BRAZILIAN AQUACULTURE

Valentim A.L.ᵃ§, Matins D.G.ᵃ, Prado F.D.ᵃ, Foresti F.ᵇ, Porto-Foresti F.ᵃ

ᵃ Biology Department, São Paulo State University (UNESP), Bauru, 17033-360, Brazil
ᵇ Morfology Department, São Paulo State University (UNESP), Botucatu, 18.600-000, Brazil

The species *Pseudoplatystoma corruscans* (pintado) and *Pseudoplatystoma reticulatum* (cachara) are found in freshwater, have important commercial characteristics and due to the high demand of these individuals, frequently the fish farmers produce hybrid lines. These study aimed to study the purity of a breading stock of these species derived from the fish farmer "Pirajuba Aquicultura". The samples were identified through morphological characteristics by the fish farmer, classifying them as pintado, cachara and hybrid. For obtaining all the genomic DNA, were removed small fragments of the fin, using the Wizard Genomic DNA Purification Kit (Promega, WI). All experiments included one DNA sample from each pure parental species of pintado and cachara as control reactions. To identify the species of the processed samples, PCR techniques were applied to examine the regions of nuclear β-globin (GLOB), nuclear recombination-activating gene 2 (RAG2), elongation factor 1-alpha (EF1α), and mitochondrial 16S ribosomal DNA (rDNA) genes, generating diagnostic electrophoretic fragments for the species and their hybrids being studied. Multiplex PCR was performed for each of the genes listed. The results from the molecular analysis showed that the total number of individuals, nine were considered pintado, five cachara, three hybrids (F1) and one characterized as hybrid post-F1. Therefore, we observed slight divergence between morphological and molecular data, where 22,2% of individuals were identified inefficiently when considering only the morphology of animals. These hybrid fish are explored by the Brazilian aquaculture most of the times without an efficient management control and can be considered a great threat to the environment and the breading stock for putting the genetic integrity of the species at risk. Such molecular tools can be used for technical control of the production and commercialization of these fish species in hatcheries, the monitoring of hybridization among natural fish stocks, and in biological conservation programs involving these species. In this context, we can conclude that the morphological data can contribute to identification fishes siluriformes, but, so there is high reliability of results, is necessary to use other efficient methodologies, as proved molecular analysis.

Keywords: Pseudoplatystoma corruscans; Pseudoplatystoma reticulatum; hybrid; moleculars markers; PCR-Multiplex

*These authors have contributed equally to this work.
§Corresponding author: Aline Leão Valentim. Tel.: +55 14 32231560; Cel.: +55 14 981339396
Email address: aline_valentimm@hotmail.com

EFFECT OF GLYPHOSATE ON ZEBRAFISH (*Danio rerio*) LARVAE

Vasconcelos A.C.N.[a, *, §], Murgas L.D.S.[b, *], Streit Jr. D.P.[a, *]

[1]*Departament of Aquaculture, Federal University of Rio Grande do Sul, Porto Alegre, Post Code 91540-000, Brazil*
[2]*Departament of Veterinary Medicine, Federal University of Lavras, Lavras, Post code 37200-000, Brazil.*

Glyphosate is an herbicide widely used on the agricultural production in many countries. The products based on this compost are highly toxic to people and animals and many studies have shown that, in different organisms, this pesticide can affect the survival, fertility and genetic composition of populations (Benachour & Seralini, 2009; Gasnier *et al.*, 2009; Souza Filho *et al.*, 2013; Webster *et al.*, 2014; Lopes *et al.*, 2014). An experimental model that has stood out in the scientific area in fields as toxicology and teratogenicity is zebrafish (*Danio rerio*) (Kari *et al.*, 2007). This is mainly due to its sequenced genome and the genetic similarity with humans. This study examined the influence of exposure of zebrafish embryos to different concentrations of glyphosate (0, 5, 35, 65 and 95 µL/L) during embryonic development. We used a completely randomized design with four replicates. In each replicate 120 embryos were distributed individually in containers with a total capacity of 0.5 mL. Glyphosate was previously added to water and the embryos were exposed to this environment from the early embryonic development until complete 96 hours after fertilization. The survival rates, larval size, malformation and epidermal pigmentation were checked at the end of the experiment. The glyphosate addition reduced the length larval, survival rate and caused malformations and depigmentation of the larva treated with concentrations of 65 and 95 µL/L. According Paganelli *et al.* (2010) glyphosate causes an increase in the activity of retinoic acid in the early hours of development. This acid controls the growth pattern of the early stages of the animal and any change in its quantity, modify the enzymatic metabolism, thus causing, embryonic malformation (Niederreither & Dollé, 2008). The depigmentation can be explained by impaired antioxidative activity of some cells caused by glyphosate (Heu *et al.*, 2012), making this product able to cause a discoloration of the pigment melanin since it is susceptible to bleaching by oxidizing agents. These results clearly show that at certain concentrations, glyphosate causes morphological changes in embryos, confirming its potential risk to human health, since it is possible to extrapolate the results.

Embryos. Herbicide. Teratogenic effect. Toxicity.

[*] These authors have contributed equally to this work.
[§] Corresponding author. Tel.: + 553788241259
E-mail address: anacarina.vasconcelos@hotmail.com

PATHOGEN-SPECIFIC TRADE-OFFS IN HOST TOLERANCE AND SUSCEPTIBILITY PHENOTYPES

Antczak P.§, **Williams D.***, **Olohan L.***, **Kay S.***, **Fang Y.***, **Gregory R.***, **King K.***, **McAndrew B.*****, **Dixon P.****, **Jeney G.**¶**, Falciani F.**§, **Jeney Z.**¶**, and Cossins A.R.***

Centre for Genomic Research, University of Liverpool, Liverpool L69 3BX, UK
§ Centre for Computational Biology and Modelling, Institute of Integrative
Biology, University of Liverpool, Liverpool L69 3BX, UK
¶ HAKI, Szarvas, 5540 Hungary
**** Institute of Aquaculture, University of Stirling, FK9 4LA Scotland, UK*
*** CEFAS, Weymouth Laboratory, Weymouth Dorset DT4 8UB, UK*

Host responses to virulent pathogens can either reduce the burden of invading pathogens (resistance) or reduce the wider negative effects of pathogens on host fitness (tolerance). Whilst the immune basis of resistance is well understood, the mechanistic basis of tolerance is unexplored. Using cDNA microarrays, and head kidney preparations, we have compared the genome-wide gene responses of 6-fold replicated tolerant (T) and susceptible (S) families of carp exposed to either viral (koi herpes virus, CyHV-3) or bacterial (*Aeromonas* sp.) pathogens. T/S contrasts revealed over 900 responding genes, of which 189 were evident in both pathogen challenges (FDR<0.01). T/S differences in infected animals were particularly evident for down-regulated genes, and this applied to genes of protein synthesis and turnover, and immune function. For the bacterial infection we noted a distinctive up-regulation of haemoglobin (Hb) gene probes in T families but the reverse for S. By contrast, viral challenge Hb transcripts were more downregulated in S than in T families. This is consistent with divergent responses between T/S and between pathogens in blood flow or haematopoesis. Direct comparison of uninfected controls for the two pathogens showed inverse T/S ratios indicating that genetic selection favours very different gene abundance ratios. Thus, the host tolerance phenotype involves a wide range of molecular responses, but selection by the two pathogens induced very different tolerance phenotypes in hosts, which likely reflect distinctive trade-offs between tolerance to other energetic traits. Finally we show that gene responses discovered here can be used to predict to 90% the T/S status of individual specimens, in genetic selection experiments.

Keywords: Disease tolerance, bacteria, virus, gene responses

NUTRITIONAL REGULATION NEUROPEPTIDE Y EXPRESSION IN BRAIN OF GILTHEAD SEA BREAM (*Sparus aurata*)

Babaei S.[a], Sáez A.[b], Caballero-Solares A.[c], Metón I.[b], Fernández F.[c], Baanante I.V.[b, §]

[a]Fisheries Departament, Faculty of Marine Sciences, Tarbiat Modares University (TMU), Noor, Mazandaran, 64414-356, Iran
[b]Departament de Bioquímica i Biologia Molecular, Facultat de Farmàcia, Universitat de Barcelona, Joan XXIII 27-31, Barcelona, 08028, Spain
[c]Departament d'Ecologia, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, Barcelona,08028, Spain

Endocrine systems are well known as primary regulators of growth in vertebrates. Neuropeptide Y (NPY), a 36-amino acid peptide, regulates a variety of physiological functions. Among the brain orexigenic signaling molecules, NPY is the most powerful central enhancer of appetite and has a key role to stimulate food intake in vertebrates. Expression of NPY is mostly distributed in the central nervous system. Examination of the role of dietary conditions in regulating endocrine activity in fish may provide practical information on the physiological consequences of alterations in diet composition and dietary conditions and evolution of the regulation of hormone production in vertebrates. In the present study, we analyzed the effect of diet composition on NPY gene expression in the brain of gilthead sea bream (*Sparus aurata*). During 37 days two groups of fish were fed *ad libitum* with either a high protein/low carbohydrate (HLL) or a low protein/high carbohydrate (LLH) diet. Another group of fish was submitted to starvation for the same time period. A 68 bp nucleotide fragment corresponding to *S. aurata* NPY mRNA was isolated by RT-PCR using primers designed from conserved regions for NPY in other vertebrates, and total RNA isolated from brain of *S. aurata*. Oligonucleotides designed from the 68 bp sequence of *S. aurata* NPY were used for subsequent quantitative real-time RT-PCR assays performed on RNA isolated from brain samples of the experimental fish. Starvation increased NPY mRNA levels in brain samples compared to fed fish. Among fed fish, those supplied with HLL diet exhibited the lowest NPY expression levels. Our findings suggest that starvation and low efficient diets promote an increase in NPY expression in the brain of *S. aurata*.

§ Corresponding author. Tel.: +34 934024521; Fax: +34 934024520;
E-mail address: baanantevazquez@ub.edu

IDENTIFICATION OF TWO FERRITIN SUBUNIT GENES FROM *Haliotis rufescens* AND ANALYSIS OF THEIR EXPRESSION PATTERNS DURING EARLY DEVELOPMENT, GROWTH AND PATHOGEN CHALLENGE

Cárcamo C.[a], Coba de la Peña T.[b], Brokordt B.[a], Winkler F.[a][§]

[a]*Laboratorio de Fisiología y Genética Marina (FIGEMA), Centro de Estudios Avanzados en Zonas Áridas & Departamento Biología Marina, Universidad Católica del Norte, Coquimbo 1781421, Chile*
[b]*Instituto de Ciencias Agrarias, CSIC, Madrid 28006, Spain*

Ferritin is a ubiquitous protein that plays a pivotal role in iron homeostasis. Ferritin was described to be involved in several physiological processes in mollusks, such us growth, shell formation and immunity. In this study, we characterized two ferritin homologs in the red abalone *Haliotis rufescens*, a mollusk species of importance for aquaculture purposes in northern Chile and that is highly valued in the international market. Two ferritin subunits (*Hrfer1* and *Hrfer2*) were cloned from *H. rufescens* by rapid amplification of cDNA ends (RACE). *Hrfer1* cDNA is a 807-bp clone containing a 515–bp partial-open reading frame (ORF) that corresponded to a novel ferritin subunit in *H. rufescens*. *Hrfer2* cDNA was a 711-bp full-length clone containing a 516-bp ORF that corresponded to a previously reported ferritin subunit. A putative iron responsive element (IRE) was identified on the 5'-untranslated region of *Hrfer2*. The deduced protein sequences possessed the domains characteristic of known ferritin subunits. *Hrfer1* exhibited 65% sequence identity with *Hrfer2* and both genes shared 99% identity with ferritin subunits of *H. discus hannai*. Gene expression analyses in adult tissues using quantitative real-time PCR revealed that expression level of *Hrfer1* was much higher in gonad and digestive gland than in other organs. Expression of *Hrfer2* was highest in these two organs and in gills. *Hrfer1* and *Hrfer2* expression in mantle tissue was significantly higher in fast-growing than in slow-growing individuals. Expression of both genes also increased significantly during larval development (6h, 24h, 72h and 6 days after fertilization) and this increment was higher in *Hrfer1* than in *Hrfer2*. Both ferritin genes also showed augmented expression in hemocytes after challenge with the bacterium *Vibrio splendidus*, although with different profiles and magnitudes. *Hrfer1* expression was induced 2, 12 and 24 h after bacterial challenge. The highest induction was observed at 12 h. *Hrfer2* expression was induced from 2 to 12 h after challenge. These results suggest that both *Hrfer1* and *Hrfer2* are likely to play important roles in several fundamental biological processes in *H. rufescens*, including iron homeostasis, growth, early development, shell formation and immune defense against bacterial infections.

Keywords: Ferritin, Haliotis, growth, development, immunity

[§] Corresponding author. Tel.: 56-512209794; Fax: 56-512209814   ;
E-mail address: fwinkler@ucn.cl   ;

DIFFERENTIAL EXPRESSION OF *Salmo salar* CONSIDERING RESISTANCE AGAINST *Piscirickettsia salmonis*, USING THREE STATISTICAL METHODS

Dettleff P., Martínez V.

FAVET-INBIOGEN, Faculty of Veterinary Science, University of Chile, Santiago, Post code 8820808, Chile.

Piscirickettsia salmonis generate an aggressive bacterial disease that has caused major economic losses in the aquaculture industry of Atlantic salmon. However, the studies related with resistance to this bacteria are scarce. In this study, we perform a RNA-seq analysis of the response to *P. salmonis* of fish belonging to families with different resistance background, on head kidney and liver, two key target of the dissease. We perform a *de novo* assembly of the transcriptome of head kidney and liver using Trinity, obtaining a total of 16,614 annotated genes, with 81,791 GO terms. For the differential expression analysis between susceptible and resistant group in each tissue, we applied three different statistical methods: edgeR, DESeq and baySeq. We deffine as differentially expressed those genes that were detected in at least two of the three algorithms (FDR<0.01). The comparison of genes detected by DESeq, baySeq and edgeR showed a final list of 230 differentially expressed genes in at least two algorithms on head kidney, with 61% increased on susceptibles and 39% on resistant. In the case of liver, a final list of 669 differentially expressed genes in at least two algoristm was detected, with 72% of the increased on susceptible, while the 28% was increased in resistant. When compare head kidney and liver, a total of 46 genes were differentially expressed in both tissues, showing a tissue-specific response. Among the differentially expressed genes on each tissue, we found genes related with oxidative stress; regulation of apoptosis; pathogen recognition; oxygen transport; iron transport; inflamatory response; complement system and acuthe phase response. An exacerbated transcriptional response was observed on susceptibles, with an acute phase response increased. However, these response is not protective, generating uncontrolled inflammatory response that produces tissue damage. The resistant individuals present a controlled response, that could be more efficient in eliminating the bacteria, together with protect the cells of the oxidative damage, allowing to survive and decrease the bacterial load. This study shows how the differential responses to the bacteria affect the survival, providing a better understanding of disease resistance on Atlantic salmon.

Keywords: salmon, Piscirickettsia salmonis, disease resistance, RNA-seq

COMPARATIVE GENE EXPRESSION AND HISTOLOGICAL ANALYSIS OF SKIN RESPONSE TO INJURY IN TWO CONGENERIC FLATFISH WITH STRIKING SKIN MORPHOLOGICAL DIFFERENCES, THE BRILL (*Scophthalmus rhombus*) AND THE TURBOT (*Scophthalmus maximus*)

Estêvão J.[a,b][§], Millan A.[b], Pardo B.G.[b], Fernández C.[b], Rubiolo J.[b], Herrera M.[c], Gómez-Tato A.[d], Ronza P. [b,e], Cabaleiro S.[f], Quiroga M.I.[e], Power D.M.[a], Martínez P.[b]

[a] *Centre for Marine Sciences (CCMAR), Universidade do Algarve, Campus de Gambelas, 8005-139 Faro, Portugal*
[b] *Departamento de Genética, Facultad de Veterinaria, Universidad de Santiago de Compostela, Campus de Lugo, 27002 Lugo, Spain*
[c] *IFAPA Centro Agua del Pino, Cartaya, 21459 Huelva,Spain*
[d] *Departamento de Geometría y Topología, Facultad de Matemáticas, Universidad de Santiago de Compostela, 15782 Santiago de Compostela, Spain*
[e] *Departamento de Ciencias Clínicas Veterinarias, Universidad de Santiago de Compostela, 27002 Lugo, Spain*
[f] *Cluster de la Acuicultura de Galicia (CETGA), Punta de Couso s/n, 15965, Aguiño (Ribeira), A Coruña, Spain*

The skin, the largest organ of fish's body and primary barrier against external agents and plays a crucial role in protecting against environmental aggressions and is decisive in communication between individuals. Cutaneous lesions are common in fish and create an open door for entry of infectious agents and can create osmotic stress, which can be life threatening. Turbot (*Scophthalmus maximus*) and brill (*Scophthalmus rhombus*) are two closely related congeneric species that have striking differences in their skin, since turbot has tubercles and brill has elasmoid scales. In this work, histological and gene expression analyses were performed in order to analyze the morphological and genetic differences in the skin response between the two species. For that, skin scraping areas (72h after injury) from three biological replicates of each species were compared with normal skin areas from the same individuals. A histological examination was carried out in both species to evaluate the lesional pattern and correlate it to the gene expression data. For the gene expression analysis in damaged skin from turbot and brill, a previously reported 4x44k Agilent turbot microarray was employed, after evaluating its suitability for brill by preliminary bioinformatics analysis. Skin-related genes found in previous studies (1564 sequences) were taken as a reference to screen the turbot database and the oligo-probes in the microarray. Among them, 584 (~37%) were present in the turbot database (E-value < $9x10^{-20}$) and 326 (~21%) contained oligo-probe in the microarray, a notable result considering that the database was especially enriched with immune-related organs. Genes with significant fluorescent signal in the microarray (>200 fluorescence units) were identified within species (turbot: 17217; brill: 13556) and between species (11038). Among them, 1803 differentially expressed genes (t-test P-value p<0.005; FC <-2 and >2) were detected in brill, 1511 in turbot, and 955 showed differences between species, respectively. Our results strongly suggest the suitability of the turbot microarray for gene expression analysis in brill and show remarkable differences in skin response and regeneration to injury between the two species.

Keywords: Skin, Brill, Turbot, Oligo-microarray, Skin histology

[§] Corresponding author. Tel.: + 351 969605603; E-mail address: a39292@ualg.pt

DIFFERENTIALLY EXPRESSED GENES IN *IN VITRO* STIMULATED *Ruditapes philippinarum*-HAEMOCYTE AND *Perkinsus olseni*-TROPHOZOITE: UNVEILING HOST-PARASITE INTERACTION IN PERKINSOSIS

Hasanuzzaman A.F.M.[a, b, *], Robledo D.[a, *], Gómez-Tato A.[c], Álvarez-Dios J.A.[d], Harrison P.[e], Fernández-Boo S.[f], Cao A.[f], Villalba A.[f], Pardo B.G.[a, *], Martínez P.[a, §]

[a]*Departamento de Genética, Universidad de Santiago de Compostela, Lugo, 27002, Spain*
[b]*Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna, 9208, Bangladesh*
[c]*Dpto. de Geometría y Topología. Universidad de Santiago de Compostela, Santiago de Compostela, 15782, Spain*
[d]*Dpto. de Matemática Aplicada. Universidad de Santiago de Compostela, Santiago de Compostela, 15782, Spain*
[e]*Department of Genetics, Evolution and Environment. University College London, London, 1 WC1E 6BT, United Kingdom*
[f]*Centro de Investigacións Mariñas. Cons. Medio Rural e do Mar da Xunta de Galicia, Vilanova de Arousa, 36620, Spain*

Perkinsosis, caused by intracellular protozoan parasite *Perkinsus olseni*, is deadly hindering the production of Manila clam (*Ruditapes philippinarum*). Understanding the molecular mechanisms involved in *R. philippinarum-P. olseni* genetic interaction through transcriptomic analysis would aid to develop strategies for controlling this parasitosis. In this study, RNA samples from *in vitro* stimulated Manila clam-haemocytes by molecular signals from trophozoites, zoospores and extracellular products of *P. olseni* along a time-course experiment were obtained in three replicates per condition to study gene expression of host-parasite interaction. A RNA-seq Illumina lane was used to characterize their respective transcriptomes and to develop an oligo-microarray tool for gene expression analysis. Transcriptome were assembled using Trinity and CAP3, and a total of 33,182 and 47,590 unique transcripts were identified in the clam-haemocyte and *P. olseni*, respectively. From this information, two custom 8x15K Agilent oligo-microarrays were designed for the host and the parasite. Using RNA-seq data we performed a preliminary analysis of differentially expressed genes in host and parasite. Among annotated genes, clam-haemocytes showed 687 up-regulated and 2688 down-regulated. The high amount of immune-related down-regulated genes, including those related to innate response, like lectin, complement, interferon, and cytokine-related genes, suggests parasite-induced suppression of host-immunity. However, up-regulation of some key genes related to processes involved in antigen presentation, cell signaling, serine protease inhibition and peptidase activation in inflammatory cells demonstrates induction of host cell-mediated defence factors in response to signals from stimulated parasite-molecules. Regarding *P. olseni* trophozoites, we detected 682 up-regulated and 478 down-regulated annotated genes. Among up-regulated; proteases, antioxidants and hydrolases could be related to the process of virulence-mechanism, while others, down-regulated, like some proteinases, oxidoreductases and hydroxylases may be an indicator of pathogenicity-suppressing character of clam-haemocyte. Notably; substantial modulation (up and down) of several enzymes related to apoptosis, serine protease activity, lysosomal activity, hydrolase activity and ubiquitin-modification in stimulated haemocyte and parasite-trophozoite implicates molecular-response variation along the infection-progression. Such simultaneous analysis on gene-expression profiles in clam-haemocyte and trophozoite provides valuable information for further *in vitro* and *in vivo* study to understand the genetic basis of clam response to Perkinsosis.

Keywords: Manila clam, perkinsosis, host-parasite interaction, RNA-seq, differentially expressed genes

[*]These authors have contributed equally to this work. [§] Corresponding author. Tel.: +34982822428; Fax: +34982822428; E-mail address: paulino.martinez@usc.es

TRANSCRIPTOME CHARACTERIZATION AND SNP DISCOVERY IN SPECIES OF SERRASALMIDAE: GENETIC SUBSIDIES FOR THE BRAZILIAN AQUACULTURE

Hashimoto D.T.[a, §], Hata M.E.[a], Sato L.S.[a], Jorge P.H.[a], Mastrochirico-Filho V.A.[a], Martínez P.[b], Foresti F.[c], Porto-Foresti F.[d]

[a] *Center of Aquaculture, University of São Paulo State, Jaboticabal, 14884-900, Brazil*
[b] *Department of Genetics, University of Santiago de Compostela, Lugo, 27002, Spain*
[c] *Department of Morphology, University of São Paulo State, Botucatu, 18618-970, Brazil*
[d] *Department of Biology, University of São Paulo State, Bauru, 17033-360, Brazil*

Species of Serrasalmidae, pacu *Piaractus mesopotamicus*, pirapitinga *Piaractus brachypomus* and tambaqui *Colossoma macropomum*, represent the major group of native fish intended for local breeding programs in Brazil, due to their market acceptance (national and international) and highest levels of production. However, the genetic information available for these species is scarce. The present study was performed to sequence the liver transcriptome and characterize intra and interspecific SNPs (single-nucleotide polymorphisms) in pacu, pirapitinga and tambaqui, using the 454-Roche platform. The construction of cDNA libraries were obtained from the total RNA extracted of liver of 10 individuals for each species (collected from different fish farms and rivers in Brazil). In total, we obtained 277,596, 212,813 and 192,373 sequences for *C. macropomum*, *P. mesopotamicus* and *P. brachypomus*, respectively; with average size of 616 bp. Bioinformatic analyzes (software CLC Genomics Workbench) allowed the establishment of 7,290, 4,217 and 3,797 contigs, for *C. macropomum*, *P. mesopotamicus* and *P. brachypomus*, respectively. The pairwise assembly (between the species) resulted in 8,273 (*P. mesopotamicus* x *P. brachypomus*), 12,431 (*C. macropomum* x *P. mesopotamicus*) and 11,714 contigs (*C. macropomum* x *P. brachypomus*). In relation to the SNPs discovery, we found 1,107 (*C. macropomum*), 450 (*P. mesopotamicus*) and 416 (*P. brachypomus*) intraspecific SNPs. Moreover, 2,430 (*C. macropomum* x *P. mesopotamicus*), 2,080 (*C. macropomum* x *P. brachypomus*) and 811 (*P. mesopotamicus* x *P. brachypomus*) interspecific SNPs were discovered, of which 69 were characterized as validated SNPs. In general, 95,83% of the contigs with SNPs were identified by similarity search in BlastX (nr), of which 91,66% were classified in GO (gene ontology) categories: Biological process (50,82%), Cellular component (21,45%), Molecular function (27,73%). Functional classification of the SNPs showed that 14,74% were located in 5'UTR (untranslated region), 23,16% in 3'UTR and 62,10% in cds (coding sequence), of which 76,27% were synonymous and 23,73% were non-synonymous. The expect result is that our data could be used in the Brazilian aquaculture industry, which will accelerate the genetic improvement and increase the productivity of this important group of fish.

[§] Corresponding author. Tel.: + 55 16 3209-7477;
E-mail address: diogo@caunesp.unesp.br

DIFERENTIAL EXPRESSION OF GROWTH HORMONE (GH) IN HETEROTIC CROSSBREEDS FROM CHITRALADA AND RED STIRLING STRAINS OF NILE TILAPIA (*Oreochromis niloticus*)

Herkenhoff M.E.[a, §], Dias M.A.D.[b], Hilsdorf A.W.[c], Pinhal D.[a]

[a] *Department of Genetics, São Paulo State University, Botucatu - SP, 18618-970, Brazil*
[b] *Department of Animal Sciences, Federal University of Lavras, Lavras, 37200-000, Brazil*
[c] *Unit of Biotechnology, University of Mogi das Cruzes, Mogi das Cruzes,08780-911, Brazil*

The Nile tilapia (*Oreochromis niloticus*) is an economically important farmed fish in different countries and particularly in Brazil. Crossbreeding in this species has been successfully performed between Red-Stirling and wild type Chitralada strains. This crossing has generated a heterotic crossbred, which embodies both the superior growth performance of Chitralada strain with red colouration (preferable by consumers) of Red-Stirling strain. Although crossbreeding is a usual strategy to produce species with this so-called hybrid vigor, the molecular mechanisms of heterosis including genes that govern biological pathways to produce particular phenotypic effects remain largely unknown. Fish genetics and genomics research has shown the direct and indirect interference of several genes in animal performance, particularly GH (growth hormone)/IGF (insuline-like growth factor) and other related genes. Since GH stimulates growth and cell proliferation in animals, several studies have been carried out to trace GH effects in the growth and weight gain of farm animals, including fish. The objective of this study was to evaluate the correlation between growth performance and gene expression. For this purpose, morphometric parameters and the expression of the GH gene were assessed in adult male and female (N=27). Chitralada and Red-Stirling strains (controls) as well as crossbred individuals were checked for morphometric parameters such as weight and total length from three to seven months old. Subsequently quantitative PCR (RT-qPCR) was performed in brain tissues and expression profiles analysed in the software REST. Nile tilapia growth was low and constant from the 3rd to 5th month, matching to a low GH expression in this period. A growth peak was clearly observed in the 6th month for both crossbred and controls. However in the 6th month crossbred have shown a 2-fold higher GH expression in comparison to Chitralada and Red Stirling, which reflected in the crossbred increased growth at the 7th month. Although several genes are predicted to control growth, these findings suggest that crossbred heterosis is associated to a differential expression in GH genes. A complementary analysis of IGF-I, IGF-II and miostatin (Mstn) gene expression is currently under development and may help to further clarify the molecular basis of heterosis in fish.

Keywords: heterosis; growth hormone (GH); Real time PCR; Nile tilapia

§ Corresponding author. Tel.: + 55 (14) 3880-0363; Fax: + 55 (14) 3815-3131
E-mail address: herkenhoff@ibb.unesp.br

GENERATION OF GENOMIC RESOURCES FOR ATLANTIC STURGEON USING NEXT GENERATION SEQUENCING

Hori T.[1], Stannard J.[2], Plouffe D.[1], Buchanan J.[2], Fast M.[3]

[1]The Center for Aquaculture Technologies Canada Souris, PE, Canada C0A 2B0
[2]The Center for Aquaculture Technologies San Diego, California, USA, 92121
[3]Department of Pathology and Microbiology, Atlantic Veterinary College, University of PEI
550 University Avenue, PE, Canada C1A 4P3

Caviar producing sturgeon is a high-value species and over the past decade sturgeon aquaculture has grown. However, market demand and growth may have negative impacts on production due to the need for higher throughput, which can strain the physiological limits of the fish and lead to greater susceptibility to pathogens and external stressors. A broader knowledge of sturgeon biology and genetics will be required to maximize culture and to fulfill the market demand for sturgeon products. Unfortunately, there is a paucity of genomic information available for sturgeon species, and thus the generation of these resources may help to identify high-quality broodstock, facilitate optimization of culture conditions, identify biomarkers for real-time health monitoring, and enable differentiation from wild sources for traceability. In collaboration with the Atlantic Veterinary College at the University of Prince Edward Island (UPEI), the Center for Aquaculture Technologies Canada (CATC) used a whole-transcriptome shotgun sequencing (RNA-seq) approach designed to identify Atlantic sturgeon (*Acipenser oxyrhynchus*) genes that respond to lipopolysaccharide (LPS) stimulation. A reference transcriptome assembly for Atlantic sturgeon spleen was generated with a custom bioinformatic pipeline using over 750,000,000 pair-end Illumina reads. The reference assembly was comprised of ~ 400,000 cDNAs, had N50 = 1212 bp and average contig length of 742.36 bp. Further, we were able to properly re-map ~ 70% of the 750,000,000 pair-end reads to the reference. This transcriptome was used for the identification of sturgeon LPS-responsive genes such as interleukin-8, cathelicidin, major histocompatibility complex (MHC) I and II. In addition, existing pipelines were used to identify 3 million single-nucleotide polymorphisms (SNPs) for this emerging aquaculture species. Due to the duplicated nature of the Atlantic sturgeon, following read mapping, consensus calling and SNP identification, we used an in-house custom filtering algorithm and reduced the SNPs to a collection of ~ 50,000 high quality markers. Further studies are under way to validate the LPS-responsive genes and SNPs.

Keywords: sturgeon, RNA-seq, SNPs, LPS, transcriptome

IDENTIFICATION AND EXPRESSION PROFILES OF IFABP PARALOGS IN GILTHEAD SEA BREAM (*Sparus aurata*) AND EUROPEAN SEA BASS (*Dicentrarchus labrax*)

Kaitetzidou E.[a, b], Chatzifotis S.[a], Antonopoulou E.[b], Sarropoulou E.[a, §]

[a] *Institute of Marine Biology, Biotechnology and Aquaculture, Hellenic Centre for Marine Research, P.O. Box 2214, 71003 Heraklion, Greece.*
[b] *Laboratory of Animal Physiology, Department of Zoology, School of Biology, Aristotle University of Thessaloniki, 54124 Thessaloniki, Greece.*

Intestinal fatty acid binding protein (IFABP or FABP2) is a cytosolic transporter of long chain fatty acids, which is mainly expressed in cells of intestinal tissue. Fatty acids in teleosts are an important source of energy for growth, reproduction and swimming as well as a main ingredient in the yolk sac of embryos and larvae. In the present study, two paralogs of IFABP –IFABP alpha and IFABP beta- were identified in two fish species important for the Mediterranean aquaculture, i.e. gilthead sea bream (*Sparus aurata*) and European sea bass (*Dicentrarchus labrax*). Despite the high similarity displayed by their nucleotide and amino acid sequences and the identical organization in 4 exons, paralogs were mapped to different chromosomes/ linkage groups supporting our hypothesis that the identified transcripts are true paralogs. This was also supported by phylogenetic analysis using IFABP sequences of 26 teleost fish species; the constructed tree revealed two principal clades, corresponding to each paralogue. Synteny analysis further showed that IFABP genes share common neighboring genes in 10 teleosts. Both synteny and phylogenetic analysis are pinpointing to the hypothesis that IFABP paralogs are originated from a single ancestor gene after genome duplication. Differential expression analysis of the IFABP paralogs in the intestine after fasting and refeeding experiment in the studied species, revealed their implication in metabolism. Additional expression studies in 7 developmental stages of gilthead sea bream and European sea bass detected IFABP paralogs relatively early in the embryonic development. Furthermore, the different expression pattern between alpha and beta paralogs during the development of both species may indicate possible complementary or separated roles for the paralogs. Thus, the possible involvement of IFABPs in lipid absorption and metabolism may be considered as an additional tool in genomic approach offering insights in the growth farmed teleosts.

Keywords: FABP2, Paralogs, Expression, Fasting and Refeeding, Development

§ Corresponding author. Tel.: 00302810337753
E-mail address: sarris@hcmr.gr

RESPONSE OF GENES INVOLVED IN LIPID METABOLISM OF ATLANTIC SALMON FAMILIES FED TO PARTIAL PLANT OIL-DIET.

Lam N.[ab], Cordova V.[ab], Ulloa P.[c], Araneda C.[a], Di Genova A.[d], Maass A.[d], Iturra P.[b]

[a]*Producción Animal, Fac. Cs. Agronómicas, Universidad de Chile, Santiago, 8820808, Chile.*
[b]*Genética Humana, Fac. de Medicina, Universidad de Chile, Santiago, 8380453, Chile.*
[c]*Lab. Biología del Desarrollo, Facultad Ciencias Biológicas, UNAB, Santiago, 8370146, Country*
[d]*Mathomics, Center for Mathematical Modeling, Universidad de Chile, 8370456, 7t, Santiago, Chile*

Nowadays, the salmon industry is using diets with a major proportion of protein and oil of plant products making of the aquaculture more sustainable.

In this work, the growth response in 35 Atlantic salmon families fed diet including 50% rape-seed oil (VO) and a control diet including fish oil (FO) was evaluated. After feed period (60 days), the heritability of Weight Gain was calculated in 0.35. The Breeding Value (PBV) for this trait was predicted in each family and separately for FO and VO diets. A PBV ranking was constructed for each diet. Some families fed VO diet changed position in the ranking when compared with its position in the FO diet ranking. A new ranking was built with families fed VO considering the magnitude of change of PBV ranking compared with FO diet.

In order to explore the genes and metabolic pathways underlying variation in fish growth as a response to the VO diet, two families with the mayor difference in favor of PBV (FPBV) and two families with the greatest difference against the PBV (APBV) were selected for analyzing of the liver gene expression pattern by RNAseq.

Total RNA was extracted from the liver from twelve individuals from selected families, and individual libraries were prepared and sequenced with Illumina HiSeq. Using the package edgeR RNAseq analysis revealed 260 genes differentially expressed ($p > 0.05$, FDR>0.05) between the families of FPBV and APBV. The enriched analysis identified the GO terms as monocarboxylic acid metabolism, response to oxidative stress and fatty acid biosynthesis. The pathways analysis identified several genes participated of the unsatured fatty acid bio-synthesis and the PPAR (peroxisome proliferator-activated receptors) pathways.

To complement our results, some important genes as *acac, acsl4, elovl6, cpti, fasn, Δ6fad*α, *fabp3, cyp7a1, pparg and nr2b2* and genes *ppara*, *srebp1* and *apoa4*α were selected to analyze the expression levels by qPCR in individuals from FPBV and AFPBV families fed VO diet compared with FO diet. We observed that FPBV families have a different gene expression pattern in response to a diet rich in vegetable oils.

[§] Corresponding author. Tel.: +   5629785854
E-mail address: + nlam@u.uchile.cl

LONG TERM FEEDING OF VEGETABLE OIL BASED DIETS AFFECTS MUSCLE CELLULARITY AND THE EXPRESSION OF GROWTH-RELATED GENES

Lopes G.[a], Castro L.F.C.[a, b], V. Sousa[a, c], Valente L.M.P.[a, c, *]

[a] CIIMAR/CIMAR, Interdisciplinary Centre of Marine and
Environmental Research, Porto, 4050-123, Portugal
[b] Department of Biology, FCUP, Faculty of Sciences
University of Porto, Porto, 4169-007, Portugal
[c] ICBAS-UP, Instituto de Ciências Biomédicas de Abel Salazar, Porto, 4050-313, Portugal

Texture is one of the most important aspects related with fish quality. Being a sensory attribute of flesh capable of influencing consumers' acceptance, texture assumes a major role in species with high economic value, like Senegalese sole.

Several intrinsic and extrinsic attributes are capable of influencing flesh texture, and fish diet is one of those. The sustainable growth of the aquaculture sector depends on finding alternative sources for aquafeeds, reducing the use of fish meal (FM) and fish oil (FO). Vegetable oils (VO) are a feasible alternative to FO, but have significant impacts on flesh quality. Most studies evaluated the impact of such diets on the nutritional value of the fillet, but few focused on organoleptic attributes and even less on textural properties.

Textural properties can be accessed through muscle cellularity and gene expression. The aim of this work was to evaluate the effect of increasing dietary levels of VO (50%, VO50 and 100%, VO100) in muscle cellularity and gene expression of Senegalese sole juveniles fed the experimental diets for 140 days.

The total substitution of FO by VO (VO100) significantly increased the cross sectional area (CSA) of Senegalese sole ($P<0.05$) and reduced the number of white muscle fibers/mm2 compared to the other treatments. On the other hand, fibers area (µm2) of fish fed VO100 was significantly higher than those fed the CTR and VO50. The dietary inclusion of VO had no effect on fish length and weight neither on the total number of fibers per CSA.

The relative expression of 13 genes related to muscle differentiation and growth, standardized with 2 reference genes, were studied. No statistical differences were reported for the expression of myogenic regulatory genes or the insulin growth factor II (igf-II) in fish fed the VO diets compared to the CTR. However, the expression of the growth factor igf-I was significantly higher in fish fed VO100 contributing to a higher white fiber area in this fish.

Keywords: Solea senegalensis, Vegetable oil, Texture, Muscle cellularity, Myogenesis

Corresponding author. Tel.: +351 223401825; Fax: +351 223390608;
E-mail address: lvalente@icbas.up.pt;

GENE EXPRESSION OF IMMUNE GENES IN SKIN OF *Salmo salar* INTERACTING WITH SESSILE *Caligus rogercresseyi*

Lozano I.[a,] , Neumann K.[a] , Cichero D.[b] , Schroeder G.[b], Martínez V.[a,*]

[a] *FAVET-INBIOGEN-Facultad de Veterinaria, Universidad de Chile, Santiago, 8820000, Chile*
[b] *R&D, Blue Genomics SPA, Puerto Varas, 8820000, Chile*

Caligus rogercresseyi commonly called sea louse is an endemic copepod ectoparasite, which parasites farmed *Salmo salar*. This parasitism generates great economic losses, predisposition to secondary infections and welfare issues. Understanding the biology behind the mechanisms involved in the immune response of the host is important for selecting individuals bearing different resistance genetic backgrounds.

Fish skin acts as a mechanical barrier and represents a metabolically active tissue; the immune response of the skin is due to the skin´s complex structure, mucus and cell composition. In early stages of sea lice infestation the skin response is expected to explain to what extent the fish is susceptible or resistant to the parasite. Early innate immune response of the skin of susceptible and resistant salmonids to *L. salmonis* had been reported previously, however, this is not the case for the interaction between *Caligus rogercresseyi* and *Salmo salar*. In order to investigate the gene expression levels for key genes, such as **Matrix metalloproteinase-13**, **Beta-2-microglobulin**, **Metalloreductase STEAP**, **CD4**, **MHCII**, **Transferrin, Arachidonate 5-lipoxygenase**, **Galectin-9** and **Prostaglandin D synthase** involved in L. salmonis response. We perform an *in vivo* challenge test of *Salmo salar* with the pathogen *Caligus rogercressy* comprising different families from the AQUAGEN breeding program. We characterize the skin immune response in naïve fish (2 days before the challenge start); 9 days post-challenge T (1) and 21 days post-challenge T (21), both in naïve tissue as well as in attachments sites where the host-parasite interaction occurred.

Our results show a comparable response of *Salmo salar* when interacting with *Caligus rogercressyi*. For example, in louse attachment sites an early pro-inflammatory response was observed in genes; Arachidonate 5-lipoxygenase (LOX-5) and CD4-1, which significantly increased at 9 days post-challenge. The metalloproteinase-13 gene was significantly up regulated at 21 days post-challenge; B2M and MHCII expression significantly decrease in naïve skin at 21 dpi. Galectin-9 and Prostaglandin D synthase were significantly down regulated in the louse attachment sites at 21 dpi. Transferrin showed a bi-phasic transcriptomic response, first the gene was down regulated and its expression increased significantly at 21 days post-challenge at attachment sites.

The results show that the transcriptomic response in skin of *Salmo salar* is complex, evolving slowly as the parasite became adults, when most of the skin damage is observed. Moreover, it appears that the response of the host is quite modest in the sessile stages, possibly due to the fact that *Caligus* fed mainly on mucus. Some of the results following the full transcriptomic assessment will be presented.

Keywords: in vivo challenge, Caligus rogercresseyi, gene expression, fish skin, Atlantic salmon.

[*]These authors have contributed equally to this work.
[§] Corresponding author. Tel.: + 56-22-9785675 ; Fax: + 56 29785641
E-mail address: vmartine@u.uchile.cl

IMMUNE RESPONSE STUDY IN BLOOD OF ATLANTIC SALMON FED WITH DIETS SUPPLEMENTED WITH SEAWEED: AN ANTIVIRAL NUTRIGENOMICS APPROACH

Lozano I.[a], Wacyk J.[a], Carrasco J.[b], Cortez-San Martin M.[c]

[a] *Facultad de Agronómia, Laboratorio de Genética y Acuicultura, Universidad de Chile, Santiago, 8820000, Chile*
[b] *I&D Salmon division, BioMar Chile S.A, Puerto Montt, 548000, Chile*
[c] *Facultad de Química y Biología, Laboratorio de Virología Molecular, Universidad de Santiago de Chile, Santiago, 8320000, Chile*

The most important pathogens that affect aquatic animal farming are viruses, and viral diseases continue generating significant economic losses in global aquaculture; Veterinary drug treatments have long been used as a strategy to control viral diseases, however their success is questionable added to the fact their used can negatively impact on the environment and human health. An alternative solution is the use of natural products as they tend to be more biodegradable than synthetic molecules and they are less likely to generate a resistance (Reverter et al., 2014). Marine macro algae contain bioactive compounds that are a natural alternative therapy to drugs, and red macro algae are considered as a source of new anti-viral compounds (Vo et al., 2011; Mohamed et al., 2011).

Having this in mind, the aim of this study was to evaluate red macro algae as functional feed ingredient in Atlantic salmon diet by measuring changes in transcription of antiviral genes. Fish were fed 56-days with nine diets containing either *Pyropia columbina* or *Gracilaria chilensis* at 0.1, 1 and 10 g kg^{-1} and a control diet containing no marine algae. Blood samples were collected at 2,4 and 8 weeks. We extracted the RNA of white blood cells and variation in the transcription of key genes associated with antiviral response in *Salmo salar* was analyzed by qRT-PCR.

Candidates genes previously reported to play important roles in innate immune responses of *Salmo salar* against virus infectious were selected: INF gamma, Mx, and IL6; cathelicidin an antimicrobial peptides reported to be able to kill bacteria, fungi, viruses and parasites (Broekman et al., 2013) and lysozyme a cationic protein that is involved in a broad range of defense mechanisms including antiviral activity (Mai et al., 2009).

Interestingly, the diets including red seaweed supplementation significantly induced the Mx transcription in *Salmo salar* blood leukocytes. Lysozyme, cathelicidin, INFɣ and IL6 showed a stable transcription levels however INFɣ, an important antiviral marker showed a slight increase in response to the diet.

Keywords: Gene expression, Atlantic salmon, antiviral immune responses, fish leucocytes, Salmo salar, Mx.

[*]These authors have contributed equally to this work.
[§] Corresponding author. Tel.: + 56-2-9785793 ; Fax: + ;
E-mail address: + jwacyk@u.uchile.cl ;

TRANSCRIPTOME CHARACTERIZATION AND MARKER DISCOVERY IN MEAGRE *Argyrosomus regius*

Manousaki T.[a], Tsakogiannis A.[a,b], Lagnel J.[a], Estévez A.[c], Mylonas C.C.[a], Duncan N.[c], Tsigenopoulos C.S.[a§]

[a] *Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Centre for Marine Research (HCMR), Heraklion, 71500, Greece*
[b] *Department of Biology, University of Crete, Heraklion, 71003, Greece*
[c] *Institut de Recerca I Tecnologia Agroalimentáries (IRTA), Sant Carles de la Rapita, 43540, Tarragona, Spain*

Meagre (*Argyrosomus regius*), a teleost fish of the family Sciaenidae, is a recently recruited species in the industry of Mediterranean aquaculture. The species has a great potential to increase its share into the market given its large size, fast growth, good processing yield and low fat content. The number of biological studies on this fish has been continuously increasing whereas genomic information is practically non-existent. Transcriptome sequencing through RNA sequencing (RNA-Seq) is one of the most widely used approaches to understand various biological aspects of unexplored species like meagre. We obtained transcriptome information of *A. regius* using the Illumina HiSeq2500 platform (2x100bp) from muscle and liver tissues of sixteen juvenile fish coming from five families. The aim of this study was two-fold. First, based on more than 300 million paired reads we created a reference transcriptome for meagre in order to obtain a backbone that can support future research studies regarding physiological, immunological and other analyses. Second, transcriptome data permitted the identification of polymorphic molecular markers within the coding regions of the genome (single nucleotide polymorphisms-SNP and simple sequence repeats-SSR) to be used in future population genetics, genomic and expression studies and finally to set the ground for understanding growth and other traits for this economically important species.

Keywords: meagre, transcriptome, RNA-Seq, SNP & SSR discovery

[§] Corresponding author. Tel.: +30 2810337854; Fax: +30 2810337870
E-mail address: tsigeno@hcmr.gr

GENOME EDITING OF YELLOWTAIL *Seriola quinqueradiata* MUTAGENESIS OF YELLOWTAIL USING GENOME EDITING TECHNOLOGY.

Okamoto H.[a*§], Shimada Y.[a*], Yamaguchi T.[a], Sakuma T.[e*], Yamamoto T.[e], Nishiki I.[c], Iwasaki Y.[c], Fujiwara A.[c*], Yoshida K.[b], Goto R.[d], Matsubara T.[d], Inoue N.[a], Nagoya H.[a], Usuki H.[a], Gen K.[b], Mushiake K.[b], Kobayashi T.[f], Fukuda M.[f]

[a]*National Research Institute of Aquaculture, FRA, Kamiura, Saiki, Oita 879-2602, Japan*
[b]*Seikai National Fisheries Research Institute, FRA, Nunoura, Goto, Nagasaki 853-0508, Japan*
[c]*National Research Institute of Fisheries Science, FRA, Yokohama, Kanagawa 236-8648, Japan*
[d]*South Ehime Fisheries Research Center, Ehime University, 25-1 Ainan-cho, Minami-Uwa, Ehime 798-4206, Japan*
[e]*Graduate school of science, Hiroshima University, 1-3-1 Kagamiyama, Higashi-Hiroshima, Hiroshima 739-8526, Japan*
[f] *Fisheries Research Agency (FRA), Yokohama, Kanagawa 220-6115, Japan*

Genome editing technology using artificial nucleases has been developed in many fish species. Genome editing is not only used to analyze gene function in biological studies, or identify genes responsible for disease in model organisms, but also to identify commercial genetic traits for genomic breeding in aquaculture. We report here the deletion of nucleotide sequence in the tyrosinase (*tyr*) gene using genome editing in yellowtail (*Seriola quinqueradiata*), the main aquaculture fish species in Japan. *tyr* is one of the genes responsible for albinism. Loss of this gene function was expected to result in a decrease in melanin synthesis, and a subsequent reduction or elimination of black pigments in organisms. We designed recognition sites for transcription activator-like effector nucleases (TALEN) in the *tyr* gene. Hatching larvae were obtained following microinjection of TALEN RNA at the one-cell stage in yellowtail eggs. We selected two candidates after screening using a T7 endonuclease I (T7EI) assay. One larva exhibited a reduction in black body pigment; another exhibited almost complete elimination of pigmentation in the retina and the whole body. The *tyr* gene DNA sequences were amplified from each of the two fish and 30 clones were randomly selected from the PCR products. Nucleotide deletions, of varying lengths, were observed in all clones of the 30 PCR products. This study demonstrates the potential use of genome editing technology in genomic breeding for marine aquaculture.

*These authors have contributed equally to this work.
§ Corresponding author: Tel.:+81-972-32-2125; Fax:+81-972-32-2293
E-mail address: hiokamot@affrc.go.jp

A FLAT OYSTER DATABASE FROM GENOMIC AND EXPRESSED SEQUENCES TAG OBTAINED FROM HAEMOCYTES INFECTED WITH *Bonamia ostreae,* REPETITIVE ELEMENTS AND OLIGO-MICROARRAY DEVELOPMENT

Pardo B.G.[a][§], Álvarez-Dios J.A.[b], Gómez-Tato A.[c], Vera M.[a,d], Bello X.[e,f], Bartolomé C.[e,f], Maside X.[e,f], Carlsson J.[g], Carlsson N.[g], Cao A.[h], Villalba A.[h], Martínez P.[a]

[a] *Departamento de Genética, Universidad de Santiago de Compostela, Lugo, 27002, Spain*
[b] *Dpto. de Matemática Aplicada, Universidad de Santiago de Compostela, 15781, Spain*
[c] *Dpto. de Geometría y Topología, Universidad de Santiago de Compostela. 15782, Spain*
[d] *Department de Biologia, Universitat de Girona, 17071 , Spain*
[e] *Medicina Xenómica, CIMUS, Universidade de Santiago de Compostela, 15782, Spain.*
[f] *Xenómica Comparada de Parásitos Humanos, IDIS, Santiago de Compostela, Spain.*
[g] *School of Biology & Environmental Science. University College Dublin, Ireland.*
[h] *Centro de Investigacións Mariñas, Xunta de Galicia, Aptdo E-36620, Spain*

The flat oyster, *Ostrea edulis,* is one of the most appreciated farmed oysters in Europe but also in the United States and Canada. The demise of flat oyster culture in major production areas is mainly due to bonamiosis caused by the protozoan *Bonamia ostreae.* Due to the economical and ecological importance of flat oyster, genomic data are eagerly needed for devising genetic improvement strategies aimed to control this parasitosis, however, very scarce genomic information is available in public databases. In this study transcriptome and genomic information were obtained using 454 pirosequencing and was compiled into a browseable database referred to as OedulisDB, which consisted in two sets of 10,318 and 7,159 unique contigs representing the oyster's genome (WG) and *de novo* haemocyte transcriptome (HT) after challenges with *B. ostreae.* Expressed sequence tags (ESTs) were annotated with their putative functions, and sequences related to immune response and several novel key immune genes were identified. The two datasets were also used to analyse the repetitive fraction (i.e. Transposable Elements (TEs) and Short Tandem Repeats (STRs)) of the oyster genome and haemocyte transcriptome. A total of 1,083 sequences were identified as TE-derived, which corresponded to 4.0% of WG and 1.1% of HT. These elements were mainly assigned to the Penelope order of retrotransposons, and to the Helitron and TIR DNA-transposons. For STRs, the most frequent motifs identified in WG were tetranucleotides while trinucleotides were in HT. Forty identified microsatellite loci (20 from each database) were selected for technical validation. Success was much lower among WG than HT microsatellites (15% vs 55%). Transcriptome information was also applied to design the first oligo-microarray in flat oyster enriched with immune sequences from haemocytes. Finally, the combination of transcriptome and genomic information allowed the identification amongst the deepest contigs of putative *in silico* single-nucleotide polymorphisms (SNPs) for the flat oyster. The results here reported will aid to enrich our understanding about the immune response of *O. edulis* against bonamiosis and to provide resources to support future breeding programs and to manage genetic resources of natural flat oyster beds.

Keywords: flat oyster*,* bonamiosis*, database, repetitive elements,* oligo-microarray

[§] Corresponding author. Tel.: +34982822428; Fax: +34982822428;
E-mail address: belen.gomez@usc.es

NEXT-GENERATION SEQUENCING TECHNOLOGIES AS STARTING POINT FOR THE STUDY OF THE IMMUNE RESPONSE IN TURBOT (*Scophthalmus maximus*)

Pereiro P., Figueras A., Novoa B.[§]

Instituto de Investigaciones Marinas (IIM-CSIC), Vigo, Spain

Turbot is an important aquaculture resource both in Europe and Asia. However, until the last few years, there was little information on gene sequences available in public databases. Currently, one of the main problems affecting the culture of this flatfish is mortality due to several pathogens, especially viral diseases which are not treatable. In order to identify new genes involved in immune defense, we conducted 454-pyrosequencing of the turbot transcriptome after different immune stimulations. Our results provided a rich source of data (55,404 contigs and 181,845 singletons) for discovering and identifying new genes, which served as a basis for microarray construction, gene expression analysis and for identification of genetic markers to be used in several applications. A specific turbot microarray enriched in immune-related genes was designed using this information. This tool was used for analyzing the transcriptome profiles associated to the VHSV infection or DNA vaccination in turbot, as well as the differences between vaccinated and non-vaccinated fish after viral infection. The overall analysis of all this information provided interesting information about genes implicated in the defense mechanisms against viral diseases and let us to focus the attention on some specific genes to be further characterized and studied in detail. This is the case, for example, of type I interferons (*ifn1* and *ifn2*) or Nk-lysin (*nkl*). Type I interferons (IFNs) are considered the main cytokines directing the antiviral immune response in vertebrates but, interestingly, whereas *ifn1* reflected a clear and typical antiviral activity, *ifn2* was not able to induce this response and its role could be more related with the immune regulation, being involved mainly in the inflammation process. On the other hand, *nkl*, an antimicrobial cationic peptide produced mainly by cytotoxic T lymphocytes and natural killer cells, was observed to be associated to the resistance against VHSV infection and this molecule could be considered as a VHSV-resistance marker.

Keywords: turbot, transcriptome, 454-pyrosequencing, microarray, immune genes, viral diseases

[§]Corresponding author. Tel.: +34 986 231 930; Fax: +34 986 292 762
E-mail address: beatriznovoa@iim.csic.es

TRANSCRIPTOME PROFILING AND DIFFERENTIAL GENE EXPRESSION IN MUSSELS EXPOSED TO *Prorocentrum lima*, A DINOFLAGELLATE PRODUCING DSP TOXINS

Prego-Faraldo M.V.[a*§], Flórez-Barrós F.[b*], Fernández-Tajes J.[c*], Eirín-López J.M.[d*], Méndez J.[a*]

[a] *Department of Cell and Molecular Biology, Universidade da Coruña, A Coruña, 15071, Spain*
[b] *Centre for Nephrology, University College London, London, WC1E 6BT, UK*
[c] *Wellcome Trust Centre For Human Genetics, University of Oxford, Oxford, OX3 7BN, UK.*
[d] *Department of Biological Sciences, Florida International University, North Miami, FL 33181, USA*

Okadaic acid (OA) and dinophysistoxins (DTX) constitute the main Diarrhetic Shellfish Poisoning (DSP) toxins found European coasts. These biotoxins are produced by dinoflagellates of the genera Dinophysis and Prorocentrum during Harmful Algal Bloom episodes (HABs). Given their lipophilic nature, OA and DTX can be accumulated on different tissues from filter-feeder organisms, including commercial shellfish species that can transfer these toxins to human consumers. Upon exposure to these toxins, humans typically experience the DSP syndrome, characterized by nausea, abdominal pain and diarrhea. Studies carried out on mammalian cell lines have demonstrated the potential of OA to induce apoptosis, cytoskeleton disruption and cell cycle alterations. On the contrary, it seems that different marine organisms (notably bivalve molluscs) have developed mechanisms able to alleviate the harmful effects associated with constant exposure to these biotoxins in the oceans. Given the little knowledge about these mechanisms and their potential relevance for toxicology and management purposes, the present work studied the molecular mechanisms potentially involved in the tolerance to DSP toxins in bivalve molluscs. With that in mind, suppression subtractive hybridization libraries were constructed from digestive gland, gill and haemolymph tissues from the mediterranean mussel *Mytilus galloprovincialis* following a 4 day exposure to relatively high concentrations of the OA-producing dinoflagellate *Prorocentrum lima* (20.000 cel·L^{-1}). Six forward and six reverse subtractive libraries were constructed, allowing to sequence, assembly and functionally annotate at least 200 clones from each library. Differentially expressed genes were subsequently identified from library comparisons, enabling the analysis of temporal gene expression patterns using quantitative PCR. Our results revealed that gene expression profiles in response to OA vary significantly depending on different tissues. Surprisingly, temporal analyses of gene expression data revealed that DSP toxins elicit a stronger genetic response at low concentrations and short exposure times. Overall, this work provides an initial step towards better understanding the molecular mechanisms mediating the response and tolerance to OA and DTX biotoxins in bivalve molluscs. Those include a list of potential candidate genes for future analyses.
This work has been funded by the project AGL 2012-30897 (Spanish Ministry of Economy and Competitivity).

Keywords: okadaic acid, dinophysistoxins, bivalves, suppression subtractive libraries, gene expression

*These authors have contributed equally to this work.
§ Corresponding author. Tel.: +34 981 167 000 (2030); Fax: +34 981 167 065
E-mail address: veronica.prego@udc.es

microRNA IDENTIFICATION AND CHARACTERIZATION IN TURBOT (*Scophthalmus maximus*)

Robledo D.[a*§], Martín A.P.[b*], Álvarez-Dios J.A.[c], Pardo B.G.[d], Martínez P.[d]

[a] *Departamento de Genética, Facultad de Biología (CIBUS), Universidad de Santiago de Compostela, Santiago de Compostela, 15782, Spain*
[b] *Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), Rosario, S2002LRK Argentina*
[c] *Departamento de Matemática Aplicada, Facultad de Matemáticas, Universidad de Santiago de Compostela, Santiago de Compostela, 15782, Spain*
[d] *Departamento de Genética, Facultad de Veterinaria, Universidad de Santiago de Compostela, Lugo, 27002, Spain*

MicroRNAs (miRNAs) are abundant short (~22 nucleotides) non-coding RNAs highly conserved in vertebrates. miRNAs are post-transcriptional regulators of mRNA and play an important role in the regulation of many fundamental developmental and functional processes in eukaryotes. miRNAs frequently represent the central nodes of regulatory networks, providing fine-tuning to gene expression of many transcripts. So, miRNA expression patterns can be especially relevant for understanding biological functions since they may capture the expression variation of hundreds of mRNA. Currently, there are 1,637 miRNAs identified in public databases in 9 teleost species (miRBase v. 21), which reflects how little has been done in fish miRNA characterization. Here, we present the identification and characterization of miRNAs in turbot, a flatfish species with aquaculture interest in Europe and China. A pool of tissues coming from different turbot development stages was sequenced for identifying small RNAs, leading to the discovery of 292 putative mature miRNAs. A higher number of microRNAs have only been described in *Salmo salar* (498) and *Danio rerio* (350) according to miRBase. Among the mature miRNAs identified, 246 formed 123 5p- and 3p- pairs. These 292 miRNAs accounted for a total of 3,457,300 reads. miR-10b-5p and miR-146a-5p were the two most expressed miRNAs representing 33% of the total reads with over half a million reads each. Ninety-eight miRNAs presented more than 1000 reads and 246 more than 10. All the mature turbot miRNAs were aligned against the draft turbot genome. Some aligned to different genomic positions, for example mir-199a was placed in four different genomic locations, corresponding to the same intron of different paralogs of the dynamin gene. Genomic analysis revealed 23 clusters of two miRNAs, 11 of three and 1 of four miRNAs. Also, 42 miRNAs were found to be located in intronic regions. The genomic information and annotation of the microRNAs were consistent with an origin by duplication in many cases. Turbot precursor sequences for every miRNA were obtained using the turbot genome and precursor sequences of other fish species. Furthermore, a set of 15 highly expressed microRNAs involved in different biological processes was selected for qPCR analysis during turbot metamorphosis.

Keywords: Turbot, microRNA, qPCR, metamorphosis

[*]These authors have contributed equally to this work
[§] Corresponding author. Tel.: +34981516965
E-mail address: diego.robledo@usc.es

NEXT GENERATION SEQUENCING, *DE NOVO* ASSEMBLY, AND EXPRESSION ANALYSIS OF GONADAL TRANSCRIPTOMES IN *Acipenser naccarii*

Robles F., Bakkali M., Molina-Luzón M.J., García Zea A.J., Martín-Blázquez R., Navajas-Pérez R., De la Herrán R., Ruiz Rejón C.

Department of Genetics, Facultad de Ciencias, University of Granada, Granada, 18071, Spain

We carried out Illumina sequencing of two cDNA libraries from the gonads of two males and two females of the sturgeon *Acipenser naccarii* —both belonging to 4-5 years old specimens. Proof of the quality of the resulting 73,927,492 testis and 72,170,178 ovary library reads are the facts that only 0.039% of their 147,558,646,70 bases were undetermined (N), the average GC content (46.71%) was similar for both testis and ovary libraries (46.17% and 47.24% respectively), and over 75% of the bases did satisfy the Q30 (78.34% testis and 86.24% ovary).

The reads were assembled into 652,358 sequences with lengths ranging from 35 to 15,730 bases (N50=670). The average GC content of the assembled sequences was 42.4% and BLASTX against the NCBI non-redundant protein database (nr) resulted in 586,017 annotated sequences.

Of the assembled reference transcriptome, 9,238 gene transcripts and undetermined sequences were 'sex-specific', of which 89.4% were found only in the testis transcriptome and the remaining 10.6% only in the ovary. Analysis by GO terms assigned biological processes to 6,836 of these sequences, 6,098 of which were 'testis-specific' and the remaining 738 'ovary-specific'.

In this preliminary effort aimed at identifying potential sturgeon sex-specific markers, we screened the 'sex-specific' sequences for genes known to be involved in sex determination and sexual development in vertebrates. After primer design and quantitative PCR analysis of the expression levels of the selected potential sturgeon sex-specific markers, we found the expression of the *Dmrt1* and the *steroidogenic factor 1* gene to apparently be exclusive, thus specific markers, of at least the 4-5 years old male specimens of *A. naccarii*.

We also looked for microsatellite repeats within the set of assembled sequences that had sex-related annotations and obtained 780 microsatellite-containing sequences; 257 of which had dinucleotide motives, 288 had trinucleotide motives and 235 had tetranucleotide motives (the average length of the microsatellites was 15 to 16 bases). Of all of them, microsatellite-flanking primers were designed on 30 sequences. The levels of polymorphism were then analyzed in a sample of 26 *A. naccarii* specimens (10 males, 10 females and 6 sex-undifferentiated).

Keywords: Acipenser naccarii, transcriptome sequencing, gonad, differential gene expression, microsatellites

*These authors have contributed equally to this work.
§ Corresponding author. Tel.: +34-958249704; Fax: + 34-958244073
E-mail address: carmelo@ugr.es

NUTRITIONAL REGULATION OF KEY GENES INVOLVED IN MYOGENESIS IN GILTHEAD SEA BREAM (*Sparus aurata*)

Sáez A.[a], Caballero-Solares A.[b], Silva-Marrero J.[a], Fernández F.[b], Baanante I.V.[a], Metón I.[a§]

[a]*Departament de BioquímicaiBiologia Molecular, Facultat de Farmàcia, Universitat de Barcelona, Joan XXIII 27-31, Barcelona, 08028, Spain.*
[b]*Departament d'Ecologia, Facultat de Biologia, Universitat de Barcelona, Diagonal 645, Barcelona, 08028,Spain.*

Myogenesis is an essential event for proliferation, differentiation, migration and fusion of myoblasts during skeletal muscle development. The myogenic regulatory factors MyoD2 and myogenin are essential for the regulation of these events. In the present study, we analysed the expression of MyoD2, myogenin and insulin-like growth factor 1 (IGF-1) in skeletal muscle of gilthead sea bream (*Sparus aurata*) juveniles submitted to different nutritional conditions. For a period of 37 days fish were starved or fed daily *ad libitum*. Total RNA was obtained from skeletal muscle and the concentration, purity and RNA integrity was determined in each sample. Gene expression levels were determined by quantitative real time RT-PCR. Feeding increased the mRNA expression levels of MyoD2, myogenin and IGF-1 in the muscle of *S. aurata* compared to starved fish. The higher increase corresponded to MyoD2, suggesting that MyoD2 expression in skeletal muscle could be a sensible biomarker of nutritional condition in *Sparus aurata*.

Keywords: MyoD2, myogenesis, nutritional status, Sparus aurata

[§] Corresponding author. Tel.: +34 934024521;Fax: + 34 934024520
E-mail address: imeton@ub.edu

GENETIC AND MOLECULAR ANALYSIS OF PHOSPHORUS HOMEOSTASIS REGULATORY GENES. A KEY NUTRIENT FOR AQUACULTURE SUSTAINABILITY

Suarez-Bregua P.[a,b§], Torres-Nuñez E.[a], Prober D.[c], Cerdá-Reverter J.M.[d], Guerreiro P.M.[e], Du S.J.[f], Cañestro C.[g], Rotllant J.[a]

[a] *Aquatic Molecular Pathobiology Group. Marine Research Institute (IIM- CSIC) Vigo, 36208, Spain*
[b] *Department of Biochemistry, Genetics and Inmunology, University of Vigo, Vigo, 36310, Spain.*
[c] *Biology Division, California Institute of Technology, Pasadena, 91125, USA*
[d] *Department of Fish Physiology and Biotechnology, Institute of Aquaculture Torre de La Sal (IATS-CSIC), Castellón,12595, Spain*
[e] *Comparative Molecular Endocrinology Group, Centre of Marine Science (CCMAR), University of Algarve, Faro, 8005-139, Portugal*
[f] *Department of Molecular and Cellular Biology, University of Maryland, Baltimore, 21201, USA*
[g] *Department of Genetics, University of Barcelona, Barcelona, 08007, Spain*

Phosphorus, in the form of inorganic phosphate (Pi), is one of the most important macronutrients for all organisms, including fish. Its deficiency has implications not only for hard tissues, where it is responsible for defective mineralization of bones, leading to skeletal malformation, but also for disturbances of intermediary metabolism especially energy metabolism, leading to impairment of growth. However the endocrine mechanisms for regulation of Pi balance in fish have largely been overlooked. Nowadays, world fish production is the fastest growing animal food sector internationally and the main objectives in fish farming are improvement of the foods used and the reduction of nutrients excreted in the water. In commercial fish culture systems Pi enriched diets are generally used to avoid skeletal malformations, ensure health and increase growth. However, excess levels can have harmful effects on fish, leading endocrine dysfunction, ectopic calcification and eventually death. Additionally, the excess of unused/excreted Pi in the effluents from these culture systems turns intensive fish farming in a major source of eutrophication and a consequent change in the aquatic system. Therefore, the reduction of the outputs of these dissolved wastes it will be a key element for the long-term sustainability of aquaculture around the world. One of the fundamental points to achieve this goal is to characterize and understand the molecular mechanisms that regulate Pi homeostasis in fish, which ultimately will contribute to the equilibrium between the requirements for optimal physiology, fast growth and reduced environmental impact. We have isolated and characterized a new parathyroid hormone (PTH) family member, named PTH4 that it is undoubtedly involved in bone mineral homeostasis. We have found that this new neuropeptide is synthesized by two clusters of neurons located in lateral hypothalamus. Functional studies using a stable PTH4 transgenic zebrafish line and morpholino oligonucleotide knockdown have revealed PTH4 as a powerful regulator of bone mass accrual acting on phosphate homeostasis. Our results, therefore, define a new neural brain-to-bone pathway involving efferent neural signal from hypothalamus to receptors on bone cells controlling phosphate homeostasis. Acknowledgements: This work was funded by the Spanish Science and Innovation Ministry project ALG2011-23581. JR. Paula Suárez Bregua was supported by Campus Do Mar-Xunta de Galicia PhD fellowship.

Keywords: Phosphate, diet, PTH4, neuropeptide, bone homeostasis

[*]These authors have contributed equally to this work.
§ Corresponding author. Tel.: + 34 986231930 ext. 289; Fax: +34 986292762
E-mail address: paulasuarez@iim.csic.es

EXPRESSION PATTERN ANALYSIS OF GENES INVOLVED IN GONAD DEVELOPMENT IN TURBOT (*Scophthalmus maximus*)

Taboada X.[a], Martínez P.[b], Adrio F.[c], Viñas A.[a][§]

[a] *Department of Genetics, University of Santiago de Compostela, Santiago de Compostela, 15782, Spain*
[b] *Department of Genetics, University of Santiago de Compostela, Lugo, 27002, Spain*
[c] *Department of Cell Biology and Ecology, University of Santiago de Compostela, de Compostela, 15782, Spain*

Turbot (*Scophthalmus maximus*) is an appreciated flatfish species in European aquaculture with a high commercial value. This species has also been recently introduced in China where turbot farming suffered a rapid development. As a result, the estimated global annual production in 2012 was 77,117 tons. One of the main objectives of turbot industry is to control sex ratio, a central demographic parameter that influences the structure of populations and determines their reproductive potential. Furthermore, this species shows extreme growth rate dimorphism between sexes, females reach commercial size 3–4 months before males, explaining the interest of the industry in produce all-female populations and therefore the importance of understanding sex determination and differentiation mechanism. Although a QTL analysis identified the major sex determining region in linkage group 5 and three minor in LG6, LG8 and LG21, genetic basis of these processes remain unknown. In order to broaden the understanding in this field we have analyzed the expression of some genes involved in sex (*amh*, *cyp19a*, *foxl2* and *vasa*) through *in situ* hybridization with riboprobes in the developing gonad of the turbot, at 130 days post-fertilization (dpf), 180dpf, and two years old specimens of both sexes. Results obtained are mostly in accordance with the data reported in other fish species. We found expression in both sexes for *amh*, *foxl2* and *vasa* genes. However, expression of *amh* (*anti-Mullerian hormone*), which is involved in testis differentiation in mammals, was higher in males and in females was higher *foxl2* (*forkhead box l2*), which is considered a good *marker of* ovarian differentiation; whereas an extremely high expression was detected in both sex for *vasa*, a germ cell marker. On the other hand, *cyp19a* (*cytochrome P450, family 19*) expression was restricted to ovary. This gene encodes aromatase enzyme that is responsible for the aromatization of androgens to estrogens. Finally, *in situ* hybridization of these genes were combined with inmunolabeling of PCNA (Proliferating cell nuclear antigen). The expression of these genes in different development stages in ovary and/or testis seems to confirm their involvement in the gonad development in turbot

Keywords: Sex determination, sex differentiation, gonad, turbot

[§] Corresponding author. Tel.: + 34881816918
E-mail address: anamaria.vinas@usc.es

SEQUENCING AND *DE NOVO* ASSEMBLY OF THE DIGESTIVE GLAND TRANSCRIPTOME IN *Mytilus galloprovincialis* AND ANALYSIS OF DIFFERENTIALLY EXPRESSED GENES IN RESPONSE TO DOMOIC ACID

Ventoso P.[a], Martínez-Escauriaza R.[a], Sánchez J.L.[a], Pérez-Parallé M.L.[a], Blanco J.[b], Triviño J.C.[c], Pazos A.J.[a§]

[a] *Departamento de Bioquímica y Biología Molecular, Instituto de Acuicultura, Universidad de Santiago de Compostela, Santiago de Compostela, 15782, Spain.*
[b] *Centro de Investigacións Mariñas, Xunta de Galicia, Pedras de Corón s/n Apdo 13, Vilanova de Arousa, 36620, Spain*
[c] *Sistemas Genómicos, Ronda G. Marconi 6, Paterna (Valencia), 46980, Spain*

The mussel *Mytilus galloprovincialis* is an important aquaculture resource in Spain. Bivalve molluscs are filter feeding species that can accumulate biotoxins in their body tissues during harmful algal blooms. Amnesic Shellfish Poisoning (ASP) is caused by species of the diatom genus *Pseudo-nitzchia*, which produces the toxin domoic acid. The *M. galloprovincialis* digestive gland transcriptome was *de novo* assembled based on the sequencing of 12 cDNA libraries, six obtained from control mussels and six from mussels naturally exposed to domoic acid. The mussels were collected from rafts in the Ría de Vigo, Spain. Illumina paired-end sequencing produced 705,558,488 reads (676,169,399 filtered reads) and the average read length was 100 bp. The percentage of reads with mean sequence quality ≥ Q30 was 95.04%. After *de novo* assembly with Trinity and Oasis 94,727 transcripts were obtained which ranged from 450 to 15,385 bp with an average length of 1,015 bp and a N50 length of 761 bp. The assembled transcripts were clustered (homology > 90%) to reduce redundancy, thus 69,294 unigenes were obtained. Furthermore digital gene expression analysis was performed in the digestive gland of *M. galloprovincialis* following exposure to domoic acid. Differential gene expression was calculated using the DESeq2 algorithm and genes were considered to be significantly differentially expressed if the absolute fold change was > 1.5 and the p-value was < 0.05. A total of 1,158 differentially expressed unigenes were detected (686 up-regulated and 472 down-regulated). Finally a study of functional enrichment was performed using annotations obtained from the genes differentially expressed, 66 Pfam families were found to be significantly (p<0.05) enriched. Among these enriched domains we found: C1q domain, sulfotransferase domain, aldo/keto reductase family, carboxylesterase family and major facilitator superfamily. Some of these families contain genes involved in toxin metabolism and detoxification processes. In conclusion this study provides a high quality reference transcriptome of *M. galloprovincialis* digestive gland and identifies potential genes involved in response to domoic acid in *M. galloprovincialis*. Acknowledgements: This work has been supported by the Spanish Ministry MINECO and the FEDER Funds of the EU under the project AGL2012-39972-CO2.

Keywords: Mytilus galloprovincialis, transcriptome, differential expression, domoic acid, ASP

§ Corresponding author. Tel.: + 34-881816056; Fax: + 34-881816047 ;
E-mail address: antonioj.pazos@usc.es ;

SOYBEAN MEAL INCLUSION IN ATLANTIC SALMON (*Salmo salar L.*) DIETS IS ASSOCIATED WITH CHANGES IN THE PRODUCTION OF INFLAMMATORY MARKERS AND INTESTINAL NUTRIENT TRANSPORTERS

Wacyk J.[*a], González S.[a], Mercado L.[b], Schmitt P.[b], Romero J.[c], Opazo R.[c]

[a]Departamento de Producción Animal, Universidad de Chile, Santiago, 8820808, Chile.
[b]Insituto de Biologia, Universidad Catolica de Valparaiso, Curauma, Chile
[c]Instituto de Nutricion y tecnología de los alimentos, Universidad de Chile, 7830490, Chile.

High inclusion of soybean meal in salmon diets is associated with reductions in fish growth performance and inflammatory responses in the digestive tract. Many of the studies studying the effect of the inclusion of soybean meal have looked at this non-infectious inflammatory condition which has been described to affect the posterior part of the digestive tract of fish. This, despite the fact most of the nutrients are absorbed in the anterior section of this organ. To gain further understanding of the effects of plant protein use in carnivorous fish diets, the objective of the present study was to evaluate the effects of soybean meal and fermented soybean meal inclusion in salmon diets kept in fresh water over growth performance parameters, changes in inflammatory markers as well as transcriptional changes of nutrient transporter in the proximal intestine of fish. Five hundred and forty Salmo salar juveniles (average weight 50 g) were randomly distributed in 9 tanks (150 L), supplied with 10 L/min (14 °C) of fresh water. Fish were fed to apparent satiety, three times daily, 6 days a week, during a period of 70 days with either control (fish meal based) or experimental diets (30% inclusion of regular or fermented soybean meal). Intestinal tissue samples were collected at 6 time points throughout the study at days 0, 2, 10, 20, 50 and 70. In the intestine of fish fed regular soybean meal base diets increasing levels of IL-1 beta were detected via immunohistochemistry, when compared with fish fed the control diets. Intestinal cells positive for IL-1 beta were detected as early as 10 days and statistical differences were observed among treatments starting at 20 days. Data on other inflammatory markers as well as nutrient transporter gene expression is being generated.

GENETIC ARCHITECTURES AND RESPONSE TO SELECTION

Álvarez-Castro J.M.[a, §]

[a] Department of Genetics, University of Santiago de Compostela, Lugo, 27002, Spain

The genetic architecture of a trait in a population is entailed by the network of loci in which there is variability underpinning phenotypic variance. Such network involves possible interactions between or among alleles that may occur within, between or among loci. The response of a trait to phenotypic (natural or artificial) selection is known to depend upon its genetic architecture, particularly upon interactions, which influence the additive variance and may drive temporary stasis periods in the face of a high selection effort. Also genomic selection may be improved by taking into account the presence of interactions in the genetic architectures of traits of importance for livestock production. In this communication, the Natural and Orthogonal Inter Actions (NOIA) model of genetic effects is presented as a unification and generalization of previous theoretical developments to handle complex genetic architectures and recent proposals involving NOIA for applying it to livestock production are reviewed.

Keywords: Genetic architecture, genetic effects, interactions, selection response, genomic prediction

§ Corresponding author. Tel.: + 34982822426
E-mail address: + jose.alvarez.castro@usc.es

SALMON AND SNP CHIPS: RAPID GENOMIC TECHNOLOGICAL TRANSFER AND IMPLEMENTATION OF GENOMIC SELECTION IN A NORTH AMERICAN *Salmo salar* BREEDING PROGRAM

Ang K.P.[a,*,§], Elliott J.A.K.[a,*], Powell F.[a,*], Schaeffer L.R.[b,*], Boulding E.G.[c,*]

[a] Kelly Cove Salmon Ltd., Cooke Aquaculture Inc., Saint John, E2K 1K7, CANADA
[b] Department of Animal and Poultry Science, University of Guelph, Guelph, N1G EC4, CANADA
[c] Department of Integrative Biology, University of Guelph, Guelph, N1G EC4, CANADA

Within the past year Kelly Cove Salmon Ltd. (KCS) has made a rapid transition from a traditional family-based salmon breeding program to a modern breeding program using genomic selection. The KCS breeding program required custom technology transfer because they are required, by legislation, to use only one strain of Atlantic salmon, the Saint John River Aquaculture Strain (SJR), in their marine net pens in Atlantic Canada. SJR was founded from the North American subspecies so fewer genomic resources have been developed. In collaboration with the University of Guelph, KCS is developing a custom 50K SNP chip (AquaGen strain and CIGENE) and low density SNP assay (Agena) to provide the necessary high throughput platforms to incorporate genomic technology into our Atlantic Canadian operations. Achieving these two key objectives is required to effectively implement genomic selection into the KCS breeding program making it much more effective, especially with respect to resistance to diseases and parasites which are not possible to measure on candidate broodstock held in freshwater. Currently in year 2 of the project the use of microsatellites to determine the parentage of communally-reared juveniles has been almost entirely replaced by the use of low density SNP assays. Further, SNP data from a HD chip was used to select which candidate broodstock to spawn late in year one. Genomic selection for traits potentially including saltwater growth, late maturity and resistance to BKD, ISA and sea lice will be used to select candidate broodstock in subsequent years. The logistics of being industrial co-lead on such an ambitious technology transfer project with many industry, government, and academic collaborators will be discussed.

Keywords: Atlantic salmon, genomic selection, industry perspective, Salmo salar, SNP,

*These authors have contributed equally to this work.
§ Corresponding author. Tel.: +1 506-755-1328; Fax: +1 506 694-1381
E-mail address: keng.pee.ang@cookeaqua.com

BIO-ECONOMIC MODELING: A KEY TOOL TO EVALUATE THE BENEFITS OF GENOMIC SELECTION IN ATLANTIC SALMON

Araneda M.E.[a,§], Yáñez J.M.[a,b], Lhorente J.P.[a], Moreno J.M.[a]

[a]*Department of bio-economy, Aquainnovo S.A., Puerto Montt, 5480000, Chile*
[b]*Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, 8820808, Chile*

Breeding objectives for Atlantic salmon should include traits related to growth and disease resistance. In this regard, two of the main diseases affecting Chilean salmon farming are caused by *Piscirickettsia salmonis* and *Caligus rogercresseyi*. It has been stated that genomic-enabled evaluations (i.e. genomic selection, GS) for disease resistance will have a favorable impact on the genetic progress for these traits. However, there are few studies aimed at evaluating the risk and uncertainty of implementing GS for Atlantic salmon from an economic perspective. The aim of this study was to evaluate the risk of implementing GS for resistance to *P. salmonis* and *C. rogercresseyi* using a bio-economic model. We developed a model including the effects of temperature, photoperiod and individual weight. The model was calibrated by historical observations of Atlantic salmon production, scientific literature, market and technical parameters. We assumed that the production system was based on 318 cages with a maximum capacity of 70,000 tons cycle^{-1}. A Limit Reference Point (LRP) of US\$3.81 kg^{-1} for the scenario without GS was considered. This indicator was evaluated through Monte Carlo simulation using 2,000 iterations. Technological and economic variables were considered as sources of uncertainty. Results showed that the implementation of GS can reduce production costs by 3.30% (\$0.115 kg^{-1}) compared to the scenario without GS. By implementing GS, the risk of exceeding the limit reference point is 26.1%. However, without GS the economic risk increases 46.2%.The cost associated with genotyping accounted for only 0.54% of the total cost. The incorporation of GS helped to reduce total mortality by 3.34% in one cycle. Specific biomass growth rate increased from 0.67% to 0.73% day^{-1} on average and feed conversion efficiency decreased from 1.49 to 1.47. Production increased by 12.6% (0.209-0.235 kg/smolt/cycle). These results demonstrate the economic feasibility of incorporating genomic selection schemes to improve disease resistance in Atlantic salmon in Chile and also point to the importance of bio-economic modeling for the incorporation of new technologies.

Keywords: Bio-economy, genomic selection, risk, uncertainty, Atlantic salmon

[§]Corresponding author. Tel.: (56-65) 22 04 317
E-mail address: maraneda@aquainnovo.com

GENOME WIDE ASSOCIATION ANALYSIS FOR RESISTANCE TO *Caligus rogercresseyi* IN ATLANTIC SALMON (*Salmo salar* L.)

Correa K.[a,b], Lhorente J.P.[b], López M.E.[b,c], Figuera R.[b], Bassini L.[b,c], Di Genova A.[d], Maass A.[d], Davidson W.[e], Yáñez J.M.[a§]

[a] *Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, 8820808, Chile*
[b] *Aquainnovo, Puerto Montt, 5503032, Chile*
[c] *Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago, 8820808, Chile*
[d] *Laboratory of Bioinformatics and Mathematics of the Genome, Center for Mathematical Modeling, Universidad de Chile, Beauchef 851, Santiago, 8370456, Chile*
[e] *Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby,V5A1S6, Canada*

Caligus rogercresseyi is considered one of the main parasites affecting profitability of the Chilean salmon industry. The objective of this study is to determine association between Single Nucleotide Polymorphisms (SNPs) and resistance to *Caligus rogercresseyi* in Atlantic salmon.

2301 fish belonging to 118 families were experimentally infested with *C. rogercresseyi* (chalimus stage) and kept under controlled conditions. After six days fish were euthanized and sessile caligus were count in fins of all fish. Fin clip samples were taken for DNA extraction. Samples were genotyped with a 50K SNP Axiom® myDesign™Genotyping Array.

Genome-wide association analysis was conducted using the GenABEL software. A polygenic model (*Family-Based Score Test for Association)* was used. Resistance was considered as the total count of caligus in fins. Bonferroni correction was used to control the false positive rate.

2277 samples and 47922 SNPs passed all quality control criteria. Estimated heritability (h^2) obtained with genomic information matches the one obtained from pedigree (0.117). We did not find evidence of genome-wide significant markers after multiple testing corrections. Only one marker at chromosome 21 was significant at a chromosome-wide level. The proportion of the phenotypic variance explained by this marker is 0.008.

There is only one chromosome-wide significant marker that is associated to resistance against *Caligus rogercresseyi* in this population of Atlantic salmon. This marker explains a small proportion of this variation, suggesting that this trait has a highly polygenic component. A genomic selection approach will be more suitable to incorporate molecular information to accelerate the genetic improvement for this trait.

Keywords: Atlantic salmon, Caligus rogercresseyi, resistance, association, SNP

§ Corresponding author. Tel.: + 56-2-29785533
E-mail address: jmayanez@uchile.cl

GENOME WIDE ASSOCIATION ANALYSIS REVEALS GENETIC ARCHITECTURE OF THE RESISTANCE TO *Piscirickettsia Salmonis* IN ATLANTIC SALMON (*Salmo salar* L.)

Correa K.[a,b], Lhorente J.P.[b], López M.E.[b,c], Figueroa R.[b], Bassini L.[b,c], Di Genova A.[d], Maass A.[d], Davidson W.[e], Yáñez J.M.[a,b§]

[a] *Facultad de Ciencias Veterinarias y Pecuarias, Universidad de Chile, Santiago, 8820808, Chile*
[b] *Aquainnovo, Puerto Montt, 5503032, Chile*
[c] *Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago, 8820808, Chile*
[d] *Laboratory of Bioinformatics and Mathematics of the Genome, Center for Mathematical Modeling, Universidad de Chile, Beauchef 851, Santiago, 8370456, Chile*
[e] *Department of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, V5A1S6, Canada*

Piscirickettsia salmonis is considered one of the most important pathogens in the Chilean salmon farming industry. The objective of this study is to determine association between Single Nucleotide Polymorphisms (SNPs) and resistance to *Piscirickettsia salmonis* in Atlantic salmon.

2600 fish belonging to 118 families were experimentally infected with *P. salmonis* and were kept under controlled conditions. The day of death for each fish was registered and fin samples were taken for DNA extraction. At day 40 all survivor fish were euthanized. Samples were genotyped with a 50K SNP Axiom® myDesign™Genotyping Array.

Genome-wide association analysis was conducted using the GenABEL software. Linear regression, logistic regression, and Cox proportional hazards models were used. Resistance was considered as a binary trait and as time to death trait. Bonferroni correction was used to control the false positive rate.

2391 samples and 49688 SNPs passed all quality control criteria. Estimated heritability obtained with genomic information matches the one obtained from pedigree (0.18). Two markers were significant at genome-wide level and 5 markers were significant at chromosome-wide level. The genome-wide and two of the chromosome-wide significant markers are located in a 800kb region of chromosome Ssa1. The other markers were located in different regions of chromosome 1 and 17.The proportion of the phenotypic variance explained by each of these markers is 0.02-0.03.

There are two genome-wide significant markers associated to resistance against *P. salmonis.* The markers found explain a small proportion of the phenotypic variation of the trait. Thus, a genomic selection approach will be suitable to include molecular information to accelerate the genetic progress for this trait.

Keywords: Atlantic salmon, Piscirickettsia salmonis, resistance, association, SNP

[§] Corresponding author. Tel.: +56-2-29785533
E-mail address: jmayanez@uchile.cl

ALLELE SPECIFIC EXPRESSION ON LIVER AND HEAD KIDNEY OF *Salmo salar* WITH DIFFERENTIAL SUSCEPTIBILITY TO THE CHALLENGE WITH *Piscirickettsia salmonis*

Dettleff P. and Martínez V.

FAVET-INBIOGEN, Faculty of Veterinary Science, University of Chile, Santiago, Post code 8820808, Chile.

The allele specific expression (ASE) determines the existence of imbalances in the expression of one allele relative to the other. This analysis is possible using deep sequencing of mRNA obtained from different tissues and susceptibility background. The aim of this study is to identify SNPs that present ASE in Atlantic salmon challenged with *Piscirickettsia salmonis,* based on large scale challenge test for this disease. A *de novo* assembly was obtained using a sample of individuals of different families, representing the population under analysis. In this study, samples of liver and head kidney of susceptible and resistant individuals to the disease were used for RNA-seq analysis, as obtained throughout the challenge test. SNPs present in the transcripts of the two groups were identified, determining heterozygotes in the population and the number of reads for each allele. To identify ASE, the deviation of the expected ratio 50:50 of the expression of each allele was evaluated using a Chi-square test (FDR <0.01). Subsequently, the SNPs with ASE in each sample were compared, identifying those SNPs with ASE in both groups and tissues. Additionally the SNPs that present ASE exclusively on susceptible or in resistance group were determined. A total of 4063 heterozygotes SNPs were identified. Of these, 170 SNPs present ASE in both tissues and in both groups, present on 59 different transcripts. To evaluate the molecular and biological function of these genes a Gene Ontology analysis was conducted. The results show that genes with ASE in all individuals are associated with metabolic processes of organic substances; nitrogen compounds metabolic processes and biosynthetic processes. Additionally 174 SNPs (in 62 transcripts) were identified with ASE within the susceptible group. In the case of resistant group, a total of 102 SNPs (in 43 transcripts) with ASE exclusively in this group were obtained. The SNPs with ASE of these transcripts could be of importance in disease resistance against this bacterium, due to the fact that they could be used in marker-assisted selection for tolerance against this pathogen. Still, further studies are required in order to understand how these *cis* acting factors explain disease resistance in practice. Funding: FONDECYT 1120608, CONICYT scholarship for PhD.

Keywords: salmon, allele specific expression, SRS, RNA-seq

MICROSATELLITE POLYMORPHISM IN GROWTH HORMONE GENE AND ITS ASSOCIATION WITH PRODUCTION PERFORMANCE OF NILE TILAPIA STRAINS

Dias M.A.D.[a,c], Bueno-Filho J.S.S.[b], Jaser S.K.K.[c], Freitas R.T.F.[a], Hilsdorf A.W.S.[c,§]

[a]*Department of Animal Sciences, Federal University of Lavras, Lavras, 37200-000, Brazil*
[b]*Department of Exact Sciences, Federal University of Lavras, Lavras, 37200-000, Brazil*
[c]*Unit of Biotechnology, University of Mogi das Cruzes, Mogi das Cruzes,08780-911, Brazil*

Crossbreeding using *O. niloticus*, Red-Stirling (GG1) and Chitralada (GG2) strains were carried out aiming to assess the association between microsatellite (STR) polymorphism on the promoter and intron-1 of growth hormone gene (GH) loci and performance characteristics. Seven genetic groups (parental, ½ Chitralada and reciprocal, ¾ Chitralada and reciprocal, and 7/8 Chitralada from Chitralada dam) were evaluated by weight, standard length, body height and width. The function *lmer ( )* of the package *lme4* of R software were used to estimate the model effects. We considered as variation sources the fixed effects: sex, contemporary group of age and the animal initial weight class; and random: genetic group, *STR*-Promoter and *STR*-Intron. Six alleles were found in *STR*-Promoter ranging from eighteen to twenty-one possible genotypic combinations. The alleles 191 and 196 from *STR*-Promoter comprise 80% of the total alleles in assessed individuals, whereas 196/196, 191/196 and 196/201 genotypes were found more often. Three alleles were observed in *STR*-Intron, of which allele 198 were not found in the GG1 group and the homozygote genotype 198/198 were present only in the group 7/8 from chitralada dam. Performance differences were detected during crossbreeding when using GG1 and GG2 as dams. Assessing the molecular diversity in relation to the genetic groups, we observed that 94,61% of total variation is due to the individual variation, with low average level of inbreeding (F_{IS} = 0,021). The *STR*-Promoter and *STR*-Intron loci were important variation sources for evaluated characteristics (except for weight in *STR*-Íntron), independently of sex, initial weight class and genetic group effects. For *STR*-Promoter locus the genotypes 181/181, 181/191 and 196/206 showed mean higher performance, and the genotypes 196/201, 201/201 and 191/196 the lower performance. For *ST*R-Intron locus, the highest performance genotypes were 202/206 and 198/206, with superiority bias towards genotypes having allele 198. The polymorphisms found in STR-Promoter and STR-Intron of the GH gene of *O. niloticus* are associated to performance characteristics. Alleles with lower number of tandem repetitions were associated to higher performances. The observed association suggests that the polymorphisms found herein may be incorporated into Marked-Assisted Selection program.

Keywords: Oreochromis niloticus, STR, Polimorphism, Growth, GH

*These authors have contributed equally to this work
§Corresponding author. Tel.: + 55 11 4798-7210; Fax: +55 11 4798-7106
E-mail address: wagner@umc.br

GENETIC DIVERSITY ASSOCIATED WITH MICROSATELLITE MARKERS IN GROWTH HORMONE GENE AND NEUTRAL LOCI OF THE NILE TILAPIA (*Oreochromis niloticus*)

Dias M.A.D.[a§]**, Freitas R.T.F.**[a]**, Arranz S.E.**[b]**, Villanova G.V.**[b]**, Hilsdorf A.W.S.**[c]

[a] *Departmentof Animal Science, Universidade Federal de Lavras, Lavras, 37200-000, Brazil*
[b] *Plataforma de Biotecnología Acuática, Instituto de Biologia de Rosario, Rosario, S2000, Argentina*
[c] *Department, IUniversidade de Mogi das Cruzes, Mogi das Cruzes, 08735-400, Brazil*

Understand the genetic basis of variation in traits related to growth in *Oreochromis niloticus* is important to the aquaculture industry. The Growth Hormone gene is considered a highly conserved and crucial growth-regulating gene in species and strains of Nile tilapia found worldwide. To successfully use them in breeding programs, they first need to be genetically characterized. In this study, regions on GH1 gene of *O. niloticus* were genotyped, aligned and compared across four strains (UFLA, GIFT, Chitralada and Red-Stirling). Two microsatellites (*STR*) were identified in the putative promoter (position -693 / -679, called STR-Promoter, motif (ATTCT) 8) and intron 1 (position +140/+168, called STR-Intron, motif (CTGT)7) regions. The strains were genetically characterized using 10 noncoding microsatellite loci (UNH828, UNH829, UNH009, UNH005, UNH103, UNH104, UNH123, UNH203, UNH866, and GM672) and two microsatellites located in the promoter and first intron of the *GH1*. The STR-Promoter (6 alleles) and STR-Intron (3 alleles) loci were polymorphic in the four studied strains. A total of 88 alleles with high polymorphism among the studied strains were identified by analysis of 10 noncoding *STR* loci, except for the UNH-005 locus (with allele 159, private in UFLA and GIFT strains) and the UNH-866 locus (with allele 167, private in the Red-Stirling strain, and with alleles 171 and 177, private in the UFLA and Chitralada strains, respectively). Genetic diversity was measured as mean expected heterozygosity and numbers of alleles, which were 4 and 0.60 (GIFT), 3.5 and 0.71 (UFLA), 4.5 and 0.57 (Chitralada), and 2.5 and 0.42 (Red-Stirling), respectively. The UFLA and GIFT strains are less genetically divergent (D_{EST} = 0.10) and have similar structure in relation to Chitralada and Red-Stirling strains (D_{EST} = 0.32 and 0.33; 0.45 and 0.47, respectively), with the latter being the most distant (D_{EST} = 0.59). UFLA strain was genetically characterized and presented as an important source of variability adapted to regions with mild temperatures. Validation and association studies with performance characteristics of STR-Promoter and STR-Intron loci should be performed to confirm the potential of these loci as possible quantitative trait locus on marker-assisted selection programs.

Keywords: genetic variability; tilapia strains; population genetics; GH1;STR

§ Corresponding author. Tel.: +55 (35)9198-0382
E-mail address: marconepre@yahoo.com.br;

MYOD-2 SNPs IN GILTHEAD SEABREAM *Sparus aurata* L.: POTENCIAL GENETIC VARIANTS LINKED TO BODY WEIGHT

García-Fernández C.*, Sánchez J.A.*, Blanco G.*[§]

Laboratory of Aquaculture Genetics, Genetic Area, Department of Functional Biology, Faculty of Medicine, University of Oviedo, 33006 Asturias, Spain

Maximizing growth rate is a key objetive to increase culture productivity by achieving a more profitable cost/benefit balance. Growth is a productive trait with a complex genetic control where many genes with very different effect are involved. Among the wide range of available candidate growth genes in gilthead seabream, we focused on *MyoD-2*. This gene is linked to growth traits due to its key role in the differentiation and development of muscular tissue. The main goal of the present study was the identification of putative MyoD-2 SNPs associated with changes in body weight.

Three SNPs (*SaMyoD-2_A785C, SaMyoD-2_C1982T* and *SaMyoD-2_A2031T)* were identified through a 1.963bp sequencing of *MyoD-2* gene [AF478569.1] in a subset of 30 seabream (classified as unrelated by microsatellite markers).These SNPs were located in promoter region, non-coding intron 1 and coding exon 2, respectively.

The relationships between SNPs and body weight were evaluated by SNP genotyping of 53 breeders from two broodstocks (A:18♀ - 9♂; B:16♀ - 10♂) and 389 offspring divided into two groups (slow- and fast-growth) with significant differences at 18 months of development (A18Slow: N=107, A18Fast: N=103, B18Slow: N=92 and B18Fast: N=87) [Borrell et al. (2011), Aquaculture 314: 58-65]. Haplotype and diplotype were reconstructed from genotype data by Phase 2.1 software. Differences among means of different diplotypes were calculated by one-way ANOVA followed by post-hoc Tukey test. Association analysis indicated that single SNP did not show significant effect on body weight. However, when the analysis is carried out considering diplotype data it was observed that the ACA/CCA diplotype was linked to lower body weight. This allelic SNP combination always showed the lowest mean body weight, differing statistically ($P<0.05$) in three (A18Slow, A18Fast & B18Slow) of the four groups tested. Specially, ACA/CCA diplotype recorded a maximum decrease of 14-18% on mean body weight.

Although further studies are needed to validate the role of these 3 SNPs as markers for body weight, the polymorphism-trait association established in this work creates promising expectations on the use of these variants as genetic tool for future giltead seabream breeding programs.

Keywords: MyoD-2; SNP; growth; gene assisted selection; gilthead seabream (Sparus aurata)

[*]These authors have contributed equally to this work.
[§] Corresponding author. Tel.: + 985106262 ; Fax: + 985103534
E-mail address: gloriablanco@uniovi.es

PRELIMINARY RESULTS OF THE TRAINING PANEL SNP´S AND PHENOTYPIC VARIABILITY IN THE MUSSEL *Mytilus galloprovincialis.*

Gallardo J.[a], Prieto P.[a], Arriagada D.[a], Espinoza C.[a], Hermida M.[b], Martínez P.[b] & Faúndez V.[a, §]

[a] *Departamento de Ingeniería Ambiental y Recursos Naturales, Universidad Católica Santísima Concepción, Concepción, 4030000, Chile.*
[b] *Departamento de Genética, Universidad de Santiago de Compostela, Lugo, 27002, España.*

Mytilus galloprovincialis is a species of great economic interest within the region of Bio-Bio, Chile. In this context, it is currently running the Innova Chile (13IDL2-23408), which seeks to create the technological basis for the selection of families with better performance in terms of survival and growth project. To this are being developed in parallel two lines of work of which previous results are reported:

First, a search of SNPs was carried out using mass sequencing (Illumina, RAD_seq) in 8 individuals to characterize the genome. The sequencing results showed a total of 54.2 million reads, with an average from 6,025,896 for each individual. Following this, the analysis pre-processing and assembly against the reference genome of *Mytilus galloprovincialis* previously described was conducted. The total number of candidates SNP´s was 6,382. The subsequent statistical analysis allowed to determine the existence of variables 2,680 points higher reliability. With these results we have a large genomic basis for the search for QTL economic importance within the species.

On the other hand, two economically important traits were analyzed in F1 families in order to assess the phenotypic variability within the populations studied. The characters were the growth rate and survival rate. Among the results obtained it has been observed that there are significant differences in growth character, both in length and wet weight factor. The largest segment size, has an average of 27.3 (± 5) mm shell length whereas segment families smaller size, has an average of 17.3 (± 4.1) mm, with a difference 57.4%. On the weight, the higher segment has an average of 2.43 (± 1.09) g of wet biomass, while segment families lower weight, has an average of 0.834 (± 0.51) g, with a difference of 191.6%. However, considering the survival factor in families, they only achieve a survival rate of 0.12%. In this context it is necessary to evaluate economic factor is more relevant in a commercial scale production.

Keywords: Genomics, selection, Rad_seq, improvement, SNPs

[§] Corresponding author. Tel.: + 56-41-2345043 .
E-mail address: vfaundez@ucsc.cl

IDENTIFICATION OF GROWTH-RELATED QUANTITATIVE TRAIT LOCI AND HIGH-RESOLUTION GENETIC LINKAGE MAPS USING SIMPLE SEQUENCE REPEAT MARKERS IN THE KELP GROUPER (*Epinephelus bruneus*)

Kessuwan K.[a,e], Kubota S.[a], Liu Q.[a,f], Sano M.[a,c], Okamoto N.[a], Sakamoto T.[a], Yamashita H.[b], Nakamura Y., Ozaki A.[d§]

[a] *Faculty of Marine Science, Tokyo University of Marine Science and Technology, 4-5-7 Konan, Minato-ku, Tokyo 108-8477, Japan*
[b] *Ehime Research Institute of Agriculture, Forestry and Fisheries, Fisheries Research Center, 5516 Shitaba, Uwajima-shi, Ehime 798-0104, Japan*
[c] *National Research Institute of Fisheries Science, Fisheries Research Agency, 2-12-4 Fukuura, Kanazawa-ku, Yokohama, Kanagawa 236-8648, Japan*
[d] *National Research Institute of Aquaculture, Fisheries Research Agency, 422-1 Nakatsuhamaura, Minamiise-cho, Watarai-gun, Mie 516-0193, Japan*
[e] *Coastal Fisheries Research and Development Bureau, Department of Fisheries, 50 Kaset Klang Jatujak, Bangkok 10900, Thailand*
[f] *Center for Marine Ranching Engineering Science Research of Liaoning, Dalian Ocean University, 52 Heishijiao Street, Dalian 116023, China*

To conduct a genetic breeding program of a kelp grouper, reference family and marker-assisted selection (MAS) based on the high density of genetic linkage maps and quantitative trait loci (QTLs) are important. Herein, we constructed a high-resolution genetic linkage map using 1,055 simple sequence repeat (SSR) markers in an F_1 family. Genome-wide and chromosome-wide significances of growth-related QTLs (body weight: BW and total length: TL) were detected using non-parametric mapping, Kruskal-Wallis analysis, simple interval mapping (IM) and a permutation test (PT). Two stages and two families of fish were used to confirm the QTLs region. Ultimately, 714 SSR markers were matched that evenly covered the 24 linkage groups. 509 and 512 markers were identified in the female and male maps. The genome lengths was approximately 1,476.45 cM and 1,370.39 cM and covered 84.66% and 83.21% of the genome, with an average interval of 4.1 cM and 4.0 cM, in females and males, respectively. One major QTL affecting BW and TL was found on linkage group EBR17F that identified for 1% of the genome-wide significance and accounted 14.6–18.9% and 14.7–18.5% of the phenotypic variance and several suggestive QTL with 5% chromosome-wide significance were detected on 8 linkage groups. Furthermore, the confirmed results of the regions harboring the major and suggestive QTLs showed consistent significant experiment-wide values of 1%, 5% and a chromosome-wide value of 5%. We anticipate that the high resolution of genetic linkage map and growth-related QTLs found in this study could be applied to find candidate genes, will be powerful tools for a future MAS breeding program and may provide further insights into the genetic control of growth traits in the kelp grouper.

§ Corresponding author. Tel.: +81-599-66-1830; Fax: +81-599-66-1962
E-mail address: aozaki@affre.go.jp

DNA sequence Variation of common carp myostatin - a candidate growth gene

Kohlmann K.[a*, §], Kersten P.[a*], Gela D.[b*], Irnazarow I.[c*], Füllner G.[d*]

[a] *Department of Ecophysiology and Aquaculture, Leibniz-Institute of Freshwater Ecology and Inland Fisheries, Berlin, 12587, Germany*
[b] *South Bohemian Research Center of Aquaculture and Biodiversity of Hydrocenoses, University of South Bohemia in České Budějovice, Vodňany, 38925, Czech Republic*
[c] *Institute of Ichthyobiology and Aquaculture in Golysz, Polish Academy of Sciences, Chybie, 43-520, Poland*
[d] *Fisheries Department, Saxon State Agency for Environment, Agriculture and Geology, Königswartha, 02697, Germany*

Myostatin (MSTN), or Growth/Differentiation Factor-8 (GDF-8), belongs to the Transforming Growth Factor-β (TGF-β) superfamily. It has been identified as a candidate growth gene in livestock including fish that might be used in marker assisted selection to accelerate genetic gains. In the context of a performance test, individuals originating from four German and Polish mirror strains and one Czech scaly strain of common carp (*Cyprinus carpio*) were randomly chosen and examined for MSTN sequence variation. The common carp MSTN gene has an open reading frame (ORF) of 2662 bp and consists of three exons (376, 371, and 381 bp) and two introns (691 and 843 bp). So far, six single nucleotide polymorphisms (SNPs) arranged in two alleles were detected. In contrast, insertions/deletions could not be found. SNP1 (A/C at nucleotide position 363 of the ORF) and SNP6 (G/A at nucleotide position 2323 of the ORF) were located in exon 1 and exon 3, respectively. Both SNPs were synonymous coding for threonine and arginine, respectively. SNP2 (C/T at nucleotide position 1618 of the ORF), SNP3 (C/A at nucleotide position 1672 of the ORF), SNP4 (T/C at nucleotide position 2158 of the ORF), and SNP5 (A/G at nucleotide position 2160 of the ORF) were all located in intron 2. Both alleles occurred in mirror as well as in scaly common carp. For routine genotyping of common carp MSTN by PCR-RFLP methods, five restriction enzymes were identified that cover all six SNPs. Future studies should be directed to examine possible associations between the MSTN sequence variation and growth performance of common carp.

*These authors have contributed equally to this work.
§ Corresponding author. Tel.: + 49 30 64181634; Fax: + 49 30 64181663
E-mail address: kohlmann@igb-berlin.de

IDENTIFICATION AND MAPPING OF A SCAR MARKER LINKED TO A LOCUS INVOLVED IN SHELL PIGMENTATION OF THE PACIFIC OYSTER *Crassostrea gigas*

Li Q.[§], Ge J., Kong L.

Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China

The Pacific oyster (*Crassostrea gigas*) is one of the most important farmed oysters world-wide, ranking first in production among all other cultured aquatic animals. Recently, with the improvement of growth rate and survival, the visual perception trait of Pacific oyster, i.e., shell and mantle pigmentation, has attracted more and more attention. To identify QTL that may be helpful in selective breeding for shell pigmentation in the Pacific oyster, bulked segregant analysis in combination with amplified fragment length polymorphism (AFLP) technique was used to identify associated fragments of shell pigmentation. Additionally, associated AFLP fragments were converted into PCR-based sequence-characterized amplified region (SCAR) marker and genetic mapping of SCAR marker was constructed.

An F1-segregating population exhibiting a bimodal distribution of shell pigmentation intensity was obtained by crossing two wild oysters with opposite shell pigmentation. Genomic DNA from nine individuals with lightest shell pigmentation and nine individuals with darkest shell pigmentation were equally pooled for AFLP screening. In bulked segregant analysis, 6 out of 225 selective primer pair combinations produced 7 polymorphic fragments tightly associated with shell pigmentation across the segregating population. All 7 AFLP markers were almost specific to one group and segregated in the ratio of 1:1 expected for a dominant marker in the full-sib family. The 7 AFLP markers were mapped onto a single linkage group that spanned a region of 11.4 cM, and 80% of phenotypic variance could be explained by this locus. In conversion of the seven fragments into SCAR markers, only one was successfully converted into a co-dominant SCAR marker, named SP-170. This SCAR marker was at a distance of 27 cM from the microsatellite marker ucdcg184.

In conclusion, a SCAR marker linking to a major gene/QTL controlling the shell pigmentation was identified for the Pacific oyster. This SCAR marker shows promise in identifying breeders with the major gene underlying shell pigmentation. A further characterization of this locus may ultimately help in the positional cloning of the shell pigmentation gene and the better understanding of shell pigmentation variation in this species.

Keywords: Pacific oyster; Shell pigmentation; AFLP; SCAR

[§] Corresponding author. Tel.: +86-532-82031622; Fax: +86-532-82031765
E-mail address: qili66@ouc.edu.cn

GENOMICS: MARKER ASSISTED SELECTION GENOME-WIDE SEARCH FOR QTL FOR GROWTH TRAITS OF SALINE TILAPIA (*Oreochromis* spp.)

Lin G.[a,b], Yue G.H.[a§]

[a] *Molecular Population Genetics, Temasek Life Science Laboratories Ltd, Singapore, 117604, Singapore*
[b] *School of Biological Sciences, Nanyang Technological University, Singapore,* 639798, *Singapore*

Mozambique tilapia and its hybrids (including the red tilapia) are a representative group of euryhaline cichlids in aquaculture. Growth rate is one of the important and desired traits in aquaculture. Mapping QTL for growth is an essential step towards marker-assisted breeding with the purpose of growth enhancement. QTL mapping for growth traits (ie. Body weight, Total length and Standard length) was conducted in a full sib F2 family consisting of 522 individuals with 125 microsatellite markers covering the whole genome. Significant QTL for these growth traits were mapped on linkage groups 9, 10, 15 and 22 at 90 dph and 180 dph explaining 1.6 to 10.3% of phenotypic variations. The significant QTL for growth traits are being fine-mapped to identify genes for growth traits. The data that result from this study provides a useful resource in the marker-assisted selection of saline tilapia breeding programmes.

Keywords: Oreochromis spp., growth, saline tilapia, RNAseq, QTL

[§] Corresponding author. Tel.: +65 68727405; Fax: +65 68727000
E-mail address: +genhua@tll.org.sg

DEVELOPMENT OF GENOMIC TOOLS IN SENEGALESE SOLE: TRANSCRIPTOME ASSEMBLY, ANNOTATED DATABASE, MICROARRAY AND GENOME DRAFT

Manchado M.[b§], Benzekri H.[b], Seoane P.[b], Bautista R.[c], Sánchez J.J.[d], Cousin X.[e], Planas J.V.[f], Rebordinos L.[g], Claros M.G.[b,c]

[a] *IFAPA Centro El Toruño, Consejeria de Agricultura, Pesca y Medio Rural, 11500 El Puerto de Santa María, Cádiz, Spain*
[b] *Departamento de Biología Molecular y Bioquímica, Universidad de Málaga, Málaga, 29071, Spain*
[c]*Plataforma Andaluza de Bioinformática, Universidad de Málaga, Málaga, 29071, Spain*
[d] *Campus de Ciencias de la Salud, Instituto Nacional de Toxicología y Ciencias Forenses (INT), La Laguna, Spain*
[e] *IFREMER, Laboratoire d'Ecotoxicologie, Place Gaby Coll, BP 7, 17137 L'Houmeau, France.*
[f] *Departament de Fisiologia i Immunologia, Facultat de Biologia, Universitat de Barcelona and Institut de Biomedicina de la Universitat de Barcelona (IBUB), 08028 Barcelona, Spain*
[g] *Laboratorio de Genética. Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, Polígono del Río San Pedro, 11510, Puerto Real, Cádiz, Spain*

The Senegalese sole is an economically important flatfish species in fisheries and aquaculture. However, the genomic resources in this species are still scarce. Hence, the transcriptome and genome have been investigated. For transcriptome analysis,more than 1,800 millions reads from different larval and adult tissues were *de novo* assembled resulting in 701,767 tentative transcripts. Orthology analysis using zebrafish as reference identified at least 45,063 putative different transcripts, 18,738 of which were reconstructed with a complete ORF. Moreover, cross-species comparison with the closely-related species *S. solea* and other teleosts identified a set of 14,451 putative transcripts for sole- or lineage-specific genes. As a result, a reference transcriptome including 59,514 transcripts was defined and used to print a sole oligonucleotide microarray containing 43,303 probes. Moreover, a search of molecular markers identified a total of 266,434 SSRs and 337,315 SNPs in the transcriptome. These data and the complete annotation of the transcriptome is available for browsing and downloading at SoleaDB (http://bit.ly/SoleaDB). For genome analysis, ~3.000x10^6 raw reads (including single, paired-end and mate-pair reads from both 454/Roche and Illumina platforms) were processed. Sequences were cleaned using SeqTrimNext, assembled with Ray, reconciled with Gam-NGS, scaffolded with SOAPdenovo2 and SSPACE, and finally gaps closed with Gap closing tool of SOAPdenovo2. Several in-house scripts were developed for contig and scaffold validation and mapping. The 132,712 contigs obtained provide 34,176 scaffolds with a N50 of 85,602 nt. The whole draft genome was ~600 Mb in size and the longest scaffold was 638 kb in length. Mapping of scaffolds onto *Cynoglossus semilaevis* draft genome located 95% of scaffolds (569 Mb in total) onto 21 chromosomes. *In silico* comparison of genetic map markers and scaffold positioning confirmed a linkage correspondence higher than 90%. Moreover, the amplification of 111 predicted SSR markers distributed throught the chromosomes confirmed the accuracy of the assembly obtained. These results confirmed the high similarityo f both flatfish genomes and represent new powerful tools for genomic analysis in *S. senegalensis*. This research was funded by AQUAGENET project program INTERREG IVB SUDOE (ERDF) as well as INIA and EU through the ERDF 2014-2020 "Programa Operativo de Crecimiento Inteligente (RTA2013-00023-C02-01).

Keywords: Sole, transcriptome, genome, draft, database, microarray

§ Corresponding author. Tel.: +34 671532088; Fax: + 34956011324
E-mail address: + manuel.manchado@juntadeandalucia.es

DISCOVERY OF PARALOGOUS REGIONS IN THE COHO SALMON GENOME USING REDUCED REPRESENTATION SEQUENCING

Martínez V.[§], Bravo C., Barra V., Machuca A., Patel A.

FAVET-INBIOGEN. Faculty of Veterinary Science, University of Chile, Santiago, 8820808, Chile.

Several species of salmonids have an increase probability of sequence similarity in different regions of the genome. The sequence data obtained from such regions can be very similar, thus making it difficult to disentangle estimates of genetic variation. Due to the increase importance of the Coho salmon for the Chilean aquaculture, it is necessary to develop genomic resources aimed at implementing genomic selection for traits that are difficult to measure such as disease resistance. We characterize regions of the genome for SNP discovery and paralogous assessment using a reduced representation sequencing approach. Coho salmon eggs were obtained from individual females obtained from the AQUAGEN breeding program. Eggs were fertilized using irradiated UV sperm and after 300 UTA, hatching appears to be anomalous. Embryos showed external deformities, indicative of the haploid syndrome. DNA was extracted from embryos and digested using a HaeIII restriction enzyme. Pair-end libraries for each individual were prepared, and the sequence was obtained in a Miseq instrument. Reads were trimmed using stringent quality control measures and then mapped onto a *de novo* assembly using a single library sequenced at a high depth using as a guide the rainbow trout genome. High quality reads were mapped using bowtie2 (10 million per sample). About 80% of the reads mapped back to the selected contigs. Samtools were used to call variants. After filtering, QC (>30) and read depth (>10 and <100), 70K high quality SNPs were called in the samples. Paralogous regions appear to be very important; since 25 to 40% appear to be maped as heterozygous in the samples. The regions are consistent between dams. This is the first study aimed at characterizing the genome of commercial populations of Coho salmon. The long term objective of the project is to generate a draft sequence using a number of important individuals from the breeding population and thereafter design SNP platforms for measuring the effects of selection due to production, mapping loci associated with disease resistance, alongside transcriptomic analysis. FONDEF: ID14I10090.

Keywords: Coho salmon, Paralogous sites, SNP variation,

[§] Corresponding author. Tel.: +56963060306; Fax:+ 56 22 9785641
E-mail address: vmartine@u.uchile.cl

A QTL FOR RESISTANCE TO INFECTIOUS PANCREATIC NECROSIS IN RAINBOW TROUT

Moen T.[a§], Ødegård J.[a], Baranski M.[b], Lien S.[c], Santi N.[a]

[a] *AquaGen, Trondheim, 7462, Norway*
[b] *Nofima, Ås, 1430, Norway*
[c] *Norwegian University of Life Sciences, Ås, 1430, Norway*

A genome scan was performed in order to identify QTL for resistance to infectious pancreatic necrosis (IPN) in rainbow trout. A pilot disease challenge was first performed, comparing a commonly used salmon-derived virus isolate to an isolate derived from rainbow trout, and identifying the isolate from rainbow trout as the most virulent. This isolate was used for challenge-testing 46 full-sib groups (8,683 individuals) of rainbow trout from the AquaGen population, employing a cohabitant challenge model. The overall mortality rate was 93 %, although approximately 20 % of the mortalities appeared not to be due to IPN. The 20 most- and the 20 least IPN-resistant individuals from each full-sib group (excluding animals displaying signs of not having tolerated the transition to solid feed) were genotyped using a 55k Affymetrix SNP-chip, developed for rainbow trout partly based on whole-genome sequence data from AquaGen individuals. One major QTL was identified, represented by four experiment-wide significant SNPs (p-value of most significant SNP = 2.5×10^{-8}) located within a 2.2 cM region on the female genetic map. The mean survival rates of genotyped animals homozygous for the low- and high-resistance alleles were 0.17 and 0.45, respectively. The QTL was confirmed in an additional challenge test, employing the West Buxon IPN strain in a cohabitant model. The location of the QTL within the rainbow trout genome indicates that this QTL is not a homologue of the major QTL for IPN-resistance in Atlantic salmon. The QTL has allele frequencies highly suitable for marker-assisted selection, and has been implemented in AquaGen's breeding programme since 2014.

Keywords: trout, QTL, IPN, MAS, disease resistance

§ Corresponding author. Tel.: + 47 976 24 263; Fax: + 47 73 54 62 91
E-mail address: thomas.moen@aquagen.no

EPITHELIAL CADHERIN DETERMINES RESISTANCE TO INFECTIOUS PANCREATIC NECROSIS IN ATLANTIC SALMON

Moen T.[a, §], Torgersen J.[a], Santi N.[a], Davidson W.S.[b], Baranski M.[c], Ødegård J.[a], Kjøglum S.[a], Velle B.[d], Kent M.[d], Lubieniecki K.P.[b], Isdal E.[e,] Lien S.[c]

[a] AquaGen, Trondheim, 7462, Norway
[b] Simon Fraser University, V5A 1S6, Canada
[c] Nofima, Ås, 1430, Norway
[d] Norwegian University of Life Sciences, Ås, 1430, Norway
[d] Vaxxinova, 5006, Norway

Infectious pancreatic necrosis virus (IPNV) is the cause of one of the most prevalent diseases in farmed Atlantic salmon (*Salmo salar*). A quantitative trait locus (QTL) has been found to be responsible for most of the genetic variation in resistance to the virus. Here we describe how a linkage disequilibrium-based test for deducing QTL allele was developed, and how it was used in order to produce IPN-resistant salmon, leading to a 75 % decrease in the number of IPN outbreaks in the salmon farming industry. Furthermore, we describe how whole-genome sequencing of individuals with deduced QTL genotypes was used in order to map the QTL down to a region containing an epithelial cadherin (*cdh1*) gene. In a co-immunoprecipitation assay, the Cdh1 protein was found to bind to IPNV virions, strongly indicating that the protein is part of the machinery used by the virus for internalization. Immunofluorescence revealed that the virus co-localizes with IPNV in the endosomes of homozygous susceptible individuals but not in the endosomes of homozygous resistant individuals. A putative causal single nucleotide polymorphism was found within the full-length *cdh1* gene, in phase with the QTL in all observed haplotypes except one; the absence of a single, all-explaining DNA polymorphism indicates that an additional causative polymorphism may contribute to the observed QTL genotype patterns. Cdh1 has earlier been shown to be necessary for the internalization of certain bacteria and fungi, but this is the first time the protein is implicated in internalization of a virus.

Keywords: trout, QTL, IPN, MAS, disease resistance

§ Corresponding author. Tel.: + 47 976 24 263; Fax: + 47 73 54 62 91 .
E-mail address: thomas.moen@aquagen.no

SEQUENCING AND ASSEMBLY OF A 10 Mb REGION OF THE ASIAN SEABASS GENOME CONTAINING GROWTH-ASSOCIATED QTLS

Ngoh S.Y.[1,2], Thevasagayam N.M.[1], Prakki S.R.S.[1], Orbán L.[1,3,4] , Shen X.Y.[1]

1) Reproductive Genomics Group, Strategic Research Program, Temasek Life Sciences Laboratory, Singapore 117604; 2) Nanyang Technological University, Singapore 639798; 3) Department of Animal Sciences and Animal Husbandry, Georgikon Faculty, University of Pannonia, 8360 Keszthely, Hungary; 4) Centre for Comparative Genomics, Murdoch University, 6150, Australia

The Asian seabass is an important marine aquaculture species that has been cultured and/or selected for over 30 years in Southeast Asia and Australia. However, the genome information of this species, which plays a crucial role in the selective breeding, is currently limited. As a pilot study for our ongoing Asian Seabass Genome Project, we have generated a 3D BAC pool (38,400 clones) and screened it using 22 microsatellite DNA markers located on the seabass linkage group 2 (LG2) which has been shown earlier to contain a growth-related QTL region[1]. A total of 86 positive clones were detected. Based on an existing physical map, 72 of them corresponding to 22 BAC pools covering a substantial portion of LG2 were sequenced by Illumina MiSeq technology. In order to improve the assembly, we then co-assembled the scaffolds from each MTP with error-corrected PacBio reads that were produced for *de novo* sequencing of the Asian seabass genome[2]. The number of the assembled scaffolds for each MTP dropped substantially and the final total assembly size increased to over 9.7 Mb. A total of 257 unique genes were identified from the hybrid assembly. Functional and pathway analysis revealed eleven genes potentially responsible for growth traits, and their tissue-specific expression patterns were investigated. This study provided nearly 10 Mb (1.4 %) of the Asian seabass genome (700 Mb), which i) helped the first understanding of its genome characterization; and ii) provided useful information on further improve the assembly and annotation of our ongoing Asian seabass genome project.

Keywords: Asian seabass, QTL region sequencing, growth, association studies, comparative mapping

§ Corresponding author. Tel.: +65/6872-7413 (L.O.); +65/6872-7414 (X.Y.S.); Fax: + 65/6872-7007; E-mail address: + laszlo@tll.org.sg (L.O.); xueyan@tll.org.sg (X.Y.S.)

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CONVENIENT PHYSICAL GENOME MAP OF FLOUNDER. A PRACTICAL WHOLE-GENOME RADIATION HYBRID MAP OF JAPANESE FLOUNDER

Okamoto H.[a§], Aoki J.[b], Nozaki T.[b], Ozaki A.[c], Uji S.[c], Fuji K.[d],
Sakamoto T.[d], Okamoto N.[d], Kawai J.[e], Araki K.[b§], Ekker M.[f§]

[a]National Research Institute of Aquaculture, FRA, Kamiura, Saiki, Oita 879-2602, Japan
[b]National Research Institute of Aquaculture, FRA, Tamaki, Watarai, Mie 519-0423, Japan
[c]National Research Institute of Aquaculture, FRA, Minami-Ise, Watarai, Mie 516-0193, Japan
[d]Faculty of Marine Science, Tokyo University of Marine Science and Technology,
Tokyo 108-8477, Japan
[e]Preventive Medicine and Diagnosis Innovation Program (PMI), RIKEN,
Suehiro-cho, Tsurumi-ku, Yokohama 230-0045, Japan
[f]CAREG, University of Ottawa, Ottawa K1N 6N5, Canada

The Japanese flounder (*Paralichthys olivaceus*), or olive flounder, is one of the most commercially important fish in Japan with a production size only behind that of yellowtails, red seabream, bluefin tuna and pufferfish (torafugu). We previously reported the preliminary whole-genome radiation hybrid (RH) map of the flounder. However, the previous map had wide regions devoid of markers in many linkage groups (LG). For example, LG9 had the longest (225 centiRays (cR);estimated size: 6,800 kb) no-marker region (NR). Numerous wide NR in each LG make it difficult to find a small haplotype blocks containing a commercial trait during marker-assisted selection (MAS) breeding. Therefore, we re-mapped many new short tandem repeat (STR) markers in LGs to make NRs as small as possible. We also performed mapping of EST markers to facilitate synteny analysis with other model fish organisms. The total size of the new RH map was 35,845 cR and the average breakpoint frequency (ABF) was estimated at 20 kb/cR (the old map indicated a total size of 23,659 cR and the ABF was 30 kb/cR). We placed 1,192 markers including 886 STR markers, 196 cloned genes, 109 EST markers and one telomere marker on the new map; while the old map had 780, 609, 171, 0 and 0 of these markers, respectively. The average distance between each marker was 590 kb for all markers and 800 kb for only STR markers. We succeeded in making NRs small, and in distributing makers evenly in each LG. The new RH map is intended to be convenient and useful for performing MAS breeding of flounders.

§ Corresponding author: Tel.: +81-972-32-2125; Fax: +81-972-32-2293
E-mail address: hiokamot@affrc.go.jp, arakin@affrc.go.jp, mekker@uottawa.ca

MULTI-GENE SCREENING APPLICATION FOR MUTAGENIZED POPULATIONS. APPLICATION OF MUTANT SCREENING FOR MULTI-GENES USING NEXT GENERATION SEQUENCING TECHNOLOGY FROM MUTAGENIZED POPULATIONS

Okamoto H.[a]§, Ishikawa T.[a], Nishiki I.[b], Iwasaki Y.[b], Kai W.[b], Fujiwara A.[b]

*[a]Kamiura branch of National Research Institute of Aquaculture,
FRA, Kamiura, Saiki, Oita 879-2602, Japan
[b]National Research Institute of Fisheries Science, FRA, Yokohama, Kanagawa 236-8648, Japan*

We developed a new application of random mutagenesis for an aquaculture species, fugu (*Takifugu rubripes*). The application involved intraperitoneal injection of a chemical mutagen into mature males to cause mutagenesis in the genome of sperm. The mutation ratio of this method was the same as that of another common method employed for small experimental fish, medaka and zebrafish, which involves exposing mature males to mutagen-containing rearing water for several hours, over several days. Usually, over five thousand experimental fish are screened to obtain a target mutant for selection by targeting induced local lesions in genomes (TILLING). Fugu, however, is much larger than the commonly-used experimental fish, and so requires greater investment for feeding and rearing, and has a longer time to maturity of more than two to three years. Consequently, TILLING is harder to perform on fugu and thus has never been successfully accomplished in the species to date. One method of overcoming TILLING difficulties in aquaculture fish is to select one mutant for one gene from fewer than one thousand mutagenized fish, wherein the mutant gene is a member of a responsible gene family related to one trait. From approximately eight hundred mutagenized individual fugu, we screened a mutated nucleotide in nine members of one gene family (presumed to be responsible for morphogenesis of teeth) using next generation sequencing (Ion PGM™) and Access Array™ systems, and the software CLC Genomics Workbench for analyzing sequence data. This application was successful for identifying mutated nucleotides and mutants, and could help to perform TILLING for breeding of aquaculture fish.

§ Corresponding author: Tel.: +81-972-32-2125; Fax: +81-972-32-2293
E-mail address: hiokamot@affrc.go.jp

INTEGRATION OF GENOMIC, RNASEQ AND QTL RESOURCES IDENTIFIES VARIATION IN CANDIDATE GENES FOR GROWTH IN TURBOT (*Scophthalmus maximus* L.)

Robledo D.[a], Fernández C.[b], Hermida M.[b], Sciara A.[c], Arranz S.E.[c], Álvarez-Dios J.A.[d], Caamaño R.[e], Álvarez-Castro J.M.[b], Nettelblad C.[f], Martínez P.[b], Bouza C.[b§]

[a] *Departamento de Xenética, Facultade de Bioloxía (CIBUS), Universidade de Santiago de Compostela, Santiago de Compostela, 15782, Spain*
[b] *Departamento de Xenética, Facultade de Veterinaria, Universidade de Santiago de Compostela, Lugo, 27002, Spain*
[c] *Instituto de Biología Molecular y Celular de Rosario (IBR-CONICET), Rosario, S2002LRK, Argentina*
[d] *Departamento de Matemática Aplicada, Facultad de Matemáticas. Universidad de Santiago de Compostela. 15782 Santiago de Compostela, Spain*
[e] *Cluster de Acuicultura de Galicia (Punta do Couso), Aguiño-Ribeira, 15695, Spain*
[f] *Department of Information Technology, Uppsala University, SE-751 05 Uppsala, Sweden*

Turbot is a marine flatfish of commercial importance in Europe and China, the main goal of genetic breeding programs in this species is improving growth traits. Previous genomic studies have detected several growth-related quantitative trait loci (QTL), of interest for marker assisted selection. In this study, integrative genomics was applied to dissect the genetic basis of growth traits in turbot. RNAseq was carried out to analyze muscle and liver transcriptomes under nutritional stress to look for genes involved in growth regulation. Muscle and liver cDNA samples from fastened and control turbot were sequenced using Illumina HiSeq. A total of 36,793,041 paired-end reads were generated, accounting for 20,447 genes after alignment against the draft turbot genome. Muscle and liver transcriptomes were used to look for SNP variation at growth-related genes, which were anchored to structural QTL-mapping by means of the recently assembled turbot genome as a way to tackle the genetic basis behind phenotypes. Sixty-four genes were found to be close to growth QTL associated markers. Forty-five genes-SNPs were selected based on the availability of feasible SNPs, the proximity of QTL growth markers and functional relevance for growth, including the insulin growth factors 1 and 2, leptin receptor, myostatin1, parvalbumin1 or growth hormone receptor2, which were associated with growth traits in other vertebrates and teleosts. 60% of these SNPs were located within untranslated regions, 31% in coding sequences and the rest in putative splicing regions derived from RNAseq assembly, and thus putative candidates for functional changes underlying growth phenotypes. Technically feasible SNPs (96%) were validated in a wild Atlantic population, 91% of them were polymorphic (mean unbiased gene diversity=0.30, range:0.06-0.51; mean minimum allele frequency=0.22, range:0.03-0.50) and all loci were in Hardy-Weinberg equilibrium. Linkage mapping of informative SNP and association with phenotypic variation for growth traits, including a search for epistatic effects, were performed using a large set of farmed families. These markers were also analyzed to look for adaptive diversity in wild and domestic populations of turbot. Integrative genomics has successfully identified SNP markers in growth-related genes suitable for population and family association studies, as a basis for marker assisted selection strategies.

Keywords: turbot, RNAseq, QTL, SNP, growth-related genes

[§] Corresponding author. Tel.: 34982822431
E-mail address: mcarmen.bouza@usc.es

COMPILATION OF QTL FOR RESISTANCE TO BACTERIAL, PARASITIC AND VIRAL DISEASES IN TURBOT (*Scophthalmus maximus*)

Rodríguez-Ramilo S.T.[a], de la Herrán R.[b], Ruiz Rejón C.[b], Hermida M.[c], Fernández C.[c], Pereiro P.[d], Novoa B.[d], Figueras A.[d], Bouza C.[c], Toro M.A.[e], Martínez P.[c§], Fernández J.[a]

[a] *Departamento de Mejora Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Ctra. Coruña Km 7.5, 28040 Madrid, Spain*
[b] *Departamento de Genética, Facultad de Ciencias, Universidad de Granada, Avenida Fuentenueva s/n, 18071 Granada, Spain*
[c] *Departamento de Genética, Facultad de Veterinaria, Universidad de Santiago de Compostela, Campus de Lugo, 27002 Lugo, Spain*
[d] *Instituto de Investigaciones Marinas, CSIC, Eduardo Cabello 6, 36208 Vigo, Spain*
[e] *Departamento de Producción Animal, ETS Ingenieros Agrónomos, Universidad Politécnica de Madrid, Ciudad Universitaria, 28040 Madrid, Spain*

The culture of turbot is a well-established process. However, several infectious diseases caused by bacteria, parasites and viruses represent one of the most relevant limiting factors, causing severe economic losses. Consequently, one of the main objectives of genetic breeding programs in turbot industry is to reduce disease-related problems (mortality, slow growth, etc.). The objective of this study was to identify specific and general disease-resistance genomic regions related to the main pathogens for turbot culture. For this purpose, a comparison between QTL detected for resistance and survival time to the bacteria *Aeromonas salmonicida* (furunculosis), the parasite *Philasterides dicentrarchi* (scuticociliatosis) and the haemorrhagic septicaemia virus (VHS) was carried out. Eleven full-sib families of approximately 100 individuals each were analysed. Four families were evaluated for resistance to furunculosis, 3 families were tested for VHS, and 4 families were analysed for scuticociliatosis. All individuals were analysed with the same consensus genetic map. Several linkage groups (LG1, LG5 and LG6) showed QTL associated to the response to the three pathogens, suggesting genomic regions involved in general immunity. Notwithstanding, some disease specific genomic regions were also detected for furunculosis (LG12 and LG18), scuticociliatosis (LG3, LG7, LG10 and LG23) and VHS (LG8, LG20 and LG21). Additionally, other linkage groups shared common QTL for furunculosis and scuticociliatosis (LG4, LG9, LG11, LG13 and LG16), and for scuticociliatosis and VHS (LG2, LG15 and LG17). No QTL involved in the resistance/survival to any of the three pathogens were detected in LG14, LG19 and LG22. A gene mining approach identified candidate genes related to general or specific immunity. These findings will aid to develop marker assisted selection programs to improve turbot production.

Keywords: quantitative trait loci, molecular marker, general immunity, specific immunity, marker assisted selection

§ Corresponding author. Tel./Fax: + 34 982822428
E-mail address: paulino.martinez@usc.es

VALIDATION OF GROWTH-RELATED QTL FOR MARKER ASSISTED SELECTION IN TURBOT (*Scophthalmus maximus*)

Sciara A.A.[a], Rodríguez-Ramilo S.T.[b], Hermida M.[a], Gómez-Tato A.[c], Fernández J.[b], Bouza C.[a], Martínez P.[a§]

[a] *Departamento de Genética, Facultad de Veterinaria, Universidad de Santiago de Compostela, Campus de Lugo, 27002 Lugo, Spain*
[b] *Departamento de Mejora Genética Animal, Instituto Nacional de Investigación y Tecnología Agraria y Alimentaria, Ctra. Coruña Km 7.5, 28040 Madrid, Spain*
[c] *Departamento de Geometría y Topología, Facultad de Matemáticas, Universidad de Santiago de Compostela, Santiago de Compostela, 15782, Spain*

Classical familiar breeding programs have improved growth rate in turbot. However, marker assisted selection may further increase growth rate, because it is possible to identify specific genomic regions explaining a significant proportion of the phenotypic variance of such trait. Moreover, marker assisted selection could provide a more efficient selection of genetic variants that, otherwise, could be lost in classical breeding programs. The implementation of marker assisted selection requires markers tightly linked to the causal mutation(s). The increase of turbot genomic resources during the last years has enabled the construction of linkage maps of appropriate density to detect growth-related QTL. The objective of this study was to validate previously detected molecular markers associated to growth related traits in turbot. Eighteen full-sib families derived from breeding programs were genotyped for 39 markers at 11 linkage groups to look for association to weight, length and Fulton's condition factor. The results indicate that 25 markers out of the 39 analysed showed significant association in at least one family and for at least one trait. In addition, all the considered linkage groups comprised at least one marker with significant association. Twenty markers out of the 39 analysed jointly explained 47.5% of the phenotypic variance of weight; 18 markers explained up to 57.7% of the phenotypic variance for length; and only one marker was associated with Fulton's condition factor, explaining 1.2% of the phenotypic variance. The explained phenotypic variance when considering allelic variants, instead of markers, was higher (87.3% for weight, 89.1% for length, and 11.4% for Fulton's condition factor). The conclusion is that a set of markers and alleles highly associated with growth in turbot is now available. In practical terms and due to the sparse distribution of growth-related QTL in turbot across the genome, several markers from different linkage groups should be used to increase genetic gain in selection programs.

Keywords: growth, explained phenotypic variance, MAS, breeding program, molecular marker

[§] Corresponding author. Tel./Fax: + 34 982822428
E-mail address: paulino.martinez@usc.es

WHOLE GENOME SEQUENCING AND PRELIMINARY ANALYSIS IN TURBOT, *Scophthalmus maximus*

Shao C.W., Chen S.L.[§]

Yellow Sea Fisheries Research Institute, CAFS, Key Laborator yfor Sustainable Development of Marine Fisheries, Ministry of Agriculture, Qingdao 266071, China.

A whole-genome shotgun sequencing strategy and Illumina Genome Analyser sequencing technology were used for turbot (*Scophthalmus maximus*) genome sequence. Six paired-end sequencing libraries were constructed with insert sizes of about 170 base pairs (bp), 500 bp, 800bp, 2 kb, 5 kb and 20 kb were constructed. In total, we generated more than 84 Gb of usable sequence which is equal to 120-fold coverage of the whole female-genome. The genome was assembled by the SOAP*denovo* software and the results show that the assembly size of 568 Mb in total with a GC content of 43.41%. The genome assembly with a contig N50 is 12 kb and scaffold N50 is 6.2 Mb with the largest length scaffold is 19.68 Mb. We then assessed the assembly quality by ESTs. The result shows that more than 90% of which can be detected in the assembled scaffolds. Besides, we identified a total of 70 Mb repeat sequence containing the DNA repeats, LINE, SINE, LTR and other repeats, which represent 12.32% of the whole genome. We used three gene-prediction methods (cDNA-EST, homology based and *ab initio*) to identify protein-coding genes and then built a consensus gene set by merging all of the results. We predicted 21,134 genes, with a mean coding sequence size of 1,605 bp and an average of 9 exons per gene. We then constructed 22 pseudo-chromosomes with a total of 541 Mb based on high-resolution genetic map; each chromosome comprised an average of 25.86 Mb and the Chr.1 is the largest chromosome (33.15 Mb). We next constructed a phylogenetic tree using turbot, Japanese flounder, tongue sole, medaka, stickle-back, takifugu, tetradon and tilapia with an out-group of zebrafish. The result showed that the turbot have a relatively closer phylogenetic relationship in other two flatfish species with a divergence time of 78 MYA between turbot and tongue sole and 65 MYA between turbot and Japanese flounder. The construction of turbot genome map in this study will provide an excellent resource for future molecular breeding efforts such as genome selection.

Keywords: turbot, *Scophthalmus maximus, whole genome sequencing,* pseudo-chromosomes, *phylogenetic tree*

[§]Corresponding author: Tel: 008653285844606; Fax: 008653285811514
E-mail address: chensl@ysfri.ac.cn

POLYMORPHISMS IN MYOSTATIN GENE AND ASSOCIATIONS WITH GROWTH TRAITS IN *Ancherythoculter nigrocauda*

Sun Y.H.[a,b], Li Q.[a*], Tong J.G.[c*], Wang G.Y.[a], Zhu D.M.[a], Chen J.[a], Li P.[a]

[a] Wuhan Aquaculture Science Research Institute, Wuhan 430207, China;
[b] Wuhan Academy of Agricultural Science Technology, Wuhan 430071, China;
[c] State Key Laboratory of Freshwater Ecology and Biotechnology, Institute of Hydrobiology, the Chinese Academy of Sciences, Wuhan 430072, China

Myostatin (*MSTN*) is a member of the transforming growth factor-β superfamily that negatively regulates skeletal muscle development and growth. In the present study, partial genomic fragments of *MSTN* were screened for single nucleotide polymorphisms (SNPs) in two commercial hatchery *Ancherythoculter nigrocauda* populations. Two SNPs (g.935A>T and g.958A>G) in intron 1 and two synonymous SNPs (g.1129T>C, g.1336T>C), one non-synonymous SNP (g.1289G>A) in exon 2 were identified. Genotyping by direct sequencing of PCR products for these five SNPs was performed in the two populations. Association analyses showed that one synonymous SNP (g.1129T>C) in the two populations were significantly associated with total length (*TL*), body length (*BL*), body height (*BH*) and body weight (*BW*). And g.1289G>A was significantly associated with body height (*BH*) and body weight (*BW*) in the six-month population. Haplotype analyses revealed that fish with the genotype combinations TC/TC or TC/GA showed better growth performance. Our results demonstrated that the SNP in *MSTN* may have positive effects on growth traits, and suggested that *MSTN* could be candidate gene for marker-assisted selection (MAS) in *A.nigrocauda*.

Keywords: Myostatin (MSTN); Single nucleotide polymorphisms (SNPs); Ancherythoculter nigrocauda; Growth traits; Association analysis.

[*] Corresponding author. Tel.: +86-27-88118801 (Q.L.); +86-27-68780751 (J.T.)
Fax: +86-27-88115862 (Q.L.); +86-27-68780123 (J.T.)
E-mail address: +xfsckj@163.com (Q.L.); jgtong@ihb.ac.cn (J.T.)

GENOME WIDE ASSOCIATION STUDY IDENTIFIES SNPS ASSOCIATED WITH EARLY GROWTH PERFORMANCE IN ATLANTIC SALMON (*S. Salar*)

Tsai H.Y.[a*§], Hamilton A.[b*], Guy D.R.[b*], Tinch A.E.[b*], Bishop S.C.[a*], Houston R.D.[a*]

[a] *The Roslin Institute and Royal (Dick) School of Veterinary Studies,*
The University of Edinburgh, Midlothian EH25 9RG, UK.
[b] *Landcatch Natural Selection Ltd., 15 Beta Centre, Stirling*
University Innovation Park, Stirling, FK9 4NF, UK.

The genetic architecture of growth traits in farmed Atlantic salmon is of scientific and commercial interest. A high density Affymetrix SNP array (~132K verified SNPs) was recently developed by our group. This array was utilized to perform a genome-wide association study (GWAS) on a farmed Atlantic salmon (*S. salar*) population (n = 626) with measurements of body weight and length at approximately one year post-hatch. Heritability estimates were obtained using both the pedigree-based and genomic relationship matrix model, and these consistently showed a moderate to high heritability for the growth related traits (0.55-0.60). However, our GWAS results indicated that the genetic factors affecting body weight and length of individuals were largely polygenic. The most significant markers (p~10^{-5}) were analysed to identify proximal candidate genes. In conclusion, although the individual SNP effects were relatively minor, most of the genetic variation in these key traits was captured by our SNP array. In addition, a further project is underway, to assess the association of these markers with similar traits in an independent salmon population.

Keywords: GWAS, salmon, growth performance

[*]These authors have contributed equally to this work
[§] Corresponding author. Tel.: + 44(0)1316519224 ; Fax: + 44(0)1316519105
E-mail address: + hsin-yuan.tsai@roslin.ed.ac.uk

TURNING AQUACULTURED TELEOSTS TO MODEL SPECIES: LINKAGE MAP CONTRUCTION FOR RED PORGY *Pagrus pagrus* USING RAD SEQUENCING

Tsakogiannis A.[a,b], Manousaki T.[a], Tsaparis D.[a], Papandroulakis N.[a], Mylonas C.C.[a], Tsigenopoulos C.S.[a§]

[a] *Institute of Marine Biology, Biotechnology and Aquaculture (IMBBC), Hellenic Centre for Marine Research (HCMR), Heraklion, 71500, Greece*
[b] *Department of Biology, University of Crete, Heraklion, 71003, Greece*

Next generation sequencing has given unprecedented possibilities regarding the study of non-model species, those with scarce or non-existent prior genomic knowledge. Most newly recruited species in aquaculture lack basic genetic information, an obstacle that can be overcome with modern sequencing technologies. A prominent target in aquaculture genomics is the identification and use of genetic markers first to build linkage maps and second to map Quantitative Trait Loci (QTL) for desirable production traits. Except gilthead sea bream, other species of the Sparidae family have also been investigated by the industry over the past decade. Their rapidly growing potential and economical significance in aquaculture urges even more for exploring the genome evolution of the group. Here, we focus on the red porgy (*Pagrus pagrus*), a relatively new species for the Mediterranean aquaculture. Using the double-digest restriction site associated DNA (ddRAD) methodology on a full-sib family, we identified hundreds of polymorphic markers widely distributed in the unexplored genome of red porgy. Employing the Illumina MiSeq platform, we sequenced a reduced genomic fraction of nearly one hundred individuals, and constructed the first linkage map for the species. Our comparative genomic analysis, suggested a high degree of conserved synteny of this linkage map to the genomes of European sea bass, tilapia, stickleback and medaka. These results in combination with our previous work on common Pandora (*Pagellus erythrinus*) show a conserved genome structure in the family Sparidae. Current study exploits the possibilities of genotyping-by-sequencing to gain insights regarding genome structure and evolution of red porgy. Further, it sets the baseline for QTL mapping of traits of interest and Marker-Assisted Selection (MAS) that will boost the aquaculture industry.

Keywords: red porgy, Sparidae, linkage map, RAD Sequencing

[§] Corresponding author. Tel.: +30 2810337854; Fax: +30 2810337870
E-mail address: tsigeno@hcmr.gr

GENOTYPING BY SEQUENCING USING NEXTseq 500 IN SELECTIVE BREEDING OF ASIAN SEABASS

Wang L.[a], Wan Z.Y.[a], Yue G.H.[a§]

[a] *Molecular population genetics group, Temasek Life Sciences Laboratory, 1 Research Link, National University of Singapore, 117604, Singapore*

Genotyping by sequencing (GBS) developed based on next-generation sequencing (NGS) provides cost-effective and high-throughput SNPs discovery and genotyping for genomics-level marker-assisted selection (MAS) in aquaculture. Here, we present a cost-effective, flexible and high-throughput GBS workflow for SNPs identification and genotyping in Asian seabass using Illumina NEXTseq 500. Firstly, GBS libraries from 192 individuals of Asian seabass were separately constructed using 200 ng high-quality genomic DNA with double digestion of restriction enzyme and were then legated with bar-coded adaptors. Equal amount of each library of 192 samples was pooled into single multiplexed library for size selection and validation. The library was then sequenced using the Nexseq 500. The raw sequencing reads were demultiplexed and then used for de novo SNPs discovery and genotyping in pipeline using Stacks software. In total, more than 50 K SNPs were identified from the 192 individuals of Asian seabass. Over 10 K SNPs were genotyped in population and can be used for population genomic analysis of Asian seabass. This GBS method is useful in MAS for aquaculture species.

Keywords: Genotyping by sequencing, marker-assisted selection, SNPs genotyping, Asian seabass

§ Corresponding author. Tel.: + 65-68727405: Fax: + 65-68727007
E-mail address: genhua@tll.org.sg

TRANSCRIPTOME AND THE POTENCIAL GENETIC GENE ANALYSIS UPON THE PHENOMINON OF ADIPOSE SURROUNDING OVARY IN CULTURED SIBERIAN STURGEON (*Acipenser baeri*)

Hu H-X.[a,b§], Dong T.[a], Dong Y.[a], Zhu H.[a,b]

[a] *Beijing Fisheries Research Institute& National Engineering Research Center for Freshwater Fishery,Beijing,100068,P.R.China*
[b] *Fishery Biotechnology laboratory of Beijing, Beijing, 100068, P.R.China*

The product of cultured sturgeon accounts for 99% of total global production, while the wild caught resources are near exhaustion. More than 80% aquaculture sturgeons were produced in China. Siberian sturgeon is one of the most popular cultured species throughout the world. Large amounts of adipose tissue surrounding individual eggs in the ovigerous folds and large lobes of fat attached to the ovary were found among farm raised Siberian sturgeon, which resulted in the failure of artificial reproduction and decreased the caviar yield. The speculated reason was the abnormal lipometabolism because of aquaculture situation. The blood lipid levels including cholesterol, triglycerides, High-density lipoprotein-C and low-density lipoprotein-C were tested and showed significant difference between fatty ovary and thinner one in Siberian sturgeon. Some genes related to lipometabolism like LPL gene *etc.* expressed also obvious different within two groups ovary. In order to find more regulated genes of this phenomenon, transcriptome analysis between adipose surrounding ovary and thinner ovary were tested. 116 different expression genes were found with 60 down regulated genes and 56 up regulated genes. It supposed to detect the related genetic genes to avoid keeping fatty ovary sturgeon in fish farm and assist the breeding of Siberian sturgeon.

Keywords: Siberian sturgeon, cultured, adipose surrounding ovary, transcriptome, breeding

§ Corresponding author. Tel.: + 0086-10-67583152 ; Fax: + 0086-10-67583152
E-mail address: huhongxiazh@163.com

MASCULINIZATION INDUCED BY ANDROGEN IN ORANGE-SPOTTED GROUPER *Epinephelus coioides*

Li S.[a], Zhang H.[b], Zhang Y.[a], Lin H.[a]

[a] *Institute of Aquatic Economic Animals and Guangdong Provincial Key Laboratory for Aquatic Economic Animals, Sun Yat-Sen University, Guangzhou 510275, China*
[b] *Marine Fisheries Development Center of Guangdong Province, Huizhou 516081, China*

Groupers are widely recognized as protogynous hermaphroditism with sex changeable from female in young age and then become male in adult. We observed the gonad development stage of orange-spotted grouper *Epinephelus coioides* cultured under artificial condition. The results show that ovarian development of the grouper originated in 9—12-month old age. Therefore, we fed fish with 17α-methyltestosterone (MT) in the 8th month before ovarian development started, and gonadal differentiation were successfully induced to be male. In addition, feeding with MT at dosage of 10 mg per kg pellets for 3 months could induce male sexuality. However, gonad development of the male individuals stopped developing into male but into female when MT feeding ended. In conclusion, the sex determination and differentiation of the grouper are not only influenced by genetic factors and reproductive endocrine, but also by the fish age, size, population, and environment factors. Artificial feeding MT hormone could not alter sex orientation into male entirely, but induce the development of testes. Young groupers would develop into female unless continuous feeding with MT is provided for inducing matured males ultimately.

Keywords: orange-spotted grouper Epinephelus coioides; 17α-methyltestosterone MT; sex differentiation; gonadal development

EXPRESSION ANALYSIS OF THE SOX9 GENE, DURING EARLY DEVELOPMENT OF *Acipenser persicus*

Ranjbar H.R.[a§], Yarmohammadi M.[b], Sadjadi M.M.[a], Kazemi R.[c]

[a] *Fisheries Department, Faculty of Natural Resources, University of Guilan, Sowmeh Sara, P.O. Box 1144, Iran*
[b] *Genetic Department, Khazar International Sturgeon Research Institute, Rasht, 41635-3464, Iran*
[c] *Physiology and Biochemistry Department, Khazar International Sturgeon Research Institute, Rasht, 41635-3464, Iran*

The SRY (sex determining region Y)-box 9 (SOX9) is a protein-coding gene that plays a crucial role in the regulation of the early development of the male gonad and organogenesis in a variety of vertebrates, including mammals and fish. Persian sturgeon (*Acipenser persicus*) is a rare valuable aquaculture species, but sex determination in the early developmental stage in particular is a major problem in today's sturgeon farming. In this study, a partial-length cDNA for SOX9, was characterized from the Persian sturgeon and examined its expression during egg to 50 day post hatching. The predicted SOX9 is 210 bp in length and encodes a 70 amino acid protein, which is 100% and 97% identical to *Acipenser baerii* and *Acipenser sturio*, respectively, and 86%, 94% and 79% identical to mouse, frog and human, respectively. During the embryo development stage, SOX9 mRNA is low but highest detectable at hatching time; then SOX9 mRNAs decrease in a gradual manner in muscle contraction stage embryos, this relatively stable expression continues during the following embryogenic stages and declines 6 day post hatching. RT-PCR analysis indicates that SOX9 mRNA is presents in many juvenile tissues, with the highest expression in the gill and pyloric. The results suggest that SOX9 plays an important role in cartilage forming and providing fundamental information on the earliest period of molecular sex determination in acipenseriformes.

Keyword: Sturgeons, Sex differentiation, early development, gene expression

[§] Corresponding author. Tel.: +989179172308; Fax: +98 1334533722;
E-mail address:Ranjbar.shilat88@gmail.com;

TRANSCRIPTOMIC STUDY OF GONADAL SEX DIFFERENCIATION IN TURBOT (*Scophthalmus maximus*) USING A SPECIES-SPECIFIC MICROARRAY ENRICHED WITH REPRODUCTION-RELATED GENES

Ribas L.[1*], Robledo D.[2*], Gómez-Tato A.[3], Viñas A.[2], Martínez P.[2], Piferrer F.[1§]

[1] Recursos Marins Renovables, Institut de Ciències del Mar-Consejo Superior de Investigaciones Científicas, Barcelona, 08003, Spain
[2] Genética, Universidad de Santiago de Compostela, Lugo, 27002, Spain
[3] Matemática Aplicada, Universidad de Santiago de Compostela, Santiago de Compostela 15781, Spain

The turbot (*Scophthalmus maximus*) is a flatfish with increasing consumer demand world-wide and therefore its aquaculture production is steadily growing. Turbot exhibits a marked sexual growth dimorphism in favor of females and thus there is interest to understand its sex determination and differentiation in order to help the production of all-female stocks. Here, we studied gene expression during sex differentiation in turbot by transcriptional analysis of gonads of sexually undifferentiated, sexually differentiating and juvenile males and females by using a species-specific microarray enriched with reproduction-related genes. We identified groups of genes preferentially expressed at different stages during sex differentiation and studied the expression patterns of 18 canonical reproduction-related genes. We found that in turbot *cyp19a1a* and *dmrt3* expression levels can be used as reliable markers of female and male differentiation, allowing the accurate prediction of phenotypic sex at 90 and 140 days post fertilization (dpf), respectively, as verified by molecular and histological analysis. With fish thus sexed, we identified a suite of 45 and 12 novel differential expressed genes (DEG) associated with ovarian and testicular differentiation, respectively. Some of these genes were previously related not only to sex differentiation, but also to other aspects of reproduction control, general metabolism, immune response and the circadian clock system. Further investigation of these genes should be addressed. In juveniles, we found ~4.000 DEG between ovaries and testes and, with the aim of identifying possible sex determining candidate genes, we mapped these DEG to the previously-identified sex- and growth-QTL markers. We found that that a larger but not significantly different amount of male-biased sex-differential transcripts were located in the linkage group 8 of turbot map. Transcripts mapped near growth-related QTLs were only found in females but not in males. In summary, this study describes the first transcriptomics of turbot gonads during sex differentiation, identifies new candidate genes and establishes two markers of early phenotypic sex.

Keywords: gonad differentiation, sex, transcriptome, microarray, marker genes

* These authors contributed equally to this study
§ Corresponding author. Tel.:+34-932309567; Fax: + 34-932309555
E-mail address: piferrer@icm.csic.es

MULTIPLE SEX-ASSOCIATED SNPS IDENTIFIED IN *Characidium gomesi* (TELEOSTEI: CHARACIFORMES) USING RAD MARKERS

Scacchetti P.C.[a], Martínez P.[b], Hermida M.[b], Robledo D.[b], Mank J.[c], Taggart J.[d], Bekaërt M.[d], Utsunomia R.[a], Pansonato-Alves J.C.[a], Oliveira C.[a], Foresti F.[a]

[a] *Instituto de Biociências de Botucatu, UNESP, Botucatu, SP, Brazil.*
[b] *Universidad de Santiago de Compostela, USC, Santiago de Compostela, Spain.*
[c] *University College London, UCL, London, England.*
[d] *University of Stirling, Stirling, Scotland.*

Several studies have demonstrated that sex determination in fish can behave like a complex trait, with different genes and environmental factors influencing this character. In addition, independent sex chromosome evolution in lower vertebrates is a well-documented feature and is probably connected to the complexity of sex determination mechanisms. In the present study a RAD sequencing sex-association study was developed in *Characidium gomesi*, a Characid fish species with a ZZ/ZW sex chromosome system. Thirty-nine libraries (20 females and 19 males) were developed and the samples barcoded, pooled and pair-end sequenced in one lane of an Illumina HiSeq 2500 sequencer that generated 2 FASTAq files with 75 Gb of sequences (~200 millions reads). In total, 284,836,820 raw reads (100 bp length) were produced. Based on significant Fst values between sexes (P < 0.05) representing a figure above 0.10 or higher, 204 loci were initially selected for further analysis. Furthermore, 26 loci were only detected in females (female-restricted). The selected loci were used as queries in a BLAST search (Evalue<10^{-5}) against the zebrafish genome and demonstrated to be allocated in single or multiple linkage groups of zebrafish. However, several loci did not show any similarities with the zebrafish genome. Moreover, every locus was also used as queries in a BLASTn and BLASTx search against the NCBI database and the results indicated similarities with several single-copy genes, microsatellite sequences and transposable elements (TEs). Finally, the putative repetitive sequences were used as queries in CENSOR searches against the Repbase and identified several classes of TEs. Although known sex-related genes were not identified in our analyses, multiple SNPs with surprisingly high Fst values were identified (very close to the maximum 33% value expected in a ZZ/ZW system) suggesting linkage to the sex determining gene in the sex chromosome pair. Among the 26 exclusive-female discovered loci, around half of them showed exclusive PCR bands in females constituting a useful tool for sexing in this species. Additionally, their putative location in the W chromosome would provide landmarks for approaching to the sex determining gene in this species. The markers obtained related to sex in *C. gomesi* will be useful to understand the evolution of sex determination in the genus *Characidium*.

Keywords: sex determination, sex chromosomes, SNPs, fish cytogenetics, Characidium

The authors have contributed equally to this work.
Corresponding author. Tel.: + (55) 14 3880-0480 ; Fax: + (55) 14 3821-3744
E-mail address: + pcardim@ibb.unesp.br

FOXL2 GENE PLAYS A DECISIVE ROLE FOR EARLY SEX DIFFERENTIATION IN BLUEGILL SUNFISH *Lepomis macrochirus* IN DIFFERENT TEMPERATURE ENVIROMENTS

Shen Z.G.[a], Wang H.P.[a], Li Y.H.[a], Yao H.[a], Eissa N.[a,b]

[a] Aquaculture Genetics and Breeding Laboratory, The Ohio State University South Centers, Piketon, Ohio, 45661, U.S.A.
[b] Aquatic Diseases Laboratory, Aquaculture Division, National Institute of Oceanography and Fisheries, Cairo, 11516, Egypt.

Bluegill sunfish are extraordinary organisms that display three special biological character-istics - complex sex determination mechanism, natural hybridization, and alternative mating tactics, which solely or interactively influence population sex ratio. The present work pro-ceeds from the most important one, sex determination, and focuses on molecular players involved in sex differentiation. Two ovarian differentiation related genes, *foxl2* and *cyp19a1a*, and one testis differentiation related gene, *dmrt1*, were detected at 7 days post-hatching (dph), which is well before the onset of morphological gonadal differentiation, indicating that these genes have already played a role before sex differentiation. The expression of *foxl2* reached the peak and was thermo-sensitive at 27 dph, which is just prior to the onset of ovarian differentiation, suggesting the importance of *foxl2* in sex differentiation. Compar-ative analysis of the expression profile of *foxl2* in different species indicates that the expres-sion level of *foxl2* or the *foxl2*/testis differentiation gene(s) ratio may be what triggers the direction of the gonads, into the female or male pathway. Histological examination displayed that proliferation of germ cells, as well as ovarian differentiation, were delayed in low tem-perature treatment, although no biased sex ratio was produced. The present work highlights the importance of the *foxl2* gene in ovarian differentiation. Further work with comparative study of the expression profile of the *foxl2* gene in species with both genetic sex determina-tion (GSD) and temperature-dependent sex determination (TSD), such as Atlantic silverside and bluegill, will shed light on the evolution of sex determination mechanisms.

GENE EXPRESSION DURING SEX DIFFERENTIATION IN CORTISOL DEFICIENT COMMON CARP, CYPRINUS CARPIO L.

Van Pelt H.[a*], Komen H.[b*$]

[a]IMARES, Institute for Marine Research and Ecosystem Studies, IJmuiden, the Netherlands
[b]Animal breeding and genomics group, department of animal sciences.
Wageningen University, Wageningen, 6700 AH, the Netherlands

Sex differentiation in fish can be seen as a battle between male and female specific gene regulatory pathways. In males, early expression of dmrt1 leads to expression of sf-1, anti-mullerian hormone (AMH) and the differentiation of sertoli cells. In females, expression of Foxl2 has been proposed as the primary signal, activating estrogen receptors and aromatase, and the differentiation of granulosa cells. In common carp, sex determination is male dominant ("XX/XY"). Interestingly, female carp that are homozygous for a mutation in *cyp17a2*, do not produce 17-alpha hydroxylase in the head kidney and hence do not produce cortisol. *Cyp17a1* expression in the gonads is normal but *cyp17a2* deficient (CYP17^{-}) female fish develop testis or intersex gonads. To investigate gene expression during sex differentiation in these carp, we sampled gonads of normal males, normal females and CYP17^{-} females during sexual differentiation. Our results show that the steroidogenic enzyme *cyp19a1* involved in the production of estradiol is approximately 10 times higher in normal females compared to normal males. The expression of *Amh* is approximately 3 times lower in females and in CYP17^{-} females, compared to normal males. Together, these results suggest a female-like development in CYP17^{-} females. However, the expression of *dmrt 1* and *sf-1* in normal males and CYP17^{-} females is approximately 5 and 6 times higher than in normal females, while the expression of *cyp19*a1 is reduced by 50% in CYP17^{-} females, compared to normal females, suggesting that testis development has started in CYP17^{-} females. A striking difference was found between the expression levels of estrogen receptors *esr2a* and *esr2b*. In male carp expression of *ers2a* and *ers2b* is higher than in females. By contrast in CYP17^{-} females, *ers2a* is overexpressed while *ers2b* is almost absent. We conclude that early expression of dmrt1 results in a gradual masculinisation of the CYP17^{-} gonad. The possible role of ACTH and cortisol in this sex reversal is discussed.

Keywords: sex determination, fish, gene expression, gonads

[*]These authors have contributed equally to this work
[§] Corresponding author. Tel.: +31317482465
E-mail address: hans.komen@wur.nl

SEXUAL DIMORPHISM EXPRESSION OF GENES IN GONADS DURING MATURITY DEVELOPMENTAL STAGES OF GREAT STURGEON *Huso huso*

Yarmohammadi M.[a§], Pourkazemi M.[a], Kazemi R.[b], Yazdani A.M.[c], Hallajian A.[b], Hassanzadeh Saber M.[a], Yousefi A.[b], Ranjbar H.R.[d]

[a] *Genetic Department, Khazar International Sturgeon Research Institute, Rasht, 41635-3464, Iran*
[b] *Physiology and Biochemistry Department, Khazar International Sturgeon Research Institute, Rasht, 41635-3464, Iran*
[c] *Rearing Department, Khazar International Sturgeon Research Institute, Rasht, 41635-3464, Iran*
[d] *Fisheries Department, Natural Resources Faculty, Guilan University, Somesara, Iran*

There is little knowledge regarding the molecular mechanisms involve in gonad differentiation in sturgeon species. In this study we investigated expression pattern of four genes previously found to be involved in vertebrates' testicular development (sox9, dmrt1, cyp17a1, and ar) together with two plasma androgen levels (11-KT and Tes), during different stages of sexual maturity in great sturgeon. The results showed that among these four genes, only cyp17a1 expressed in male gonads at stage 1 (immature), suggesting that this gene maybe applicable as sex marker in recently differentiated male great sturgeon. Sexually dimorphic patterns in other studied genes suggesting that these genes may be important for testicular development and differentiation in premature great sturgeon. Results from plasma androgens showed that at maturity stages 2, 3, and 4 androgens level were significantly higher in males compared to females. The obtained results provide a foundation for further research on sex differentiation and developing strategies for sexing of sturgeon for aquaculture.

Key word: Sturgeons, sex differentiation, gonadal development, gene expression

§ Corresponding author. Tel.: +989110338O3117; Fax: +98 1334533722
E-mail address:mahtayarmohammadi@gmail.com

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Bashir Tunde Adeniyi
Federal University of Agriculture
Abeokuta
Nigeria
adeniyibt@funaab.edu.ng

Javier Albadalejo
Illumina
CB21 6GP Cambridge
UK
jalbadalejo@illumina.com

François Allal
Ifremer
34250 Palavas-les-Flots
France
fallal@ifremer.fr

Standish Allen
Virginia Institute of Marine Science
23062 Gloucester Point
USA
ska@vims.edu

José Antonio Alvarez Dios
Universidad de Santiago de Compostela
15782 Santiago de Compostela
Spain
joseantonio.alvarez.dios@usc.es

José M Álvarez-Castro
University of Santiago de Compostela
27002 Lugo
Spain
jose.alvarez.castro@usc.es

Daiki Ando
Tohoku University
986-2102 Ishinomaki-shi
Japan
daiki870530@yahoo.co.jp

Keng Pee Ang
Cooke Aquaculture Inc.
E5H 1K1 Blacks Harbour, NB
Canada
kpa2000@hotmail.com

Alberto Arias Perez
Universidade da Coruña
15009 A Coruña
Spain
aarias@udc.es

Silvia E. Arranz Arranz
*Instituto de Biología Molecular y
Celular de Rosario (CONICET-UNR)*
2000 Rosario
Argentina
arranz@ibr-conicet.gov.ar

Anzhela Asatryan
Sevani ishkhan CJSC
14 Yerevan
Armenia
angela.asatryan@sevantrout.com

zhiyi Bai
Shanghai Ocean University
201306 Shanghai
China
zybai@shou.edu.cn

Aline Bajek
Ecloserie Marine de Gravelines Ichtus
59820 Gravelines
France
aline.bajek@ecloserie-emg.com

Håvard Bakke
Salmobreed
5003 Bergen
Norway
haavard@salmobreed.no

Matthew Baranski
Nofima
1430 Ås
Norway
matthew.baranski@nofima.no

Luca Bargelloni
University of Padova
35020 Legnaro
Italy
luca.bargelloni@unipd.it

Zubaida Basiao
Institute of Biology, College of Science,
University of the Philippines Diliman
1103 Quezon City
Philippines
zbasiao08@gmail.com

Rose Komugisha Basiita
James Cook University
4817 Townsville
Australia
basiita.komugisha@my.jcu.edu.au

Giana Bastos Gomes
James Cook University
4817 Townsville
Australia
giana.bastosgomes@my.jcu.edu.au

Costas Batargias
TEI of Western Greece
30200 Messolonghi
Greece
batc@teimes.gr

Raúl Benito Marco
IMV Technologies - Humeco
22006 Huesca
Spain
raul@humeco.net

Med Walid Benkhira
Benaissa Habib
31100 Oran
Algeria
widbfps@gmail.com

Mathieu Besson
Wageningen UR - ASG
6708 PB Wageningen
The Netherlands
mathieu.besson@wur.nl

Anastasia Bestin
SYSAAF
35042 Rennes
France
anastasia.bestin@rennes.inra.fr

Andres Blanco Hortas
Universidad de Santiago de Compostela
15706 Santiago de Compostela
Spain
abhortas@gmail.com

Carmen Bouza
University of Santiago de Compostela USC
27002 Lugo
Spain
mcarmen.bouza@usc.es

Fiona Brew
Affymetrix
HP10 OHH Wooburn Green,
High Wycombe
UK
Fiona_Brew@affymetrix.com

John Buchanan
The Center for Aquaculture Technologies
92121 San Diego
USA
dsidhpura@aquatechcenter.com

Santiago Cabaleiro
Cluster de la Acuicultura de Galicia
15965 Ribeira
Spain
secretaria@cetga.org

Laura Cal Delgado
Instituto de Investigaciones
Marinas de Vigo-CSIC
36208 Vigo
Spain
lauracal@iim.csic.es

Noa Carames
Cluster de la Acuicultura de Galicia
15965 Ribeira
Spain
cluster@cetga.org

Roberto Carvalheiro
Unesp
14884900 Jaboticabal
Brazil
rcar@fcav.unesp.br

Béatrice Chatain
Ifremer
34250 Palavas-les-flots
France
beatrice.chatain@ifremer.fr

David Chavarrías
Grupo Pescanova
36320 Redondela
Spain
dchavarrias@acuinova.com

Songlin Chen
Yellow Sea Fisheries Research Institute, CAFS
266071 Qingdao
China
chensl@ysfri.ac.cn

Jie Cheng
Max Planck Institute for Evolutionary Biology
24306 Ploen
Germany
jiecheng@evolbio.mpg.de

Raquel Coimbra
Universidade Federal Rural de Pernambuco
52171-900 Recife
Brazil
raquel@depaq.ufrpe.br

Avner Cnaani
Agricultural Research Organization
50250 Bet Dagan
Israel
avnerc@agri.gov.il

Ramón Costoya
R. Costoya S.L. Suministros de laboratorio
15008 A Coruña
Spain
rcostoya@rcostoya.com

Céline Coulombet
Ecloserie Marine de Gravelines Ichtus
59820 Gravelines
France
celine.coulombet@ecloserie-emg.com

Lara Covelo-Soto
Universidade de Vigo
36310 Vigo
Spain
laracovelo@gmail.com

Juanjo De La Cerda
Grupo Pescanova
36320 Redondela
Spain
csueiro@pescanova.es

Phillip Dettleff
University of Chile
8820808 Santiago
Chile
satryl@veterinaria.uchile.cl

Olivier Didierjean
Affymetrix
HP10 0HH Wooburn Green,
High Wycombe
UK
Olivier_Didierjean@affymetrix.com

Khanh Doan
IFREMER
34250 Palavas-les-flots
France
dqkhanh@ifremer.fr

Vuong Doi
University of South Bohemia in Ceské Budejovice
38925 Vodnany
Czech Republic
vdoi_thi@frov.jcu.cz

Jose A Domingos
James Cook University
4811 Townsville
Australia
jose.domingos1@jcu.edu.au

Fernanda Dotti do Prado
Universidade Estadual Paulista
17033-360 Bauru
Brazil
prado.fernandad@gmail.com

Rex Dunham
Auburn University
36849 Auburn University
USA
dunhara@auburn.edu

Mathilde Dupont-Nivet
INRA
78350 Jouy en Josas
France
mathilde.dupont-nivet@jouy.inra.fr

Nick Elliott
CSIRO
7001 Hobart
Australia
nick.elliott@csiro.au

Florian Enez
SYSAAF
35042 Rennes
France
florian.enez@rennes.inra.fr

Montserrat Espiñeira
Anfaco-Cecopesca
36310 Vigo
Spain
montse@anfaco.es

João Estêvão
Universidade do Algarve
8005-139 Faro
Portugal
a39292@ualg.pt

Brad Evans
Tassal Operations
7000 Hobart
Australia
brad.evans@tassal.com.au

Alicia Felip Edo
Institute of Aquaculture of
Torre de la Sal (IATS-CSIC)
12595 Castellón
Spain
afelip@iats.csic.es

Carlos Fernández
Universidad de Santiago de Compostela
27002 Lugo
Spain
carlos.fernandez.lopez@usc.es

Raquel Fernández-Cebrián
Geneaqua S.L.
27003 Lugo
Spain
raquel.fernandez@geneaqua.com

Rosa Fernández-Otero
CETMAR
36208 Vigo
Spain
rfernandez@cetmar.org

Jenyfer Fernández-Pérez
Universidade da Coruña
15071 A Coruña
Spain
jenyfer.fernandez.perez@udc.es

Jose Bento S. Ferraz
Univ. of Sao Paulo/FZEA/NAP-GMAB
13635-900 Pirassununga
Brazil
jbferraz@usp.br

Isabel Ferreiro
Stolt Sea Farm, S.A.
15707 Santiago de Compostela
Spain
l.Ferreiro@stolt.com

Fausto Foresti
UNESP - Universidade Estadual Paulista
18618-970 Botucatu, SP
Brazil
fforesti@ibb.unesp.br

Juan Carlos Francia Quiroz
Scientific University of the South (UCSUR)
18 Lima
Perú
jfrancia@cientifica.edu.pe

Carlos Franco
CDTI
28001 Madrid
Spain
carlos.franco@cdti.es

Jousepth Gallardo Hidalgo
Universidad Católica Santísima Concepción
4090541 Concepción
Chile
jgallardo@gmail.com

Zexia Gao
Huazhong Agricultural University
430070 Wuhan
China
gaozexia@hotmail.com

Carmen García Fernández
Universidad de Oviedo
33006 Oviedo
Spain
garciafernandezcarmen@gmail.com

Maíce Giovanini Ramos
São Paulo State University (UNESP)
17012535 Bauru
Brazil
maice_ramos@hotmail.com

Thomas Gitterle
SyAqua Group
10330 Bangkok
Thailand
thomas.gitterle@goldcoin-th.com

Boris Gomelsky
Kentucky State University
40601 Frankfort
USA
boris.gomelsky@kysu.edu

Sonia Gómez
Universidad de Santiago de Compostela
27002 Lugo
Spain
sonia.gomez@usc.es

Antonio Gómez-Tato
University of Santiago de Compostela
15782 Santiago de Compostela
Spain
antonio.gomez.tato@usc.es

Serap Gonen
The Roslin Institute (University of Edinburgh)
EH25 9RG Edinburgh
UK
serap.gonen@roslin.ed.ac.uk

Angel Francisco González
IIM-CSIC
36208 Vigo
Spain
afg@iim.csic.es

Kim Erik Grashei
AquaGen AS
1430 Ås
Norway
kim.erik.grashei@aquagen.no

Emma Greatorex
Affymetrix
HP10 0HH London
UK
emma_greatorex@affymetrix.com

Riho Gross
Estonian University of Life Sciences
EE-51006 Tartu
Estonia
riho.gross@emu.ee

Jorge Guerra
Universidad de Santigo de Compostela
27002 Lugo
Spain
jorge.guerra@usc.es

Alejandro Gutierrez
Roslin Institute
EH25 9RG Edinburgh
UK
apgutier@sfu.ca

Pierrick Haffray
SYSAAF
35042 Rennes
France
haffray@rennes.inra.fr

Alastair Hamilton
Landcatch Natural Selection
FK9 4 NF Stirling
UK
Alastair.Hamilton@Hendrix-Genetics.com

Abul Farah MD. Hasanuzzaman
Universidad de Santiago de Compostela
27002 Lugo
Spain
mhzaman.bd@gmail.com

Diogo Teruo Hashimoto
University of São Paulo State
14884-900 Jaboticabal
Brazil
diogo@caunesp.unesp.br

Dennis Hedgecock
University of Southern California
90089-0371 Los Angeles
USA
dhedge@usc.edu

Marcos Edgar Herkenhoff
UNESP
18618-970 Botucatu
Brazil
marcos.herkenhoff@gmail.com

Marine Herlin
Aquicultura Balear S.A.U., Grupo Culmarex
7007 Es Coll d'en Rabassa (Mallorca)
Spain
marine.herlin.absa@culmarex.com

Miguel Hermida
Universidad de Santiago de Compostela
27002 Lugo
Spain
miguel.hermida@usc.es

Jasmien Hillen
KU Leuven
3000 Leuven
Belgium
jasmien.hillen@bio.kuleuven.be

Borghild Hillestad
Salmobreed
3174 Revtal
Norway
borghild@salmobreed.no

Alexandre Hilsdorf
University of Mogi das Cruzes
08780-911 Mogi das Cruzes
Brazil
wagner@umc.br

Thommy Holmvag
Eurofins Food & Agro Testing Ålesund
NO-6057 Ellingsøy
Norway
events-eu@eurofins.com

Sho Hosoya
University of Tokyo
431-0214 Hamamatsu
Japan
ahosoya@mail.ecc.u-tokyo.ac.jp

Courtney Hough
Federation of European Aquaculture Producers and European Aquaculture Technology Platform (EU)
Brussels
Belgium
courtney@feap.info

Ross Houston
University of Edinburgh
EH25 9RG Edinburgh
UK
ross.houston@roslin.ed.ac.uk

Hongxia Hu
Beijing Fisheries Research Institute
100068 Beijing
P.R.China
huhongxiazh@163.com

Minoru Ikeda
Laboratory of Integrative Aquatic Biology
986-2242 Miyagi
Japan
ikusyu@bios.tohoku.ac.jp

Ana Insua
Universidade da Coruña
15071 A Coruña
Spain
insuax@udc.es

Kasper Janssen
Wageningen University
6700 AH Wageningen
The Netherlands
kasper.janssen@wur.nl

Zsigmond Jeney
NARIC Research Institute for Fisheries and Aquaculture
H-5540 Szarvas
Hungary
jeneyz@haki.hu

Dean Jerry
James Cook University
4810 Townsville
Australia
dean.jerry@jcu.edu.au

Harry Johansen
Akvaforsk Genetics Center
6600 Sunndalsoera
Norway
harry.johansen@afgc.no

Katrin Juling
Eurofins Genomics
85560 Ebersberg
Germany
sandramayer@eurofins.com

Elisavet Kaitetzidou
HCMR
71003 Heraklion
Greece
ekaitetz@hcmr.gr

Artūrs Kārkliņš
Daugavpils university
LV-5401 Daugavpils
Latvia
carlie@inbox.lv

Antti Kause
Natural Resources Institute Finland
FI-31600 Jokioinen
Finland
antti.kause@luke.fi

Kiyoshi Kikuchi
University of Tokyo
431-0214 Hamamatsu
Japan
akikuchi@mail.ecc.u-tokyo.ac.jp

Sissel Kjøglum
AquaGen AS
7462 Trondheim
Norway
sissel.kjoglum@aquagen.no

Klaus Kohlmann
Leibniz-Institute of Freshwater Ecology and Inland Fisheries
12587 Berlin
Germany
kohlmann@igb-berlin.de

Hans Komen
Wageningen UR - ASG
6708 PB Wageningen
The Netherlands
hans.komen@wur.nl

Lingfeng Kong
Ocean University of China
266003 Qingdao
China
klfaly@ouc.edu.cn

Balázs Kovács
Szent IstvánUniversity
H2100 Gödöllo
Hungary
Kovacs.Balazs@mkk.szie.hu

Ólafur Kristjánsson
Stofnfiskur
101 Reykjavík
Iceland
olafur@stofnfiskur.is

Peter Kube
CSIRO
7000 Hobart
Australia
peter.kube@csiro.au

Renate Kvingedal
EWOS Innovation
4327 Sandnes
Norway
renate.kvingedal@ewos.com

Monal Lal
James Cook University
QLD 4811 Townsville
Australia
monal.lal@my.jcu.edu.au

Delphine Lallias
INRA
78350 Jouy en Josas
France
delphine.lallias@jouy.inra.fr

Natalia Lam
Universidad de Chile
8820808 Santiago
Chile
nlam@u.uchile.cl

Cynthia Lawley
Illumina, Inc
94552 Castro Valley
USA
clawley@illumina.com

Daniela Leão
STAB VIDA
2815-182 Caparica
Portugal
daniela@stabvida.com

Aline Leão Valentim
*Universidade Estadual Paulista *
17012535 Bauru
Brazil
aline_valentimm@hotmail.com

Ievgen Lebeda
*University of South Bohemia
in Ceské Budejovice*
38925 Vodnany
Czech Republic
ilebeda@frov.jcu.cz

Gael Leclercq
Concepto Azul
92190 Meudon
France
gael_leclercq@yahoo.com

Irit Levinger
NRGene
740648 Ness Ziona
Israel
irit@nrgene.com

Jean Paul Lhorente
Aquainnovo S.A
5503032 Puerto Montt
Chile
jean.lhorente@aquainnovo.com

Qi Li
Ocean University of China
266003 Qingdao
China
qili66@ouc.edu.cn

Marie Lillehammer
Nofima
1431 Ås
Norway
marie.lillehammer@nofima.no

Grace Lin
Temasek lifescience Laboratories Ltd
117604 Singapore
Singapore
gracelin@tll.org.sg

Zhanjiang Liu
Auburn University
36849 Auburn
USA
liuzhan@auburn.edu

Sixin Liu
USDA/ARS
WV 25430 Kearneysville
USA
sixin.liu@ars.usda.gov

Graciliana Lopes
CIIMAR
4050-123 Porto
Portugal
gracilianalps@gmail.com

Maria Eugenia Lopez Dinamarca
Universidad de Chile
8820808 Santiago
Chile
me.lopez.dinamarca@icloud.com

Ivonne Lozano
Universidad de Chile
8820000 Santiago
Chile
ivonneok@ug.uchile.cl

Iain MacLaren-Lee
Illumina
CB10 1XL Saffron Walden
UK
ilee@illumina.com

Manuel Manchado
IFAPA
11500 El Pto Sta Maria
Spain
manuel.manchado@juntadeandalucia.e

Jovana Marjanovic
Wageningen UR - ASG
6708 PB Wageningen
The Netherlands
jovana.marjanovic@wur.nl

Francesco Maroso
Università degli Studi di Padova
35020 Legnaro
Italy
francesco.maroso@gmail.com

Victor Martinez
Universidad de Chile
8820808 Santiago
Chile
vmartine@u.uchile.cl

Paulino Martínez
Universidad de Santiago de Compostela
27002 Lugo
Spain
paulino.martinez@usc.es

Carlos Mazorra de Quero
Grupo Tinamenor, S.L.
39548 Pesués
Spain
carlosmazorra@tinamenor.es

Colin McGowan
Fisheries and Oceans Canada
K1A 0E6 Ottawa
Canada
colin.mcgowan@dfo-mpo.gc.ca

Kristian Meier
Aarhus University
DK-8830 Tjele
Denmark
kristian.meier@mbg.au.dk

Bruna Mendonça
São Paulo State University
17011-060 Bauru
Brazil
bruna.bmendonca@hotmail.com

Adrián Millán
Geneaqua S.L.
27003 Lugo
Spain
adrian.millan@geneaqua.com

Yuki Minegishi
Tohoku university
986-2242 Onagawa, Oshika, Miyagi
Japan
y.minegishi@m.tohoku.ac.jp

Rodger Miranda
AquaInnovo S.A.
5480000 Puerto Montt
Chile
rmiranda@aquainnovo.com

Jon Mkrtchyan
Sevani ishkhan CJSC
14 Yerevan
Armenia
jon.mkrtchyan@sevantrout.com

Thomas Moen
AquaGen
7462 Trondheim
Norge
thomas.moen@aquagen.no

Hooman Moghadam
Nofima
1431 Ås
Norway
hooman.moghadam@nofima.no

Rebeca Moreira
IIM - CSIC
36208 Vigo
Spain
rebecamoreira@iim.csic.es

Jessica Moss Small
Virginia Institute of Marine Science
23062 Gloucester Point
United States
jamoss@vims.edu

Jose C Mota Velasco G
Landcatch Natural Selection
FK9 4NF Stirling
Scotland
jose.mota-velasco@hendrix-genetics.com

Maria Raquel Moura Coimbra
Universidade Federal Rural de Pernambuco
52171-900 Recife
Brazil
raquel@depaq.ufrpe.br

Masamichi Nakajima
Japan
981-8555 Sendai
Japan
mnkjm@bios.tohoku.ac.jp

Uthairat Na-Nakorn
Kasetsart University
10900 Bangkok
Thailand
ffisurn@ku.ac.th

Roberto Neira
Universidad de Chile
7560755 Santiago
Chile
rneirar@gmail.cl

Carl Nettelblad
Uppsala University
75237 Uppsala
Sweden
carl.nettelblad@it.uu.se

Ashie Norris
Marine Harvest
14 churchtown
Ireland
ashie.norris@marineharvest.com

Jorgen Odegard
AquaGen
NO-1432 Aas
Norway
jorgen.odegard@nmbu.no

Victor Øiestad
Akvaplan-niva 36620
Vilanova de Arousa
Spain
victor@akvaplan.niva.no

Hiroyuki Okamoto
*National Research Institute
of Aquaculture, FRA*
879-2602 Saiki
Japan
hiokamot@affrc.go.jp

Simion Omasaki
Wageningen University
6700 Ah Wageningen
The Netherlands
simion.omasaki@wur.nl

Münevver Oral
University of Stirling
FK9 4LA Stirling
UK
mo19@stir.ac.uk

László Orbán
Temasek Life Sciences Laboratory
117604 Singapore
Singapore
laszlo@tll.org.sg

Mónica Otero
Universidad de Santiago de Compostela
27002 Lugo
Spain
monicaafrica.otero@usc.es

Luis Outeiriño
Comercial Hospitalaria Grupo 3 SL
36350 Nigran
Spain
louteirino@chgrupo3.com

Akiyuki Ozaki
National Research Institute of Aquaculture
516-0193 Minamiise-cho,
Watarai-gun, Mie
Japan
aozaki@affrc.go.jp

Mª Angel Palacios
Piszolla, S.L.
37800 Alba de Tormes
Spain
m.angel@piszolla.com

Leonidas Papaharisis
Nireus Aquaculture SA
19400 Koropi
Greece
l.papaharisis@nireus.com

Belén G. Pardo
Universidad de Santigo de Compostela
27002 Lugo
Spain
belen.gomez@usc.es

Alok Patel
University of Chile
8820808 Santiago
Chile
aloksu@gmail.com

Antonio J. Pazos
Universidad de Santiago de Compostela
15782 Santiago de Compostela
Spain
antonioj.pazos@usc.es

Carolina Penaloza
Roslin Institute
EH25 9RG Edinburgh
UK
v1cpeal@exseed.ed.ac.uk

David Penman
University of Stirling
FK9 4LA Stirling
UK
d.j.penman@stir.ac.uk

Patricia Pereiro
IIM-CSIC
36208 Vigo
Spain
patriciapereiro@iim.csic.es

Luis Antonio Pérez Carrasco
*Acuinuga (Acuicultura y
Nutrición de Galicia S.L.)*
15220 Bertamirans
Spain
info@acuinuga.com

María Pérez Rodríguez
CETMAR
36208 Vigo
Spain
mperez@cetmar.org

Ricardo Perez-Enriquez
*Centro de Investigacioines
Biologicas del Noroeste, S.C.*
23096 La Paz
México
rperez@cibnor.mx

Francesc Piferrer
Institute of Marine Sciences
8003 Barcelona
Spain
piferrer@icm.csic.es

Danillo Pinhal
UNESP - São Paulo State University
18618970 Botucatu
Brazil
dlpinhal@ibb.unesp.br

Pedro Pinto
STAB VIDA
2825-182 Caparica
Portugal
pedropinto@stabvida.com

Debbie Plouffe
*The Center for Aquaculture
Technologies Canada*
COA 2BO Souris, PEI
Canada
dplouffe@aquatechcenter.com

Supawadee Poompuang
Kasetsart University
10900 Bangkok
Thailand
supawadee.p@ku.ac.th

Fabio Porto-Foresti
Faculdade de Ciências / UNESP
17033-360 Bauru
Brazil
fpforesti@fc.unesp.br

María Verónica Prego-Faraldo
University of A Coruña
E15071 A Coruña
Spain
veronica.prego@udc.es

Laureana Rebordinos
University of Cadiz
11510 Cadiz Puerto Real
Spain
laureana.rebordinos@uca.es

Clint Rhode
Stellenbosch University
7600 Stellenbosch
South Africa
clintr@sun.ac.za

Ana Riaza-Carcamo
Stolt Sea Farm, S.A
15707 Santiago de Compostela
Spain
a.riaza@stolt.com

Nick Robinson
Nofima
1432 Ås
Norway
nick.robinson@nofima.no

Diego Robledo
Universidad de Santigo de Compostela
15782 Santiago de Compostela
Spain
diego.robledo@usc.es

Maria Rowena Romana-Eguia
Southeast Asian Fisheries
Development Center
1940 Rizal
Philippines
mreguia@seafdec.org.ph

Gil Ronen
NRGene
7403648 Ness-Ziona
Israel
gil@nrgene.com

Paolo Ronza
Universidad de Santiago de Compostela
27002 Lugo
Spain
paolo.ronza@usc.es

Juan Rubiolo
Universidad de Santiago de Compostela
27002 Lugo
Spain
ja.rubiolo@usc.es

Carmelo Ruiz-Rejon
Universidad de Granada
18071 Granada
Spain
rherran@ugr.es

Carlos Saavedra
IATS - Consejo Superior de
Investigaciones Científicas
12595 Ribera de Cabanes (Castellon)
Spain
saavedra@iats.csic.es

Alberto Sáez Arteaga
University Barcelona
8028 Barcelona
Spain
albertosaeza@ub.edu

Eric Saillant
The University of Southern Mississippi
39564 Ocean Springs
USA
eric.saillant@usm.edu

Takashi Sakamoto
Tokyo University of Marine
Science and Technology
1088477 Tokyo
Japan
takashis@kaiyodai.ac.jp

Laura Sánchez
Universidad de Santiago de Compostela
27002 Lugo
Spain
lauraelena.sanchez@usc.es

Jose Luis Sánchez López
Universidad de Santiago de Compostela
15782 Santiago de Compostela
Spain
joseluis.sanchez@usc.es

Elena Sarropoulou
HCMR
71003 Heraklion
Greece
sarris@hcmr.gr

María Saura
INIA
28040 Madrid
Spain
saura.maria@inia.es

Xueyan Shen
Temasek Life Sciences
Laboratory,Singapore
117604 Singapore
Singapore
xueyan@tll.org.sg

Anders Skaarud
GenoMar
250 Oslo
Norway
anders.skaarud@genomar.com

Ashraf Suloma Soliman
Cairo Univ
12613 Giza
Egypt
suloma2001@agr.cu.edu.eg

Anna K. Sonesson
Nofima
1431 AAs
Norway
anna.sonesson@nofima.no

Giovanna Elizabeth Sotil Caycho
Universidad Nacional Mayor de San Marcos / Univ. Científica del Sur / Instituto del Mar del Perú
4 Callao
Perú
gsotil@gmail.com

Paula Suárez-Bregua
Marine Research Institute (IIM-CSIC)
36208 Vigo
Spain
paulasuarez@iim.csic.es

Xoana Taboada
Universidad de Santigo de Compostela
15782 Santiago de Compostela.
Spain
xoana.taboada@usc.es

Rilke Tadeu Fonseca de Freitas
Universidade Federal de Lavras
37200000 Lavras
Brazil
rilke@dzo.ufla.br

Ingunn Thorland
Akvaforsk Genetics Center AS
6600 Sunndalsøra
Norway
ingunn.thorland@afgc.no

Alejandro Tola Alvarez
Genomar AS
554 Oslo
Norway
alejandro.alvarez@genomar.com

Jiingou Tong
Institute of Hydrobiology of CAS
430072 Wuhan
China
jgtong@ihb.ac.cn

Miguel Angel Toro
ETS Ingenieros Agrónomos, Universidad Politécnica de Madrid
28040 Madrid
Spain
miguel.toro@upm.es

Hsin-Yuan Tsai
The Roslin Institute, University of Edinburgh
EH25 9RG Edinburgh
UK
hsin-yuan.tsai@roslin.ed.ac.uk

Costas Tsigenopoulos
Hellenic Centre for Marine Research
71500 Heraklion
Greece
tsigeno@hcmr.gr

Kostas Tzokas
Andromeda Group
26504 Rion Achaias
Greece
ktzokas@andromedagroup.gr

Tsubasa Uchino
Tokyo University of Marine Science and Technology
108-8477 Tokyo
Japan
wingin.side88@gmail.com

Luísa Valente
CIIMAR
4050-123 Porto
Portugal
alhambra.rd@hotmail.com

Roger Vallejo
USDA-ARS
25430 Kearneysville
USA
roger.vallejo@ars.usda.gov

Marc Vandeputte
INRA/Ifremer
F-34250 Palavas les Flots
France
marc.vandeputte@jouy.inra.fr

Ana Carina Vasconcelos
Universidade Federal do Rio Grande do Sul
91540000 Porto Alegre
Brazil
aninha-nv@hotmail.com

Jesús Vázquez
Xenética Fontao
27210 Lugo
Spain
jesus.vazquez.perez@xunta.es

Sergio Vela Avitúa
AFGC - NMBU
6600 Sunndalsøra
Norway
sergio.vela@afgc.no

Manuel Vera
Universidad de Santigo de Compostela
27002 Lugo
Spain
manuel.vera@usc.es

Alain Vergnet
Ifremer
34250 Palavas-les-flots
France
alain.vergnet@ifremer.fr

Rodrigo Vidal
Universidad de Santiago
9160000 Santiago
Chile
ruben.vidal@usach.cl

Gabriela Villanova
National University of Rosario - CONICET
2000 Rosario
Argentina
gvillanova@fbioyf.unr.edu.ar

Beatriz Villanueva
INIA
28040 Madrid
Spain
villanueva.beatriz@inia.es

Ana Viñas
Universidad de Santiago de Compostela
15782 Santiago de Compostela
Spain
anamaria.vinas@usc.es

Marnik Vuylsteke
GNOMIXX
9000 Gent
Belgium
marnik.vuylsteke@telenet.be

Jurij Wacyk
University of Chile
8820808 Santiago
Chile
jwacyk@u.uchile.cl

Yan Wang
Hainan University
570228 Haikou
China
wy2005@163.com

Weimin Wang
Huazhong Agricultural University
430070 Wuhan
China
wangwm@mail.hzau.edu.cn

Le Wang
Temasek life sciences laboratory
117604 Singapore
Singapore
smartdoit@126.com

Hanping Wang
The Ohio State University
45661 Piketon
USA
wang.900@osu.edu

Federico Winkler
Universidad Católica del Norte
1781421 Coquimbo
Chile
fwinkler@ucn.cl

Curry Woods
University of Maryland
20742 College Park
USA
curry@umd.edu

John Woolliams
The Roslin Institute,
University of Edinburgh
EH25 9RG Midlothian
UK
john.woolliams@roslin.ed.ac.uk

José Yáñez
Universidad de Chile
8820808 Santiago
Chile
jmayanez@uchile.cl

Suthajaree Yenmak
Kasetsart University
10900 Bangkok
Thailand
syenmak@gmail.com

Grazyella Yoshida
Universidade Paulista Júlio Mesquita Filho
14883-380 Jaboticabal
Brazil
grazyoshida@hotmail.com

Mª Asunción Cao
CIMA - Centro de Investigacións Mariñas
36620 Vilanova de Arousa
Spain
asuncion.cao.hermida@xunta.es

Susana Darriba Couñago
INTECMAR - Consellería do
Medio Rural e do Mar
36611 Vilagarcía de Arousa
Spain
sdarriba@intecmar.org

José-Miguel Fuentes-González
CIMA - Centro de Investigacións Mariñas
36620 Vilanova de Arousa
Spain
jose.miguel.fuentes.gonzalez@xunta.es

Ziniu Yu
South China Sea Institute of Oceanology,
Chinese Academy of Sciences
510301 Guangzhou
China
carlzyu@scsio.ac.cn

Gen Hua Yue
Temasek Lfe Sciences Laboratory
117604 SG
Singapore
genhua@tll.org.sg

Kyall Zenger
James Cook University
4810 Townsville
Australia
kyall.zenger@jcu.edu.au

Quanqi Zhang
Ocean University of China
266003 Qingdao
China
qzhang@ouc.edu.cn

Manuel Antonio García Tasende
Xunta de Galicia
15781 Santiago de Compostela
Spain
manuel.garcia.tasende@xunta.es

Richard Le Boucher
IMV Technologies
61300 L'Aigle
France
maria.pzrz@gmail.com

Fátima Linares
CIMA - Consellería do Medio Rural e do
Mar - Centro de Investigacións Mariñas
36620 Vilanova de Arousa
Spain
fatima.linares.cuerpo@xunta.es

José Molares
Consellería do Medio Rural e do Mar
15701 Santiago de Compostela
Spain
jose.molares.vila@xunta.es

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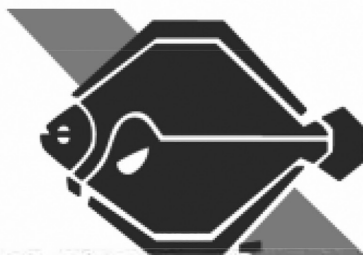
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