42nd Scientific Symposium of the UJNR Aquaculture Panel

Genetics in Aquaculture

NOAA Southwest Fisheries Science Center
8901 La Jolla Shores Drive
La Jolla, CA
October 1, 2014

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Aim of the Symposium

Exciting new developments in genetics and genomics contribute significantly to advances in aquaculture production today and will be of even greater importance in the future. Genetic improvements through selective breeding, genetics and health management, understanding genetic interactions of wild and cultured stocks and genetics and climate change are all research priorities of the Japanese Fisheries Research Agency, the National Oceanic and Atmospheric Administration and the United States Department of Agriculture. The primary focus of this symposium will be on solving production problems faced by the aquaculture industries of the two nations using genetic and genomic approaches. This will facilitate development of more competitive new and existing aquaculture industries. Selective breeding has a long history of improving production traits in many livestock species and is having progressively greater impacts in aquaculture. Enhancing genetic improvement through application of genomics can significantly accelerate this process. In health management, genetic approaches to improve resistance, enhance immune response, better understand pathogens and improve vaccines are all tools to strengthen the aquaculture industry. Genetics and genomics will also prove to be valuable tools in addressing the effects of climate change on aquaculture species. This symposium will include a workshop component with discussion on new scientific approaches and their potential application in collaborative research efforts to resolve key bottlenecks and facilitate industry expansion in Japan and the United States.

Program

Registration 8:30 - 9:00

Opening Session and Orientation to the Southwest Fisheries Science Center

Welcome
Kristen Koch, Acting Deputy Director, Southwest Fisheries Science Center 9:00 - 9:10

Aim of the Symposium
Michael Rust, NOAA Fisheries Office of Aquaculture, United States Panel Chair 9:10-9:20

Plans, Challenges and Opportunities for Warm Water Aquaculture and the Role of the Southwest Fisheries Science Center
Russ Vetter, Southwest Fisheries Science Center 9:20-9:40

Keynote Session

(Moderators: Paul Olin and Junya Higano)

Strategy for breeding study of fisheries organisms by FRA
Hiroyuki Okamoto, National Research Institute of Aquaculture, Fisheries Research Agency
9:40-10:10

Selective breeding research at USDA ARS
Jeff Silverstein, US Department of Agriculture, Agriculture Research Service
10:10-10:40

Genomes and Tools

(Moderators: Russ Vetter and Satoshi Watanabe)

Assessment of global population genetics of *Seriola lalandi*: Implications for genetic management in aquaculture
John Hyde, Southwest Fisheries Science Center 10:40-11:00

The de novo draft assembly of the yellowtail, *Seriola lalandi*, genome
Catherine Purcell, Southwest Fisheries Science Center 11:00-11:20

Identification of Quantitative Trait Loci (QTL) and Marker-Assisted Selection (MAS) by using genomics information in yellowtail (*Seriola quinqueradiata*)
Akiyuki Ozaki, National Research Institute of Aquaculture, Fisheries Research Agency
11:20-11:40

Examining host-pathogen interactions at mucosal surfaces reveals novel molecular targets for columnaris disease intervention
Ben Beck, US Department of Agriculture, Agriculture Research Service 11:40-12:00

Lunch Break: 12:00 – 13:00

Physiology and Manipulation

(Moderators: Mark Drawbridge and Masayuki Minakawa)

The genetics and breeding of highly fecund marine aquaculture species
Dennis Hedgecock, University of Southern California 13:00-13:20

Assessment of fertilization ability of cryopreserved sperm in fish using interspecific hybridization
Yukinori Shimada, National Research Institute of Aquaculture, Fisheries Research Agency
13:20-13:40

Genetic and physiological studies of health and fitness in aquaculture-reared California yellowtail, *Seriola lalandi*
Nick Wegner and Catherine Purcell, Southwest Fisheries Science Center 13:40-14:00

Demands for infertility of cultured marine fishes and study of UV irradiation at developmental stages in Japanese flounder
Toshiya Yamaguchi, National Research Institute of Aquaculture, Fisheries Research Agency
14:00-14:20

RNAseq analysis of early larval development in Seriola lalandi with emphasis on differential development of digestive system in fast and slow growing groups
Vincent Buonaccorsi, Juniata College 14:20-14:40

Break 14:40-15:00

Ecosystem Based Management of Aquaculture

(Moderators: Emily Trentacoste and Yasuji Sakai)

Exploring the genetic risks to natural populations by escaped cultured marine fish: a reintroduction to the OMEGA model and next steps
Kristen Gruenthal, NOAA Northwest Fisheries Science Center 15:00-15:20

Suspended culture of Asari clam, *Venerupis philippinarum*, and their roles in the ecosystem
Junya Higano, National Research Institute of Aquaculture, Fisheries Research Agency
15:20-15:40

Interpreting the microbial ecology found within marine fish
Jessica Blanton, Scripps Institution of Oceanography 15:40-16:00

Development of integrated multi-trophic aquaculture using sea cucumber
Satoshi Watanabe, National Research Institute of Aquaculture, Fisheries Research Agency
16:00 -16:20

Open Discussion: Development and support for research collaborations 16:20 – 16:50
Moderator/s: Mike Rust, Fuminari Ito

Science Symposium Closing
Fuminari Ito, Fisheries Research Agency, Japan Panel Chair 16:50 – 17:00

Symposium Reception: One on one discussions continue 18:30 – 20:00
Surfside, Scripps Institution of Oceanography (see below)

Symposium Reception
Surfside, Scripps Institution of Oceanography

List of Participants

Beck, Benjamin	USDA Agricultural Research Service (ARS)
Ben-Aderet, Noah	Scripps Institution of Oceanography (SIO)
Bess-Stimpert, Jessica	NOAA Southeast Fisheries Science Center
Blanton, Jessica	SIO
Buonaccorsi, Vincent	Juniata College
Gruenthal, Kristen	NOAA Northwest Fisheries Science Center
Hedgecock, Dennis	University of Southern California
Higano, Junya	Fisheries Research Agency
Hyde, John	NOAA Southwest Fisheries Science Center (SWFSC)
Ito, Fuminari	Japan Panel Chair, FRA
Jones, Robert	NOAA Fisheries Office of Aquaculture (AQC)
Minakawa, Masayuki	FRA
Olin, Paul	California Sea Grant
Ozaki, Akiyuki	FRA
Okamoto, Hiroyuki	FRA
Purcell, Catherine	SWFSC
Rust, Mike	AQC
Sakai, Yasuji	Former Japan Panel Chair
Shimada, Yukinori	FRA
Silverstein, Ahn	
Silverstein, Jeffrey	USDA ARS
Smith, Elizabeth	University of San Diego
Stuart, Kevin	Hubbs-SeaWorld Research Institute
Sylvia, Paula	SWFSC
Trentacoste, Emily	AQC
Vetter, Russ	SWFSC
Watanabe, Satoshi	FRA
Wegner, Nick	SWFSC
Yamaguchi, Toshiya	FRA

List of Abstracts

Examining host-pathogen interactions at mucosal surfaces reveals novel molecular targets for columnaris disease intervention

Benjamin H Beck* and Eric Peatman

Columnaris disease, caused by the bacterial pathogen *Flavobacterium columnare*, is a major problem globally and leads to tremendous losses of freshwater fish, particularly in intensively farmed aquaculture species. Despite its widespread importance, our understanding of *F. columnare* infectious processes remains limited. Specifically, little is known regarding the mechanisms controlling pathogen adhesion and replication on host mucosal surfaces and how these may differ between resistant or susceptible fish. Utilizing next-generation sequencing-based RNA-Seq we profiled the channel catfish gill transcriptome following columnaris infection in columnaris disease resistant and susceptible families to examine transcriptional differences in the gill before pathogen exposure and at early timepoints following a columnaris challenge. The results revealed a consistent pattern of basal immune polarization between resistant and susceptible fish prior to challenge, including key differences in expression of genes linked to mucin abundance and composition, lysozyme, and a rhamnose-binding lectin; a potential receptor for *F. columnare*. We propose that the disparate mucosal signatures exhibited between resistant and susceptible fish are a critical component in determining disease outcomes in infected fish. The position of these molecular actors on ectopic mucosal surfaces make them accessible for use as prognostic indicators of disease fitness, and highly amenable to modulation through dietary or topical prophylactic or therapeutant approaches.

Interpreting the microbial ecology found within marine fish

Jessica M Blanton* and Eric E Allen

The natural microbial communities found within vertebrate digestive tracts are composed of a multitude of different microorganisms, collectively known as the microbiome. Recent work in humans and model organisms has shown that the microbiome is a dynamic and vital element of host health—contributing to nutrition and digestion processes, as well as immune system development. Technological advances in sequencing now allow us to look at these communities as a whole, promoting the use of ecological theory to understand how these fish-microbiome systems are structured. Here we investigate the microbiome of wild fishes by looking at species local to the Southern California Bight: the sport fish and aquaculture target *Seriola lalandi* (California yellowtail) the forage fish *Scomber japonicus* (Pacific chub mackerel) and the omnivorous *Atherinops affinis* (Topsmelt silverside). Using high throughput sequencing, we analyze the microbial composition of the intestinal contents of individual fish. Initial results confirm that fish microbiomes have distinct taxonomic signatures unique from the environment, including the presence of key host-associated taxa such as segmented filamentous bacteria (SFB), the phyla Tenericutes, and the phyla Spirochaetes. Furthermore, differences between the communities associated with the epithelial and the lumenal regions indicate the importance of spatial organization within the gut environment. These results demonstrate early steps and methods to characterize marine fish gut microbiomes within an ecological framework. Ultimately, this knowledge can be applied to understanding the importance of the gut microbiome to fish health in aquaculture and ecosystems management.

RNAseq analysis of early larval development in Seriola lalandi with emphasis on differential development of digestive system in fast and slow growing groups

Catherin Purcell, Andrew Severin, Vince Buonaccorsi*, and John Hyde

Seriola lalandi is considered a prime candidate for aquaculture development in southern California. In the U.S., hatchery production of both *S. lalandi* and *S. rivoliana* is rapidly growing but has been hindered by a propensity for deformities and growth heterogeneity developed during larval and early juvenile stages that limit the production capacity and efficiency. The causes of, and solutions for the growth variation and high deformity rates remain unresolved. Our aim here is to characterize development of physiological systems important to aquaculture, and understand differences in gene expression between size classes that correlate with size heterogeneity. We performed an RNAseq experiment to characterize differences in gene expression between fast- and slow-growing larval *S. lalandi* at 2, 7 and 17 days post hatch. Each developmental stage was represented by three biological replicates, and each biological replicate was comprised of mRNAs drawn from a pool of 10 individuals. An average of 60 million reads per replicate were obtained from Illumina HiSeq sequencing and subjected to differential gene expression (DGE) analysis. Genes of related function were sorted into clusters, and those that were found at high frequency in the DGE set were identified. Here we focus on patterns of development related to the digestive system, highlighting progressions with larval stage and variation between size classes. Fish in the smaller size class developed more slowly, with heavier reliance on amylase and lipases. By 17dph faster growing fish transitioned heavily to chitinase, indicating a dietary shift to *Artemia*, with upregulated carbohydrate, as well as alkaline and acidic proteinase metabolic systems.

Exploring the genetic risks to natural populations by escaped cultured marine fish: a reintroduction to the OMEGA model and next steps

Kristen M Gruenthal*

Aquaculture programs have the potential to create myriad negative fitness effects when cultured fish come in contact with wild conspecifics. Physical contact may result in fragmentation, competition, and disease transmission, while reproduction between cultured and wild fish may cause inbreeding or outbreeding depression, reduced genetic variability, domestication, and/or genetic swamping in the mixed population (wild plus cultured; Tringali et al. 2007; Waples et al. 2012). Reduced genetic diversity, a gene pool swamped with hatchery alleles, and the introduction of non-native or maladapted genes, for instance, compromise the adaptive potential of a mixed population, making it less fit or able to respond to stochastic environmental change (Tringali et al. 2007; Waples et al. 2012).

The rapid development worldwide of offshore marine finfish aquaculture has raised concerns, in significant part, due to the genetic impact escaped cultured fish may have on natural populations. Marine fish escape for a variety of reasons, such as improper mesh size or holes from normal wear and tear in net pen cages; fish transfer among cages (e.g. during grading or harvesting); high wind and sea conditions during storms; and cage breaches by large predators. When evaluating the genetic threat(s) escapes may pose, several factors merit consideration, including but not limited to the genetic structure and phenotypic variability of the

species in the wild; size of the local (affected) wild population relative to the magnitude, frequency, and survival rate of escapes; age and growth characteristics of the wild and cultured populations; encounter rate between wild and cultured fish; type of breeding program at the farm, including broodstock selection and breeding protocols; and the likelihood of genetic drift and/or domestication resulting from hatchery breeding and rearing practices (Tringali et al. 2007; Waples et al. 2012).

Unfortunately, little experimental information is available to reliably assign risk to population fitness due to escapes, and because scientific data is lacking, escape standards are largely theoretical or qualitative rather than quantitative. To address this technical and scientific barrier and advance sustainable US marine aquaculture, the NOAA Fisheries Office of Aquaculture (OAQ) fostered a research initiative aimed at exploring the genetic interactions between wild fish and escapes and how to minimize the impact. First, a comprehensive white paper was created by Waples et al. (2012) to synthesize what is known about the genetic risks associated with marine aquaculture. Next, the Offshore Mariculture Escapes Genetics Assessment (OMEGA) model was developed by NOAA Fisheries and ICF International to simulate, identify, and quantify these risks. Outputs from OMEGA describe the influence escapes may have on survival and fitness in the mixed population over time (per Ford 2002). Ultimately, OMEGA is intended to provide insight into factors affecting risk, help identify research priorities, explore options for design or modification of culture programs, and inform policy and management decisions related to mitigating these genetic impacts. To realize this goal, the current focus is on fostering domestic and international collaborations to develop model scenarios, evaluate model parameters, and validate the model with data from current and planned marine aquaculture operations.

The genetics and breeding of highly fecund marine aquaculture species

Dennis Hedgecock*

Aquaculture genetics and breeding has been dominated by a focus on selection, largely as a result of its transformative success in improving salmon and rainbow trout. However, application of selection to highly fecund marine species faces challenges not present in species with lower fecundity: higher likelihood of random genetic drift, owing to sweepstakes reproductive success, higher genetic loads, and most important, larger non-additive components of genetic variance in yield-related traits. Crossbreeding offers an alternative strategy for improving highly fecund species with these characteristics. For example, the Pacific oyster shows dramatic heterosis (hybrid vigor) for yield and can be improved through crossbreeding of inbred lines, as demonstrated in corn, rice, and other major crops. In addition to improving yield, crossbreeding provides a means for improving triploid oysters and the tetraploid lines used to produce triploid seed. Heterosis should be measured in other highly fecund aquatic species. The Pacific oyster also shows, not unexpectedly, strong inbreeding depression for yield characteristics, particularly mortality of early life stages. High early mortality (type-III survivorship) is a consequence of strong natural selection against inviable genotypes that are likely generated as a by-product of high fecundity. Selection for early life-history traits or for survival in the face of diseases or environmental challenges must account for this strong background selection. Finally, unequal reproductive contributions by broodstock (sweepstakes reproductive success) reduce effective population sizes and increase genetic drift in closed

aquaculture populations, which then have increased risk of losing genetic diversity and fitness, owing to inbreeding depression. Crossbreeding programs, because they are based on controlled, systematic inbreeding, can prevent inbreeding depression from reaching farmed populations. Progress in crossbreeding can be accelerated, moreover, by application of genomic science to a global, mechanistic understanding of physiological and metabolic processes underlying production traits.

Suspended culture of Asari clam, *Venerupis philippinarum*, and their roles in the ecosystem

Junya Higano*, Nariaki Inoue, Natsuki Hasegawa, Yuka Ishihi, Yoshimi Fujioka, Masahiro Kuno, Daisuke Asao, Megumu Yamaguchi, Yoshitaka Imai, and Setsuo Kobayashi

Asari clam, *Ruditapes philippinarum*, is one of the most important fisheries species in Japan. Most of the clam's production depends on wild catch although the culture production in tidal flats is occupied only 3 to 4 % of the total production. The suspended culture of Asari clam is not prevalent yet in Japan and any other countries. In order to develop the practical method of the suspended culture, the culture experiment was performed at an oyster culture raft in Ohnoura Bay, Toba City, Mie Prefecture. Prior to the culture experiment, for the collection of natural seed of Asari clam, 60 x 30cm Nylon mesh bags with 2x3 mm mesh size were set on the tidal sand flat in Ohnoura Bay. Fine gravels and Care-Shells (oyster shell processed materials) were put in mesh bags. The mesh bag could collect more than 100 individuals/bag with the medium size of 20mm in shell length after several month. For suspended culture, these seeds were put in plastic containers (41x31x14 cm). In each container 150 seeds (average shell length was 22mm, wet weight was 2.0g) were put with fine gravels and Care-Shells (oyster shell processed materials) in five different ratio of these substrates, namely 0, 20, 50 80 100% Care-Shell contents, setting to 6 cm in thickness. The containers were suspended in 2m from the water surface. The culture started on April 18, 2011. After the five month culture, average SL and WW of the clams reached 33 to 35mm and 8.3 to 9.7g, and the survival rate was higher than 90%. Total wet weight of clams in the containers having started from 300g reached 1,100 to 1,300g. In terms of substrates, 50 % of Care-Shell contents or over maintained the pore water pH of more than 8.0 during the culture period. The growth of the clams in 50 and 80 % of Care-Shell contents seemed to be better result, but not significant. The growth of Asari clam in suspended culture showed much higher than on the tidal flat in Ise Bay. The results suggest that suspended culture of the clam has a potential for commercial clam production both on the high growth and survival rates.

According to the filtration experiment, the clam can filtrate 1.5 to 2.5 L/hr/g soft tissue dry weight. On the other hand, chlorophyll concentration adjacent the net pens of the red sea bream culture in Gokasho Bay, Mie showed in the range of 1.7-27.5μg-Chl/L 2 - 8 m beneath the surface. By the estimation of nitrogen budget around the suspended culture of the clam, one kg of the clam has ability to absorb 4 – 200 mg-Nitrogen/day depending on the chlorophyll concentration. The harvesting the clam with increased one kg corresponds to 3.4g nitrogen removal from the system. It is suggested that the introduction of the suspended clam culture has positive ecological impacts in terms of controlling water quality and biological production.

Assessment of global population genetics of *Seriola lalandi*: Implications for genetic management in aquaculture

John R Hyde* and Catherine Purcell

On the west coast of the United States, the California yellowtail, *Seriola lalandi*, is considered a great candidate for mariculture due to its high market demand and value. While most yellowtail production relies on capture of wild juveniles and fattening in offshore pens, successful spawning and rearing techniques for yellowtail have been developed at the Hubbs-SeaWorld Research Institute (HSWRI) with the intent to translocate reared individuals to offshore pens to raise to market size. As aquaculture for yellowtail grows here and around the world, the impact of unintentional releases on wild populations has become an increasingly important issue. As such, most future aquaculture projects will likely require a genetic analysis component for the permitting process. This study aims to examine the genetic diversity of yellowtail over both a global scale and more regionally in the California-Mexico region to develop a baseline of the genetic variability in wild populations, and to evaluate locally adapted traits for broodstock selection. A total of 260 specimens collected around the Pacific and in the Atlantic, and 755 specimens collected in the northeast Pacific were evaluated using 16 nuclear microsatellite markers. Overall genetic population structure was highly significant at both the global and regional scale (F_{ST} = 0.0858 and 0.0091, respectively). Pairwise comparisons indicated four distinct groups at the global scale: northeast Pacific, northwest Pacific, south Pacific, and South Africa. However, the pairwise results at the regional scale were not as clear, but may indicate differences between yellowtail found nearshore versus offshore. Combined phylogentic analyses using two mitochondrial and four nuclear genes strongly support at least 3 evolutionarily distinct groups among the global samples, supporting previous taxonomic hypotheses that *S. lalandi* is a complex of three regional species (*S. aureovittata, S. dorsalis, S. lalandi*). By creating the framework for a global genetic diversity monitoring program, substantial progress can be made towards establishing yellowtail as a commercially viable aquaculture species on the west coast.

Strategy for breeding study of fisheries organisms by FRA

Hiroyuki Okamoto*

Selective and cross breeding of ornamental fish (goldfish, carps) is thought to have started several hundred years ago in Japan, but breeding of edible marine fish has just started in about a half of hundred years. Recent progresses of seed production techniques and of molecular biological techniques are increasing expectations to genetic improvement of cultured species, that is facing high price of feeds, high cost for disease prevention, low farm gate price and many other problems. For effective and efficient promotion of aquatic breeding studies which lead to accurate measures against problems in actual culture sites, the Fisheries Research Agency of Japan announced "Strategy for breeding study of fisheries organisms" on March 2013, through several discussions and cooperation with prefectural institutes, universities, governmental officials and private corporations. The strategy consists of two parts, namely, "Present situation of research and development of aquatic breeding study" and "Direction and policy for promotion of aquatic breeding study". We introduce the latter parts of the strategy in this paper.

As a fundamental concept, we should decide on priority in industrially large –scale and widely spread species, while strains adapting to regional environments are expected to be made by each prefectural institute. Development of fundamental technology, evaluation methods of traits and preservation methods of genetic resources are thought to be necessary as schemes for promotion of aquatic breeding. In addition, improving cooperative system of R&D, provisions for protection of intellectual property and consideration to natural environment are thought to be necessary to enhance improved strains in actual culture sites.

Identification of Quantitative Trait Loci (QTL) and Marker-Assisted Selection (MAS) by using genomics information in yellowtail (*Seriola quinqueradiata*)

Akiyuki Ozaki*, Jun-ya Aoki, Kazuo Araki, Kazuki Akita, Satoshi Kubota, Takashi Koyama, Takashi Sakamoto, Kazunori Yoshida, Takurou Hotta, Tsutomu Noda, Hirotaka Mizuochi, Yasuhiro Shima

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.

Benedenia infections caused by the monogenean fluke ectoparasite *Benedenia seriolae* seriously impact marine finfish aquaculture. We have discovered the evidence that contributes to detailing the phenotypic resistance to Benedenia disease in yellowtail (*Seriola quinqueradiata*). Two putative quantitative trait loci (QTL) associations, of medium to large effect of with Benedenia disease resistance, were localized to linkage groups Squ2 and Squ20. Finding the QTL region strongly supports the potential for success of marker-assisted selection (MAS) for resistance to Benedenia disease. The aim of this study was to confirm the QTL significant region and compare the susceptibility depend on the difference of linkage disequilibrium (LD) block for Benedenia disease resistance in yellowtail, for use in MAS to increase the rate of genetic improvement for this trait.

Two major QTL regions (*BDR-1, BDR-2*) were employed for MAS in F_1 siblings from QTL selection candidates to produce F_2 full-sib families. F_1 siblings were placed in two groups which are according to whether or not they have inherited the QTL significant LD about Benedenia disease resistance. Two crossbreed types of F_2 full-sib families were established by one-on-one crossing from F_1 siblings. The families were putative resistant families (R-families) whose parents were selected for their inheritance of QTL significant LD block, and the other families were putative susceptive families (S-families) whose parents were selected for not having inherited the QTL significant LD block.

In artificial infection experiment, F_2 R-family and F_2 S-family were placed in each tank with hatched *B. seriolae*. We had performed infection experiment in 6 times. All infection experiment

showed the same result, the F_2 R-family fish had significantly fewer parasites than the F_2 S-family fish. In natural infection condition, all eight rounds were showed the significant results. The F_2 R-family fish had significantly fewer the number of parasites than the both F_2 S-family fish and the F_1 progeny from the wild parental fish.

We employed LD blocks based on QTL significant region for MAS, and confirmed their effects in subsequent F_2 full-sib families. Furthermore, it means QTL significant regions are identified from these wild ancestors to the breeding population. It would be possible to introgression certain properties of the wild population in the breeding population. These results indicate that it is possible to rapidly develop domesticated strains having commercially important traits by MAS in aquaculture.

Genetic and physiological studies of health and fitness in aquaculture-reared California yellowtail, *Seriola lalandi*

Catherine Purcell*, Nicholas Wegner*, and John R Hyde

The California yellowtail (*S. lalandi*) is a likely candidate for the future development and expansion of offshore commercial aquaculture in the southern California region. However, larval rearing methods for this and other *Seriola* species are still unreliable, resulting in highly variable survival rates and prevalence of physical malformations. The causes of, and solutions for, the variability and abnormalities in the larvae remain largely unresolved. Several factors have been associated with these abnormalities, such as nutritional imbalances (in the larvae and/or broodstock), culture conditions (e.g. light, temperature, salinity), and genetic background of the parents. To better understand the connection between individual brood fish and abnormal offspring, and to gain insight into possible causes of the growth variation and the deformities, we conducted genetic analyses of samples of juvenile fish exhibiting deformities and extreme size variation. Juvenile *Seriola lalandi* (43 to 50 days post hatching) were collected from three production runs at HSWRI in the summer/fall of 2012.Three different types of juvenile fish were collected: 1) small and large fish (representing juveniles with different growth rates, 2) deformed fish (this included various abnormalities), and 3) randomly sampled fish (not sorted). Genetic samples from the 21 broodstock individuals were also collected. Genetic markers (16 nuclear microsatellites already optimized for this species) were used to evaluate parent-progeny relationships to test whether observed fitness traits are associated with pedigree. In addition, sustained swimming capacity and oxygen consumption rates were measured for randomly sampled juvenile yellowtail (16 – 19 cm fork length) as additional fitness measures and compared to the performance of wild caught juveniles. Analyses are ongoing, but genetic analyses of juveniles sampled from HSWRI show significant differences in parental contribution between offspring with deformities, growth variation, and the randomly sampled offspring. Aquaculture-reared yellowtail also showed significantly lower maximum sustainable swimming speeds and higher oxygen consumption rates than wild caught individuals, indicating reduced fitness in farm-raised fish. Understanding the genetic and physiological processes contributing to yellowtail seed stock quality is of considerable value to hatchery and aquaculture facilities rearing this species. Information gained in this study will be used to increase hatchery production, which will facilitate the culture of yellowtail at a much larger and cost-effective scale.

The de novo draft assembly of the yellowtail, *Seriola lalandi*, genome

Catherine Purcell*, Andrew Severin, and John R Hyde

Seriola species (*S. dumerili, S. lalandi, S. rivoliana, S. quinqueradiata*), collectively known as amberjacks, are fish of particular interest to the growing aquaculture industry due to their high value, forming a billion dollar plus component of the sashimi industry. Of these species, the native California *Seriola lalandi*, is considered a prime candidate for aquaculture development in southern California. In developing aquaculture for this species, methods to improve culture efficiency and effectiveness are of great interest. Genetic resources have been developed and used extensively in agriculture and livestock to improve product quality and quantity, and only more recently have these approaches been applied to select aquaculture species (e.g. rainbow trout, tilapia, catfish, flounder, and Atlantic cod). Previously technology costs were prohibitive for many species; however accessibility has improved through decreasing sequencing costs and enhanced bioinformatics analyses. With the improved accessibility to this technology, we are working to develop genetic resources for *S. lalandi* to help improve aquaculture techniques. In a collaborative effort between the SWFSC and Genome Informatics Facility at Iowa State University, we have created a de novo draft assembly of the genome for *Seriola lalandi*. DNA sequencing was conducted on juvenile *S. lalandi* using mate-paired and paired-end reads with the Illumina HiSeq 2500, resulting in approximately 1.2 billion raw reads, for estimated 160X coverage of the 685Mb genome. Two programs were used to assemble the sequencing reads, ALLPATHS-LG (v1.1) and MaSuRCA. MaSuRCA resulted in a greater number of scaffolds (n = 86,357) than ALLPATHS-LG (n = 2,460), and similarly more contigs (n = 102,628 and n = 40,683, respectively). However, the longest scaffold size was nearly three times larger using MaSuRCA; 14,669,447 bp versus 5,745,343 bp. The N50 scaffold length was also greater with MaSuRCA (N50 = 2,145,274) than with ALLPATHS-LG (N50 = 900,636), as was the N50 contig length (N50 = 171,508 and 36,582, respectively). Genes were annotated using Maker2. A web portal (Genome Browser) to directly view the genome, gene annotations, gene models, and markers was also created to provide a publically available resource. This study represents the first draft genome for *Seriola lalandi*. When correctly applied, this genomic approach has the potential to improve broodstock selection through identification of genes underlying complex and/or economically important traits, characterization of variation (both beneficial and detrimental), and marker-assisted selection for this and other *Seriola* species. Investment in the genetic resources for *Seriola* will contribute to making aquaculture practices more economically viable, and will help improve domestic seafood production for these valuable finfish.

Assessment of fertilization ability of cryopreserved sperm in fish using interspecific hybridization

Yukinori Shimada*, Hiroyuki Okamoto, Hiroyuki Nagoya, and Toshiya Yamaguchi

Yellowtail, *Seriola quinqueradiata*, is the most farmed species in Japan. However, most of yellowtail farming depends on wild seedling because artificial seedling production of this species is not easy. Fisheries Research Agency 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 high growth) in yellowtail. Fish farming, including yellowtail, has been threatened with the loss of strain by fish disease and red tide because most of them are farmed in sea cages. Therefore, it is important to develop novel preservation method that is independent of

biological preservation, and to be able to restore the preserved strain whenever. In this species we are planning to develop 1) cryopreservation of sperm, 2) cryopreservation of germ cell (e.g. spermatogonia and oogonia) and 3) production method of eggs using the germ cell transplantation in fish.

As the first step, we developed the cryopreservation method of sperm in yellowtail, and assessed its fertilization ability using eggs of other fish species, longtooth grouper *Epinephelus bruneus* , collected easier (but in case of our group). Yellowtail sperms were obtained from three males. Those sperms were prepared to cryopreserved-sperm as soon as possible based on different concentration of dimethyl sulfoxide (DMSO) solution (1, 5, 10, 15 and 20%) with artificial seminal plasma (ASP), respectively. Active sperm after thawing was assessed by rate of sperm motility and duration of sperm motility (observation of max. 10 minutes) after 24 hours. We performed interspecific hybridization between longtooth grouper and yellowtail, and then fertilization ability of cryopreserved-sperm was calculated by the rate of fertilization, embryogenesis and hatching. Hatched larvae were fixed with 70% ethanol after nuclear DNA treatment, and assayed ploidy of longtooth grouper, yellowtail and their hybrid using flow cytometer.

Cryopreserved-sperm of 10% DMSO/ASP solution was showed in the highest sperm motility as compared with those of 1-20% DMSO/ASP solutions, and it corresponds to about 80% of the pre-freeze. On the other hands, those of 1 and 5% DMSO/ASP solutions were showed in the aggregation of sperm after thawing, and their rates of sperm motility were very low (less than 3%).

The hybrid between longtooth grouper and yellowtail was developed and hatched. The rates of fertilization, embryogenesis and hatching in hybrid were 39.5, 2.5 and 0.8%, respectively, while those in longtooth grouper were 51.5, 20.9 and 19.9%, respectively. Hybrid was shown in lower rates of embryogenesis and hatching than longtooth grouper, but hybrid on rate of fertilization was comparatively high. Relative genome size in hybrid was shown being diploid according to intermediate value between both species. That is, it is suggested that cryopreserved-sperm of yellowtail can be assessed its fertilization ability by rate of fertilization. Thus, it is possible to assess fertilization ability of sperm using easily egg-collected fish species in other species.

Selective breeding research at USDA ARS

Jeffrey T Silverstein*

There is great potential for intensifying aquaculture production. Selective breeding to improve production efficiency is an important tool and the potential to modify performance traits through selective breeding is considerable. The development of successful selective breeding programs for aquaculture is a long-term commitment. While the gains from selective breeding are permanent and cumulative, initiation of breeding programs often require government support in the early stages, with regular input from industry. The aquaculture program within the US Department of Agriculture, Agricultural Research Service has supported development of numerous selective breeding programs for food fish and shellfish species using a variety of methods including mass selection, family selection and crossbreeding both intra-specific crossbreeding-different strains, same species; and interspecific cross-breeding-different

species. I will highlight aspects of the development of genetic improvement programs for catfish, rainbow trout, Atlantic salmon, yellow perch, striped bass and oysters. While growth performance is a trait of primary concern in most programs, other traits have gained importance, too. With catfish, the recent development of methods for mass production of the hybrid catfish (blue catfish male x channel catfish female) has raised the importance of reproductive performance. Atlantic salmon selective breeding programs are some of the most advanced in the world (Norway, Chile, Iceland, Scotland), however, due to their listing as endangered species in North America, continent of origin has become a fundamental criterion for breeding. Disease resistance, the ability to withstand specific pathogens and have higher survival, has been a critical focus for rainbow trout and other species as well. Product aspects such as greater fillet yield are being targeted; and the possibility to selectively breed for greater ability to convert and deposit long chain n-3 fatty acids is an intriguing target for selective breeding, too. In addition to reviewing progress in these projects, I will discuss some of the research and breeding strategies that have been adopted recently to maximize industry relevance and to incorporate genomic information into our genetic improvement programs.

Plans, Challenges and Opportunities for Warm Water Aquaculture and the Role of the Southwest Fisheries Science Center

Russ Vetter*, John R Hyde and Cisco Werner

Each region of the US EEZ provides unique challenges and opportunities for marine aquaculture. Access to markets, regulatory frameworks, competing ocean uses, water quality and prevalence of extreme weather events are all part of considerations for the development of a successful industry. The southwest portion of the western US coastline and adjoining waters in Mexico are characterized by: a. minimal continental shelf habitat, b. low terrestrial runoff and nutrient loading, c. reliable water exchange via currents, upwelling and tidal mixing, and d. low prevalence of extreme weather events. Natural features of the southwest US coupled with a diverse and educated urban population provides economic opportunities for the marketing of high value aquaculture products. It also requires strict adherence to the highest standards of seafood quality and a minimum of adverse environmental impacts. Siting of aquaculture facilities in federal waters (beyond 3 miles) provides access to high quality habitat but is limited by the availability of locations suitable for moorings.

The Southwest Fisheries Science Center (SWFSC) is interested in partnering with private entrepreneurs, academic researchers, and State and international regulatory partners to explore opportunities to increase seafood supply, economic activity and job creation while minimizing the impacts to the marine ecosystem. The SWFSC and academic partners are particularly strong in Fisheries Oceanography, Protected Resources Monitoring, Biotechnology and Economics. In this presentation we will discuss current and planned research opportunities and how they relate to site selection, impacts evaluation and brood stock improvement. The California Cooperative Fisheries Investigations (CalCOFI) has maintained an ocean-observing program that has routinely sampled water conditions in the California Current since 1949 and is providing baseline information and predictive modeling data for site selection and evaluation of impacts. Likewise the Southern California Ocean Observing System (SCOOS) is providing real-time observations for impacts evaluation. Along with site impacts, successful aquaculture must document and maintain natural genetic structure and genetic diversity while addressing issues

associated with disease, parasitism and artificial diet in culture. Genomic approaches are being developed for abalone species and *Seriola* species to provide a baseline record of natural genetic variance and a means of monitoring genetic changes in brood stocks. Economic studies focus on individual economics but more important evaluate systemic impacts and also explorations of the "transfer effect", the ecological and protected resources consequences of our reliance on wild capture seafood imports. Protected resources surveys provide information on migratory pathways and areas of high occupancy and can be used to guide site selection and monitor interactions via passive and active acoustics.

Development of integrated multi-trophic aquaculture using sea cucumber

Satoshi Watanabe*, Masashi Kodama, Joemel G Sumbing, and Ma JH Lebata-Ramos

Continuous intensification of aquaculture production has brought about environmental issues associated with eutrophication worldwide. Environmental deterioration such as hypoxia and sulfide production due to water and sediment eutrophication by aquaculture effluent has been problematic, entailing sporadic disease outbreaks and fish kills. Integrated multi-trophic aquaculture (IMTA) is one of the promising measures for sustainable aquaculture and a supplementary income source to the fish farmers. IMTA is a polyculture system that integrates culturing of fed species (e.g. finfish) the main commodity, organic extractive species (e.g. deposit and filter feeding benthos) and inorganic extractive species (e.g. seaweed). We have been conducting a research to establish IMTA techniques for small-scale coastal fish farmers in the Philippines, with sea cucumber (*Holothuria scabra*, commonly known as sandfish), as the key species. Sandfish commands the highest price in tropical sea cucumber species. Nitrogen (N) budget of sandfish in polyculture with milkfish (*Chanos chanos*) and Elkhorn sea moss (*Kappaphycus alvarezii*), both of which are commonly cultured in the Philippines, was estimated using a simple closed box model.

Information on stocking density, stocking size, mortality, growth, feed ration, feed assimilation, NH_4-N production and NH_4-N absorption of these species was obtained from a series of experiments and existing literatures. Culturing conditions assumed as follows: 26 g milkfish were cultured in a 5 x 5 x 4 m cage at the stocking density of 36.7 ind/m^3 with the initial feeding ration of 10% of body weight which was gradually decreased to 4% over time; 10 g sandfish were cultured in a 5 x 5 x 0.3 m cage hanged under the milkfish cage to trap particulate N waste (i.e. feces and leftover feed) from the milkfish culture at the stocking density of 35 ind/m^2; the stocking weight of Elkhorn sea moss line culture was 10 kg; culturing period was 200 days.
It was estimated that milkfish culture cumulatively produced 145 kg of particulate N, and milkfish and sandfish together produced 60 kg of NH_4-N in 200 days of culture. Daily assimilation rate of the particulate N by sandfish ranged 3.4 - 12.4%, and 6.4% of the particulate N was estimated to be removed by sandfish in 200 days of culture. Daily absorption rate of NH_4-N by Elkhorn sea moss increased exponentially with time and reached 100% on the 125 days of culture. Cumulative NH_4-N was estimated to be depleted by 162 days of culture. For complete utilization of particulate N by the end of culture, sandfish stocking density should be 805 ind/m^2, which is 200 times as high as that in existing sandfish aquaculture operations.

Demands for infertility of cultured marine fishes and study of UV irradiation at developmental stages in Japanese flounder

Toshiya Yamaguchi* **and Koichi Okuzawa**

Various kinds of marine fish species are produced in Japan. There has been increasing needs for the technique to sterilize farmed fish, because the sterilization of cultured fish could protect the improved strains, prevent undesired gonadal development that often result in the deterioration of meat quality, and prevent possible negative genetic impact on wild populations. In this way, sterilization of farmed fish is benefitial not only to the aquaculture industry but also for the environmantal conservation. However, the applicable techniques for sterilization has not been established in cultured marine fish.

The germ cell has an important role to transmit the genomic information to the next generation. It is known that the germ cells develop into sperm in males and eggs in females and they starts with germ lineage specification during early embrionic development and concludes in adulthood with gamete differentiation. Therefore, in this study, we try to destroy the germ cells using ultraviolet (UV) irradiation on fertilized egg of Japanese flounder, *Paralichthys olivaceus*.

We examined the effects of irradiation of fertilized eggs on the hatching rate. The intencity of UV ranged from 0 to 200 mJ (Joule) and the timing of irradiation were set at 4, 12, 28, 52 hours after fertilization. The UV was applied by UV cross linker. Furthermore, the hatched larvae were reared until 55 days after hatching (dah), and the juveniles were fixed in Bouin's solution, dehydrated in graded ethanol, embedded in paraffin, sectioned serially at 5 μm, stained hematoxylin-eosin, and observed gonads using light microscope. We also examined the expressions of both *vasa* and *sycp3* (germ cell marker) mRNA in gonads using *in situ* hybridization that indicated the presence of germ cells in the gonads.

The hatching rate decreased as the intencity of UV radiation increased. The microscopic observation of the gonads revealed the abnormal gonads in the UV-irradiated group. The expression of *vasa* and *sycp3* mRNA was observed in the gonads of the control group, which indicates presence of germ cells. On the other hand, the expression of *vasa* and *sycp3* mRNA was not detected in the gonad area of some of the UV irradiation individual. These results indicate that germ cells may be deleted by UV irradiation of fertilized egg, which in turn the flounder may became infertile.