

**United States-Japan  
Natural Resources Panel on Aquaculture (UJNR)  
43<sup>rd</sup> Scientific Symposium**

“Evaluation of the impact of breeding organisms on the ecosystem  
and aquaculture industry”

November 10-11, 2015  
Nagasaki



**Program and Abstracts**



## **Program**

The 43rd Scientific Symposium of UJNR Aquaculture Panel

### **Evaluation of the impact of breeding organisms on the ecosystem and aquaculture industry**

Date:

|             |             |   |
|-------------|-------------|---|
| November 10 | 15:00-17:30 | Poster Session                                  |
| November 11 | 9:30-17:30  | Oral Session, Poster Session during lunch break |

Venue:

Lecture room, 2F General education and research building, Bunkyo Campus, Nagasaki University

#### Aim of the Symposium

Breeding research will be of great help with the improvement of aquaculture technology. The utilization of wild broodstock/seeds does not result in genetically improved stocks, and the importance of genetics and breeding studies has increased to meet various needs. Although genetic improvement is common in agriculture and the resulting benefits unquestioned, there are few selectively bred commercially available strains in the aquaculture industry except in inland aquaculture.

Moreover, we should pay attention to the ecological impacts of breeding technology. At the same time selection may increase risks when selected animals escape and breed with wild stocks unless proper safeguards are put into place. Integration of scientific knowledge of the benefits and risks can help contribute to genetic improvement programs. Our UJNR activity should deepen discussions on the issue of genetics/breeding studies, which is expected to contribute to the development of the aquaculture industry in both countries of Japan and United States.

This Symposium consists of oral session and poster session. In oral session, we will deal with the subjects relating to 'genetics and breeding' such as: the impact of breeding organisms on the ecosystem and aquaculture industry; selective breeding techniques; bioinformatics, etc. In poster session, we will present and discuss broader topics relating to Aquaculture.

## **Tuesday, November 10<sup>th</sup>, 2015**

Registration 14:00-17:30

### **Poster Session**

Poster presentation 15:00-17:30

## **Wednesday, November 11<sup>th</sup>, 2015**

Registration 9:00-12:00

### **Oral Session**

#### **Opening Session.**

(Moderators: J. Higano & M. Rust)

#### **Welcome to Nagasaki University**

Atsushi Hagiwara (Dean of the Graduate School of Fisheries and Environmental Sciences,  
Nagasaki University) 9:30- 9:35

#### **Aim of the Symposium**

Fuminari Ito (Japan Chair, Fisheries Research Agency) 9:35- 9:50

#### **Session I. Breeding technique**

(Moderators: S.Watanabe & A. Fuller)

1. Appropriate conditions for the production of triploidy induced by cold shock in yellowtail

*Seriola quinqueradiata*

Yukinori Shimada (National Research Institute of Aquaculture, FRA) 9:50-10:15

2. Potential Application of Germplasm Preservation in Breeding Programs for Molluscan Shellfish Aquaculture and Restoration

Huiping Yang (University of Florida) 10:15-10:35

3. Improving Aquaculture Production in *Halotis* Species Through the Development of a Genomic Toolkit

Catherine Purcell (NOAA Fisheries) 10:35-10:55

4. Culture Protocols and Production of Triploid Purple-Hinge Rock Scallops

Paul Olin (California SeaGrant) 10:55-11:15

#### **Session II. Genetic improvement 1**

(Moderators: A. Ozaki & B. Bosworth)

5. Big Data in Agriculture and the USDA/ARS Initiative

Jeffrey Silverstein (USDA Agricultural Research Service) 11:15-11:35

6. Signature of artificial selection in a breed of coho salmon *Oncorhynchus kisutch*

Sho Hosoya (Fisheries Laboratory, University of Tokyo) 11:35-12:05

Group Photo 12:05-12:10

Lunch Break 12:10-13:10

7. Genetic Selection in Animals Using Pedigree, Phenotypic, and Genomic Information  
Shogo Tsuruta (University of Georgia) 13:10-13:40
8. Exploring Transcriptomic Patterns in Slow- and Fast-Growing *Seriola dorsalis* Larvae  
Catherine Purcell (NOAA Fisheries) 13:40-14:00

### Session III. Risk evaluation of escaped fish

(Moderators: K. Ikuta & B. Iwamoto)

9. Modeling the Variable Effects of Using Wild and Cultured Broodstock on the Fitness Risk Due to Escaped Farmed Fish  
Kristen Gruenthal (NOAA Fisheries) 14:00-14:20
10. Did farmed Coho salmon *Oncorhynchus kisutch* that escaped during the earthquake and tsunami disaster of 2011 interbreed with native Masu salmon *Oncorhynchus masou*?  
Kei Sasaki (Tohoku National Fisheries Research Institute, FRA) 14:20-14:45
11. Evaluation of the tsunami impact on the genetic diversity of the marbled flounder *Pseudopleuronectes yokohamae* in Sendai Bay, Miyagi, Japan  
Yuki Minegishi (Tohoku Ecosystem-Associated Marine Sciences, Tohoku University) 14:45-15:10
12. Competition between Atlantic salmon (*Salmo salar*) and Japan's native salmonids.  
Kazuo Araki (National Research Institute of Aquaculture, FRA) 15:10-15:35
- Break 15:35-15:50

### Session IV. Genetic improvement 2

(Moderators: M. Ototake & H. Yang)

13. Hybrid Striped Bass National Breeding Program: Research Towards Genetic Improvement of a Non-Model Species  
Adam Fuller (USDA Agricultural Research Service) 15:50-16:10
14. Production of Benedenia-resistant Yellowtail (*Seriola quinqueradiata*) Families –A Preliminary Approach to the Candidates–  
Tsutomu Noda (Seikai National Fisheries Research Institute, FRA) 16:10-16:35
15. Coho Salmon Broodstock Development: A Case Study of the Domsea Coho (1977 to 2015)  
Bob Iwamoto (Spring Salmon LP) 16:35-16:55
16. Development of Improved Catfish Germplasm at the Warmwater Aquaculture Research Unit, USDA-ARS  
Brian Bosworth (USDA Agricultural Research Service) 16:55-17:15
- Discussion 17:20-17:50  
(Moderators: F. Ito & M. Rust)
- Closing remarks (Michael B. Rust, U.S.A. Panel Chair) 17:50-18:00



**Abstracts with Annotated Bibliography of the 43<sup>rd</sup>  
Scientific Symposium of UJNR Aquaculture Panel**

## **Titles of Oral Presentation**

1. Appropriate conditions for the production of triploidy induced by cold shock in yellowtail *Seriola quinqueradiata* (Yukinori Shimada, National Research Institute of Aquaculture, FRA)
2. Potential Application of Germplasm Preservation in Breeding Programs for Molluscan Shellfish Aquaculture and Restoration (Huiping Yang, University of Florida)
3. Improving Aquaculture Production in *Haliotis* Species Through the Development of a Genomic Toolkit (Catherine Purcell, NOAA Fisheries)
4. Culture Protocols and Production of Triploid Purple-Hinge Rock Scallops (Paul Olin, California SeaGrant)
5. Big Data in Agriculture and the USDA/ARS Initiative (Jeffrey Silverstein, USDA Agricultural Research Service)
6. Signature of artificial selection in a breed of coho salmon *Oncorhynchus kisutch* (Sho Hosoya, Fisheries Laboratory, University of Tokyo)
7. Genetic Selection in Animals Using Pedigree, Phenotypic, and Genomic Information (Shogo Tsuruta, University of Georgia)
8. Exploring Transcriptomic Patterns in Slow- and Fast-Growing *Seriola dorsalis* Larvae (Catherine Purcell, NOAA Fisheries)
9. Modeling the Variable Effects of Using Wild and Cultured Broodstock on the Fitness Risk Due to Escaped Farmed Fish (Kristen Gruenthal, NOAA Fisheries)
10. Did farmed Coho salmon *Oncorhynchus kisutch* that escaped during the earthquake and tsunami disaster of 2011 interbreed with native Masu salmon *Oncorhynchus masou*? (Kei Sasaki, Tohoku National Fisheries Research Institute, FRA)
11. Evaluation of the tsunami impact on the genetic diversity of the marbled flounder *Pseudopleuronectes yokohamae* in Sendai Bay, Miyagi, Japan (Yuki Minegishi, Tohoku Ecosystem-Associated Marine Sciences, Tohoku University)
12. Competition between Atlantic salmon (*Salmo salar*) and Japan's native salmonids (Kazuo Araki, National Research Institute of Aquaculture, FRA)
13. Hybrid Striped Bass National Breeding Program: Research Towards Genetic Improvement of a Non-Model Species (Adam Fuller, USDA Agricultural Research Service)
14. Production of Benedenia-resistant Yellowtail (*Seriola quinqueradiata*) Families –A Preliminary Approach to the Candidates– (Tsutomu Noda, Seikai National Fisheries Research Institute, FRA)
15. Coho Salmon Broodstock Development: A Case Study of the Domsea Coho (1977 to 2015) (Bob Iwamoto, Spring Salmon LP)
16. Development of Improved Catfish Germplasm at the Warmwater Aquaculture Research Unit, USDA-ARS (Brian Bosworth, USDA Agricultural Research Service)



## O-1

### **Appropriate conditions for the production of triploidy induced by cold shock in yellowtail *Seriola quinqueradiata***

Yukinori Shimada\*<sup>1</sup>, Hiroyuki Nagoya<sup>1</sup>, Hiroyuki Okamoto<sup>1</sup>, Toshiya Yamaguchi<sup>1</sup>, Nariaki Inoue<sup>1</sup>, Takachi Ishikawa<sup>1</sup>, Kazuhisa Hamada<sup>2</sup>, Kazuharu Nomura<sup>3</sup>, Hironori Usuki<sup>1</sup>, Kazunori Yoshida<sup>4</sup>

<sup>1</sup>National Research Institute of Aquaculture, FRA, Tsuiura, Kamiura, Saiki, Oita 879-2602, Japan,

<sup>2</sup>National Research Institute of Aquaculture, FRA, Komame 330, Ootsuki, Kochi 788-0315,

<sup>3</sup>National Research Institute of Aquaculture, FRA, Nakatsuhamaura 422-1, Minamiise, Mie 516-0193, <sup>4</sup>Seikai National Fisheries Research Institute, FRA, Nunoura 122-7, Tamanoura, Goto, Nagasaki 853-0508

\*E-mail: yukinoris at affrc.go.jp

In yellowtail *Seriola quinqueradiata*, FRA aims to contribute to the aquaculture industry through promoting of the artificial seedling production of the strain having economic traits (e.g. resistance to disease and rapid growth). However, in terms of preventing genetic contamination and breeding strategy, the development of reliable infertility method is desired. We focused on the induction of triploidy because triploidy is expected being infertile so far. In this study, we estimated a releasing time of the second polar body, and then examined the appropriate condition induced triploidy by cold shock. A releasing time of the second polar body in fertilized eggs reared at 20°C was investigated by histological sections using samples of fertilized eggs, fixed by Bouin solution at 5, 10 and 15 min after fertilization. The second polar body was observed to be released during 5–10 min after fertilization. Two factors (i.e. water temperature and duration of the cold shock) were examined to maximize the triploidy rate. Ploidy was determined by DNA contents using flow cytometry. The triploid rate was the highest (100%) in 10 min at 3°C. The hatching rate of this condition was 0.2–34.9% when the hatching rate of control (i.e. non-treatment fertilized eggs) was 13.3–45.2%. In the future, we verify the usefulness as an infertility method by investigating sex ratio, fertility and growth performance of triploidy.

**Keywords:** yellowtail, *Seriola quinqueradiata*, triploid, cold shock

#### **Annotated Bibliography of Key Works**

W. R. Wolters, G. S. Libey, and C. Larry Chrisman (1982). Effect of Triploidy on Growth and Gonad Development of Channel Catfish. Transactions of the American Fisheries Society 111: 102-105.

Diploid and triploid in channel catfish *Ictalurus punctatus* were reared in indoor tanks. Triploids were significantly heavier than diploids at 8 months of age and older. Triploid female and male had smaller gonads with altered histology. Triploids converted feed more efficiently, and may provide greater profits in commercial culture than diploids.

S. K. Allen, Jr. and J. G. Stanley (1978). Reproductive Sterility in Polyploid Brook Trout, *Salvelinus fontinalis*. Transactions of the American Fisheries Society 107 (3): 473-478.

Red blood cell size were measured in brook trout (*Salvelinus fontinalis*) from Phillips Hatchery in Maine to investigate naturally occurring polyploid sterility. In eight brook trout in which gonads were lacking or undeveloped, the red blood cells were large, suggesting polyploidy. The average size of the red blood cells in other sterile fish fit into the normal range but all of the eight fish appeared to have some red blood cells that were polyploid. All polyploids appeared to be mosaics, containing diploid, triploid, or pentaploid cells. The cause of the polyploidy was not determined but may have been caused by the inadvertent exposure of the eggs to low temperatures after fertilization.

T. J. Benfey (1999). The Physiology and Behavior of Triploid Fishes. Reviews in Fisheries Science 7(1): 39-67.

Induced triploidy is widely accepted as the most effective method for producing sterile fish for aquaculture and fisheries management. Artificially produced triploids generally differ from conspecific diploids in three fundamental ways: they are more heterozygous, they have larger but fewer cells in most tissues and organs, and their gonadal development is disrupted to some extent. Despite these basic biological differences, triploids are similar in most respects to diploids when examined at the whole animal level. The only clear differences relate to the effects of impaired gametogenesis on the reproductive physiology and behavior of triploids, especially in females. Other apparent differences include reduced aggressiveness, occasional specific morphological abnormalities, and inferior performance when reared under suboptimal conditions. The causes of these latter two problems are poorly understood but must be addressed if triploids are to be used more extensively.

## O-2

### **Potential Application of Germplasm Preservation in Breeding Programs for Molluscan Shellfish Aquaculture and Restoration**

Huiping Yang

*School of Forest Resources and Conservation, Institute of Food and Agricultural Sciences,  
University of Florida, 7922 NW 71st Street, Gainesville, FL 32653,*

*\*E-mail: [huipingyang.at.ufl.edu](mailto:huipingyang.at.ufl.edu)*

Germplasm are the genetic materials of germ cells including gametes, embryos, or larvae. Preservation of germplasm is usually achieved through cryopreservation. The technology of cryopreservation has been applied for human artificial reproduction as a clinical treatment for infertility, and for livestock as a tool for breeding programs worldwide. For fish and shellfish, cryopreservation has been studied in more than 200 species for preservation of natural resources and conservation of endangered species. For molluscan shellfish aquaculture, this technology can have the following potential applications: **1) Preservation of specific lines or strains.** Ongoing breeding programs have yielded specific strains and lines, such as disease resistant oysters. Cryopreservation can be used to preserve these valuable strains and provide gametes for assistance of breeding programs. **2) Preservation of natural wild populations.** The cryopreserved germplasm of natural populations will act as a repository of genetic diversity and allow for the continued adaptive genetic variation for aquaculture populations through infusion of new material from wild populations. In addition, a germplasm repository of wild populations can provide easy access as study materials for researchers. **3) Creation of self-fertilization inbred lines.** Inbred lines are one of the most valuable resources for breeding programs, but difficult to produce and require years of repeated crossing of brothers and sisters or backcrossing. Most bivalves are protandrous, beginning life as males and changing into females as they age. Therefore, with the techniques of non-lethal sperm collection and cryopreservation, self-fertilized lines can be created by using cryopreserved sperm and oocytes from the same individual after sex reversal; **4) Preservation of sperm from tetraploid oysters.** Triploid-tetraploid technology is probably the most promising in oyster aquaculture because of the superior traits of triploids. Cryopreservation of sperm from tetraploids can extend the commercialization of triploid-tetraploid technology by the sale of frozen sperm, and provide cost savings and security for maintaining tetraploids. In addition, cryopreservation of tetraploids produced each year can offer benefit for maintenance of the tetraploid populations. **5) Assistance for creation of mutant lines.** Mutant breeding is an effective approach for creation of new strains or lines, but seldom used in animal breeding programs because of its low efficiency. Recently, a new approach called TILLING (Targeting Induced Local Lesions in Genomes) was developed in plants for creation of mutant lines. To use this new technique for animal mutant breeding, sperm cryopreservation is an absolutely required technique. So far this approach has been applied to zebrafish and puffer fish. With the genome sequencing accomplished in oysters, establishment of mutant lines by TILLING and sperm cryopreservation will benefit the aquaculture and research on oyster functional genetics, and **6) Assistance of aquaculture hatchery practice for regular and hybrid seed production.** The cryopreserved germplasm materials can function as a

reservoir to meet the need for regular and hybrid seed production practice. For example, the hard clam hybrid offspring of *Mercenaria mercenaria* with *Mercenaria campechiensis* showed fast growth and higher survival.

### **Annotated Bibliography of Key Works**

Polge, C., A. U. Smith, A. S. Parkes. 1949. Revival of spermatozoa after vitrification and dehydration at low temperatures. Vol 164:4172. Pp. 666.

This is the first paper to report the successful cryopreservation and cold-dry of sperm from fowl by using glycerol as cryoprotectant. The finding in this one-page short report opened the door for human sperm cryopreservation which is now a major clinical treatment for infertility, and bull sperm cryopreservation which is now a huge industry for its breeding programs worldwide. For fish, the first report was published after this report in 1953 in herring (Blaxter, J. H. S. 1953. Sperm storage and cross-fertilization of spring and autumn spawning herring. *Nature* 172, 1189 – 1190), and this preliminary communication showed that it was possible to cross-fertilize the two spawning ‘types’ of herring *Clupea harengus* found in the north-east Atlantic by using the cryopreserved sperm.

Yang H., Y. Wang, X. Guo, T. R. Tiersch. 2015. Production of inbred larvae through self-fertilization using oocytes and cryopreserved sperm from the same individuals after sex reversal in eastern oyster *Crassostrea virginica*. *Aquaculture Research*, Vol 46:9. Pp. 2153-2165.

The authors in this study reported the production of self-fertilization larvae in eastern oyster *Crassostrea virginica* for the first time, and demonstrated the feasibility of creating self-fertilized inbred lines by use of non-lethal sperm collection and cryopreservation. In this study, small (~1 year old) and large (~2–3 years old) oysters were biopsied for sperm collection. Survival of the biopsied oysters after 1 year was 50% for small oysters and 17% for large oysters, and sex reversal was observed bidirectional (from female to male and also from male to female). Oocytes were collected from sex-reversed females, and self-fertilized with cryopreserved sperm. Of the 24 cryopreserved samples, 14 individuals had  $\leq 1\%$  fertility when crossed with oocytes from unrelated females, indicating that the cryopreserved sperm had reduced fertility. The other 10 individuals had a fertility of  $39 \pm 25\%$  when crossed with oocytes from unrelated females (non-selfing), but showed a significantly lower success of self-fertilization ( $12 \pm 16\%$ ) ( $P = 0.008$ ), while aliquots of the same oocytes had a fertilization of  $83 \pm 11\%$  when crossing with fresh sperm. Larvae were produced in the self-fertilized families (12–94% of the fertilized oocytes), and survived to eyed-larvae stage at days 11–14. Genotyping with 9 microsatellite markers confirmed that the larvae resulted from self-fertilization in four families.

Yang H., E. Hu, R. Cuevas-Urbe, J. Supan, X. Guo, T. R. Tiersch. 2012. High-throughput sperm *Crassostrea virginica* through systematic evaluation of the factors in process of cryopreservation. *Aquaculture*, Vol 344-349. Pp. 223–230.

This study provided a reliable protocol for sperm cryopreservation in the eastern oyster *Crassostrea virginica* with potential for high-throughput processing. In this study, DMSO yielded the highest post-thaw motility at a cooling rate of 20 °C/min when thawed at 30 or 40 °C among the three tested cryoprotectants. Further evaluation of cooling rates of 10, 15, 20, 25 and 30 °C /min showed that 20 or 25 °C /min yielded the highest post-thaw motility ( $34 \pm 5\%$ ) and fertility

( $77 \pm 12\%$ ) for French straws and CBS straws ( $28 \pm 3\%$  and  $69 \pm 14\%$ ). Equilibration times of 10 to 60 min did not cause significant differences in post-thaw motility when freezing with 10% DMSO at a cooling rate of 25 degrees C/min. Also, sperm concentrations ranging from  $1 \times 10^8$  to  $1 \times 10^9$  cells/ml at freezing did not cause significant differences in post-thaw motility. Finally, after thawing, sperm cryopreserved from 16 males with this protocol showed  $58 \pm 24\%$  fertility (from 18 to 86%) for French straws, and  $54 \pm 21\%$  fertility for CBS straws (from 18 to 95%). Overall, this research provided an outline template for developing a basic protocol for sperm cryopreservation for any other molluscan shellfish species.

Fuller B, Lane N, Benson E. 2004. Life in the Frozen State, CRC Press, New York. Pp 672.

Cryobiology is a research field encompassing disciplines including mathematics, biophysics, cell biology, molecular biology, and metabolism physiology. This book brings together the knowledges about cryobiology from these aspects into one platform for readers to understand this research, and is probably the first major textbook on cryobiology. It includes four themes with a total of 23 chapters, and the four themes are Fundamental Aspects, Life and Death at Low Temperatures, Freezing and Banking of Living Resources, and Medical Applications.

Tiersch T. R. and C. C. Green. 2011. Cryopreservation in Aquatic Species, World Aquaculture Society, Baton Rouge, LA. Pp. 1034.

This is the 2<sup>nd</sup> version of the book published electronically by the World Aquaculture Society ([www.was.org](http://www.was.org)). The first version is the volume 7 in the series of publication "Advances in World Aquaculture" published in 2000. The revised version is designed as a comprehensive single compendium of information on cryopreservation in aquatic species, and provide a broad overview of the principles, procedure and perspectives which are necessary for development and application of the cryopreservation technology. It includes 101 chapters (compared to the 55 in the first edition) organized into 11 sections. This book can assist with teaching, research and program development, and is available for readers to purchase through the World Aquaculture Society with a very affordable price for students and researchers.

## Improving Aquaculture Production in *Haliotis* Species Through the Development of a Genomic Toolkit.

Catherine Purcell\*<sup>1</sup>, Andrew Severin<sup>2</sup>, Paula Sylvania<sup>1</sup>, Nick Wegner<sup>1</sup>, Luis Rodriguez<sup>1</sup>, and John Hyde<sup>1</sup>

<sup>1</sup>Southwest Fisheries Science Center, NOAA Fisheries, 8901 La Jolla Shores Dr., La Jolla, CA 92037, Genome Informatics Facility, 206 Science I, Iowa State University, Ames, Iowa 50011

\*E-mail: [Catherine.Purcell@noaa.gov](mailto:Catherine.Purcell@noaa.gov)

Commercial abalone aquaculture has greatly expanded over the past decade, becoming a thriving global industry valued at over \$100 million USD. Abalone is one of the few species where culture production dominates the global market as a result of increasing demand and declining natural stocks from overexploitation and disease. U.S. abalone production is also growing due to high market value and demand. Most farms operating in California utilize three native west coast species: red abalone (*Haliotis rufescens*), green abalone (*H. fulgens*), and pink abalone (*H. corrugata*). These species differ in commercially important traits that are key to culture expansion in California and improved production efficiency (e.g., growth rate, disease resistance, thermal tolerance). Next generation sequencing has opened the door for extensive and explorative genetic research on abalone, and several studies were recently published examining transcriptomic data for several species including the domestically important red abalone, *H. rufescens* (Franchini et al. 2011; Bester-Van der Merwe et al. 2013; De Wit and Palumbi 2013). Recently, researchers working with the SWFSC used restriction site associated DNA sequencing (RAD-Seq) methods to identify genome-wide SNP markers in *H. fulgens* and to examine population structure in wild populations (Gruenthal et al. 2014). However, transcriptomic and RAD-mapping analyses were also limited by the paucity of genomic information available for abalone; without knowledge of the genomic structure it is very difficult to ascertain coverage depth in these studies (Franchini et al. 2011; Arai and Okamura 2013). We are working to create the first *de novo* abalone genome assembly using sexed *H. rufescens* samples, generate tissue specific transcriptomes for *H. rufescens*, and conduct comparative genomic analyses with other commercially important California abalone. Comparative analyses will include *H. fulgens*, *H. corrugata*, and endangered white (*H. sorenseni*) and black (*H. cracherodii*) abalone. Genomic, transcriptomic, and comparative analyses will improve our understanding of sex-determination, thermal/environmental preference, disease resistance, hybridization outcomes, and local adaptation, especially for commercially important California abalone. This will enable identification of candidate genes of interest and marker development for marker assisted selection to improve aquaculture practices in the U.S. abalone industry and elsewhere. This research will also help guide restoration and wild stock enhancement along the west coast for these species, including for the endangered white and black abalone.

### **Annotated Bibliography of Key Works**

Gruental, K., Witting, D., Ford, T., Neuman, M., Williams, J., Pondella, D., Bird, A., Caruso, N., Hyde, J., Seeb, L., and Larson, W. (2014). Development and application of genomic tools to the restoration of green abalone in southern California. *Conservation Genetics*, 15: 109-121.

The authors describe the use of next generation sequencing technology to develop an extensive set of markers to test population structure and effective population size in green abalone in Southern California. In this study, RADSeq (restriction site associated DNA sequencing) was used to generate millions of short sequences, from which, many thousands of SNPs may be identified that span a greater proportion of the genome compared to previous types of marker development. A total of 1209 polymorphic SNPs were developed from this sequencing. While the extensive set of markers did not detect population structure in green abalone in the range that was sampled, they were able to estimate an effective population size ( $N_e$ ) of 1,100-3,600 individuals. Importantly, this work generated valuable genomic resources that can be used to further build the set of tools available to study *Haliotis* species.

Franchini, P., van der Merwe, M., and Roodt-Wilding, R. (2011) Transcriptome characterization of the South African abalone *Haliotis midae* using sequencing-by-synthesis. *BMC Research Notes*, 4: 59.

This study describes the use of applying next generation sequencing technology to develop molecular tools for a South African abalone species, *Haliotis midae*. They use the Illumina Genome Analyzer II to generate 25 million sequences. Using the transcriptome sequences, they did a de novo assembly that resulted in 27,761 contigs with an average length of 260 bp. Importantly, although abalone have a relatively poor representation in genome databases (likely due to their large genome size), a good number of the contigs had BLAST matches to known annotated genes in Genbank; with a stringent e-value set, 16.8% of the contigs had a homologous BLAST match against Genbank. These sequences were assigned to functional categories using GO and KOG databases. The authors were also able to use this data to identify thousands of SNPs, and out of those, they developed 420 primer sets.

De Wit, P., and Palumbi, S. (2013) Transcriptome-wide polymorphisms of red abalone (*Haliotis rufescens*) reveal patterns of gene flow and local adaptation. *Molecular Ecology*, 22: 2884-2897.

The authors of this study tested whether signals of environmental selection could be detected in samples of red abalone (*Haliotis rufescens*) collected from three locations in California: Monterey Bay, Sonoma, and north of Cape Mendocino. These particular areas are especially distinct in terms of their temperature, aragonite saturation, exposure to hypoxia stress, and disease pressure; as such, the authors hypothesized that genes related to shell biomineralization, resistance to hypoxia, temperature tolerance, and resistance to pathogens would show the strongest signals of local adaptation. The authors tested this by conducting RNA-Seq analyses on mantle tissue of 39 red abalone individuals from the above locations. A total of 21,579 SNPs were genotyped for each individual, and out of these 691 showed significant differentiation. From this set of 691, 163 loci could be identified through BLAST annotation; many of these genes had functions related to biomineralization, energy metabolism, heat-, and disease- or hypoxia-tolerance. These genes are now candidates for further studies to look for signals of local adaptation.

## O-4

### Culture Protocols and Production of Triploid Purple-Hinge Rock Scallops

Paul G. Olin

California Sea Grant, UCSD / Scripps Institution of Oceanography, 133 Aviation Blvd., Suite 109, Santa Rosa CA 95403

\*E-mail: [polin.at.ucsd.edu](mailto:polin.at.ucsd.edu)

The goal of this ongoing research and outreach is to expand the West Coast shellfish industry through creation of triploid seed and demonstration of efficient culture methods for the native purple-hinge rock scallop (*Crassadoma gigantea*). Shellfish aquaculture is a low trophic level means of seafood production that provides many benefits to coastal communities and the environment, while at the same time increasing the supply of locally produced safe and nutritious seafood. There is a strong desire to develop native species for aquaculture development to diversify the shellfish industry and help to avoid concerns often voiced today about the use of non-native species. While new native species of shellfish for aquaculture are highly sought after, there are also genetic concerns associated with rearing native species for aquaculture using hatchery-reared seed that may have undergone significant domestication selection or been produced from distant broodstock populations. This may occur as a normal consequence of rearing in the hatchery environment or through highly directed selection, crossbreeding, or other means to genetically change the production characteristics of the organism. These risks are significant and must be addressed to realize the potential for growth of the U.S. west coast shellfish industry. Issues associated with potential genetic risk to wild rock scallop populations could be resolved through the creation of tetraploid scallop stocks which could be mated to diploids producing 100% triploid offspring, or by using chemical means. Scallops were successfully spawned and cultured at the Taylor Shellfish hatchery in Washington State and at the Bodega Marine Laboratory of UC Davis in Bodega Bay, California. Growth and survival of larvae was highly variable among batches and populations. Despite broodstock maintained in common conditions and similar larval dietary rations. Causes for this variability, and generally low larval survival are being investigated. Initial efforts to culture scallop larvae relied mainly on *C-Isochrysis sp.* These efforts produced weak larvae with low survival and nutrition was identified as a limiting factor early on. Subsequent efforts relied on a mixed diet of *C-Isochrysis sp.*, *Nannochloropsis sp.*, *Pavlova sp.*, *Chaetoceros sp.*, and *Thallasiosira sp.* The optimal timing for production of 3N scallops by inhibition of second polar body extrusion using 6-DMAP has been determined to be a 20 min treatment, 55-60 min post fertilization at 17° C. The optimal dosage of 6-DMAP for production of 3N scallops by inhibition of second polar body extrusion has been determined to be 425 uM 6-DMAP.



### **Annotated Bibliography of Key Works**

Bourne, N. C. A Hodgson and J. N. C. White. 1989 A manual for Scallop Culture in British Columbia. Canadian Technical Report of Fisheries and Aquatic Sciences. No. 1694 Department of Fisheries and oceans, Biological Sciences Branch, Pacific Biological Station, Nanaimo, BC  
A comprehensive manual for hatchery production of scallops.

The hatchery culture of bivalves: a practical manual. FAO

<http://www.fao.org/docrep/007/y5720e/y5720e09.htm>

A comprehensive manual for hatchery production of bivalve shellfish

Mortalities in a hatchery of the great scallop, *Pecten maximus*, . University of Bergen, Department of Biology Bergen Norway, 2004.

A bacteriological study was carried out in a scallop hatchery in western Norway. The hatchery had suffered severe mortalities during the larval stages, and prophylactic use of antibacterial agents was necessary to produce larvae. A number of bacterial strains were isolated from the hatchery. A challenge test was performed with the isolates. Six of the strains caused mortalities not statistically different from *Vibrio pectenica*, a known pathogen from France. From 16S rDNA analysis on these strains, the phylogenetic tree indicated two groups of apparent pathogens; one strain that resembles the Alteromonas/Pseudoalteromonas group, and a cluster of strains that resemble the species *Vibrio splendidus*. Since the antibacterial agent used in the hatchery was chloramphenicol, which is now banned in Norway and Europe, application of alternative antibacterial agents were investigated. In this study the minimum inhibitory concentration (MIC) values of chloramphenicol, florfenicol, flumequinone, the combination trimethoprim/sulfadiazine, oxytetracycline, oxolinic acid and Pyceze on bacterial strains isolated from scallop larvae were investigated. Based on these MIC values, procedures for the treatment of scallop larvae with antibacterial agents were evaluated. Since the therapeutic procedures will be used in a marine environment, the antagonizing effect of seawater on some of the antibacterial agents was measured. For flumequinone and trimethoprim/sulfadiazine the average MIC values increased significantly when using seawater with salinity of 25ppt compared to 2% NaCl.

Caines, J; Crocker, K. 1999. Hatchery production of sea scallop spat (*Placopecten magellanicus*) in Newfoundland, Canada

A commercial hatchery for production of sea scallop spat was commissioned in 1995 at Belleoram, Newfoundland, by the provincial Department of Fisheries and Aquaculture. Four annual production seasonal cycles have been completed to date, where spat have been transferred to grow-out at shellfish farms. The annual yield from the hatchery has been 2-25% of the planned target of 20 million spat per year. This presentation will report our experiences in 1998 regarding algal culture, broodstock conditioning, larval growth, spat settlement and growth, and bacteriological monitoring of water quality. Algal culture: Algae (flagellates and diatoms) were grown in a continuous culture system purchased from Seasalter Shellfisheries in England. The intent was to provide scallop larvae and spat with a diet of mixed species of varied lipid and carbohydrate content. The following flagellates and diatoms were cultured in 500-L polyethylene bags under continuous light at 22 degree C with the addition of CO<sub>2</sub>: *Isochrysis galbana*, *T.Iso*, *Pavlova lutheri*, *Tetraselmis suecica*, *Chaetoceros calcitrans*, *C. ceratosporum*, *C. muelleri*,

*Thalassiosira pseudonana*, *T. weissflogii*, and *Chroomonas salina*. The growth rate was approximately 0.30 and 0.35 divisions/day for the flagellates and diatoms respectively.

[Meng, Qinglei; Bao, Zhenmin; Wang, Zhaoping; Wang, Shi; Hu, Jingjie. 2012. Growth and Reproductive Performance of Triploid Yesso Scallops \(\*Patinopecten yessoensis\*\) Induced by Hypotonic Shock. \*Journal of Shellfish Research\* 31.4 \(Dec 2012\): 1113-1122.](#)

The successful induction of triploid embryos or larvae has been performed in *Patinopecten yessoensis* during the past 2 decades. However, no research has been reported about the performance of triploid *P. yessoensis* cultured in the field. This study induced triploidy in *P. yessoensis* by hypotonic shock and compared the growth and reproductive performance of triploids and diploids reared under commercial conditions for up to 24 mo. The main results of this study are as follows: Triploid scallops were smaller in size and weight compared with diploids and had a retarded absolute growth rate (AGR). After 24 mo. of cultivation, the mean shell height, shell length, shell width, and body weight of triploids were 9%, 10%, 9%, and 25% less than diploids, respectively ( $P < 0.01$ ). Although normal in sex ratio, the reproductive potential of triploids was significantly reduced. Only 87% of the triploids exhibited sex-discernible gonads during the breeding season. None of the male triploids spawned, and the percentage of female spawners among the triploid population was only 27% of that for the diploid population. The relative fecundity of triploid females was only 4% of diploid females. Triploid eggs produced mostly aneuploid larvae and had an extremely small chance of generating viable offspring when fertilized by sperm from diploid males. Most aneuploid larvae died before the D-shaped stage, and no survival exceeded 7 days. The potential to yield viable offspring from the triploid population was estimated to be only 4% 10<sup>-6</sup> of that of the diploid population. Despite the growth disadvantage of triploids, this study may support, in part, the energy reallocation hypothesis, because triploid AGR was similar to diploid AGR (2% variance) during the sexual maturation season. Our results also indicate that there would be no growth advantage, but instead a disadvantage, for triploid *P. yessoensis* growing at the experiment site. In addition, this research provides the first evidence that viable triploid molluscs can be induced by hypotonic shock, of which the practical and evolutionary implications are also discussed.

[Cogswell, A. T., Roach, S. E., MacDonald, B. W. 2006. Triploid bay scallops \(\*Argopecten irradians\*\): induction methodology, early gonadic development and growth. \*Department of Fisheries and Oceans, Dartmouth, NS \(Canada\). Science Branch. Canadian technical report of fisheries and aquatic sciences/Rapport technique canadien des sciences halieutiques et aquatiques\* 2635. \(2006\).](#)

Triploid (3N) Pacific oysters (*Crassostrea gigas*) account for more than 50% of total oyster production in the US. The success of this species has created an interest in producing triploids of other commercial shellfish species, including scallops, clams and mussels. Here, we report on 3N induction trials with the bay scallop, *Argopecten irradians*. The most commonly employed 3N induction technique involves exposing early embryos to chemicals (e.g., Cytochalasin B (CB)). CB inhibits the release of the second polar body immediately following fertilization, causing retention of both pairs of female chromosomes in addition to the male chromosome set. It does this by disrupting actin polymerization. Four concentrations of CB were evaluated for ability to produce 3N larvae. The efficacy of current and proposed 3N induction techniques (i.e., 4N x 2N crosses) and the commercial potential of 3N bay scallops are discussed.

[Ruiz-Verdugo, CA; Allen, S K, and ; Ibarra, A M. 2001. Family differences in success of triploid induction and effects of triploidy on fecundity of catarina scallop \(\*Argopecten ventricosus\*\). \*Aquaculture\* 201.1-2 \(Sep 14, 2001\): 19-33.](#)

Mass induction of triploidy in the catarina scallop (*Argopecten ventricosus*) results in low success in the percentage of triploids produced. To understand whether this is a treatment effect affecting all eggs equally, families were individually induced to triploidy with cytochalasin-B (CB), comparing their survival from egg to D-larvae and spat, and the percent of triploidy within families. Differences in percent triploidy success were evident between families, obtaining some with no triploids, and some with high triploidy. Among the possible causes for these differences are quality of eggs, different developmental rates, and differences in susceptibility to the treatment (CB or DMSO) itself. Regardless of those differences, overall triploidy production was increased by inducing individually eggs of each scallop rather than in mixed egg batches. In the first experiment, it was improved by 17%, and in the second experiment by 42%, as indicated by the weighted mean of triploids among the families, and when compared with previous results with this same species, where triploidy success was 58%. In a second experiment with three different families, the growth and fecundity of triploid and diploid catarina scallop were evaluated. The growth superiority of triploids was confirmed. The results indicated that triploid catarina scallop had a significantly reduced fecundity when compared with diploid scallop. The reduced fecundity appears to be mostly of a random nature, possibly associated with a reduced capability to produce balanced gametes. Whereas the successful production of tetraploid catarina scallop from fertile triploid scallop is in principle possible, the low number of eggs shed by triploid catarina scallop could diminish that success rate, even more if single triploid females are required to optimize tetraploid induction.

## Big Data in Agriculture and the USDA/ARS Initiative

Jeffrey Silverstein

Southeast Area, US Department of Agriculture, Agricultural Research Service, P.O. Box 225,  
Stoneville MS 38776

\*E-mail: [jeff.silverstein@ars.usda.gov](mailto:jeff.silverstein@ars.usda.gov)

In recent years, technological achievements have permitted the relatively inexpensive and rapid production of vast amounts of data. The large and often complex datasets produced in the scientific sphere demand new approaches to gain value and to turn data into information. Management of the growth in the volume, variety, and velocity of data is often referred to as the 'Big Data problem'. The Agricultural Research Service of the U.S. Department of Agriculture (USDA/ARS) has long been a strong, science-based, problem solving agency. In the past, our computational infrastructure has primarily been based on meeting administrative needs and security requirements, whereas computational, analytical, and sharing of scientific data were regarded as secondary priorities. The Big Data problem has required a reassessment of scientific computing needs. In February 2013, we held a workshop led by ARS scientists to assess scientific data needs, and this resulted in a \$25 million initiative to develop the USDA/ARS capacity to collect, share, and analyze Big Data. Our Big Data initiative contains three elements. First, we have developed a dedicated scientific research network (SCInet) which will leverage Internet2 to facilitate large scale transfer of research data at high-speeds with low latency. Second, we have constructed a high-performance computing (HPC) system with high memory, high processing capacity and the potential to burst computational workloads to commercial resources when necessary. SCInet and the HPC have been largely constructed and connected in the first 18 months of the initiative. Finally, we are developing a virtual research support core of individuals who will provide scientific computational and informatics support. These individuals will work with agency scientists to facilitate specific projects and will also develop standardized solutions and training for common challenges.

Key steps of this initiative, 1) determining the needs of the agency, 2) developing a plan to connect more than 90 locations in the agency, 3) building the technical capacity in our labs, and 4) controlling costs due to restrictive budgets are challenges that will be shared and discussed.

## O-6

### **Signature of artificial selection in a breed of coho salmon *Oncorhynchus kisutch***

Sho Hosoya<sup>1\*</sup>, Kiyoshi Kikuchi<sup>1</sup>, Hiroshi Nagashima<sup>2</sup>, Junichi Onodera<sup>2</sup>, Kouichi Sugimoto<sup>2</sup>, Kou Satoh<sup>2</sup>, Keisuke Matsuzaki<sup>2</sup>, Masaki Yasugi<sup>3</sup>, Atsushi J. Nagano<sup>3,4</sup>, Akira Kumagayi<sup>2</sup>, Kenichi Ueda<sup>2</sup>, Tadahide Kurokawa<sup>5</sup>

<sup>1</sup>Fisheries Laboratory, University of Tokyo, <sup>2</sup>Miyagi Prefecture Fisheries Technology Center, <sup>3</sup>Center for Ecological Research, Kyoto University, <sup>4</sup>JST PRESTO, <sup>5</sup>Tohoku National Fisheries Research Institute

\*E-mail: [ahosoya.at.mail.ecc.u-tokyo.ac.jp](mailto:ahosoya.at.mail.ecc.u-tokyo.ac.jp)

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 kisutch*), 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. The two populations originated from the hatchery at Lower Kalama, WA, USA. Eyed eggs were introduced to Japanese National Research Institute of Aquaculture in 1978. Subpopulation was transferred to Inland Fisheries Experimental Station, Miyagi Prefecture Fisheries Technology Center in 1980. Two selected lines were established in 2000 from two different year classes: six and ten selected females were crossed to three unselected males each. The second and the third selection were done in 2003 and 2006, respectively. In 2009, individuals from the selected population were randomly crossed within the population (F<sub>3</sub>). The fourth selection was done in 2013 (F<sub>4</sub>). Genetic analysis was done using the F<sub>3</sub> and the size comparison using the F<sub>4</sub>. We compared fork length between the unselected and the F<sub>4</sub> 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. From the F<sub>3</sub> selected and the unselected population, 100 individuals were randomly chosen and a genome-wide set of SNPs was collected by ddRAD-seq. Output reads were first quality trimmed and survived reads at both paired ends were mapped on the rainbow trout genome individually, and analyzed variations using Stacks (ver 1.35). Close to 166,000 SNP sites were detected, out of which approximately 1,100 were considered reliable, and were used for subsequent analysis. Multidimensional scaling 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 57 sites. We also performed GWAS and detected 19 loci where *P*-values were less than 0.01. 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. As a next step, we are planning to implement genomic selection into the breeding program. This work was supported by AFFRC, Japan.

**Key words:** coho salmon, *Oncorhynchus kisutch*, artificial selection, body size, ddRAD-seq, marker assisted s

## O-7

### Genetic Selection in Animals Using Pedigree, Phenotypic, and Genomic Information

Shogo Tsuruta<sup>1\*</sup>, Daniela, A. L. Lourenco<sup>1</sup>, Yutaka Masuda<sup>1</sup>, Breno Fragomeni<sup>1</sup>, Ignacio Aguilar<sup>2</sup>, Andres Legarra<sup>3</sup>, Ignacy Misztal<sup>1</sup>

<sup>1</sup>Department of Animal and Dairy Science, The University of Georgia, Athens, GA, 30602, USA, <sup>2</sup>Instituto Nacional de Investigacion Agropecuaria, Canelones, 90200, Uruguay, <sup>3</sup>INRA, UMR1388 GenePhySE, Castanet Tolosan, 31326, France

\*E-mail: [shogo.at.uga.edu](mailto:shogo.at.uga.edu)

Genetic selection of quantitative traits in animals has been performed for over a century in the United States. In the beginning, artificial selection was conducted using only phenotypes. Later, pedigree information was added, from parent-offspring relationships, siblings, and families to all available information among related animals. Around the same time, advanced statistical methods were developed (e.g., regression analysis, least square analysis, and BLUP) to estimate genetic parameters and to predict breeding values for economically important traits. As a result, animal productions have dramatically increased in the last half-century. In the last two decades, we has contributed to various livestock industries, including dairy cattle, beef cattle, swine, chicken, goats, sheep, horses, bees, and fish. We have analyzed genetic components in animal production, reproduction, and disease traits using our own computer programs in collaborations with USDA, breed associations, breeding companies, and other institutes all over the world. Those programs have been used by breed associations and breeding companies in routine genetic evaluations. Recently, we have developed a method called single-step genomic BLUP (ssGBLUP) to predict genomic breeding values by combining pedigree, phenotypes, and genotypes (SNP markers). With ssGBLUP, we found that we could increase the accuracy of genomic breeding values in dairy and beef cattle, pigs, and chickens by 10-30% compared with traditional breeding values. This methodology has been expanded to use a large number of genotyped animals (> 1 million). The application programs are available on our website at <http://nce.ads.uga.edu/projects/programs/~>. Genomic selection has a greater advantage when more genotyped animals are available, because predictions are more accurate and more complex models can be applied. Also, if animals are genotyped in the earlier stage of their life, genetic progress can be accelerated and genetic gains can be maximized. These methodologies are applicable to other organisms, such as farming fish, shellfish, anadromous fish (salmon), and even plants.

#### Annotated Bibliography of Key Works

Misztal, I., S. Tsuruta, T. Strabel, B. Auvray, T. Druet, and D. Lee. 2002. BLUPF90 and related programs (BGF90). Proc. 7th WCGALP, Montpellier, France.

Aguilar, I., I. Misztal, D. L. Johnson, A. Legarra, S. Tsuruta, and T. J. Lawlor. 2010. A unified approach to utilize phenotypic, full pedigree, and genomic information for genetic evaluation of Holstein final score. J. Dairy Sci. 93:743-752.

Tsuruta, S., I. Misztal., I. Aguilar, and T. J. Lawlor. 2011. Multiple-trait genomic evaluation of linear type traits using genomic and phenotypic data in Holsteins. J. Dairy Sci. 94:4198-4204.

Lourenco, D. A. L., S. Tsuruta, B. O. Fragomeni, Y. Masuda, I. Aguilar, A. Legarra, J. K. Bertrand, D. Moser, and I. Misztal. 2015. Genomic evaluation using single-step genomic BLUP in American Angus. J. Anim. Sci. 93:2653-2662.

Fragomeni, B. O., D. A. L. Lourenco, S. Tsuruta, Y. Masuda, I. Aguilar, A. Legarra, T. J. Lawlor, and I. Misztal. 2015. Use of genomic recursions in single-step genomic BLUP with a large number of genotypes. J. Dairy Sci. 98: 4090-4094.

The authors have been working on estimation of genetic parameters and prediction of breeding values for economically important traits in animals. They have recently developed a method called single-step genomic BLUP (ssGBLUP) to predict genomic breeding values by combining pedigree, phenotypic, and genomic information and have implemented the method to their existing computer (BLUPF90) programs (e.g., BLUP, REML, and Gibbs Sampler) to use SNP marker information. Recently, they published several studies of ssGBLUP to maximize accuracy and minimize bias in genomic breeding values, applying this methodology to dairy cattle, beef cattle, pigs, and chickens. Currently, they have been working on how to include a large number of genotyped animals (> 1 million) in genomic evaluation. Breed associations and breeding companies that have been using their software are now planning to introduce ssGBLUP to routine evaluations. The same or similar methodology can be applied to fish breeding. The BLUPF90 family of programs and the manual are available at <http://nce.ads.uga.edu/~>.



## O-8

### Exploring Transcriptomic Patterns in Slow- and Fast-Growing *Seriola dorsalis* Larvae.

Catherine Purcell\*<sup>1</sup>, Andrew Severin<sup>2</sup>, Vince Buonaccorsi<sup>3</sup>, Mark Drawbridge<sup>4</sup>, Kevin Stuart<sup>4</sup>, and John Hyde<sup>1</sup>

<sup>1</sup>NRC Postdoctoral Fellow, Southwest Fisheries Science Center, NOAA Fisheries, 8901 La Jolla Shores Dr., La Jolla, CA 92037, <sup>2</sup>Genome Informatics Facility, 206 Science I, Iowa State University, Ames, Iowa 50011, <sup>3</sup>Juniata College, 1700 Moore St. Huntingdon PA 16652, <sup>4</sup>Hubbs Seaworld Research Institute, 2595 Ingraham Street, San Diego, CA 92109

\*E-mail: [Catherine.Purcell@noaa.gov](mailto:Catherine.Purcell@noaa.gov)

*Seriola* species have traditionally been a major component of global commercial and recreational fisheries, and in recent years, aquaculture value of these fish has grown into a ~\$1.3 billion industry. The California yellowtail (*Seriola dorsalis*) is a strong candidate for development of offshore commercial aquaculture in southern California and neighboring Baja California. Although production from broodstock populations has been successful, it has not yet reached a sustainable level where it can satisfy the aquaculture demand, largely due to difficulties from highly variable survival and growth rates through the larval stages. Given the extremely fast growth and the major physical changes that occur during the earliest life stages, one way to examine variability in survival and growth is at the gene and molecular levels across those early-developmental periods. To improve our understanding of the molecular processes underlying development, we examined RNA-Seq profiles for several early life stages of yellowtail, categorized as either slow- or fast-growing. Gene expression was measured in three replicates of pooled larval samples at 2, 7, and 17 days post hatch for these two growth categories. Using the Illumina platform, an average of sixty million reads was obtained per replicate; genes of related function were sorted into clusters, and those found at high frequency in the differential gene expression set were identified. Differences in molecular pathways, biological processes, and gene regulating patterns between the two fitness groups were examined. There were many differentially expressed genes across developmental stages and between the fitness groups, for example, genes involved in oxidative phosphorylation pathways revealed interesting patterns both across developmental stages and between slow- and fast-growing larvae. Results of these analyses will be presented.

#### Annotated Bibliography of Key Works

Kocmarek, A., Ferguson, M., and Danzmann, R. (2014) Differential gene expression in small and large rainbow trout derived from two seasonal spawning groups. BMC Genomics, 15: 57.

In this study, the authors wanted to identify genes that showed similar expression patterns between large and small rainbow trout from different spawning seasons in two types of tissue: liver and white muscle. The goal in identifying these genes was to be able to develop growth-related markers for use in breeding programs. They hypothesized that 1) genes related to carbohydrate and lipid metabolism, energy production, insulin, and growth factors would be down-regulated in both tissue types in the small fish, 2) that genes involved in cytoskeletal structuring would be

down-regulated in small fish, while myostatin will be up-regulated, and 3) that liver-specific lipid binding, cytoplasmic components, signaling, and transcription would be up-regulated in small fish. They observed that genes related to immune function were up regulated in large fish; suggesting that enhanced growth is associated with enhanced immune function. They also found that genes related to transcription, translation, and protein production were up regulated in small fish (from Sept.) in white muscle, which supports patterns previously detected in liver tissue. This indicates that protein production in small fish may not be translating effectively into finished proteins. This study was able to identify patterns of differential gene expression in small and large rainbow trout; this will enable future studies to delve deeper into the genes related to differences in growth.

Meyer, E., and Manahan, D. (2010) Gene expression profiling of genetically determined growth variation in bivalve larvae (*Crassostrea gigas*). The Journal of Experimental Biology, 213: 749-758.

The authors compared gene expression patterns in larvae of the Pacific oyster (*Crassostrea gigas*) that exhibited slow- and fast-growth (these larvae were produced from experimental crosses). Based on a previous transcriptome-wide analysis, a set of 181 candidate genes for growth heterogeneity were analyzed with the goal of understanding the biological processes underlying the differential growth rates. Of the genes identified by GenBank, ribosomal proteins were the most abundant, comprising 50% of the total genes with 17 different ribosomal protein genes. The genes included nine components of the large ribosomal subunit, and eight components of the small ribosomal subunit. Some of these genes were up-regulated in the fast-growing larvae ( $n = 6$ ), while others were up-regulated in the slow-growing larvae ( $n = 11$ ). Since ribosome biogenesis is a significant metabolic cost in cell proliferation, any changes in this pathway would likely have a large effect on the overall energy metabolism. The authors hypothesize that in the slow-growing larvae there may be a high metabolic cost to synthesizing and degrading excess ribosomal protein copies resulting from the higher expression of those genes.

Salem, M., Silverstein, J., Rexroad III, C., and Yao, J. (2007) Effect of starvation on global gene expression and proteolysis in rainbow trout (*Oncorhynchus mykiss*). BMC Genomics, 8: 328.

The authors of this study used microarrays to identify genes and pathways involved in the starvation response and protein turnover in rainbow trout, and to identify metabolic adaptations that occur in the liver during these starvation periods. This study was of interest because, as the authors point out, examining changes in metabolism during starvation is an effective way to identify relationships between metabolic pathways and body processes. The experiments showed down-regulated expression of genes involved in protein biosynthesis in the starved fish, but no significant changes in protein catabolic pathways, and a slight increase in 20S proteasome activity. Responses in the liver to starvation included an overall decline in gene expression associated with decreasing tissue metabolism, a reduction in protein synthetic capacity, an impairment of ATP-biosynthesis, and lower expression in hepatic lipid and fatty acid transport.

Moran, D. (2007) Size heterogeneity, growth potential and aggression in juvenile yellowtail kingfish (*Seriola lalandi* Valenciennes). Aquaculture Research, 38: 1254-1264.

In this study, the authors are describing the occurrence of size heterogeneity and aggressive behaviors in cultures of *Seriola lalandi*; this was done to examine the effectiveness of size-grading

in reducing aggression and mortality, and increasing growth rates. To do this, graded and ungraded juveniles were compared for various measures of aggression and growth, and a RNA/DNA ratio was used as a measure of growth rate. The authors found that size heterogeneity became more pronounced at 12 days post hatch (dph) when *Artemia* are offered as a food source. While the large and aggressive juveniles only accounted for 8% of the population, the small grade juveniles that received the aggression accounted for 42% of the population. In the ungraded treatment, this aggression was associated with mortality for most small fish. However, even without aggression, the small-grade juveniles did not gain weight or increase their RNA/DNA ratio after 12 days. The authors believe that these small fish appear to be on a degenerative developmental strategy without any influence of the larger aggressive fish.

## Modeling the Variable Effects of Using Wild and Cultured Broodstock on the Fitness Risk Due to Escaped Farmed Fish

Kristen M. Gruenthal\*<sup>1</sup>, Jason D. Volk<sup>2</sup>, Gregory R. Blair<sup>2</sup>, Gavin Key<sup>3</sup> and Michael B. Rust<sup>1</sup>

<sup>1</sup>NOAA Fisheries Office of Aquaculture, Northwest Fisheries Science Center, 2725 Montlake Blvd E, Seattle WA, 98112, USA, <sup>2</sup>ICF International, 710 Second Avenue Suite 550, Seattle, WA 98104, USA, <sup>3</sup>Kampachi Farms, LLC, 1 Keahole Point Road, Kailua Kona, HI 96740

\*E-mail: [kristen.gruenthal@noaa.gov](mailto:kristen.gruenthal@noaa.gov)

The rapid development of offshore marine aquaculture worldwide has raised concerns due in part to the potential for various negative ecological and genetic impacts if and when farmed fish escape and mix with wild conspecifics. Impacts due to interbreeding between wild fish and escapees may result in reduced genetic diversity within and among populations and a loss of fitness. Loss of genetic diversity and fitness compromise the adaptive potential of a mixed (wild plus cultured) population, making it potentially less able to respond to stochastic changes or long-term trends (e.g. climate change) in environmental conditions. Risk to wild population fitness is a function of 1) the number of escapees relative to wild census size, 2) the genetic difference between escapees and wild fish, and 3) the fitness of escapees relative to wild fish. There is little scientific data, however, that reliably assigns the risk to population fitness due to escapes. The Offshore Mariculture Escapes Genetics Assessment (OMEGA) model simulates the risk posed by escaped farmed fish to wild population fitness. OMEGA is intended to provide insight into the variables affecting risk, help identify information gaps and research priorities, inform policy and management decisions, and explore options for design or modification of culture programs, such that the aquaculture industry itself can and will drive improvements in culture techniques and sustainability. Current work continues to be directed at making OMEGA fully operational and includes fostering external collaborations to develop model scenarios, evaluate model parameters, and validate the model with data from current and planned domestic and international aquaculture operations. To illustrate OMEGA's application, we present results from evaluations of a theoretical sablefish (*Anoplopoma fimbria*) aquaculture program and a practical pilot project for Almaco jack (*Seriola rivoliana*) in Hawaii. Sablefish are a highly-prized groundfish with white flesh found along the North Pacific Rim. Commercial culture of the species currently occurs in Washington State, USA, and British Columbia, Canada. The OMEGA scenarios for sablefish were originally developed for model verification and include using a domesticated broodstock to produce up to 10,000 mt of fish stocked among 50 offshore cage sites. Kampachi Farms is a leader in sustainable US marine aquaculture systems development and hatchery methods. Recently, the group has engaged in iterative open-ocean pilot projects directed at expanding offshore farming of almaco jack to a commercial scale near Kailua Kona, HI, USA, and elsewhere. The OMEGA scenario for Kampachi uses wild broodstock and stocks F1 juveniles in tethered cages located approximately 11 km offshore of Kona, with production of about 7 mt per year for two years. Escape scenarios for both species varied from low to high base leak rates, cage failure probabilities, and catastrophic cage failure probabilities. Whereas the high escapes scenario for sablefish resulted in a significant impact to wild population fitness, even a total loss of almaco jack had a negligible effect. Key differences

in the simulated fitness risk associated with each program included broodstock source (domesticated or wild), offshore location, and the scale (size and longevity) of the operation.

### **Annotated Bibliography of Key Works**

Baskett, M.L., Burgess, S.C., Waples, R.S., 2013. Assessing strategies to minimize unintended fitness consequences of aquaculture on wild populations. *Evolutionary Applications* 6:1090-1108.  
Baskett et al. (2013) address various factors associated with the management of cultured populations and model their potential fitness impacts should farmed fish escape into the wild. Factors assessed include the origin and level of domestication in the cultured stock (i.e. degree of maladaptation), induced sterility in the cultured stock, and the magnitude and frequency of escapes (e.g. continuous low level leakage versus rare catastrophic events). Results indicate that, up to a point, the magnitude of the fitness impact rises as the maladaptation of escaped cultured fish increases; an extremely maladapted, non-local origin cultured population may actually have effects similar to a weakly diverged stock. Second, sterilization reduces unintended fitness consequences rapidly. Finally, it is more effective to reduce low-level leakage than guard against sporadic large-scale escape events.

Ford, M.J. 2002. Selection in captivity during supportive breeding may reduce fitness in the wild. *Conservation Biology* 16:815-825.

Escaped cultured fish may cause a potential loss of fitness in the wild, if breeding occurs between escapees and wild conspecifics. This drop in fitness is associated with a difference in the optimum trait values for hatchery and natural environments. The single-trait phenotypic fitness model in Ford (2002) describes how mean phenotype values of the mixed population (captive plus wild fish) may shift relative to the optimum values for each environment, based on the presence/absence and amount of gene flow (interbreeding) between the cultured escaped and wild fish. The overall fitness effect depends on the magnitude of the difference in optimum trait value, trait heritability, and selection pressure against domestication in the wild, as well as habitat capacity, magnitude and frequency of escape events, wild and captive population demographics, and the potential for interaction between wild fish and escapees. Ford (2002) was used most notably in Pacific Northwest salmonids, where the All-H Analyzer, or AHA, model helped the Hatchery Scientific Review Group explore the potential fitness consequences of supplementing wild populations and of cultured fish straying into wild populations.

Hindar, K., Ryman, N., Utter, F., 1991. Genetic effects of cultured fish on natural fish population. *Canadian Journal of Fisheries and Aquatic Sciences* 48:945-957.

Hindar et al. (1991) represents one of the original manuscripts reviewing the genetic impact escaped farmed fish may have on wild populations and is one of the most often cited. The authors recommend several strategies for protecting the genetic integrity of wild populations, many of which remain the focus of aquaculture programs today. These strategies include improved containment technology and recovery, sterilization of the culture stock, and better breeding practices, coupled with monitoring the genetic contribution of escaped fish to the mixed population.

Waples, R.S., Hindar, K., Hard, J.J., 2012. Genetic risks associated with marine aquaculture. NOAA Technical Memorandum NMFS-NWFSC-119. US Department of Commerce NOAA NMFS, Washington, D.C.

Waples et al. (2012) is a comprehensive overview of the potential genetic impacts to wild populations associated with marine aquaculture. As such, the authors “provide managers with a better understanding of the genetic effects of marine aquaculture on natural populations,” with the intent of informing policy and management decision-making. The document synthesizes relevant information and provides key references, identifies research priorities, provides a risk assessment framework, and gives recommendations for monitoring and evaluation toward sustainable marine aquaculture development in the US. Waples et al. (2012) focuses on commercial aquaculture of marine finfish but presents it in light of decades of research on salmon hatcheries and marine stock enhancement.

## O-10

### **Did farmed Coho salmon *Oncorhynchus kisutch* that escaped during the earthquake and tsunami disaster of 2011 interbreed with native Masu salmon *Oncorhynchus masou*?**

Kei Sasaki\*, Hideki Nikaido, Daisuke Muraoka, Yoshiro Okada, Tadahide Kurokawa

Miyako Station, Tohoku National Fisheries Research Institute, FRA

\*E-mail: keis.at.affrc.go.jp

Sea farming of coho salmon (*Oncorhynchus kisutch*), a species that migrates to waters off Hokkaido for feeding but does not naturally spawn in Japan has been conducted around the “Sanriku” coast in Japan since 1975. In recent years, production has remained at over 10,000 tons per year. Due to the effects of the Great East Japan Earthquake and tsunami of March 11, 2011, several million farmed coho salmon escaped into the Northeast Pacific Ocean off Tohoku, northeastern Japan. In the fall of that year, sexually mature coho salmon migrated up rivers in this area. Farmed coho salmon that migrated up rivers to breed may have affected the genetic material of native salmon species and result in weakened populations. Especially, there is strong concern that coho salmon may cross with the native masu salmon (*Oncorhynchus masou*); it is known that hybrids of these species are able to survive (while the ability to survive is low for crosses with chum salmon). Assuming that hybrids are present, it is unclear how many years they will need to mature. However, based on the maturation age of both species, the possibility that hybrids will return in spring 2014 is considered to be high. We conducted visual checks of masu and coho salmon landed at a fish market during May to September 2014 (approx. 2,000 individuals). We selected 30 masu, and 5 coho salmon (all individuals were sampled during the survey) judged from appearance for analysis. Furthermore another sample of individuals (n=9) that were caught at the same place and were considered to potentially be crosses were also examined in the laboratory. For the morphological comparison, we compared the number of rays of each fin, and the number and length of the gill rakers. We conducted sequence analysis of the intron C of growth hormone 1 (GH-1). PCR amplified product of masu salmon (256bp) is 34bp less than that of coho salmon (290bp) using a primer set with 17bp adapter sequence for the fluorescent label. Therefore, it is possible to distinguish both species. The amplicon is seen in both if there is a hybrid. As a result of the morphological comparison and DNA fragment analysis, hybrids were not confirmed, and all the individuals that were initially considered to be potential hybrids were judged to be masu salmon. Therefore based on the current survey, the impact on the genetic resources of masu salmon is considered to be low. However, three of five of coho salmon were of the 2011 brood and their gonads were developed (although there is no conclusive evidence that these coho salmon individuals were derived from the escaped coho salmon during the 2011 earthquake). Therefore it is necessary to carefully monitor the occurrence of hybrids in the short-term future.

**Key words:** farmed coho salmon, *Oncorhynchus kisutch*, escaped, masu salmon, *Oncorhynchus masou*, hybrids, DNA fragment analysis

## O-11

### **Evaluation of the tsunami impact on the genetic diversity of the marbled flounder *Pseudopleuronectes yokohamae* in Sendai Bay, Miyagi, Japan**

Yuki Minegishi<sup>1\*</sup>, Minoru Ikeda<sup>1</sup>, Yutaka Kurita<sup>2</sup>, Hiroyuki Togashi<sup>2</sup>, Yukinori Nakane<sup>2</sup>, and Akihiro Kijima<sup>1</sup>

<sup>1</sup>Tohoku Ecosystem-Associated Marine Sciences, Graduate School of Agricultural Science, Tohoku University, <sup>2</sup>Tohoku National Fisheries Research Institute, FRA

\*E-mail: y.minegishi at m.tohoku.ac.jp

Evaluating genetic diversity is a fundamental step for appropriate stock management. The information on genetic diversity such as population structure, migration rate and population size helps identify management units and translocation ranges, and monitoring those alterations provides the insights into the population vulnerability and viability. At the same time, genetic diversity is influenced by environmental and demographic changes. Direct effects involve population bottleneck and varied migration between populations, and indirect ones cause changes in habitat structure and community composition. Therefore, genetic diversity should be carefully investigated, especially where dramatic disturbances have occurred. Marbled flounder *Pseudopleuronectes yokohamae* is a commercially valued fish overall Japan. Due to the benthic lifestyle including low dispersal ability, this species possibly consists of multiple management units. The population in Sendai Bay is considered to form a single management unit, and its spawning ground has been protected for stock conservation. However, this population is expected to have been strongly affected by the great tsunami occurred along the Pacific coast in North Japan in 2011, and consequently the genetic diversity could be changed. Thus, the temporal genetic variation of the Sendai Bay population needs to be investigated and the geographic range of the management unit should be determined based on the genetic population structure inferred together with other Pacific Ocean populations. To develop a genomic resource, a 200-bp library was prepared using the genomic DNA of a single specimen of the marbled flounder collected in Sendai Bay. IonTorrent PGM sequencing was performed up to  $35.4 \times$  coverage (23.7 gigabases), and *de novo* assembly generated a total of 525,502 contigs ( $N_{50} = 1994$ ) and 10,732,070 unassembled reads. We then designed a total of 331,368 primer pairs for 86,732 unique microsatellite sequences detected in the unassembled reads. Among the arbitrarily selected 96 pairs that were experimentally tested, sixteen pairs were characterized as novel microsatellite loci applicable for population genetic analyses of this species. Temporal change in genetic diversity after the tsunami impact was investigated using thirteen populations collected in Sendai Bay from April 2012 to February 2014 ( $N = 807$ ). Nine populations from the Pacific Ocean coasts ( $N = 596$ ) were also analyzed for comparisons. Genotyping was performed at the newly developed sixteen microsatellite loci and population genetic analyses were conducted. Significant genetic divergence was found between the east and west sides of Boso peninsula, in which populations further diverged (pairwise  $F_{ST} = 0.02305 - 0.19784$ ). At the east side of Boso peninsula, the Mutsu Bay population was differentiated ( $F_{ST} = 0.03829$ ,  $P < 0.001$ ) and gene flow was observed over the wide area from Onagawa Bay to Choshi including Sendai Bay. The genetic variability of the Sendai Bay population did not show any dramatic changes in time ( $H_E = 0.5061 - 0.5463$ ) and was relatively higher compared to those of



the western populations of Boso Peninsula ( $H_E = 0.3817 - 0.4557$ ). It is thus suggested that the tsunami impact on the Sendai Bay population of the marbled flounder is considered to be minor but a long-term monitoring may be needed.

**Key words:** Marbled flounder, *Pseudopleuronectes yokohamae*, genetic diversity, management units, population structure

### **Annotated Bibliography of Key Works**

Banks, S.C., G.J. Cary, A.L. Smith, I.D. Davies, D.A. Driscoll, A.M. Gill, D.B. Lindenmayer, R. Peakall. 2013. How does ecological disturbance influence genetic diversity? Trends Ecol. Evol., 28, 670-679.

Environmental disturbance underpins the dynamics and diversity of many of the ecosystems of the world, yet its influence on the patterns and distribution of genetic diversity is poorly appreciated. We argue here that disturbance history may be the major driver that shapes patterns of genetic diversity in many natural populations. We outline how disturbance influences genetic diversity through changes in both selective processes and demographically driven, selectively neutral processes. Our review highlights the opportunities and challenges presented by genetic approaches, such as landscape genomics, for better understanding and predicting the demographic and evolutionary responses of natural populations to disturbance. Developing this understanding is now critical because disturbance regimes are changing rapidly in a human-modified world.

Waples, R.S., A.E. Punt, J.M. Cope. 2008. Integrating genetic data into management of marine resources: how can we do it better? Fish Fish., 9, 423-449.

Molecular genetic data have found widespread application in the identification of population and conservation units for aquatic species. However, integration of genetic information into actual management has been slow, and explicit and quantitative inclusion of genetic data into fisheries models is rare. In part, this reflects the inherent difficulty in using genetic markers to draw inferences about demographic independence, which is generally the information of the greatest short-term interest to fishery managers. However, practical management constraints, institutional structures and communication issues have also contributed to the lack of integration. This paper identifies some of the organizational, conceptual and technical barriers that have hampered full use of genetics data in stock assessment and hence fishery management and outlines how such use could be enhanced.

## O-12

### Competition between Atlantic salmon (*Salmo salar*) and Japan's native salmonids.

Kazuo Araki\*, Tetuji Masaoka, Hiroyuki Okamoto, Hiroyuki Nagoya, Hidefumi Nakamura, Kouji Muto and Toshiya Suzuki

National Research Institute of Aquaculture, FRA  
224-1, Hiruda, Tamaki-cho, Watarai-gun, Mie, 516-0423, Japan  
\*E-mail: arakin.at.affrc.go.jp

AquaAdvantage Salmon are a strain of Atlantic salmon that have been genetically modified by the integration of Chinook salmon growth hormone gene and an anti-freezing protein promoter derived from ocean pout. This strain grows more rapidly ( $> 2\times$ ) than other farmed Atlantic salmon. Issuance of a draft Environmental Assessment (EA) and preliminary Findings Of No Significant Impact (FONSI) represent the first step in the U.S. Food and Drug Administration (FDA) evaluation of AquaAdvantage Salmon. The FDA's preliminary finding is that an approval of this application, under the specific conditions proposed in the application, would not have a significant impact on the US environment. The National Environmental Policy Act of 1969 (NEPA) requires the FDA and other federal agencies to perform such assessment whenever a major federal action is taken. If the US government approves the commercial application of AquaAdvantage Salmon, there is a possibility that genetically modified (GM) salmon will be imported to be farmed in Japan. To date, Atlantic salmon have been farmed at a single case in Japan, in Aomori Prefecture (1983~1988). As a result, there is little information describing the biology and ecology of Atlantic salmon in Japan. To address this, we documented the maturation of Atlantic salmon in Japan, and evaluated the potential for hybridization and competition with Japan's native salmonids. We demonstrated that seasonal water temperature is very important for Atlantic salmon maturation in Japan. Additionally, we artificially crossed Atlantic salmon and native salmonids. Almost all hybrid embryos stop development at the mesoderm induction stage. However, low numbers of Atlantic salmon and native char (*Salvelinus leucomaenis*) hybrids, confirmed by RFLP, have survived for 2 years. We cultured different lifestages of Atlantic salmon and several native salmonids in the same aquarium and evaluated the occurrence of competition for food and space, and changes in weight. Competition differed depending on the combination of salmonid species and life stage. Among juveniles, all native salmonids attacked by Atlantic salmon. During the adult stage, Amago (*Oncorhynchus masou ishikawae*) and Biwa trout (*Oncorhynchus masou rhodurus*) divided habitat and did not compete with Atlantic salmon. Among all life-stages, Japanese char and masu salmon (*Oncorhynchus masou*) attacked Atlantic salmon, and Atlantic salmon decreased in weight. Our data suggest that Atlantic salmon may not pose a competitive threat to Japan's native salmonids.

**Keywords:** Atlantic salmon (*Salmo salar*), Japan, native salmonids, competition

### **Annotated Bibliography of Key Works**

Du SJ, Gong ZY, Fletcher GL, Shears MA, King MJ, Idler DR, Hew CL. 1992 Growth enhancement in transgenic Atlantic salmon by the use of an "all fish" chimeric growth hormone gene construct. *Biotechnology (N Y)*. 10(2):176-81.

We have developed an "all fish" growth hormone (GH) chimeric gene construct by using an antifreeze protein gene (AFP) promoter from ocean pout linked to a chinook salmon GH cDNA clone. After microinjection into fertilized, nonactivated Atlantic salmon eggs via the micropyle, transgenic Atlantic salmon were generated. The presence of the transgene was detected by polymerase chain reaction (PCR) using specific oligonucleotide primers. A number of these transgenic fish showed dramatic increases in their growth rate. At one year old, the average increase of the transgenic fish was 2 to 6 fold and the largest transgenic fish was 13 times that of the average non-transgenic control.

E. J. Deitch, G. L. Fletcher, L. H. Petersen, I. A. S. F. Costa, M. A. Shears, W. R. Driedzic and A. K. Gamper 2006. Cardiorespiratory modifications, and limitations, in post-smolt growth hormone transgenic Atlantic salmon *Salmo salar*. *Journal of Experimental Biology* 209, 1310-1325

In recent years, there has been a great deal of interest in how growth hormone (GH) transgenesis affects fish physiology. However, the results of these studies are often difficult to interpret because the transgenic and non-transgenic fish had very different environmental/rearing histories. This study used a stable line of size-matched GH Atlantic salmon (*Salmo salar*) that were reared in a shared tank with controls (at 10°C, for ~9 months) to perform a comprehensive examination of the cardiorespiratory physiology of GH transgenic salmon, and serves as a novel test of the theory of symmorphosis. The GH transgenic salmon had a 3.6 faster growth rate, and 21 and 25% higher values for mass-specific routine and standard oxygen consumption (MO<sub>2</sub>), respectively. However, there was no concurrent increase in their maximum MO<sub>2</sub>, which resulted in them having an 18% lower metabolic scope and a 9% reduction in critical swimming speed. This decreased metabolic capacity/performance was surprising given that the transgenics had a 29% larger heart with an 18% greater mass-specific maximum in situ cardiac output, a 14% greater post-stress blood haemoglobin concentration, 5–10% higher red muscle and heart aerobic enzyme (citrate synthase or cytochrome oxidase) activities, and twofold higher resting and 1.7 higher post-stress, catecholamine levels. However, gill surface area was the only cardiorespiratory parameter that was not enhanced, and our data suggest that gill oxygen transfer may have been limiting. Overall, this research: (1) shows that there are significant metabolic costs associated with GH transgenesis in this line of Atlantic salmon; (2) provides the first direct evidence that cardiac function is enhanced by GH transgenesis; (3) shows that a universal upregulation of post-smolt (adult) GH transgenic salmon cardiorespiratory physiology, as suggested by symmorphosis, does not occur; and (4) supports the idea that whereas differences in arterial oxygen transport (i.e. cardiac output and blood oxygen carrying capacity) are important determinants of inter-specific differences in aerobicity, diffusion-limited processes must be enhanced to achieve substantial intra-specific improvements in metabolic and swimming performance.

John P. Volpe, Bradley R. Anholt, and Barry W. Glickman 2001 Competition among juvenile Atlantic salmon (*Salmo salar*) and steelhead (*Oncorhynchus mykiss*): relevance to invasion potential in British Columbia *Can. J. Fish. Aquat. Sci.* **58**: 197–207

Atlantic salmon (*Salmo salar*) are routinely captured in both freshwater and marine environments of coastal British Columbia (Canada). Recent evidence suggests that this species is now naturally reproducing in Vancouver Island Rivers. Our objective was to quantify the performance of each species in intra- and inter-specific competition by assessing the competitive ability of Atlantic salmon sympatric with native niche equivalent steelhead – rainbow trout (*Oncorhynchus mykiss*). Significant behavioural differences, particularly with respect to agonism, were observed between species; however, the status of an individual as resident or challenger was the best predictor of performance. Resident fish always outperformed challengers, regardless of species. Thus, we suggest that Atlantic salmon may be capable of colonizing and persisting in coastal British Columbia river systems that are underutilized by native species, such as the steelhead.

## O-13

### Hybrid Striped Bass National Breeding Program: Research Towards Genetic Improvement of a Non-Model Species

S. Adam Fuller

Harry K. Dupree Stuttgart National Aquaculture Research Center, US Department of Agriculture, Agricultural Research Service, 2955 HWY 130 EAST, STUTTGART, AR, 72160

\*E-mail: [adam.fuller@ars.usda.gov](mailto:adam.fuller@ars.usda.gov)

The hybrid striped bass (HSB) farming industry at present relies almost totally on wild broodstock for annual production of larvae and fingerlings, and industry efforts to domesticate the parent species of the HSB (white bass WB *Morone chrysops* and striped bass SB *M. saxatilis*) have been fairly limited in scope. At the USDA-ARS HKD Stuttgart National Aquaculture Research Center (SNARC), multiple areas of research are being pursued, with the end result being to provide HSB producers with a better performing line of broodfish. Among the areas of research that are currently being pursued at SNARC include: 1) the development of genomic resources for WB and SB; 2) the molecular and physiological consequences of alternative production diets on HSB; 3) the molecular and physiological consequences of exposure to different production environments (hypoxic environments, etc); 4) research to evaluate differential susceptibility of HSB and WB families to columnaris disease; 5) the effects of diet/parental contribution/kisspeptin injection on gonad maturation, lipid composition, and fry and fingerling performance. An overview of these findings will be discussed.

#### Annotated Bibliography of Key Works

Li C, Beck BH, Fuller SA, Peatman E (2014) Transcriptome annotation and SNP discovery in white bass (*Morone chrysops*) and striped bass (*Morone saxatilis*). *Animal Genetics* 45: 885-887.

The authors present the first ever multi-tissue reference transcriptomes for striped bass (*Morone saxatilis*) and white bass (*Morone chrysops*) which are the parental species of the hybrid striped bass, a major U.S. aquaculture species. Being non-model species, this was of critical importance, as prior to this there only existed a medium-density genetic linkage map and a well-annotated ovarian transcriptome. The assembled Moronid reference transcriptomes and identified simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) should advance ongoing studies of reproduction, physiology, and immunology in these species and provide markers for broodstock management and selection.

Childress CJ, Fuller SA, Rawles SD, Beck BH, Gaylord TG, Barrows FT, McEntire ME (2015) Lysine supplementation of commercial fishmeal-free diet in hybrid striped bass *Morone chrysops* x *M. saxatilis* affects expression of growth-related genes. *Aquaculture Nutrition* DOI: 10.1111/anu.12300

The authors present a follow-up study to a study (Rawles et al. 2013) where ideal protein theory accurately predicted first-limiting amino acids and optimum lysine level for a fishmeal-free, commercial-grade diet for hybrid striped bass (HSB). In the current study, authors sought to

determine how dietary lysine supplementation of these same diets influences the expression of two genes, myostatin and myogenin, controlling myogenesis in differentially growing groups of HSB. Real-time rt-PCR results in HSB suggest that the levels of lysine added to the diet has an impact on myogenin relative to the unsupplemented diet, but no effect on myostatin. Moreover, presented data also suggests that the amount of dietary lysine supplementation influenced the ratio of myostatin/myogenin expression in HSB and that this pattern mimicked that of most of the growth, composition of growth and nutrient retention data from the authors' previous study and may therefore be a useful marker for selecting fish for improved growth performance.

Fuller SA, Farmer BD, Beck BH (2014) White bass *Morone chrysops* is less susceptible than its hybrid to experimental infection with *Flavobacterium columnare*. *Diseases of Aquatic Organisms* 109:15-22.

The authors present research regarding *Flavobacterium columnare*, the causative agent of columnaris disease, susceptibility differences between hybrid striped bass (HSB) and white bass (WB) in a series of 3 fundamental studies. In the first experiment, the authors sought to determine whether columnaris disease could be developed using a low-water flow experimental challenge in HSB using 3 levels of *F. columnare* (60-, 30-, 10- ml). Each of these treatment groups exhibited significantly different survival rates: 0, 3.3, and 13.3%, with higher survival occurring in treatment groups exposed to less bacteria. In the second experiment (30ml), both HSB and WB had a 0% survival rate, but the WB took significantly longer to reach 100% mortality. Finally in Expt 3 (10ml), no HSB survived, whereas 33% of WB survived. Compared to controls, the authors observed extensive gill damage in HSB treated with 10 ml after 24 h, which they hypothesized could have contributed to the higher mortality observed in HSB; an observation not seen on the WB gills. From these series of experiments, it is clear that HSB are more sensitive to *F. columnare*, having lower survival and more extensive histological damage compared to WB following the bacterial challenge.

Beck BH, Fuller SA, Peatman E, McEntire ME, Darwish AM, Freeman DW (2012) Chronic exogenous kisspeptin administration accelerates gonadal development in basses of the genus *Morone*. *Comparative Biochemistry and Physiology, Part A: Molecular & Integrative Physiology* 162: 265-273.

The authors present the effects of chronic administration of kisspeptins to immature and mature white bass (WB), striped bass (SB), and hybrid striped bass (HSB) to determine its effects on gonadal development in these species. The authors determined that bi-weekly injections (over 7 weeks) differentially accelerate puberty, as evidenced by increases in the prevalence of spermatozoa in the testes of juvenile fish. Also, in sexually mature fish, kisspeptin treatment led to increased gonad weight, gonadosomatic index, and spermatocrit in some white and striped bass. Additionally, mature white bass treated with kisspeptins showed an advancement in oocyte development as determined by histological examination. Importantly, the gonadal changes occurred in the absence of any photothermal manipulation or hormone injections. This description was the first report of kisspeptin-mediated pubertal initiation in fish, and the first evidence that kisspeptins could modulate gonad maturation.

## O-14

### Production of *Benedenia*-resistant Yellowtail (*Seriola quinqueradiata*) Families— A Preliminary Approach to the Candidates—

Tsutomu Noda\*<sup>1</sup>, Kazunori Yoshida<sup>1</sup>, Takurou Hotta<sup>1</sup>, Hirotaka Mizuochi<sup>1</sup>, Taro Chujo<sup>1</sup>,  
Hideaki Aono<sup>2</sup>, Akiyuki Ozaki<sup>3</sup>, Kazuo Araki<sup>4</sup>, Mitsuru Yoshimoto<sup>5</sup>, Hiroki Ogawa<sup>6</sup>,  
Yasuhiro Shima<sup>7</sup> and Yuichiro Fujinami<sup>1</sup>

<sup>1</sup>Goto Laboratory, Seikai National Fisheries Research Institute, FRA, Nunoura 122-7, Tamanoura, Goto, Nagasaki 853-0508, Japan, <sup>2</sup>Seikai National Fisheries Research Institute, FRA, <sup>3</sup>National Research Institute of Aquaculture, FRA, <sup>4</sup>Tamaki Laboratory, National Research Institute of Aquaculture, FRA, <sup>5</sup>Aquafarm, Maruha Nichiro Corporation, <sup>6</sup>Shimaura Aquafarm Branch, Maruha Nichiro Corporation, <sup>7</sup>Minami-Izu Laboratory, National Research Institute of Aquaculture, FRA

\*E-mail: [ttmnoda@affrc.go.jp](mailto:ttmnoda@affrc.go.jp)

The skin fluke *Benedenia seriolae* is a parasite specific to *Seriola* species. Its feeding on the epidermal tissues of yellowtail (*Seriola quinqueradiata*) causes external injuries that render the fish susceptible to bacterial and viral infection. Infection with this parasite is a serious problem for yellowtail aquaculture because it also inhibits fish growth. A previous study has reported the existence of an inherited *Benedenia* disease-resistance factor in yellowtail. Yellowtail families resistant to *Benedenia* disease have not yet been produced, although the production of such families for aquaculture would help to reduce not only rates of infectious disease but also the costs and labor required to eradicate *Benedenia* on the fish's body surface. Here, we examined the relationship between susceptibility and genetic variation that was described in the previous study in order to select candidate broodstock for breeding *Benedenia*-resistant yellowtail. In September 2014, we selected 961 fingerlings among 10,000 wild-caught 0-age yellowtail at an aquaculture farm on the basis of their parasite burdens (3 or fewer *Benedenia* on each selected fish: first selection treatment); the number of parasites on each of the 10,000 fish ranged from 1 to 48. These 961 selected fish were then cultured in a net cage and the number of parasites on each was counted five times between November 2014 and July 2015. The average number of parasites per fish had a wide range from 0.2 to 39.4 over this period and the overall mean was 8.9. The 160 fish with the lowest parasite burdens were selected as broodstock candidates (second selection treatment). We are now using these broodstock candidates to produce *Benedenia*-resistant F1 yellowtail families by using DNA marker-assisted-selection breeding methods.

**Keywords:** Yellowtail, *Seriola quinqueradiata*, *Benedenia* disease, Breeding

#### Annotated Bibliography of Key Works

Akiyuki Ozaki, Kazunori Yoshida, Kanako Fuji, Satoshi Kubota, Wataru Kai, Jun-ya Aoki, Yumi Kawabata, Junpei Suzuki, Kazuki Akita, Takashi Koyama, Masahiro Nakagawa, Takurou Hotta, Tatsuo Tsuzaki, Nobuaki Okamoto, Kazuo Araki, Takashi Sakamoto. 2013. Quantitative Trait Loci (QTL) Associated with Resistance to a Monogenean Parasite (*Benedenia seriolae*) in Yellowtail (*Seriola quinqueradiata*) through Genome Wide Analysis. PLoS ONE 8(6): e64987.

[doi:10.1371/journal.pone.0064987](https://doi.org/10.1371/journal.pone.0064987)

Benedenia infections caused by the monogenean fluke ectoparasite *Benedenia seriolae* seriously impact marine finfish aquaculture. Genetic variation in host has been inferred to play a significant role in determining the susceptibility to this parasitic disease. To evaluate the genetic basis of *Benedenia* disease resistance in yellowtail (*Seriola quinqueradiata*), a genome-wide and chromosome-wide linkage analyses were initiated using F1 yellowtail families (n = 90 per family) based on a high density linkage map with 860 microsatellite and 142 single nucleotide polymorphism (SNP) markers. Two major quantitative trait loci (QTL) regions on linkage groups Squ2 (BDR-1) and Squ20 (BDR-2) were identified. These QTL regions explained 32.9–35.5% of the phenotypic variance. On the other hand, the relationship between QTL for susceptibility to *B. seriolae* and QTL for fish body size were investigated. The QTL related to growth was found on another linkage group (Squ7). As a result, the authors present first genetic evidence that contributes to detailing phenotypic resistance to *Benedenia* disease, and the results will help resolve the mechanism of resistance to this important parasitic infection of yellowtail.

Kazunori Yoshida, Akiyuki Ozaki, Masahiro Nakagawa, Takuro Hotta, Jun-ya Aoki, Takashi Koyama, Kazuo Araki, Nobuaki Okamoto, Takashi Sakamoto and Tatsuo Tsuzaki. 2011. Characteristic Evaluation Method Relating to *Benedenia* Disease of Yellowtail (*Seriola quinqueradiata*). Proceedings of the forty U.S.-Japan aquaculture panel symposium (eds M. Rust, P. Olin, A. Bagwill and M. Fujitani): Pp96-99.

*Benedenia* is a parasitic disease caused in *Seriola* species by *Benedenia seriolae*. This parasite can cause growth reduction and external injuries in yellowtail, increasing the risk of secondary viral or bacterial infection. The main method of parasite removal is to soak the fish in a freshwater bath. However, this method requires a great deal of time, cost, and effort. We have been studying DNA Marker-Assisted Selection (MAS) breeding, to select for resistance to *Benedenia* disease. Three components ("Reproduction technology", "Character evaluation", and "DNA analysis") are critically important to promote MAS breeding success. We focus on one of the key components, "Characteristic evaluation method" relating to *Benedenia* disease in yellowtail.

Mushiake, K., H. Yamazaki, and H. Fujimoto. 2005. Current Situation of Technical Developments in Seed Production of Yellowtail (*Seriola quinqueradiata*) in Japan. Proceedings of the thirty-fourth U.S.-Japan aquaculture panel symposium (eds R. Stickney, R. Iwamoto, and M. Rust): Pp1-4.

The National Center for Stock Enhancement (NCSE, formerly Japan sea-farming Association), of the FRA, introduced the stock enhancement program for yellowtail (*Seriola quinqueradiata* and *Seriola lalandi*) in 1977. Technical developments in induced spawning as well as larval and juvenile rearing techniques have increased the population of this species to 1 million juveniles per year at NCSE. This project faced three major drawbacks: high mortality of larvae, cannibalism, and the smaller size of released juveniles in comparison with their wild counterparts. The high mortality of larvae was overcome by utilizing strong aeration during the early larval stage, while cannibalism was controlled by grading juveniles by size selection. The two-month delay in the spawning season of reared broodstock (the usual spawning season is late April to early May), which caused the smaller size of released juveniles, was solved by developments in advanced spawning techniques. Photoperiod and water temperature manipulations were used to produce eggs in February, thus producing yellowtail juveniles that can be released into the wild at a size similar to that of the wild stock.



## O-15

### **Coho Salmon Broodstock Development: A Case Study of the Domsea Coho (1977 to 2015)**

Robert N. Iwamoto<sup>1</sup>, Richard Towner<sup>2</sup>, James Myers<sup>3</sup>, and Greg Hudson<sup>1</sup>

<sup>1</sup> Spring Salmon LP, Rochester, WA, <sup>2</sup> Genentech Consultants, Payette, ID., <sup>3</sup>Northwest Fisheries Science Center, Seattle, WA 98112

\*E-mail:

There are few long-term studies on Pacific salmon that inform on the potential for gains by systematic selective breeding and the potential for inbreeding losses because of constrained population sizes and matings of closely related individuals. In 1977, Domsea Farms Inc., the University of Washington, and the Washington Sea Grant Program initiated a genetic selection program for coho salmon for Domsea's marine net-pen operations. Because little was known at that time regarding the genetic potential for genetic improvement in coho salmon, the program was initially designed with two central goals: 1) collect basic information (heritabilities, genetic and phenotypic correlations) on the potential for genetic improvement for such economically important traits such as smoltification, growth rate to harvest, flesh color, and reproductive fitness; and 2) using that information, develop selection and mating protocols that would maximize selection gains but minimize inbreeding. Despite significant changes in ownership, rearing environment and operations, the program has remained remarkably consistent over the past 38 years or 19 generations of selection. We have demonstrated that selection for improved growth to the smolt stage (7.1-13.1% per generation) and adult phases (43-53 g per generation) can be achieved. Overall, the growth rate of the Domsea coho salmon has improved between 3% and 8% per generation while reproductive traits such as female weight, egg weight, and survival to ponding have remained unaffected by inbreeding. The program has also demonstrated the importance of considering the potential for genotype-environment interactions when designing selection programs for specific rearing applications. While traditional genetic approaches have been demonstrably successful for this program, it is anticipated that further consideration and application of molecular approaches will help further characterize and advance this broodstock program for coho salmon.

#### **Annotated Bibliography of Key Works**

1. a. Iwamoto, R.N., Saxton, A.M., Hershberger, W.K. 1982. Genetic estimates for length and weight of coho salmon (*Oncorhynchus kisutch*) during freshwater rearing. J. Hered. 73: 187-191.

b. Saxton, A.M., Hershberger, W.K., Iwamoto, R.N. 1984. Smoltification in the net-pen culture of coho salmon: quantitative genetic analysis. Trans. Am. Fish. Soc. 113: 339-347.

c. Hershberger, W.K., Myers, J.M., Iwamoto, R.N., McAuley, W.C., Saxton, A.M. 1990. Genetic changes in the growth of coho salmon (*Oncorhynchus kisutch*) in marine net-pens, produced by ten years of selection. Aquaculture 85: 187-197.

d. Iwamoto, R.N., Myers, J.M., and Hershberger, W.K. 1990. Heritability and genetic correlations for flesh coloration in pen-reared coho salmon. Aquaculture 86: 181-190.

The papers cited above provide much of the early quantitative genetic parameters for coho salmon traits. Genetic estimates for freshwater growth, smoltification and initial saltwater survival, seawater growth, and flesh coloration for the Domsea coho salmon were derived from full- and half-sib analyses. In almost every case, the magnitude of the derived heritabilities and genetic correlations indicated that a systematic selection program would be successful.

2. Myers, J.M., Iwamoto, R.N., Teel, D., Van Doornink, D., and Hershberger, W.K. 1999. Coho salmon broodstock development---1977-1998. Ten generations of systematic selective breeding. Bull. Natl. Res. Inst. Aquacult Suppl. 1, 63-70.

The authors summarize the concept of the Domsea coho salmon broodstock program from the selection scheme to the circular mating design. They also present allozyme comparisons of the founder strain and the broodstock after 10 generations of selection and indicate that significant genetic changes had occurred in the broodstock as a result of genetic selection and drift.

3. Myers, J.M., Heggelund, P.O., Hudson, G. and Iwamoto, R.N. 2001. Genetics and broodstock management of coho salmon. In: Lee, Cheng-Sheng and Donaldson, E.M. (Eds.) Reproductive biotechnology in finfish aquaculture, Aquaculture 197:43-62.

This paper summarized the information collected on the Domsea coho salmon broodstock program after 13 generations of implementation. It discusses the results of sib trials in marine net pens and two freshwater environments and consequently the importance of considering genotype-environment interactions in broodstock programs for specific applications. It tracked theoretical (pedigree data) and genetic changes (allozyme data and individual traits) in the broodstock population.

## O-16

### **Development of Improved Catfish Germplasm at the Warmwater Aquaculture Research Unit, USDA-ARS.**

Brian Bosworth\* and Geoff Waldbieser

Warmwater Aquaculture Research Unit, USDA-ARS, P.O. Box 38, Stonville, MS 38776.

\*E-mail: [brian.bosworth@ars.usda.gov](mailto:brian.bosworth@ars.usda.gov)

Production of farm-raised catfish is the largest aquaculture enterprise in the U.S. in terms of both volume and value. The Warmwater Aquaculture Research Unit (WARU), USDA-ARS mission is to improve production efficiency of catfish and other warmwater aquatic organisms, and includes development and release of catfish germplasm improved for economically important traits. Traditionally, the channel catfish comprised nearly all U.S. farm-raised catfish production, but in the last 10 years production of the F1 hybrid between the channel and blue catfish has increased to about 50% of total production. Therefore, the WARU breeding program is focused on genetic improvement of purebred channel catfish and F1 hybrid catfish performance. The channel catfish improvement program has used a traditional selection on BLUP breeding value estimates to improve growth rate and carcass yield. We started with a diverse population of channel catfish derived from 10 commercial farms in 2006, and have evaluated over 15,000 animals from 630 full-sib families produced by 334 sires and 516 dams between 2010 and 2013. Heritabilities for growth and carcass yield are 0.33 and 0.36, respectively; and fish are selected based on an index that puts equal weight of individual breeding values for growth and family average breeding values for carcass yield. Improvements in growth have been 8-10% per generation and are in agreement with expected improvements. Increases in carcass yield, although positive, have not been as dramatic, due at least in part to the use of less accurate family average breeding values instead of individual breeding values. The hybrid improvement program has focused on evaluation, identification and selection of blue catfish sires that produce superior performing hybrid progeny. Past work on genetic improvement of blue catfish is very limited; therefore, we initiated a program to collect several blue catfish populations and evaluate performance of their purebred and hybrid progeny. Initial evaluations suggest the majority of genetic variance for growth and carcass yield in hybrid progeny is additive in nature, and that populations and individual sires that produce purebred progeny with superior growth and carcass yield also produce hybrid progeny with superior performance. We have ongoing experiments to estimate the genetic correlation between traits in the purebred and hybrid state, which will give us direction in developing purebred blue catfish which produce superior hybrid progeny. We currently use DNA markers to identify parentage in our pedigreed populations and are developing a SNP chip to be used for genomic selection. Use of genomic breeding values should improve our breeding value accuracy and rate of improvement, particularly for carcass yield. We are evaluating other traits for inclusion in our selection index, and we are collecting and cryopreserving sperm from superior channel and blue catfish sires for future use. This combination of traditional animal breeding, genomic selection and cryopreservation will result in improved catfish germplasm, improved production efficiency and greater profitability for catfish farmers.

### **Annotated Bibliography of Key Works**

Lush, J.L. 1947. Family Merit and Individual Merit as Bases for Selection. Part I. The American Naturalist 81:241-261 and 81:362-379.

No abstract available. These 2 papers detail Lush's development of the use of information on individual performance and performance of relatives (family merit) to improve accuracy of breeding value estimates and form the basis for development of selection index theory and development of BLUP based breeding programs.

Henderson, CR. 1975. Use of all relatives in intraherd prediction of breeding values and producing abilities. Journal of Dairy Science 58:1910-1916.

Commonly used cow evaluation methods apply principles of the selection index to herdmate deviations on the cow and close relatives. In contrast, best linear unbiased prediction adjusts records by best linear unbiased estimates of all fixed effects in the model and simultaneously weights those adjusted records by selection index principles. It would be advantageous to utilize all known relationships among animals in the herd in the latter method, but computations have been too laborious, requiring the inverse of the numerator relationship matrix. By a method of writing this inverse rapidly without computing the relationship matrix itself all relationships can now be used in intraherd cow evaluation. Further, tests of progeny by artificial insemination on sires used in the herd can be incorporated.

Wei, M., van der Werf, J.H.J., 1994. Maximizing genetic response in crossbreds using purebred and crossbred information. Animal Production 59:401-413.

A combined crossbred and purebred selection (CCPS) method, i.e. using crossbred and purebred information, was proposed to achieve genetic response in crossbred animals. Selection index theory was applied to establish a CCPS index. The CCPS was compared with pure-line selection (PLS) and crossbred selection (CS) methods. The genetic correlation between purebred and crossbred performance ( $r_{pc}$ ) and crossbred heritability ( $h_c^2$ ) are crucial factors in the comparison. The CCPS is always better than PLS or CS when a fixed number of purebred progeny is tested. With a fixed total number of purebred and crossbred tested progeny, CCPS is only worse than PLS for very high values of  $r_{pc}$  ( $>0.8$ ). Superiority of CCPS over PLS increases and over CS decreases with decreasing  $r_{pc}$ . The larger  $h_c^2$  is, relative to purebred heritability ( $h_p^2$ ) the more response CS and CCPS will achieve. The robustness of CCPS against inappropriate assumptions on  $r_{pc}$  and  $h_c^2$  values was investigated. The expected response is always an overestimate, and the actual response is smaller than the optimal response when  $r_{pc}$  is assumed one but the true  $r_{pc}$  is smaller. The difference between actual and optimal response increases as  $r_{pc}$  decreases but it is small for large  $r_{pc}$  values (e.g.  $<3\%$  for  $r_{pc} >0.7$ ). The expected response is smaller than the actual response when  $r_{pc}$  is large and  $h_c^2 > h_p^2$ . Finally, the actual response to CCPS is larger than the optimal response to PLS for positive values for  $r_{pc}$ . The main conclusions are: (1) CCPS method is optimal for obtaining genetic response in crossbreds; and (2) CCPS with inappropriate assumptions on  $r_{pc}$  and  $h_c^2$  values (e.g. recognizing crossbreds as purebreds) achieves more genetic response than PLS for common values of  $r_{pc}$  and crossbred heritability.

Meuwissen, T.H.E., Hayes, B.J., Goddard, M.E. 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics 157:1819-1829.

Recent advances in molecular genetic techniques will make dense marker maps available and genotyping many individuals for these markers feasible. Here we attempted to estimate the effects of z50,000 marker haplotypes simultaneously from a limited number of phenotypic records. A genome of 1000 cM was simulated with a marker spacing of 1 cM. The markers surrounding every 1-cM region were combined into marker haplotypes. Due to finite population size ( $N_e$  5 100), the marker haplotypes were in linkage disequilibrium with the QTL located between the markers. Using least squares, all haplotype effects could not be estimated simultaneously. When only the biggest effects were included, they were overestimated and the accuracy of predicting genetic values of the offspring of the recorded animals was only 0.32. Best linear unbiased prediction of haplotype effects assumed equal variances associated to each 1-cM chromosomal segment, which yielded an accuracy of 0.73, although this assumption was far from true. Bayesian methods that assumed a prior distribution of the variance associated with each chromosome segment increased this accuracy to 0.85, even when the prior was not correct. It was concluded that selection on genetic values predicted from markers could substantially increase the rate of genetic gain in animals and plants, especially if combined with reproductive techniques to shorten the generation interval.

Legarra, A. O.F. Christensen, O.F., Aguilar, I. Misztal, I. 2014. Single Step, a general approach for genomic selection. *Livestock Production Science* 166:54–65.

Genomic evaluation methods assume that the reference population is genotyped and phenotyped. This is most often false and the generation of pseudo-phenotypes is uncertain and inaccurate. However, markers obey transmission rules and therefore the covariances of marker genotypes across individuals can be modelled using pedigree relationships. Based on this, an extension of the genomic relationship matrix can be constructed in which genomic relationships are propagated to all individuals, resulting in a combined relationship matrix, which can be used in a BLUP procedure called the Single Step Genomic BLUP. This procedure provides so far the most comprehensive option for genomic evaluation. Several extensions, options and details are described: compatibility of genomic and pedigree relationships, Bayesian regressions, multiple trait models, computational aspects, etc. Many details scattered through a series of papers are put together into this paper.

## Titles of Poster Presentation

1. Whole genome re-sequencing of fugu populations (Sho Hosoya, Fisheries Laboratory, University of Tokyo)
2. Incipient transition of a sex-determining gene among closely related species of fugu.(Kiyoshi Kikuchi, Fisheries Laboratory, University of Tokyo)
3. Estimation of breeding value in model fish, guppy (*Poecilia reticulata*) and its application for selective breeding in aquaculture. (Masamichi Nakajima, Tohoku University)
4. The life table demography and population growth of the rotifer *Brachionus angularis* Gosse, from Kenya; the influence of temperature and food density. (Erick Ochieng Ogello, Nagasaki University)
5. Effect of dissolved organic matter on electrochemical removal of ammonia in recirculating aquaculture systems. (Satoshi Tada, Nagasaki University)
6. Effect of tetrodotoxin-containing diet on feeding, digestion and growth of tiger puffer, *Takifugu rubripes* juveniles. (Kogen Okita, Nagasaki University)
7. Temperature tolerance in two clonal strains of mangrove killifish *Kryptolebias marmoratus*. (Marina Yamada, Nagasaki University)
8. Effects of protozoa *Euplotes* sp. coexistence on the population growth of minute monogonont rotifer *Proales similis*. (Naoshi Wakimura, Nagasaki University)
9. Distribution of larval and juvenile greater amberjack (*Seriola dumerili*) around the Penghu islands, Taiwan. (Takamasa Hasegawa, Nagasaki University)
10. Effect of starvation on mixis induction in offspring and its genetic mechanism of the monogonont rotifer *Brachionus manjavacas*. (Shohei Kamizono, Nagasaki University)
11. Body size, Culture and fish larval ingestion on a minute rotifer, *Colurella* cf. *adriatica*. (Stenly Wullur, Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado – Indonesia)
12. Production of myostatin gene-knockout Japanese anchovies (*Eugraulis Japonicus*) using TALEN-based genome editing. (Keishi Sakaguchi, Fisheries Research Institute of Karatsu, Department of Joint Research, Faculty of Agriculture, Kyushu University)
13. Isolation and Screening of Novel Probiotic Lactic Acid Bacteria for Aquaculture. (Nguyen Thi Hue Linh, University of Miyazaki)
14. Effect of Protease Addition to EP diet on the Growth of amberjack, *Seriola dumerili*. (Yousuke Taoka, University of Miyazaki)
15. Amino Acid Profile of Thraustochytrids Cells and Potential of Application to Aquafeed. (Kenya Horii, University of Miyazaki)
16. Diurnal changes in frequency of the burst swimming behavior of adult Pacific bluefin tuna (*Thunnus orientalis*) in a land-based tank. (Akiko Tsujita, Seikai National Fisheries Research Institute, FRA)
17. Spawning frequency of Pacific bluefin tuna *Thunnus orientalis* in a land-based tank. (Ayako Suzuki, Seikai National Fisheries Research Institute, FRA)
18. Effect of timing of restricted feeding on sexual maturation in the yellowtail, *Seriola quinqueradiata*. (Kentarō Higuchi, Seikai National Fisheries Research Institute, FRA)
19. Nitrogen excretion in Pacific bluefin tuna. (Toshinori Takashi, Seikai National Fisheries Research Institute, FRA)
20. A high density genetic linkage map for yellowtail (*Seriola quinqueradiata*) containing 6,275 EST-based SNPs. (Akiyuki Ozaki, National Research Institute of Aquaculture, FRA)

## Whole genome re-sequencing of fugu populations

Sho Hosoya\*, Satoshi Tasumi, Kiyoshi Kikuchi

Fisheries Laboratory, University of Tokyo, Hamamatsu, Bentenjim 2971-4 Japan

\*E-mail: [ahosoya.at.mail.ecc.u-tokyo.ac.jp](mailto:ahosoya.at.mail.ecc.u-tokyo.ac.jp)

Historically, the prevalence of intraspecific diversity, both neutral and adaptive, in many marine species has been unclear. However, the recent advent of new technologies, in particular, high-throughput DNA sequencing is making it feasible to evaluate genetic variability at the whole-genome level. Fugu (tiger pufferfish *Takifugu rubripes*) is one of the important cultured marine fish species in East Asia. The yield of cultured fugu accounted for approximately 5 % of total marine fish aquaculture in Japan, and ranked 3<sup>rd</sup>-4<sup>th</sup> by value. In addition, this species is becoming an important target for stock enhancement because of severe declines in the wild populations. In recent years, approximately two million hatchery-born seedlings have been released each year. Tagging and releasing experiments have suggested that fugu shows homing behavior to the natal site for spawning following long-range migration, and by extension, that distinct local populations may exist. As expected, recent study using 21 microsatellite loci revealed a shallow divergence between two wild populations from Japan Sea and Pacific Ocean. Nonetheless, the detailed degrees of population and adaptive structuring remain unclear. Moreover there is virtually no information regarding the extent and magnitude of linkage disequilibrium (LD) that play essential roles in choosing marker loci for the management of brood stocks and conservation of wild populations. In this study, we resequenced wild individuals of fugu from Wakasa Bay (Japan Sea) and Mikawa Bay (Pacific Ocean) to compare the genetic diversity within and between populations. Two libraries each containing ten individuals from either of the populations were constructed for paired-end sequencing (2 x 101bp) on the Illumina HiSeq2000. We obtained 43.2M reads per sample yielding coverage of 11.4 per genome, on average. We mapped these reads on the fugu reference genome (Fugu v.5) and called single-nucleotide polymorphisms (SNPs) using BWA, Samtools and GATK software. The number of SNPs detected per individual was about 700 thousand and the SNP frequency was about 480bp per SNP. Missing SNPs because of the shallower depth were estimated as 2 to 2.5 per cent of the total SNPs for each sample. Multidimensional scaling plot clearly separated the two populations, and individuals from Ise Bay were genetically closer than those from Wakasa Bay. However, the global  $F_{st}$  (= 0.0057) was small and no outlier locus was detected by BayeScan software. These results suggest that the genetic divergence between the two populations is shallow. Linkage disequilibrium analysis was done using PLINK software. The two populations were similar in the LD state. We detected putative 3,000 LD blocks from each population but 90 per cent of them were smaller than 1kb. The mean  $r^2$  value was 0.48 between two SNPs at 100bp distance whilst that was less than 0.20 at 1kb distance. This rapid LD decay indicates these populations have maintained at relatively healthy states until recently. The expected SNP size for the implementation of genomic selection program for fugu breeding was 300k.

**Key words:** fugu, *Takifugu rubripes*, conservation, genetic diversity, single nucleotide polymorphism,  $F_{st}$ , linkage disequilibrium, genomic selection

### **Annotated Bibliography of Key Works**

Katamachi D., Ikeda M., Sato T., Suzuki S., Kikuchi K and Ojima D. 2014. Development of a multiplex PCR assay for population genetic analysis of the tiger puffer *Takifugu rubripes* using 16 microsatellite DNA loci. *Aquaculture Sci.*, 62, 55–63

The tiger puffer *Takifugu rubripes* is a marine fish species economically important to East Asia, particularly Japan. To evaluate the genetic variability and population structure of the tiger puffer in detail, we generated a multiplex PCR assay of microsatellite DNA loci, a fast and cost-effective technique that allows high-throughput genotyping. In this study, we report the development of four multiplex PCR assays for this species using 16 microsatellite DNA loci located on independent chromosomes. We ensured quality control throughout all steps of the multiplex PCR assay development, i.e., exclusion of loci detected with stuttering, allele dropouts, or null alleles. We evaluated this set of microsatellite DNA loci for polymorphisms using 113 fishes collected from three different locations in the sea around Japan. This combination of loci will prove useful for future investigations of the fine-scale population genetic structure of this species.



P-2

## **Incipient transition of a sex-determining gene among closely related species of fugu**

Risa Ieda<sup>1</sup>, Sho Hosoya<sup>1</sup>, Satoshi Tasumi<sup>1</sup>, Shigenori Suzuki<sup>2</sup>, Kiyoshi Kikuchi<sup>1\*</sup>

<sup>1</sup>Fisheries Laboratory, University of Tokyo, Hamamatsu, Bentenjim 2971-4 Japan

<sup>2</sup>Minami-Izu Laboratory, National Research Institute of Aquaculture, FRA, Shizuoka, Minami-izu 415-0156 Japan

\*E-mail: [akikuchi.at.mail.ecc.u-tokyo.ac.jp](mailto:akikuchi.at.mail.ecc.u-tokyo.ac.jp)

Sex determination in teleosts fish is often genetic, where segregation of sex-determining loci assign the phenotypic sex. In these species, sex-linked polymorphic markers can be used for sex identification, potentially providing important information regarding the management of cultured species and conservation of wild species. However, the different master sex-determining (SD) genes in different fish lineages appear to have evolved independently and have been frequently replaced by new genes. Therefore, the sex-linked markers are often specific to a few stock population of the focal species, greatly limiting use of such markers for the management of aquaculture species. To gain more insights into the transition between SD systems in teleosts, we study closely-related species of fugu belonging to genus *Takifugu*. This 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 novel 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.

## **Estimation of breeding value in model fish, guppy (*Poecilia reticulata*) and its application for selective breeding in aquaculture**

Masamichi Nakajima<sup>1\*</sup>, Toyoko Nakajima<sup>1</sup>, Syunsuke Obinata<sup>1</sup>

<sup>1</sup> Laboratory of Marine Life Science and Genetics, Graduate School of Agricultural Science, Tohoku University, Sendai, Miyagi 981-8555, Japan

\*E-mail: [mnkjm@bios.tohoku.ac.jp](mailto:mnkjm@bios.tohoku.ac.jp)

Selective breeding is one of the most important methods for the genetic improvement in not only livestock animals, but also aquatic organisms. Many varieties and strains were produced in aquatic organisms; however the most succeeded example of selective breeding in aquatic organisms is ornamental fish. In the case of quantitative traits, the example of succeeded selective breeding is scarce. In the case of the selective breeding based on phenotypic value, many cases showed inconsistent result. This is due to its inability to remove environmental and dominance effect. Therefore the method to evaluate the accurate abilities of parent is necessary. The breeding value was contrived from such circumstances. Though the breeding value possesses such importance, the applications of the breeding value in the aquaculture are not so much. The reasons why the application in aquaculture is little are 1) the large number of offspring can obtain from small number of parental fish; 2) short life cycle of target species caused difficulties to estimate breeding value in parental fish and their offspring. Although, it is hard to obtain the breeding value, the role of breeding value is very important for the efficient selection. From the above mentioned reason, it is necessary to identify the breeding value for the effective selective breeding in aquaculture. In this study, the breeding values were estimated from selection experiment for body size in the guppy, *Poecilia reticulata*, and examined the efficiency of the selection between used phenotype and breeding value. Comparison of breeding value in parental and offspring indicated significantly positive correlation. Positive correlation also observed between breeding values of parental and body size of their offspring. Selection based on breeding value showed 5% larger in body size compared with selection using phenotype. Selection based on breeding value showed lesser increment in coefficient of inbreeding compared to the selection based on phenotype. These results suggest that the breeding values are effective to the evaluating parental fish and useful for selective breeding. It is expected that the application of breeding value in the industrially important fish, such as Bluefin tuna and Japanese eel.

**Keywords:** Guppy (*Poecilia reticulata*) ; Selective breeding ; Body size ; Breeding value

## **The life table demography and population growth of the rotifer *Brachionus angularis* Gosse, from Kenya; the influence of temperature and food density**

Erick Ochieng Ogello<sup>\*1,2</sup>, Hee-Jin Kim<sup>1</sup>, Koushirou Suga<sup>1</sup> and Atsushi Hagiwara<sup>1</sup>

<sup>1</sup>Graduate School of Fisheries & Environmental Sciences, Nagasaki University, 1-14 Bunkyo, Nagasaki 852-8521, Japan, <sup>2</sup>Kenya Marine & Fisheries Research Institute (KMFRI), Kegati Aquaculture Research Station, P.O. Box 3259 - 4200 Kisii, Kenya

\*E-mail: [bb53414801.at.cc.nagasaki-u.ac.jp](mailto:bb53414801.at.cc.nagasaki-u.ac.jp)

The nature of reproduction of the Kenyan strain of *Brachionus angularis* was investigated using individual and small batch culture approaches. First, the Kenyan rotifer was identified using morphological and molecular techniques. The life-table demography and the population growth studies were conducted at three temperatures (i.e. 20, 25 and 30°C) using *Chlorella vulgaris* at three densities (i.e.  $2.5 \times 10^5$ ,  $2.5 \times 10^6$  and  $2.5 \times 10^7$  cells mL<sup>-1</sup>). The lorica length ( $85.6 \pm 3.1$  µm) and width ( $75.4 \pm 3.6$  µm) of the Kenyan sample were smaller than those of similar species cited in the literature. The phylogenetic tree grouped the Kenyan sample together with *Brachionus caudatus* and *Brachionus angularis*. However, with additional morphological data e.g. presence of two median occipital spines with either reduced or lacking sub-median spines, *Brachionus angularis* was identified as the most likely match for the Kenyan sample. The rotifers were most fecund ( $2.11 \pm 0.07$  offspring female<sup>-1</sup> day<sup>-1</sup>) and reproductive ( $8.43 \pm 0.24$  offspring female<sup>-1</sup>) at 25°C with  $2.5 \times 10^6$  cells mL<sup>-1</sup> of *C. vulgaris*. The highest intrinsic rate of natural increase ( $0.74 \pm 0.02$  d<sup>-1</sup>), specific population growth rate ( $0.49 \pm 0.01$ ), longest life expectancy at hatching ( $12.41 \pm 0.28$  d) and shortest generation time ( $2.87 \pm 0.03$  d) were also observed at 25°C with  $2.5 \times 10^6$  cells mL<sup>-1</sup> of *C. vulgaris*. However, the duration of hatching to first egg spawning was shortest ( $2.86 \pm 0.21$  h) at 30°C with  $2.5 \times 10^7$  and longest ( $8.83 \pm 0.39$  h) at 20°C with  $2.5 \times 10^5$  cells mL<sup>-1</sup> of *C. vulgaris*. In the batch cultures, the highest population density ( $255.7 \pm 12.6$  ind mL<sup>-1</sup>) and lowest ( $122.0 \pm 3.6$  ind mL<sup>-1</sup>) were realized at 25°C with  $2.5 \times 10^6$  and at 20°C with  $2.5 \times 10^5$  cells mL<sup>-1</sup> of *C. vulgaris* on day 8 respectively. There was earlier population density peaks at higher food densities ( $2.5 \times 10^7$  cells mL<sup>-1</sup> of *C. vulgaris*) regardless of temperature. In conclusion, the Kenyan strain of *B. angularis* seems to have favorable morphological and reproductive features making them suitable for aquaculture activities. The life table demography of this strain is optimal at 25°C with  $2.5 \times 10^6$  cells mL<sup>-1</sup> of *C. vulgaris*. The results of this study are relevant for improvement of the freshwater aquaculture activities. Further studies on the population growth of the rotifer are recommended using other different food types.

**Key words:** Rotifera, alga, life table parameters, fecundity, *Brachionus angularis*.

## Effect of dissolved organic matter on electrochemical removal of ammonia in recirculating aquaculture systems

Satoshi Tada\* and Shigenobu Takeda

Graduate School of Fisheries and Environmental Sciences, Nagasaki University

\*E-mail: [tdsts.goopy.at@gmail.com](mailto:tdsts.goopy.at@gmail.com)

Improvement of water treatment systems in closed recirculating aquaculture systems (RAS) is necessary for reducing total volume of culture water, which determine the size of the whole system as well as running costs. New RAS using electrochemical oxidation for removal of ammonia in culture seawater are being developed by Nagasaki Prefectural Institute of Fisheries. In this system, hypochlorous acid, which produced by electrolysis of seawater, oxidizes the bromide ions, yielding hypobromous acid. Both hypochlorous and hypobromous acids react with ammonium ion and oxidize it to nitrogen gas. Since these free chlorine and free bromine also react with dissolved organic matter (DOM), efficiency of electrochemical ammonia removal could be affected by quantity and reactivity of DOM in culture seawater. In this study, removal of total ammonia nitrogen (TAN) and DOM by additions of hypochlorous acid was investigated using culture seawater of two RAS for tiger puffer *Takifugu rubripes* (RAS-1) and kelp grouper *Epinephelus bruneus* (RAS-2). The RAS-1 comprised of a culture tank (20 m<sup>3</sup>), a solid settler, a circulation pump, a foam fractionator, an electrolysis unit, a reaction tank (for ammonia removal), and activated charcoal tank (for removal of residual chlorine). The RAS-2 has a biofiltration tank in addition to the units provided in the RAS-1. The culture seawater was filtered through Whatman GF/F filter and dispensed into seventeen replicate 250-ml amber glass bottles. Additions of chlorine (Sodium hypochlorite solution) were conducted to achieve 17 steps chlorine doses of 0~16 (or 70) mg/L as Cl<sub>2</sub>. After 20 min contact period at 25°C, free available residual chlorine and combined available residual chlorine in the sample were analyzed by the DPD method. TAN was measured using an autoanalyzer. DOM was determined as humic-like and protein-like fluorophores based on the three-dimensional excitation emission matrix spectroscopy. Concentration of TAN in the culture seawater consistently decreased along with the increase in chlorine dose and then disappeared (what is called breakpoint). In the bottles with higher levels of chlorine dose, free available residual chlorine was detected according to the excess amount of chlorine dose. Concentrations of combined available residual chlorine were very low but small increase was observed around the breakpoint. Humic-like and protein-like fluorophores showed large decrease by the addition of 1 mg/L Cl<sub>2</sub> (the lowest chlorine dose) and then kept relatively constant concentrations until the breakpoint. In the bottled added with excess amount of chlorine (higher than the breakpoint), further decreases in humic-like and protein-like fluorophores were observed. These results suggest that DOM consists of highly reactive fraction and semi-labile fraction, and the former could reduce efficiency of electrochemical ammonia removal in the culture seawater. Compare to the chlorine demand to achieve the breakpoint in DOM-free artificial seawater, the culture seawater from RAS-2 required 17~42% more chlorine to oxidize TAN, but the difference between the artificial seawater and RAS-1 culture seawater was not clear. Skin mucus of kelp grouper seems to be one of the sources of highly reactive DOM in RAS-2.

**Key words:** Recirculating aquaculture system, electrochemical oxidation, ammonia, dissolved organic matter, seawater

### **Annotated Bibliography of Key Works**

Ohwaki H., S. Yamamoto, A. Okamoto and Y. Kurokawa. 2011. Development of a large-scale closed recirculating aquaculture system for saltwater fish. Report of Industrial Technology Center of Nagasaki, 40, 52-55 (in Japanese)

For the construction of a large-scale closed recirculating system for aquaculture of saltwater fish on land, we have been developing a new seawater treatment unit using hypochlorous acid produced by electrolysis of seawater with platinum modified titanium electrode, and a closed recirculating aquaculture system with the electrochemical water treatment. Acidic water, which is a byproduct of electrolysis of seawater, can be used for the removal of dissolved CO<sub>2</sub> that accumulated in the culture water. Our computer simulation model could reproduce the flow pattern in the electrolysis unit and the estimated pH value of the out-flow water agreed well with the observation. We found that dissolved CO<sub>2</sub> in the culture water can be easily removed by bubbling of the acidic water.

## Effect of tetrodotoxin-containing diet on feeding, digestion and growth of tiger puffer *Takifugu rubripes* juveniles

Kogen Okita<sup>1\*</sup>, Tomohiro Takatani<sup>1</sup>, Osamu Arakawa<sup>1</sup>,  
Atsushi Hagiwara<sup>1</sup>, Yoshitaka Sakakura<sup>1</sup>

<sup>1</sup>Graduate School of Fisheries and Environmental Sciences, Nagasaki University

\*E-mail: jj20140049 at cc.nagasaki-u.ac.jp

Marine pufferfish contain tetrodotoxin (TTX), an extremely potent neurotoxin. We recently clarified that a tiger puffer *Takifugu rubripes* juvenile detects TTX by olfactory organ, and actively ingests and accumulates TTX not only in liver and skin but also in central nervous system. We further revealed that gene expression of some appetite peptides in the brain of hatchery-reared non-toxic fish changed by TTX-sensing and TTX-administration. In the present study, feeding trial using non-toxic and toxic diets was conducted with hatchery-reared *T. rubripes* juveniles in order to examine the relation between the appetite and TTX ingestion. A total of 120 non-toxic cultured juveniles (mean body weight of 3.6 g) were randomly divided into two groups where one group was fed with non-toxic commercial diets and the other was fed with TTX-containing diets (5.3 MU/g feed). Fish were maintained in 3 tanks (20 fish/200 l) for each group with flow through system (2 l/min) and vigorous aeration. The fish were fed with non-toxic and toxic diets to apparent satiation three times a day at 08:00, 13:00 and 17:00 hours for 28 days. All fish survived until the last day of feeding trial. There were no significant differences in the growth performance between fish fed non-toxic diets and toxic diets: total length (85.8±1.2 vs 85.1±2.7 mm), standard length (81.1±1.0 vs 80.1±1.5 mm), degree of loss of caudal fin (77.7±0.6 vs 75.5±5.5 %), body weight (18.4±1.7 vs 17.6±1.1 g), feed intake (11.7±0.6 vs 11.3±0.8 g), assimilation rate (91.1±1.6 vs 86.3±3.0 %), weight gain (14.9±1.6 vs 14.0±1.1 g), feed efficiency (127.3±7.1 vs 123.7±5.3 %). Total amount of administered and accumulated TTX in the fish fed toxic diets are 59.9±4.1 MU/fish and 33.6±2.0 MU/fish (56.4±7.1% of administered), respectively. These results indicate that TTX at this high dose may not have function as feeding stimulant for *T. rubripes* juveniles, but that feeding activity and growth of the juveniles are not inhibited by potent neurotoxin. We will further perform quantitative analysis and investigate the immunohistochemical localization of appetite peptides in the brain of TTX-sensed and TTX-administered hatchery-reared *T. rubripes* juveniles.

**Key words:** Tiger puffer, *Takifugu rubripes*, tetrodotoxin, feeding trial, appetite

**Temperature tolerance in two clonal strains of mangrove killifish,  
*Kryptolebias marmoratus***

Marina Yamada<sup>1\*</sup>, Yoshitaka Sakakura<sup>1</sup>

<sup>1</sup>Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Japan.

\*E-mail: [bb53115032.at.cc.nagasaki-u.ac.jp](mailto:bb53115032.at.cc.nagasaki-u.ac.jp)

Mangrove killifish *Kryptolebias marmoratus* is the only known self-fertilizing vertebrate. This unique species broadly distributes in coastal mangrove habitats from southern Brazil through the Caribbean Islands and Central America to North Florida. They are capable of synchronous self-fertilization, producing homozygous clones as a consequence. Our laboratory has two clonal strains, PAN-RS and DAN, which were originally collected from near Bocas del Toro, Republic of Panama and Dangriga, Belize, respectively. PDHy strain which is the hybrid of PAN-RS and DAN was produced by artificial insemination (Nakamura et al. 2008). The descendants of PDHy are divided into 4 strains, PDHyI, PDHyII, PDHyIII and PDHyIV according to the growth rate. Since Panama and Belize show different climate, we hypothesized that PAN-RS, PDHy and DAN show different temperature tolerance.

We used two clonal strains (PAN-RS and DAN) and hybrid strains (PDHyI, II, III, and IV). Fish were kept in plastic containers filled with 60 mL of 17 ppt artificial brackish water under 25 °C and photoperiod of 14L:10D. “Upper and lower thermal acclimation limits” were quantified for each strain using chronic thermal tolerance methodology (Fangue et al. 2006). All fish were kept under 25 °C for one week, and 20 fish from each strain were subjected to either increasing or decreasing water temperatures of 0.5 °C per day. This experiment was continued until all fish died. The respective chronic thermal maximum or minimum value was taken as the high or low temperature at which 50 % morbidity was observed. The respective chronic thermal maximum was significantly higher in DAN (36.6 °C) than PAN-RS (35.7 °C), (Log-rank test,  $p<0.01$ ). The respective chronic thermal maximum was 30.7 °C, 33.6 °C, 33.7 °C and 32.5 °C for PDHyI, PDHyII, PDHyIII and PDHyIV, respectively. PDHyI showed the lowest respective chronic thermal maximum among all strains. Respective chronic thermal maximum of each PDHy strain was lower than their parents. The respective chronic thermal minimum was 9.4 °C for PAN-RS and 9.6 °C for DAN, with no significant difference (Log-rank test,  $p=0.59$ ).

**Key words:** Mangrove killifish, *Kryptolebias marmoratus*, thermal tolerance, hybrid

## Effects of coexistence of protozoa *Euplotes* sp. coexistence on the population growth of minute monogonont rotifer *Proales similis*

Naoshi Wakimura<sup>1\*</sup>, Yoshitaka Sakakura<sup>1</sup>, and Atsushi Hagiwara<sup>1</sup>

<sup>1</sup>Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Japan.

\*E-mail: [bb53115036.at.cc.nagasaki-u.ac.jp](mailto:bb53115036.at.cc.nagasaki-u.ac.jp)

The rotifer *Brachionus rotundiformis* (SS-type) is commonly used as starter food for rearing small-mouthed marine fish larvae. However, several tropical marine fishes have much smaller mouth gap, hence cannot feed on the *B. rotundiformis*. To solve this problem, the minute monogonont rotifer *Proales similis* is preferred due to its smaller size than *B. rotundiformis*. However, the culture of *P. similis* is easy to be collapsed due to inability to withstand the handling stresses and environmental changes in the culture medium. In this study, we investigated the interaction between *P. similis* and the protozoa *Euplotes* sp. in the rotifer culture water. We independently cultured *P. similis*, *Euplotes* sp. and a combination of *P. similis* and *Euplotes* sp. in 144 ml glass jar containing 50 ml of seawater (22 ppt) at an initial density of 1 ind./ml at 28°C in darkness for 14 days. All the treatments were triplicated and daily fed with *Nannochloropsis oculata* at  $8.0 \times 10^5$  cells/ml without water exchange. A similar experiment was conducted separately with *Chlorella vulgaris* as food at  $2.9 \times 10^5$  cells/ml daily without water exchange. When fed with *N. oculata*, population density increased in *Euplotes* sp. in the monoculture and mixed-cultures. However, the population growth of *P. similis* in the mixed-culture decreased after 8 days and *P. similis* disappeared after 14 days of culture. There was a significant difference in the population density of either *P. similis* or *Euplotes* sp. between the monoculture and mixed-culture on day 14. When cultured with *C. vulgaris*, population density of *P. similis* decreased from day 6, while that of *Euplotes* sp. reached its highest peak on the same day. *P. similis* disappeared completely on day 14. These results suggest that the presence of *Euplotes* sp. in the *P. similis* cultures significantly suppressed the population growth of *P. similis* presumably due to stressful interactions. Furthermore, the competition between *P. similis* and *Euplotes* sp. for bacteria may also have existed. Even though we did not observe a behavioral interaction of *Euplotes* sp. and *P. similis*, the swimming speed of the *Euplotes* sp. was higher than the *P. similis*.

**Key words:** *Proales similis*, *Euplotes* sp., monoculture, mixed-culture



## Distribution of larval and juvenile greater amberjack (*Seriola dumerili*) around the Penghu Islands, Taiwan

Takamasa Hasegawa<sup>1\*</sup>, Hsin-Ming Yeh<sup>2</sup>, June-Ru Chen<sup>3</sup>, Ryo Kawabe<sup>4</sup>, Yoshitaka Sakakura<sup>1</sup>

<sup>1</sup>Graduate School of Fisheries and Environmental Science, Nagasaki University, Japan <sup>2</sup>Coastal and Offshore Resources Research Center, Fisheries Research Institute, Council of Agriculture, Taiwan <sup>3</sup>Fishery Research Institute, Council of Agriculture, Taiwan <sup>4</sup>Institute for East China Sea Research, Nagasaki University, Japan

\*E-mail: [bb53512002.at.cc.nagasaki-u.ac.jp](mailto:bb53512002.at.cc.nagasaki-u.ac.jp)

The greater amberjack *Seriola dumerili* (family Carangidae) widely distributes around the world. Because of its commercial importance, rapid growth and good adaptation to captivity, *S. dumerili* is a very important species for aquaculture in Japan. Juveniles of *S. dumerili* associate with floating objects such as drifting seaweeds. However, there is very limited knowledge about larval and early-juvenile stages of this species in the wild. In order to investigate the early life history of *S. dumerili*, firstly we validated the otolith daily increments using artificially-raised fish (11-51 days after hatching). Then, field surveys were made by R/V Hai-an, Fishery Research Institute, Council of Agriculture, Taiwan around the Penghu Islands, Taiwan, from May to August 2015. Frontal zone and drifting seaweeds were visually observed during survey, and drifting seaweeds were scooped together with associated fishes by a hand net (Φ45 cm, 3 mm mesh). Surface tows of plankton net (Φ1.3 m, 0.33 mm mesh) were conducted for 10 minutes with towing speed of 2 knots in frontal zones and other areas. At each sampling station, vertical profile of water temperature and salinity were measured by a CTD (SBE-19 plus, Sea-Bird Electronics Inc.). Fish species were identified and zooplankton abundance (mg DW·m<sup>-3</sup>) and species composition (%) were calculated. We also measured four *S. dumerili* samples caught in 2014 deposited at Penghu Marine Biology Research Center, Fishery Research Institute, Council of Agriculture, Taiwan. Increments of sagittal otolith of reared fish showed the same number as their age in days after hatching (ANCOVA, df=1, p=0.32). Relationships between otolith diameter ( $y_{\text{dia}}$ , μm) and age ( $x_{\text{day}}$ , days), and between otolith diameter ( $y_{\text{dia}}$ , μm) and TL ( $x_{\text{TL}}$ , mm), were described as following equations:  $y_{\text{dia}}=0.0003 \cdot x_{\text{day}}^{1.93}$  (r=0.991), and  $y_{\text{dia}}=0.1937 \cdot x_{\text{TL}}-0.1018$  (r=0.981), respectively. We caught a total of four larval and juvenile *S. dumerili* by surface tows, but not from drifting seaweeds. Total length of *S. dumerili* ranged from 7.4 to 42.5 mm, and age ranged from 18 to 56 days after hatching. All *S. dumerili* were caught in open water and in frontal zone, however we could not detect significant differences in zooplankton abundance between frontal zone and other station (*t*-test, p=0.87). Thecostraca was significantly abundant in the stations away from the frontal zones (*t*-test, p<0.01). In 2014, larval and juvenile *S. dumerili* were caught in January and May, and body length ranged from 5.8 mm in NL to 54.2 mm in TL. Our study indicates that *S. dumerili* spawns from April to June in 2015, and frontal zone in open water is nursery ground for *S. dumerili* around the Penghu Islands, Taiwan.

**Key words:** Greater amberjack, *Seriola dumerili*, early life, spawning ground, otolith

**Effect of starvation on mixis induction in offspring and genetic mechanism of the monogonont rotifer *Brachionus manjavacas***

Shohei Kamizono, Koushirou Suga, Yoshitaka Sakakura, Atsushi Hagiwara

Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Bunkyo 1-14, Nagasaki 852-8521, Japan.

\*E-mail: [bb53415001.at.cc.nagasaki-u.ac.jp](mailto:bb53415001.at.cc.nagasaki-u.ac.jp)

Short time starvation of neonates hatched from rotifer resting eggs induces active mixis up to the 10<sup>th</sup> generation. However, this phenomenon remains unexplained whether it can last for further generations. Also, the mode of heredity and the acquired parental characteristics inherited by the subsequent generations are not clear. In this study, we used the rotifer *Brachionus manjavacas* and *Brachionus plicatilis* to investigate the heredity of mixis induction in rotifers up to the 80<sup>th</sup> generation and the inheritance of the acquired traits by methylation of DNA. The maternal rotifers hatched from resting eggs were starved for 12 hours before determination of the acquired characteristics. The control group was not starved. A set of 8 individual rotifers were randomly selected from starved and control group. Rotifers were individually cultured in 0.2 mL of sea water (22 ppt.) in 8 well microplate at 1 individual per well until the 80<sup>th</sup> generation. The rotifers were daily provided with *Nannochloropsis oculata* suspension at  $6.0 \times 10^6$  cells/mL. The mixis induction in each generation was determined. We determine the genetic factors that changed the mixis induction rate of the parent and future generations. First, we designed the primer using partial base sequence of the methyltransferase, which was the DNA methylase which EST analysis of *B. plicatilis* performed PCR as template cDNA of *B. manjavacas*. The mixis induction of the offspring from starved parents increased until 38<sup>th</sup> generation. In addition, mixis induction during accumulated generations peaked at the 17<sup>th</sup> generation. We observed repetitions of increase and decrease of beyond the 17<sup>th</sup> generation. The amplification of the DNA fragment of the predicted size was confirmed through PCR analysis using the primer designed from the DNA methyltransferase (DNMT) gene of *B. plicatilis*. This phenomenon could be explained by the epigenetic inheritance involving methylation of the DNA. It was estimated that the DNMT gene fragment by BLAST analysis. We have been investigating this by comparing the *DNMT* gene expression level among generations.

## Body Size, Culture and Fish Larval Ingestion on a Minute Rotifer *Colurella cf. adriatica*

Stenly Wullur<sup>1</sup>, Petrus Letsoin<sup>2</sup>, Rina P. Astuti<sup>3</sup>, Atsushi Hagiwara<sup>4</sup>

<sup>1</sup>Faculty of Fisheries and Marine Science, Sam Ratulangi University, Manado - Indonesia.

<sup>2</sup>State Polytechnic of Fisheries. Tual, Southeast Maluku – Indonesia, <sup>3</sup>Gondol Research Institute for Mariculture, Bali – Indonesia, <sup>4</sup>Graduate School of Fisheries Science and Environmental Studies, Nagasaki University, Japan

\*E-mail: *miracle\_given.at.yahoo.com*

Current procedure of rearing small mouthed marine fish larvae is using the Super Small (SS) type of rotifer *Brachionus rotundiformis* as starter food during first days of larval first feeding. The *B. rotundiformis*, however, is ineffective or even unsuitable for larvae of several marine tropical fish with even smaller mouth size including, Napoleon fish (*Cheilinus undulatus*, groupers (genus *Epinephelus*), Angelfishes (family Pomachantidae). In present study, we examined the feasibility of a minute rotifer *Colurella cf. adriatica* as live food by measuring its body size, analyzing population growth and fish larval ingestion on the rotifer.

Rotifer *Colurella cf. adriatica* was isolated using a plankton net (45 µm mesh size) from an estuary in Mangket, North Minahasa, North Sulawesi, Indonesia. Water temperature and salinity at the time of sampling were 28±1 °C and 30±1 ppt, respectively. Sixty adults of the rotifer were measured for its body length and width. As comparison, body length and width of a local strain *Brachionus rotundiformis* were also measured. Population growth of *Colurella cf. adriatica* was assessed by culturing the rotifer under four densities (3, 6, 9, 12 x 10<sup>6</sup> cells/mL) of *Nanochloropsis oculata* as food source. The rotifer was cultured at salinity of 20 ppt and placed in a controlled room temperature at 25±1 °C. Water volume of the culture was 4 mL (using 3x4, multiwell plate) and the initial density of the rotifer was 1 ind./mL. Observation was made daily by counting the numbers of rotifer in each well until the density decline. Larval ingestion on the rotifer was investigated in Gondol Research Institute for Mariculture, Bali-Indonesia. Approximately 10 ind./L eggs of humpback grouper (*Cromileptes altivelis*) were transferred to four 200-L larval rearing tank. The first two tanks were fed with 10 ind./mL of rotifer *Colurella cf. adriatica* from day 2 till day 5 after hatching, while the other two tanks were left without any addition of food. All surviving larvae were harvested on day 5 and the numbers of remaining larvae were counted. Gut content of the surviving larvae was analyzed to see the presence of rotifer. Body length and width of *Colurella cf. adriatica* were distributed from 82.8-103.2 and 46.8-61.7 µm, respectively. The mean body length (95.9±3.8µm; mean± standard deviation) and width (46.8-61.7 µm) of *Colurella cf. adriatica* were significantly smaller/narrower than *B. rotundiformis* (175.2±9.2 and 123.5±7.7 µm, respectively) (*t-test*, *p*<0.05). Rotifer *Colurella cf. adriatica* grew well in all *N. oculata* treatments. The rotifer attained highest population densities on day 16 (774±167 ind./mL) and 18 (656±139 ind./mL) at *N. oculata* densities of 6 and 9x10<sup>6</sup> cells/mL, while it was reached on day 26 (646±85 ind./mL) and 34 (560±58 ind./mL) at *N. oculata* densities of 3 and 12x10<sup>6</sup> cells/mL, respectively. Humpback grouper larvae show higher survival (1.5%) on *Colurella cf. adriatica* treatment than control (0.3%) (*t-test*, *p*<0.05). By analyzing gut content of the remaining larvae,

it was found that individual of rotifer *Colurella* cf. *adriatica* presence in gut of the larvae indicating that larvae of humpback grouper ingested the rotifer.

**Key words:** Minute rotifer, *Colurella* cf. *adriatica*, body size, population growth, fish larvae, ingestion

## **Production of myostatin gene-knockout Japanese anchovies (*Engraulis japonicus*) using TALEN-based genome editing**

Keishi Sakaguchi<sup>1\*</sup>, Kanako Nakashima<sup>2</sup>, Hajime Kitano<sup>1</sup>, Naoki Nagano<sup>1</sup>, and Michiya Matsuyama<sup>2</sup>

<sup>1</sup>Fisheries Research Institute of Karatsu, Department of Joint Research, Faculty of Agriculture, Kyushu University, <sup>2</sup>Laboratory of Marine Biology, Faculty of Agriculture, Kyushu University  
\*E-mail: keishi\_s.at.agr.kyushu-u.ac.jp

Genome editing techniques such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindromic repeats (CRISPR) have attracted considerable attention in recent years since these technologies can mediate targeted and efficient genetic modifications (knockout, knock-in, and gene modification) in various organisms. Thus, targeted genome editing, which enables researchers to tailor genomic loci of interest, is one of the most promising approaches for plant, animal, and fish breeding. Nevertheless, the application of the techniques to teleost fishes have been limited almost exclusively to popular experimental fish models, such as zebrafish (*Danio rerio*) and Japanese medaka (*Oryzias latipes*). There are no literature reports on the application of these techniques to marine fish, which includes many important species for the fisheries industry. In this context, we regard the Japanese anchovy (*Engraulis japonicus*) as a most suitable candidate for genome-editing experiments in marine fish, because they have the following advantages: 1) easy rearing and breeding in a small-scale fish tank. 2) year-round and multi-year spawning under photoperiod and temperature control. 3) quick growth into mature individuals, which produce another generation of eggs, about three months. Myostatin (MSTN), previously referred to as growth differentiation factor 8 (GDF8), is a negative regulator of skeletal muscle growth. In mammals, MSTN-deficient animals resulted in an increase of skeletal muscle mass with both hyperplasia and hypertrophy. Likewise, recent studies revealed that the MSTN gene inhibits skeletal muscle growth even in fish. Thus, to produce a fish breeding model generated by genome editing, we performed targeted gene disruption of the MSTN gene in Japanese anchovies using TALEN technology. We constructed three TALEN pairs targeting the first intron of the MSTN gene and the *in vitro* transcribed RNAs of the pairs were injected into the yolk of embryos at the one-cell stage. As a result, mutant F0 embryos were obtained with a very high insertion and/or deletion (indel) mutation rate (~96.9%), and thus the F0 founders were mated with each other to produce MSTN-knockout anchovies at the F1 generation. To our knowledge, this is the most advanced study for genome editing in marine fishes. The rearing of F1-individuals and their genotyping is now in progress.

**Key words:** Japanese anchovy, *Engraulis japonicus*, myostatin, knockout, TALEN, genome editing

## Isolation and screening of novel probiotic lactic acid bacteria for aquaculture

Nguyen Thi Hue Linh<sup>1\*</sup>, Masato Otani<sup>2</sup>, Yousuke Taoka<sup>3</sup>

<sup>1</sup>Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, <sup>2</sup>Graduate School of Agriculture, University of Miyazaki, <sup>3</sup>Department of Marine Biology and Environmental Sciences, Faculty of Agriculture, University of Miyazaki  
\*E-mail: nb14005.at.student.miyazaki-u.ac.jp

Most of probiotics used in aquaculture are considered as alternative therapies for the use of antibiotics to prevent diseases in aquatic animal. Among of probiotic bacteria, lactic acid bacteria are one of potential candidate due to several strains have been isolated from fish gut as beneficial microflora and acted antagonistic against Gram-negative fish pathogens. Therefore, in this study we conducted to isolate lactic acid bacteria from fermented food as candidate strains which may be used to reduce the antibiotics using for sustainable aquaculture. The probiotic properties of isolates were surveyed. Lactic acid bacteria were isolated from samples such as fermented foods by an agar plating method using GYP and MRS media. The antagonistic activity test against fish pathogens *Lactococcus*, *Streptococcus* and *Edwardsiella* was carried out according to the method of the double layer agar method. The tolerant ability of isolates on NaCl (0%, 3%, 5%, 10%), pH (from 2 to 9), artificial gastric juice (at pH range 2-4) with pepsin and intestine juice (at pH 8) with gall powder were evaluated. Isolates were identified based on the sequences of 16 S rRNA gene (~700bp). Totally 55 strains of lactic acid bacteria were isolated from rice bran and several kinds of fermented vegetables. In antagonistic test, three isolates showed positive results against three strains of *Edwardsiella tarda*, three strains of *Streptococcus disgnactie*, three strains of *S. iniae* and three strains of *Lactococcus garvie*. These three strains of GYP 31, GYP 69 and GYP 4-20 were identified as *Lactobacillus* sp.. The relative growth of GYP 31 strain at pH range 5-8 was from 100% to 80%, at pH 2-4 and pH 9 were below 20%; while GYP 69 strain and GYP 4-20 strain only grew well at pH 5, pH 6 with 100% of the relative growth. Three strains grew well at NaCl concentration from 0-5%, 3-5% and 0-3% with the relative growth from 80% to 100%, respectively. In tolerance test on acid and artificial gastrointestinal juices, GYP 31 strain expressed the sustained ability and survival itself better than GYP 69 strain and GYP 4-20 strain. GYP 31 strain showed the highest viable count as  $1 \times 10^8$ ,  $1.4 \times 10^8$ ,  $5.6 \times 10^7$  cfu/ml in acid solution (at pH 3,5-4), artificial gastric juice at (pH 4) and intestine juice (at pH 8), respectively, although the viable count was lower than those in the control group ( $2.2 \times 10^9$  cfu/ml at pH 7). From these results, *Lactobacillus* sp.. GYP 31 strain is considered as a potential probiotic candidate in aquaculture due to its ability competition with pathogens and high tolerance in the gastrointestinal tract of fish.

**Key words:** probiotics, lactic acid bacteria, *Lactobacillus* sp., pathogen, antagonistic activity, tolerance in gastrointestinal juice

## **Effect of protease addition to EP diet on the growth of amberjack, *Seriola dumerili***

Yousuke Taoka<sup>1\*</sup>, Takayuki Minami<sup>2</sup>, Nguyen Thi Hue Linh<sup>3</sup>, and Terumi Koga<sup>4</sup>

<sup>1</sup>Department of Marine Biology and Environmental Sciences, Faculty of Agriculture, University of Miyazaki, <sup>2</sup>Miyazaki Prefectural Fisheries Research Institute, <sup>3</sup>Interdisciplinary Graduate School of Agriculture and Engineering, University of Miyazaki, <sup>4</sup>TOA Pharmaceutical CO., Ltd.  
\*E-mail: [yousuketao.at.cc.miyazaki-u.ac.jp](mailto:yousuketao.at.cc.miyazaki-u.ac.jp)

In aquaculture, the late growth of the cultured fish in winter season is serious problem for economical aquaculture. The extruder pellet (EP) has been used as an composed feed for the culture fish because EP can be produced efficiently. However, the EP is very hard to decompose it by digestive enzymes as compared with raw diet. The activities of digestive enzymes in the gastrointestinal tracts of the cultured fish significantly decrease due to the low temperature during winter season. Therefore, it is very difficult that the digestive enzymes with low activities decompose the EP diet efficiently. To dissolve this problem, in this study, the application of proteolytic enzymes from microorganisms was examined to accelerate the digestion of the EP diet for the cultured fish. Acid and alkaline proteases from microorganisms was used. The effect of pH and temperature on enzyme reaction was also investigated. Alkaline and acid protease were added to the EP diets in a 0.1M Tris-HCl buffer and 0.1M glycine-HCl buffer, respectively. The reaction mixtures were incubated at 15, 20, 25, 30 and 35°C for 180 min. The degradation of the EP diet was evaluated by weighing the solid bodies. The nitrogen concentration of the centrifugal supernatant in the reaction mixtures were determined according to the Kjeldahl method. The alkaline proteases showed stable activities at 20-35°C, and the activity at 15°C significantly decreased by 44%. The decomposition of the EP diet was enhanced by addition of both proteases at 20°C. These results indicated that the addition of proteases from microorganisms is effective to enhance the decomposition of the EP diet at low temperature. Amberjack, *S. dumerili* was fed with EP diet with or without alkaline protease (control group) for 60 days. After 60 days of rearing, the average fish body weight was higher in the group with alkaline protease as compared with that in the control group. These results showed that it is possible that the addition of protease enhance the growth rate of *S. dumerili* in winter season.

**Key words:** *Seriola dumerili*, protease, digestibility, growth rate, feed, low temperature, aquaculture

### **Annotated Bibliography of Key Works**

Satoh K. 2005. Study on improvement of composed diet for yellowtail culture. Bulletin of Oita Institute of Marine and Fisheries Science, 6, 19-77 (in Japanese)

To date, Japanese aquaculture, mainly for yellowtail *Seriola quinqueradiata*, has been developed using raw-fish as their feedstuff, such as sardine and mackerel obtained from adjacent sea, that used to be abundant and available with low cost. The reduction of sardine resources definitely

caused the necessity of composed diet in yellowtail culture. Therefore, the studies examined to improve and to improve accommodate the composed diet for yellowtails as to feedstuff, protein digestibility, feed additives, and feeding regime.



## Amino acid profile of thraustochytrids cells and potential of application to aquafeed

Kenya Horii\*, Masahiro Hayashi, and Yousuke Taoka\*\*

Department of Marine Biology and Environment Sciences, Faculty of Agriculture, University of Miyazaki

E-mail: \* [gdl2020.at.student.miyazaki-u.ac.jp](mailto:gdl2020.at.student.miyazaki-u.ac.jp), \*\* [yousuketao.at.cc.miyazaki-u.ac.jp](mailto:yousuketao.at.cc.miyazaki-u.ac.jp)

Fishmeal is used as protein sources for aquaculture feeds. However, the price is drastically increased to around 170,000/ ton in 2013 during recent 10 years because of the decrease of fish resources, anchovy. This is serious problem for sustainable aquaculture. Therefore replacement of fishmeal to another resource is urgently needed. Thraustochytrids are marine protists and classified to one of Stramenopiles. They are widely distributed in marine environment and accumulate large number of lipids in cell bodies. Therefore, thraustochytrids have attracted strong interests for production of valuable lipids as single cell oils (SCOs) such as biodiesel and omega-3 fatty acids. In the process of lipid extraction from the cultured cells, some solids (extract residue) are produced as byproducts. It is considered that this extract residue except for lipids is mainly consists of protein. From the viewpoints of industrial application of thrautochytrid cells as protein sources, we have planned to use the byproducts as resources instead of fishmeal for aquafeeds. In this study, we evaluated the recovery rate of protein in a thrausotchytrid, *Aurantiochytrium limacinum* strain mh0186 known as a docosahexaenoic acid (DHA) producer under the lipid extraction process. For selection of adequate strains for protein production, thraustochytrids were isolated from marine environment. *A. limacinum* strain mh0186 was cultured in a GY broth. The cultured cells were collected by centrifugation and lyophilized for proximate analysis. The content of protein, lipid and ash in the lyophilized cells was determined according to Kjeldahl method, Folch method and heat-ashing method, respectively. The composition of amino acid and fatty acid were analyzed by liquid chromatography-mas spectrophly (UF-Aminostation, Shimazu Co. Ltd., Japan) and gas chromatography (GC-2014, Shimazu Co. Ltd., Japan), respectively. In the process of lipid extraction, the extract residue was collected and re-lyophilized. The protein content and amino acid composition were analyzed by same method described as above. Seawater, sands, leaves, seaweed were collected for the isolation of thraustochytrids from coastal area in Miyazaki, Kumamoto and Oita, Kyushu, Japan. Thraustochytrids were isolated on a B12 Culture Agar “Nissui” plate medium by pine pollen-baiting method. The isolates were cultured in a GY broth, and the cultured cells were collected by centrifugation for analysis of the composition of amino acid and fatty acid. Isolates were identified at genus level based on the 18S rRNA sequence analysis. The content of crude protein lipid and ash per g of the cultured cell of mh0186 strain were 333 mg, 440 mg and 42 mg. On the one hand, the protein content of the extract residue obtained from 1g of the cultured cells was 226 mg (recover rate, 68 %). In both samples of the cultured cells and the extra residue, glycine, leucine, isoleucine, glutamate and arginine were mainly detected. One hundred twenty thraustochytrids strains were isolated. In some isolates including *A. limacinum* strains SR21 and mh0186, *T.aureum* ATCC34304, *Schizochytrium aggregatum* ATCC28209, glycine, leucine

glutamate, arginine and were detected as major amino acids. Strain Tak2 specifically accumulate 25 % cystathionine to total amino acids.

**Key words:** aquaculture, feeds, fishmeal, protein source, thraustochyrid, amino acid composition, byproducts.

**Diurnal changes in frequency of the burst swimming behavior of adult Pacific bluefin tuna (*Thunnus orientalis*) in a land-based tank**

Akiko Tsujita\* , Toshinori Takashi , Kentaro Higuchi , Tatsuru Kadota ,  
Koichiro Gen , Ayako Suzuki , Junpei Konishi , Keiichi Mushiake and Masakazu Oka

Research Center for Tuna Aquaculture, Seikai National Fisheries Research Institute, FRA

\*E-mail: [tsujiaki.at.affrc.go.jp](mailto:tsujiaki.at.affrc.go.jp)

In aquaculture of Pacific bluefin tuna, *Thunnus orientalis*(PBT), development of a stable system of artificially-reared fingerlings is needed to ensure the sustainability of aquaculture through a reduction on the reliance on wild captured juveniles. Therefore, we constructed two large indoor land-based PBT broodstock tanks at Seikai National Fisheries Research Institute, FRA, Japan, and are examining the environmental cues such as water temperature and photoperiod which are essential for successful and stable spawning of PBT. However, heavy mortality of adult PBTs occur in the tank due to collisions with the tank wall. It is considered that the collision deaths are associated with the burst swimming behavior. In this study, to clarify the process of the collision death, we examined diurnal changes in frequency of the burst swimming behavior of three-year-old PBTs in the land-based tanks. Nineteen three-year-old PBTs were reared in the land-based tanks (20 m in diameter, 6 m in depth) and their swimming behaviors were recorded using a video camera for six days. A day was compartmentalized into six periods defined as dawn (7:00 to 9:00), daytime-I (11:00 to 13:00), daytime-II (15:00 to 16:00), dusk (16:00 to 18:00), night-I (20:00 to 22:00) and night II (0:00 to 2:00) according to changes in illumination. We counted the frequency of the burst swimming behavior at each period from the recorded video imagery. As a result, the frequency of the burst swimming behavior at dawn was significantly higher than that in other time periods. Notably, the burst swimming behaviors were frequently observed in 30 min just after illumination (from 32 to 85 lux) during dawn. Additionally, the frequency of the burst swimming behavior of multiple PBTs in the tank like “a panic” were often observed during dawn, whereas solitary burst swimming behaviors were observed at the other time periods. These results suggest that collision deaths of PBT in the tank were caused by burst swimming behavior associated with sudden increases of light intensity during dawn.

**Key words:** Pacific bluefin tuna, collision death, burst swimming behavior, diurnal change, broodstock tank

## Interval and spawning frequency of Pacific bluefin tuna *Thunnus orientalis* in a land-based tank

Ayako Suzuki<sup>1\*</sup>, Koichiro Gen<sup>1</sup>, Tatsuru Kadota<sup>1</sup>, Kenji Saitoh<sup>2</sup>, Takuma Sugaya<sup>3</sup>, Masakazu Oka<sup>1</sup> and Keiichi Mushiake<sup>1</sup>

<sup>1</sup>Research Center for Tuna Aquaculture, Seikai National Fisheries Research Institute, FRA,

<sup>2</sup>Research Center for Aquatic Genomics, National Research Institute of Fisheries Science, FRA,

<sup>3</sup>Research Center for Marine Invertebrates, National Research Institute of Fisheries and Environment of Inland Sea, FRA.

\*E-mail: ayakos.at.affrc.go.jp

Recently, the exploitation of natural stocks of Pacific bluefin tuna *Thunnus orientalis* (PBT) has increased to dangerous levels though increased fishing pressure. The scarcity of this species and its high commercial value, together with its very high growth rates, makes it a potential candidate for marine aquaculture. Therefore, the artificial propagation and seed production techniques for PBT represent objective for sustainable utilization of this resource. Fundamental information on the spawning ecology of PBT is essential for the development of production techniques. However, this information is still largely lacking. In this study, we investigated spawning frequency of PBT by comparing mitochondrial DNA *D-loop* region haplotypes of broodstock fish, with those of fertilized eggs and hatched larvae. Broodstock PBT, fertilized egg and hatched larval samples were obtained from a land-based tank at Research Center for Tuna Aquaculture, Seikai National Fisheries Research Institute in Nagasaki, Japan. Spawning activity in a land-based tank was observed over a 98 day period during May 16 to August 28 in 2014. The sampling of fertilized eggs and hatched larvae was conducted for a total of 15 days within the 98 days. A total of 15 samples were collected for an initial consecutive 3 day period and then at approximately 1 week intervals from May 16 to August 28 in 2014. The 36 broodstock individuals and 659 eggs and hatched larvae were observed to have 3 haplotypes. These haplotypes were named A, B and C, respectively. Among the broodstock fish, haplotype B was detected at high frequencies, secondly haplotype C, and thirdly haplotype A. The number of each broodstock individuals was 32, 3, and 1, respectively. Each haplotype of eggs and hatched larvae was detected on 14 days, 1 day and 8 days, respectively, during the 15 sampling days. Haplotype B occurred in the 3 days of consecutive spawning. This is consistent with genetic or histological observations that other wild *Thunnus* species spawn multiple times and on consecutive days. These results demonstrate that PBT in a land-based tank may have the potential to spawn consecutively and multiple times.

**Key words:** Pacific bluefin tuna (*Thunnus orientalis*), spawning frequency, mtDNA *D-loop* region, haplotype

**Effect of timing of restricted feeding on sexual maturation in the yellowtail,  
*Seriola quinqueradiata***

Kentaro Higuchi<sup>1,2\*</sup>, Kazunori Yoshida<sup>1</sup>, Koichiro Gen<sup>1</sup>, Toshinori Takashi<sup>1</sup>, Kiyoshi Soyano<sup>2</sup>,  
Keiichi Mushiake<sup>1</sup>

<sup>1</sup>Seikai National Fisheries Research Institute, FRA, 1551-8 Taira-machi, Nagasaki, Nagasaki 851-2213, Japan, <sup>2</sup>Institute for East China Sea Research, Nagasaki University, 1551-7 Taira-machi, Nagasaki, Nagasaki 851-2213, Japan

\*E-mail: [higuken.at.affrc.go.jp](mailto:higuken.at.affrc.go.jp)

In aquaculture of large sized marine fish such as bluefin tuna and yellowtail, extensive amounts of fish feed are needed for the broodstock management because of their large body size. In order to save on the feeding cost for their broodstock management, development of a restricted feeding technique without affecting reproductive performance is required. However, available information about the effect of food supply on reproduction is limited in fish. In this study, we examined the effect of restricted feeding during the gonad immature and vitellogenic phases on sexual maturation in females of the yellowtail, *Seriola quinqueradiata*. Two -year -old cultured yellowtail females, which the average body weight was 5.0 kg, were divided randomly into three sea cages on November 2012 and reared until the next spawning period (April 2013) under natural conditions at the Goto station, Seikai National Fisheries Research Institute, FRA, Japan. The feeding regimes in each cage were defined as follows: control group fed to satiation three times a week throughout the experimental period, two restricted groups fed 30% of the amount of feed given to the control group (100%) during the immature (from November to January) or vitellogenic phase (from February to April), respectively. At the end of the experimental period, the average body weights were 6.6 kg in the control group, 5.8 kg in the restricted group during the immature phase and 5.9 kg in the restricted group during the vitellogenic phase, which shows that the restricted feeding reduced approximately 50% of somatic growth throughout the experimental period. Interestingly, the gonad weights in the restricted feeding group during the vitellogenic phase were low as compared with the control group and the restricted feeding group during the immature phase. Histological observations revealed that females in all groups had oocytes that completed the accumulation of yolk globules at the spawning period. However, the mean diameter of most advanced ovarian follicles in the restricted feeding group during the vitellogenic phase was significantly smaller as compared with the other groups. Furthermore, plasma estradiol-17 $\beta$  levels in the restricted feeding group during the vitellogenic phase were significantly lower as compared with the other groups at the spawning period. These results indicate that the restricted feeding during the vitellogenic phase alters the gonadal development in relationship with the plasma estradiol-17 $\beta$  levels in the yellowtail females.

**Key words**

Yellowtail (*Seriola quinqueradiata*), Sexual maturation, Restricted feeding, Broodstock management

## Evaluation of nitrogen excretion in young, immature and adult Pacific bluefin tuna (*Thunnus orientalis*) measured in the land-based tank

Toshinori Takashi<sup>1\*</sup>, Takeshi Eba<sup>2</sup>, Tadashi Imai<sup>3</sup>, Tetsuo Morita<sup>3</sup>, Yoshihisa Yamamoto<sup>3</sup>, Hiroyuki Matsunari<sup>4</sup>, Junpei Konishi<sup>1</sup>, Kazuki Kumon<sup>2</sup>, Koichiro Gen<sup>1</sup> and Masakazu Oka<sup>1</sup>

<sup>1</sup>Seikai National Fisheries Research Institute, FRA, 8-1551 Taira, Nagasaki, Nagasaki 851-2213, Japan, <sup>2</sup>Amami Laboratory, Seikai National Fisheries Research Institute, FRA, 9555 Hyouakiyahara, Oshima Setouchi, Kagoshima 894-2414, Japan, <sup>3</sup>Yashima Laboratory, National Research Institute of Fisheries and Environment of Inland Sea, FRA, 234 Yashima-higashi-machi, Takamatsu, Kagawa 761-0111, Japan, <sup>4</sup>National Research Institute of Aquaculture, FRA, 422-1 Nakatsuhamaura, Minamiise, Mie, 516-0193, Japan

\*E-mail: ttakasi at fra.affrc.go.jp

Ammonia excretion in marine teleosts accounts for 70 to 90 % of their total nitrogen excretion. Ammonia is toxic to fish and is a major factor limiting fish biomass and stocking density in intensive culture systems and aquariums. Quantification of ammonium nitrogen is important for estimating stocking biomass/density, water flow and size of the biological filter in the culture system. Several studies have suggested that ammonia excretion is affected by several factors such as species, body weight, water temperature and ration size. Although several tuna species such as Pacific bluefin tuna (PBT) and yellowfin tuna have been reared in aquariums and research facilities including Seikai National Fisheries Research Institute, there is a lack of studies about ammonia excretion in tunas. In this study, we investigated the effects of body weight and ration level on nitrogen excretion for PBT in captivity. Young ( $0.67 \pm 0.14$  kg,  $n = 50$ ), immature ( $14.4 \pm 1.88$  kg,  $n = 2$ ) and adult ( $42.9 \pm 6.5$  kg,  $n = 2$ ) PBTs were introduced to experimental land-based tanks from net cage or rearing tank, and thereafter they were acclimated to running sea water conditions. Experimental tank size varied according to fish size (20 kl for young fish; 65 kl for immature fish and 150 kl for adult fish). The PBTs were fed raw fish or artificial feed until the experiments. Before the fasting and postprandial experiments, the fish were deprived of food for 48 hours. Rearing water was sampled every 2 h for the first 12 h, and at 4 h intervals from 12 to 24 h. In the postprandial experiment, the experimental fish were fed bait fish (chub mackerel, *Scomber japonicas*, or sandlance, *Ammodytes personatus*) or artificial feed. Fish feces were collected from the tank bottom after 24 h. Determination of fecal nitrogen and concentration of ammonium, nitrite and nitrate nitrogen was carried out on each fecal and water samples. Weight-specific ammonia excretion rates of fasted fishes showed an inverse relationship with body weight (W). The relationship for total ammonia nitrogen (TAN) was:  $\text{TAN (mg N W}^{-1} \text{ d}^{-1}) = 297.4 \cdot W^{-0.36}$  ( $r^2=0.99$ ). The ammonia excretion rate at 10 kg in PBT was twice as much as it is for red seabream (unpublished data). Although postprandial ammonium excretion rate was in relation to the ration size ( $R$ , mg feed-N  $W^{-1} \text{ d}^{-1}$ ), linear regression analysis indicated that TAN excretion rates increased with ration size:  $\text{TAN (mg N W}^{-1} \text{ d}^{-1}) = 332.4 \cdot R - 179.6$  ( $r^2=0.87$ ). There was no significant difference in the rates between PBT fed the bait fish and the artificial feed. Postprandial fecal nitrogen was positively correlated with ration size. Fecal nitrogen was excreted at a level of 0.9-1.9 % for the baitfish and 0.3-1.3 % for the artificial feed. Comparing the results of PBT with other fish, these values were

lower than that of red seabream (12.8%) and pufferfish (16.1%). This study highlights the need for effective evaluation of nitrogen loading and water quality management in PBT rearing facilities and aquaculture grounds.

**Key words**

Pacific bluefin tuna (*Thunnus orientalis*), nitrogen, ammonia excretion rate, ration size

**A high density genetic linkage map for yellowtail  
(*Seriola quinqueradiata*) containing 6,275 EST-based SNPs**

Akiyuki Ozaki<sup>1\*</sup>, Jun-ya Aoki<sup>1</sup>, Kazuo Araki<sup>1</sup>, Hironori Usuki<sup>1</sup>, Kazunori Yoshida<sup>2</sup>, Tsutomu Noda<sup>2</sup>, Takuro Hotta<sup>2</sup>, Hirotaka Mizuochi<sup>2</sup>, Taro Chujo<sup>2</sup>, Yasuhiro Shima<sup>1,2</sup>,

<sup>1</sup> National Research Institute of Aquaculture, FRA, 422-1, Nakatsuhamaura, Minamiise-cho, Watarai-gun, Mie, 516-0193, Japan, <sup>2</sup> Seikai National Fisheries Research Institute, FRA, 122-7, Nunoura, Tamanoura-machi, Goto-shi, Nagasaki, 853-0508, Japan

\*E-mail: [aozaki at affrc.go.jp](mailto:aozaki@affrc.go.jp)

The marine products industry has developed as majority of the fishery, which are captured and directly using of aquatic resources. Only recently the breeding are considered as important research because available of aquatic resources are restricted gradually. The expectation of aquaculture research is getting higher in response to the prediction of aquatic resources depletion. Also the genetic improvement of economic traits are needed, it hopes apply to superior fish breeding, because artificial juvenile have a possibility to improve the phenotype for suited to aquaculture condition in every generation. We are researching practical application about selection of economic important traits from natural genetic resources using yellow tail (*Seriola quinqueradiata*) as target species. High density SNP arrays have become the tool of choice for QTL mapping, Genome-wide association studies, marker-assisted selection (MAS) and genomic selection (GS). 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. Earlier mapping studies have identified QTL for important commercial traits including parasite 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. In this study, Affymetrix SNP array was used to genotype 460 samples collected across five families from wild population in coastal waters of Goto Fukue-island. To establish EST (expressed sequence tag) -based SNP array, a cDNA library was generated from pooled RNA samples extracted from 11 tissues from a single individual. Sequencing on Roche/454 GS FLX platform generated 1,353,405 reads. The sequencing of SNP identification produced 570,846 raw reads derived from the full-length library and 456,482 raw reads derived from the 3'-anchored library derived from 5 hundred juveniles. Quality -based variant calling using CLC Genomics Workbench detected 9,356 biallelic putative SNPs in 6,025 contigs, with a minor allele frequency (MAF)  $\geq 25\%$ . A Linkage analysis was performed using application package of LINKMFEX version 2.3. This application can separate originated alleles from male or female. In order to avoid the error of genotyping, the accuracy of genotypes in their progenies was checked from parental male and female alleles. Genotype data were converted to a backcross format as though the grandparent genotype was unknown. Pairwise analysis was performed, and markers were sorted in linkage group at a minimum LOD threshold of 5.0. Linkage phases were determined retrospectively by examining the assortment of alleles among linked markers. A total of 6,275 EST-based SNPs were mapped to 24 linkage groups. The total distance covered by the male and female maps were 1,230cM and 1,031cM. 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 yellowtail genome. It is possible to rapidly develop domesticated strains having commercially important traits in yellowtail aquaculture.

**Keywords:** Yellowtail (*Seriola quinqueradiata*;) ; EST-based SNPs ; Genetic linkage map; quantitative trait loci (QTL); Affymetrix.