44th Scientific Symposium of the UJNR Aquaculture Panel

Genetics and Breeding in Aquaculture

NOAA Northwest Fisheries Science Center
2725 Montlake Blvd. East
Seattle, WA
November 1st and 2nd, 2016

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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. Climate change will present many challenges to aquacultured species and genetics and genomics will prove to be valuable tools in addressing these. We hope to encourage broad ranging discussions of some of the new approaches being developed and how they might be applied in collaborative research efforts to resolve key bottlenecks and facilitate aquaculture industry expansion in Japan and the United States.

Program

Tuesday, November 1, 2016

Registration 13:00 - 13:30

Opening Session and Orientation to the Northwest Fisheries Science Center

Welcome
John Stein, Director, NOAA Northwest Fisheries Science Center 13:30 - 13:40

Aim of the Symposium
Michael Rust, US Panel Chair, NOAA Fisheries Office of Aquaculture 13:40-13:50

Ecosystem Approach to Aquaculture
(Moderators: Paul Olin and Takuro Shibuno)

Integrated Multi-Trophic Aquaculture (IMTA) as a countermeasure for coastal oligotrophication
Satoshi Watanabe, National Research Institute of Aquaculture,
Fisheries Research and Education Agency 13:50-14:15

Interactions between shellfish aquaculture and the environment in the Northeastern US
Matt Poach, NOAA Northeast Fisheries Science Center 14:15-14:40

Land-based aquaculture for red-spotted grouper
Masatsugu Takano, Fisheries Research and Education Agency Headquarters 14:40-15:05

Using physiological tools to assess and optimize aquaculture of California Yellowtail, *Seriola dorsalis*
Nick Wegner, NOAA Southwest Fisheries Science Center 15:05-15:30

Wednesday, November 2, 2016

Breeding Part 1
(Moderators: Andrew Severin and Masatsugu Takano)

Paradigm shift in fish breeding; marker-assisted selection to genomic selection
Akiyuki Ozaki, National Research Institute of Aquaculture,
Fisheries Research and Education Agency 9:00-9:25

Genomic analyses to inform sablefish aquaculture research
Krista Nichols, NOAA Northwest Fisheries Science Center 9:25-9:50

Mutagenesis and genome editing for aquaculture fish species
Hiroyuki Okamoto, National Research Institute of Aquaculture,
Fisheries Research and Education Agency 9:50-10:15

Break 10:15-10:45

Breeding Part 2
(Moderators: Caird Rexroad and Satoshi Watanabe)

Genome-enabled selection doubles the accuracy of predicted breeding values for bacterial cold water disease resistance compared to traditional family-based selection in rainbow trout aquaculture
Roger Vallejo, US Department of Agriculture, Agriculture Research Service 10:45-11:10

Genetic improvement of Atlantic salmon (*Salmo salar* L.) and Eastern oyster (*Crassostrea virginica* Gmelin 1791) at the USDA-ARS National Cold Water Marine Aquaculture Center
Gary Burr, US Department of Agriculture, Agriculture Research Service 11:10-11:35

The Molluscan Broodstock Program: twenty years of selective breeding of the Pacific oyster on the West Coast of the US
Romain Morvezen, Oregon State University 11:35-12:00

Lunch Break 12:00-13:00

Genomics and Epigenetics, Part 1
(Moderators: Krista Nichols and Hiroyuki Okamoto)

***Seriola* genomics and the knowledge repository Serioladb.org**
Andrew Severin, Iowa State University 13:00-13:25

The first meeting of *Seriola* genomics and breeding consortium
Akiyuki Ozaki, National Research Institute of Aquaculture,
Fisheries Research and Education Agency 13:25-13:50

Uses of genetic parentage analysis in cultured California Yellowtail (*Seriola dorsalis*)
Matthew Craig, NOAA Southwest Fisheries Science Center 13:50-14:15

Break 14:15-14:45

Genomics and Epigenetics, Part 2
(Moderators: Roger Vallejo and Akiyuki Ozaki)

Past, present and future research on the Ostreid herpes virus - 1 infections in the Pacific oyster
Caroline Friedman, University of Washington 14:45-15:10

Type 1 Ostreid Herpesvirus (OsHV-1) variants in Japan
Satoshi Watanabe, National Research Institute of Aquaculture,
Fisheries Research and Education Agency 15:10-15:35

Characterization of genetic and epigenetic variation in hatchery and natural-origin steelhead, *Oncorhynchus mykiss*
Mackenzie Gavery, University of Washington 15:35-16:00

Towards a functional understanding of DNA methylation in shellfish and implications for aquaculture
Steve Roberts, University of Washington 16:00-16:25

Open Discussion: Development and support of research collaborations
Moderators: Mike Rust and Fuminari Ito 16:25-16:50

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

Symposium Reception: One on one discussions continue 17:30-19:00
Ivar's Salmon House (see below)

List of Participants

Burr, Gary	USDA Agricultural Research Service (ARS)
Craig, Matthew	NOAA Southwest Fisheries Science Center (SWFSC)
Dumbauld, Brett	ARS
Friedman, Carolyn	University of Washington (UW)
Gavery, Mackenzie	UW
Ito, Fuminari	Japan Panel Chair, Japan Fisheries Research and Education Agency, National Research Institute of Aquaculture (FRA)
Morvezen, Romain	Oregon State University
Nichols, Krista	NOAA Northwest Fisheries Science Center
Okamoto, Hiroyuki	FRA
Okumura, Takuji	FRA
Olin, Paul	California Sea Grant
Otoshi, Clete	NOAA Fisheries Office of Aquaculture (AQC)
Ozaki, Akiyuki	FRA
Poach, Matt	NOAA Northeast Fisheries Science Center
Rexroad, Caird	ARS
Roberts, Steve	UW
Rust, Mike	US Panel Chair, AQC
Severin, Andrew	Iowa State University
Shibuno, Takuro	FRA
Takano, Masatsugu	FRA, Headquarters
Vallejo, Roger	ARS
Watanabe, Satoshi	FRA
Wegner, Nick	SWFSC

44th Scientific Symposium of the UJNR Aquaculture Panel

Genetics and Breeding in Aquaculture

Symposium Abstracts

1. *Integrated multi-trophic Aquaculture (IMTA) as a countermeasure for coastal oligotrophication*

Satoshi Watanabe[*1], Natsuki Hasegawa[1], Yuka Ishihi[1], Tomomi Mizuno[2] and Junya Higano[3]

Presenting author*

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[2]Mie Prefecture Fisheries Research Institute, 3564-3 Hamajima, Shima, Mie, 517-0404, Japan,
[3]National Research Institute of Fisheries and Environment of Inland Sea, Japan Fisheries Research and Education Agency, 2-17-5 Maruishi, Hatsukaichi, Hiroshima 739-0452, Japan

Abstract
Intensification of aquaculture production has caused environmental deterioration associated with water and sediment eutrophication and consequent harmful algal bloom, hypoxia and sulfide production, resulting in sporadic mass fish kills in some parts of the world. In contrast, eutrophication has been subsiding in coastal waters of Japan due to reduced production and improved feeding efficiency of finfish aquaculture and/or enforcement of the total pollutant load control system (TPLCS, issued by the Ministry of the Environment in 1979) that regulates the allowable amount of terrestrial nitrogen/phosphorus discharge to the sea. Red seabream, *Pagrus major*, aquaculture production, for instance, peaked in the early 1990's and has been in a decreasing trend since 2000 in Japan. The levels of dissolved inorganic nitrogen (DIN, i.e. sum of NH_4^+, NO_2^-, NO_3^-) have gradually decreased, while phosphorus levels have been rather constant in Ise Bay since the 1990's, possibly causing a nutrient imbalance for primary production. Surface chlorophyll *a* levels have been in a decreasing trend since the 1990's, and the occurrence frequency of red tide has decreased by 90% since 1979 in Ise Bay. The oligotrophication (relative to the past) of coastal waters is considered to have brought about a decline in carrying capacity of the coastal environment, causing, in part, a continuous decrease in some coastal fishery resources and reduced productivity in molluscan and algal aquaculture. The national production of the Manila clam, *Ruditapes philippinarum*, for example, has continuously declined for thirty years, falling below 10% of the peak value marked in the mid 1980's. In Ariake Bay, DIN deficiency causes bleaching of cultured nori, *Pyropia yezoensis*, severely reducing the market value especially towards the end of the culture season in early spring. Integrated multi-trophic aquaculture (IMTA) is an aquaculture approach that combines culture of economically important species from different trophic levels, typically finfish, organic extractive species (e.g. bivalve) and inorganic extractive species (e.g. seaweed). Along with reinforcement of economic stability, one of the important goals of IMTA is to mitigate effluent load from

finfish culture for environmental integrity. In this study, however, we are trying to develop techniques to use IMTA to compensate for the reduced aquaculture productivity of bivalves and seaweeds associated with the coastal oligotrophication. We found that chlorophyll *a* level was high in the areas affected by red seabream aquaculture in the semi-closed Hasamaura Cove in Mie Prefecture. Cultured green alga, *Monostroma nitidum* (Japanese common name Hitoegusa), was found to have twice as high a nitrogen content in the cove than in those cultured in nearby areas without finfish aquaculture. The amount of NH_4^+-N excretion from the red seabream culture in the cove was estimated to be 7.5 t/yr. This is equivalent to the amount of N in 1872 t/yr. (wet weight) of Manila clam suspended culture production. The amount of N extracted by *M. nitidum* culture in the cove (264 kg/yr.) is equal to 3.3% of the fish NH_4^+-N. Thus, IMTA has a big potential to enhance aquaculture production by using what would otherwise be fish waste.

Annotated Bibliography of Key Works

Chopin, T., Yarish C., Wilkes, R., Belyea, E., Lu, S. and Mathieson, A. 1999. Developing Porphyra/salmon integrated aquaculture for bioremediation and diversification of the aquaculture industry. *J. Appl. Phycol.* 11: 463–472.

This is one of the first papers on IMTA (referred to as integrated culture) combining salmon cage culture and *Porphyra* (currently *Pyropia*) species culture to alleviate the seasonal nutrient depletion by using the significant loading of salmon farms, which is then valued (wastes become fertilizers) and managed (competition for nutrients between desirable algal crops and problem species associated with severe disturbances). The development of integrated aquaculture systems is a positive initiative for optimizing the efficiency of aquaculture operations, while maintaining the health of coastal waters.

Watanabe S., Kodama, M. and Fukuda, M. 2009. Nitrogen stable isotope ratio in the manila clam, *Ruditapes philippinarum*, reflects eutrophication levels in tidal flats. *Mar. Poll. Bull.* 58: 1447–1453.

The authors revealed that the nitrogen stable isotope ratio (^{15}N) in the soft tissues of the manila clam, *Ruditapes philippinarum*, could be used as an indicator of anthropogenic eutrophication levels in tidal flat environments. In addition, they found that the acid insoluble fraction of the shell organic matrix, conchiolin, could be used as a proxy for the soft tissues in ^{15}N analyses, which will result in easier storage handling and the expansion of chances for sample acquisition. Understanding the effects of anthropogenic eutrophication on coastal fisheries may help in the enhancement of fishery production by effective utilization of sewage effluents, as well as in the consequent reduction of eutrophication.

2. *Interactions between shellfish aquaculture and the environment in the Northeastern US*

Matthew Poach[1]*, Bob Alix[2], Paul Clark[2], April Croxton[2], Mark Dixon[2], Cathy Kuropat[2], Judy Li[2], Renee Mercaldo-Allen[2], Shannon Meseck[2], Lisa Milke[2], Dylan Redman[2], Julie Rose[2], Barry Smith[2], Gary Wikfors[2].

[1] US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, James J. Howard Marine Sciences Laboratory, Highlands, NJ 07732, USA.

[2] US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Milford Laboratory, Milford, CT 06460, USA.

Abstract
Opposition to new shellfish aquaculture operations in the Northeastern US frequently is based upon the premise that these commercial operations have negative environmental consequences. This perception is not consistent with the myriad of ecosystem services that are attributed to shellfish, including improving water clarity, reducing excess nutrients, providing habitat, stabilizing sediments, and serving as a food for wildlife and humans. Researchers at the Milford Laboratory have worked to quantify the complex interactions between shellfish aquaculture and the natural environment across the Northeastern United States region and beyond.

From 2008-2010 experiments were conducted in East Creek, Long Island, New York to quantify the environmental footprint of a floating upweller system (FLUPSY) within the surrounding embayment. Metrics such as dissolved nutrients, phytoplankton abundance and community composition, chlorophyll *a*, temperature, salinity, and dissolved oxygen were measured. Results of the multi-year study indicate that the FLUPSY had a very small role in ecosystem function compared with the range of natural environmental variation within the East Creek System. From 2009 to 2013 studies were conducted to measure the consequences of hydraulic dredging for clam harvest upon sediment chemistry and the benthic community in coastal Milford Connecticut. Milford Laboratory data indicated that hydraulic dredging caused initial, short-lived changes in sediment chemistry that resolved within a few weeks. Disturbance of the benthic community was minimal, and the community that inhabited this nearshore zone was naturally resilient to a dynamic physical environment.

Milford research also has quantified the environmental benefits of shellfish aquaculture. Starting in 2011, the biodeposition method was employed to measure nitrogen removal and water clarity improvements by shellfish throughout New England and on the west coast of the Korean peninsula. US study locations have included NY, CT, RI and MA and species included ribbed mussels, blue mussels, clams and oysters. Research indicated that shellfish are able to adapt to a wide range of environmental conditions, but careful site selection is needed to maximize environmental benefits of aquaculture operations. Ultimately this work demonstrates that shellfish aquaculture can have a positive role in eutrophic ecosystems. We plan to continue our work on interactions between aquaculture and the environment by expanding into multi-trophic and off-shore aquaculture systems in New England.

Annotated Bibliography of Key Works
Li, Y., Meseck, S. L., Dixon, M. S., Rivara, K., and Wikfors, G. H. (2012). Temporal Variability in Phytoplankton Removal by a Commercial, Suspended Eastern Oyster Nursery and Effects on Local Plankton Dynamics. *Journal of Shellfish Research*, *31*(4): 1077-1089.

Meseck, S.L., Li, Y., Dixon, M.S., Rivara, K., Wikfors, G.H. and Luther, G.I. (2012). Effects of a Commercial, Suspended Eastern Oyster Nursery upon Nutrient and Sediment Chemistry in a Temperate, Coastal Embayment. *Aquaculture and Environment Interactions 3*: 65-79.

Li, Y., Meseck, S., Dixon, M., Rose, J., Smith, B., and Wikfors, G. (2013). Short Term Effects of a Commercial Eastern Oyster Nursery upon Nutrient and Plankton Dynamics of a Coastal Embayment: Observations from Mesocosm Experiments. *Aquaculture Research*, In press.

Mercaldo-Allen, R., Goldberg, R., Clark, P., Kuropat, C., Meseck, S.L., Rose, J. M. 2016. Benthic Ecology of Northern Quahog Beds with Different Hydraulic Dredging Histories in Long Island Sound. Journal of Coastal Research 32(2):408-415.

Meseck, S.L., Mercaldo-Allen, R., Rose, J.M., Clark, P., Kuropat, C., Pereira, J.J., Goldberg, R. 2014. Impacts of Hydraulic Dredging for *Mercenaria mercenaria*, Northern Quahog, on Sediment Biogeochemistry. Journal World Aquaculture Society 45(3): 301-311.

Goldberg, R., Rose, J.M., Mercaldo-Allen, R., Meseck, S.L., Clark, P., Kuropat, C., and Pereira, J.J. 2014. Effects of hydraulic dredging on the benthic ecology and sediment chemistry on a cultivated bed of the hard clam, *Mercenaria mercenaria*. Aquaculture 428–429: 150–157

Goldberg, R., R. Mercaldo-Allen, J. M. Rose, P. Clark, C. Kuropat, S. Meseck, and J. Pereira. 2012. Effects of hydraulic shellfish dredging on the ecology of a cultivated clam bed. Aquaculture Environment Interactions 3:11-21.

Galimany, E., Rose, J.M., Dixon, M.S., Wikfors, G.H., 2013. Quantifying Feeding Behavior of Ribbed Mussels (*Geukensia demissa*) in Two Urban Sites (Long Island Sound, USA) with Different Seston Characteristics. Estuaries and Coasts, 1-9.

Rose, J.M., Bricker, S.B., Tedesco, M.A., Wikfors, G.H., 2014. A Role for Shellfish Aquaculture in Coastal Nitrogen Management. Environmental Science & Technology 48, 2519-2525.

Rose, J.M., Bricker, S.B., Ferreira, J.G., 2015. Comparative analysis of modeled nitrogen removal by shellfish farms. Marine Pollution Bulletin 91, 185-190.

3. *Land-based aquaculture of red-spotted grouper（Epinephelus akaara）using the closed recirculation system*

Masatsugu Takano[1]*, Tetsuo Morita[2], Tadashi Imai[2] and Yoshihisa Yamamoto[2]

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[2]National Research Institute of Fisheries and Environment of Inland Sea, Japan Fisheries Research and Education Agency, Yashima-higashimathi 234, Takamatsu, Kagawa 761-0111, Japan

Abstract
The red-spotted grouper, *Epinephelus akaara,* is a commercially expensive fish in Japan. This species is expected to be a new target fish for aquaculture. However, its culture is thought to be difficult because of its slow growth (four or five years until the fish are of commercial size).

To achieve faster growth and higher feed efficiency for the red-spotted grouper, we experimented with different water temperatures using a closed recirculation system. In higher water temperatures between 16 and 31 degrees Celsius, growth was faster; best feed efficiency was obtained at 25 degrees Celsius. We carried out similar experiments with salinity. These results reveal that it is possible to rear the red-spotted grouper to commercial size in approximately two years.

The more traditional flow-through system is expensive to maintain in optimum condition. The closed recirculation system solves this problem by reusing drainage. However, it needs to remove harmful ammonia, suspended materials and the residual material. We have improved the efficiency of two filter devices (the biological filter and the bubble separation device); these devices were built into the system. The closed recirculation system is expected to assist the expansion of land-based aquaculture and find applications in breeding research.

Annotated Bibliography of Key Works

Yamamoto, Y. and Hayase, S., 2008. Japan, Thematic regional reviews, In The future of mariculture: a regional approach for
responsible development in the Asia-Pacific region. *FAO Fisheries Proceedings*, 11, 189-198.

Yamamoto, Y. and Masaaki Kamoshida and Toshio Takeuchi, 2013. Examination of Suitable Condition of Recirculation Rate in a Closed Recirculating System for Larviculture of Red Sea Bream, *Pagrus major*. *Eco-Engineering*, 25(2), 49-58.

Tetuso Morita, 2014. Potential for circulating land farming business of red spotted grouper *Epinephelus akaara.* Aqua Culture Business, Volume: 51, Issue: 13, 14-16.

Tomohide Nambu, 2014. II-7. Seed production and aquaculture technology of red spotted grouper *Epinephelus akaara*. Nippon Suisan Gakkaishi, 80(6), 1000.

4. *Using physiological tools to assess and optimize aquaculture of California Yellowtail, Seriola dorsalis*

Nicholas Wegner*[1], Laura Schwebel[2], and John Hyde[3]

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[2]Southwest Fisheries Science Center, NOAA Fisheries, 8901 La Jolla Shores Dr., La Jolla, CA 92037, lschwebel at sandiego.edu
[3]John Hyde, Ph.D., Southwest Fisheries Science Center, NOAA Fisheries, 8901 La Jolla Shores Dr., La Jolla, CA 92037, john.hyde at @noaa.gov

Abstract:
The California Yellowtail (*Seriola dorsalis*) is a promising candidate for offshore commercial aquaculture in Southern California. However, best rearing practices for this and other *Seriola*

species are still under development, and our work has shown that yellowtail spawned and reared in captivity are typically less healthy than their wild-caught counterparts. Our research thus seeks to use various physiological tools and metrics to monitor fish health, fitness, and growth in an attempt to enhance rearing techniques. Over the past few years our work has focused on 1: Examining fitness differences between wild-caught and aquaculture-reared individuals through measures of critical swimming speed and metabolic rate, 2. Determining the adverse effects of an extremely common deformity (an uninflated gas bladder) in hatchery-reared yellowtail on their critical swimming speed, metabolic rate, growth rate, and feed conversion ratio, and 3. Investigating the potential growth and health benefits of forced exercise on early juvenile-stage yellowtail. Our results suggest that although wild-caught fish tend to show better health and metabolic efficiency, great strides in yellowtail health and fitness in aquaculture have occurred during the duration of our research, and eliminating deformities such as uninflated gas bladders greatly increases growout efficiency. While our swimming exercise studies are still underway, preliminary data suggest that a short duration of exercise (2-4 weeks) followed by growout in standard rearing tanks produces a sustained growth advantage. Such exercise regimes may thus represent not only a mechanism for improving fish health and fitness, but also a non-genetic and non-hormonal treatment to accelerate growth of fish for food or wild stock enhancement.

Annotated Bibliography of Key Works

Brown, E.J., M. Bruce, S. Pether, and N.A. Herbert (2011) Do swimming fish always grow fast? Investigating the magnitude and physiological basis of exercise-induced growth in juvenile New Zealand yellowtail kingfish, *Seriola lalandi*. Fish Physiol. Biochem., 37: 327-336.
This study investigated the effect of swimming exercise on the growth of cultured New Zealand yellowtail kingfish, *Seriola lalandi* at different water temperatures and swimming speeds. Two growth trials exposed fish to different exercise levels (determined by current speed) at 14.9° and 21.1°C. Lengths and weights were measured for each fish before and post exercise, along with metabolic rates using a respirometer. Results of these trials showed that exercise yielded a 10% increase in growth but only at low swimming speeds (0.75 BL s^{-1}) and at a temperature of 21.1°C. Experiments using a swim tunnel respirometer indicated that exercise training had no effect on metabolic scope or critical swimming speeds but it did improve swimming efficiency.

Palstra, A. P., et al. (2015). Forced sustained swimming exercise at optimal speed enhances growth of juvenile yellowtail kingfish (*Seriola lalandi*). Frontiers in Physiology 5: 506.
The swimming exercise trials in this study for *Seriola lalandi* resulted in 46% greater increases in mass and 92% larger increases in length compared to non-exercised controls. This study thus showed greater exercise benefits than those observed in the Brown et al (2012) study (above), and these differences were likely related to using more optimal exercise swimming speeds. The exercise and control groups in this study were fed equivalent rations, thus indicating the exercised fish had more efficient resource allocation, resulting in a lower feed conversion ratio (1.21 vs 1.74 for non-exercised yellowtail). These researchers thus suggest that growth rate can be greatly enhanced without increased feeding.

Palstra, A.P. and J.V. Planas (eds). (2013) Swimming Physiology of Fish: Towards Using Exercise to Farm a Fit Fish in Sustainable Aquaculture. Springer-Verlag: Berlin.
This multi-chaptered volume reviews the relevant advances in understanding the physiology of fish swimming and examines topics ranging from fish biomechanics and migrations to the

potential benefits of swimming exercise in aquaculture resulting in changes in fish growth and flesh quality. It brings together both field and laboratory studies to better understand the physiology of fish swimming with the intent to apply such findings to enhance aquaculture practices. A chapter by Davison and Herbert summarizes research activities and advances on swimming-enhanced growth.

5. *Paradigm shift in fish breeding; marker-assisted selection to genomic selection*

Akiyuki Ozaki[1]*, Kazuo Araki[1], Jun-ya Aoki[1], Yukinori Shimada[1], Hiroyuki Okamoto[1], Hironori Usuki [1], Koichi Okuzawa[1], Kazunori Yoshida[2], Tsutomu Noda[2]

[1]National Research Institute of Aquaculture, Fisheries Research Agency, 422-1, Nakatsuhamaura, Minamiise-cho, Watarai-gun, Mie, 516-0193, Japan. **E-mail:* aozaki at affrc.go.jp
[2]Seikai National Fisheries Research Institute, Fisheries Research Agency, 122-7, Nunoura, Tamanoura-machi, Goto-shi, Nagasaki, 853-0508, Japan

Abstract:
The essential conditions for DNA marker-assisted selection is development of useful resource families to evaluate phenotypes and information about genetic linkages and a large number of polymorphic genetic markers. Some of the cases have already reached a practical stage, and have been used as genetic improvement productions. This research and development are being applied to other kinds of aquaculture fish. High density SNP arrays have become the tool of choice for QTL mapping, Genome-wide association studies, marker-assisted selection (MAS). Earlier mapping studies have identified QTL for important commercial traits including disease resistance, and combining the resources of a high density genetic map with genome sequence data will facilitate the fine mapping of these loci. Otherwise traditional MAS did not result in a widespread use of DNA information in animal breeding. The main reason was that the traits of interest in livestock production were much more complex than expected: they were determined by thousands of genes with small effects on phenotype. These effects were usually too small to be statistically significant and therefore were ignored. Genomic selection (GS) assumes that all markers might be linked to a gene affecting the trait and concentrates on estimating their effect rather than testing its significance. There are technological breakthroughs that have resulted in the current wide-spread use of DNA information in animal breeding: the development of the genomic selection technology, the discovery of massive numbers of genetic markers (SNPs), and high-throughput technology to genotype animals for thousands of SNPs in a cost-effective manner.

In aquaculture species, these very stringent tests result in only the largest QTL being found. For disease resistance traits, such large QTL were detected, e.g., Japanese flounder resistant to lymphocystis disease, Atlantic salmon resistant to infectious pancreatic necrosis (IPN), yellowtail resistant to Benedenia disease. Those large QTL results were limited to disease resistance. Growth and diet fish meal feed resistant traits might be difficult to find the largest QTL, and difficult to apply for MAS. But GS have a possibility to improve such traits which are dominated by polygenic QTL. It is anticipated that future genetic breeding application will shift to GS from MAS in aquaculture.

Keywords: marker-assisted selection (MAS); Genomic selection (GS); polygenic QTL.

Annotated Bibliography of Key Works

Fuji, K., Hasegawa, O., Honda, K., Kumasaka, K., Sakamoto, T., Okamoto, N., (2007) Marker-assisted breeding of a lymphocystis disease-resistant Japanese flounder (Paralichthys olivaceus). Aquaculture 272, 291-295.

An allele of a microsatellite, Poli9-8TUF, has a dominant effect at a single major locus and is responsible for resistance to lymphocystis disease (LD-R) in Japanese flounder. We developed a new population of Japanese flounder produced by marker assisted breeding using this allele. A female that originated from the KP-B inbred line with LD-R that was homozygous for the favorable allele (B-favorable) and a male from a commercial stock bred for higher growth rate and good body shape were selected as parents. A female was selected as the LD-R-bearing parent because the recombination rate of females is lower in the region where the LD-R locus is located. As expected, the B-favorable allele was transmitted as a heterozygote to the progeny (LD-R+ population). The LD-R+ population, when tested at two commercial fish farms that had LD outbreaks, showed no incidence of LD at either farm, while a control population without B-favorable alleles (LD-R−) had incidences of 4.5% and 6.3% at the two farms. These results show that marker-assisted breeding using molecular markers linked to an economically important trait is an efficient strategy for breeding.

Moen, T., Baranski, M., Sonesson, A.K., Kjøglum, S., (2009) Confirmation and fine-mapping of a major QTL for resistance to infectious pancreatic necrosis in Atlantic salmon (Salmo salar): population-level associations between markers and trait. BMC Genomics 10:368.

Infectious pancreatic necrosis (IPN) is one of the most prevalent and economically devastating diseases in Atlantic salmon (Salmo salar) farming worldwide. The disease causes large mortalities at both the fry- and post-smolt stages. Family selection for increased IPN resistance is performed through the use of controlled challenge tests, where survival rates of sib-groups are recorded. However, since challenge-tested animals cannot be used as breeding candidates, within family selection is not performed and only half of the genetic variation for IPN resistance is being exploited. DNA markers linked to quantitative trait loci (QTL) affecting IPN resistance would therefore be a powerful selection tool. The aim of this study was to identify and fine-map QTL for IPN-resistance in Atlantic salmon, for use in marker-assisted selection to increase the rate of genetic improvement for this trait. The QTL confirmed in this study represents a case of a major gene explaining the bulk of genetic variation for a presumed complex trait. QTL genotypes were deduced within most parents of the 2005 generation of a major breeding company, providing a solid framework for linkage-based MAS within the whole population in subsequent generations. Since haplotype-trait associations valid at the population level were found, there is also a potential for MAS based on linkage disequilibrium (LD). However, in order to use MAS across many generations without reassessment of linkage phases between markers and the underlying polymorphism, the QTL needs to be positioned with even greater accuracy. This will require higher marker densities than are currently available.

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. 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.

Meuwissen TH1, Hayes BJ, Goddard ME. (2001) Prediction of total genetic value using genome-wide dense marker maps. Genetics;157(4):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 approximately 50,000 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) = 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.

6. *Genomic analyses to inform Sablefish aquaculture research*

Krista M. Nichols[1*], Andrew Jasonowicz[2], Giles Goetz[3], J. Adam Luckenbach[3], Frederick W. Goetz[2]

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Abstract:
The marine fish species, Sablefish (*Anoplopoma fimbria*, black cod), are long-lived, migratory species native to the west coast of the U.S. Sablefish is an ideal species for aquaculture because of their high market value and fast growth in culture. The development of the species for aquaculture will benefit from genomic technologies for a number of objectives, including: 1) an understanding of the genetic stock structure in the wild, from which progenitor broodstock is developed, 2) an understanding of the genetic basis of important production traits, and 3) the development of comprehensive genotyping assays for screening sablefish in culture. We discuss in this presentation our findings of genetic stock structure in wild populations, and future directions in genome sequencing and genomics of quantitative traits. Using Restriction Site Associated DNA sequencing (RADseq), we identified more than 100,000 SNPs in >400 individuals collected from a U.S. West Coast surveys from the Bering Sea in Alaska to southern California. After filtering for genetic analyses, 2661 SNPs were used to assess population structure and test for signatures of natural selection and association with environmental variables. Our results show a lack of population structure and adaptive variation in Sablefish, and are suggestive of a single panmictic population that is likely the result of a complex juvenile life history and long range movements as adults. Our current studies now focus on understanding the genetic basis of sexually dimorphic growth in Sablefish, and the development of a refined genome assembly to provide a reference for genomic studies in this species.

Annotated Bibliography of Key Works

Jasonowicz, A.J., F.W. Goetz, Giles W. Goetz and K.M. Nichols. 2016. Love the one you're with: genomic evidence of panmixia in the sablefish (*Anoplopoma fimbria*). *Canadian Journal of Fisheries and Aquatic Sciences* (in press)
This study examines genetic stock structure in Sablefish along the U.S. West Coast and Alaska using RADseq, and finds no evidence for population differentiation.

Luckenbach, J.A., W.T. Fairgreve. 2016. Gonadal sex differentiation and effects of dietary methyltestosterone treatment in Sablefish (*Anoplopoma fimbria*). *Fish Physiology and Biochemistry* 42: 233-248.
An important characterization of sex differentiation in Sablefish, and identification of the developmental time period sensitive to sex reversal by dietary testosterone.

Rondeau, E.B., A.M. Messmer, D.S. Sanderson, S.G. Jantzen, von Schalburg, K.R., Minkley, D.R., Leong, J.S., Macdonald, G.M., Davidsen, A.E., Parker, W.A., Mazzola, R.S., Campbell B. and B.F. Koop. 2013. Genomics of sablefish (*Anoplopoma fimbria*): expressed genes, mitochondrial phylogeny, linkage map, and identification of a putative sex gene. *BMC Genomics* 14: 452.
An important paper providing evidence for the sablefish sex determining gene, which will be important in understanding genetic sex control in sablefish. This paper also provides the first draft genome sequence that we are currently improving with additional sequence data, and provides the first comprehensive genomic resource for this fish species.

7. *Mutagenesis and genome editing for aquaculture fish species*

Hiroyuki Okamoto

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Abstract:
There is increasing interest in manipulating the function of target genes to achieve production gains in agriculture and aquaculture. Although transgenic techniques are often used, there are several alternatives. Of these, the two primary methods are chemical mutagenesis, including TILLING (Targeting Induced Local Lesion IN Genomes), and genome editing, also termed new breeding technology (NBT). Chemical mutagenesis has been widely used in agriculture, but there have been few reported successes in aquaculture. TILLING has been applied to experimental fish such as zebrafish and medaka. In these instances, researchers have subjected adult fish to a bath of strong alkylating agents (e.g., N-ethyl-N-nitrosourea (ENU)). Exposure to the chemical mutagen induces single nucleotide substitutions in the genomes of all cells, but particularly in the gametes. Because the body size of most intensively cultured food fish is much larger than these experimental species, it is not practical to use whole-body baths of ENU solution. To address this, methods have been developed to induce mutagenesis by soaking eggs or sperm in ENU solution or injecting ENU solution into the abdominal cavity of ripe males. Whereas chemical mutagens induce genome-wide random mutations, artificial nuclease for genome editing technology such as CRISPR/Cas can target mutations to a specific locus with random-length deletion or specific nucleotide sequence replacement. Genome editing is preferred when genes or candidate genes that are associated with desirable traits have been identified. In the absence of this information, or if there is a need to survey for alternative phenotypes, chemical mutagenesis is the preferred method. To date, few candidate genes have been identified for commercially important traits such as growth, disease resistance, and food conversion efficiency. This is because such traits are controlled by complex gene networks, with each gene having a very small effect individually. Thus, it is expected that improvements in our understanding of gene functions related to commercial traits could be made by further investment in mutagenesis and gene editing research.

Keywords: mutagenesis, TILLING, genome editing, NBT, CRISPR/Cas

Annotated Bibliography of Key Works

Satoshi Ansai, Masato Kinoshita (2014) Targeted mutagenesis using CRISPR/Cas system in medaka, *Biology Open* , 3: 362-371; doi:10.1242/bio.20148177
Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated (Cas) system-based RNA-guided endonuclease (RGEN) has recently emerged as a simple and efficient tool for targeted genome editing. In this study, we showed successful targeted mutagenesis using RGENs in medaka, *Oryzias latipes*. Somatic and heritable mutations were induced with high efficiency at the targeted genomic sequence on the *DJ-1* gene in embryos that had been injected with the single guide RNA (sgRNA) transcribed by a T7 promoter and capped RNA encoding a Cas9 nuclease. The sgRNAs that were designed for the target genomic sequences without the 5′

end of GG required by the T7 promoter induced the targeted mutations. This suggests that the RGEN can target any sequence adjacent to an NGG protospacer adjacent motif (PAM) sequence, which occurs once every 8 bp. The off-target alterations at 2 genomic loci harboring double mismatches in the 18-bp targeting sequences were induced in the RGEN-injected embryos. However, we also found that the off-target effects could be reduced by lower dosages of sgRNA. Taken together, our results suggest that CRISPR/Cas-mediated RGENs may be an efficient and flexible tool for genome editing in medaka.

M Kuroyanagi, T Katayama, T Imai, Y Yamamoto, S Chisada, Y Yoshiura, T Ushijima, T Matsushita, M Fujita, A Nozawa, Y Suzuki, K Kikuchi and H Okamoto (2013) New approach for fish breeding by chemical mutagenesis: establishment of TILLING method in fugu (*Takifugu rubripes*) with ENU mutagenesis, *BMC Genomics*, 14:786
Background: In fish breeding, it is essential to discover and generate fish exhibiting an effective phenotype for the aquaculture industry, but screening for natural mutants by only depending on natural spontaneous mutations is limited. Presently, reverse genetics has become an important tool to generate mutants, which exhibit the phenotype caused by inactivation of a gene. TILLING (Targeting Induced Local Lesions IN Genomes) is a reverse genetics strategy that combines random chemical mutagenesis with high-throughput discovery technologies for screening the induced mutations in target genes. Although the chemical mutagenesis has been used widely in a variety of model species and also genetic breeding of microorganisms and crops, the application of the mutagenesis in fish breeding has been only rarely reported.

Results: In this study, we developed the TILLING method in fugu with ENU mutagenesis and high-resolution melting (HRM) analysis to detect base pair changes in target sequences. Fugu males were treated 3 times at weekly intervals with various ENU concentrations, and then the collected sperm after the treatment was used to fertilize normal female for generating the mutagenized population (F1). The fertilization and the hatching ratios were similar to those of the control and did not reveal a dose dependency of ENU. Genomic DNA from the harvested F1 offspring was used for the HRM analysis. To obtain a fish exhibiting a useful phenotype (e.g. high meat production and rapid growth), fugu myostatin (Mstn) gene was examined as a target gene, because it has been clarified that the mstn deficient medaka exhibited double-muscle phenotype in common with MSTN knockout mice and bovine MSTN mutant. As a result, ten types of ENU-induced mutations were identified including a nonsense mutation in the investigated region with HRM analysis. In addition, the average mutation frequency in fugu Mstn gene was 1 mutant per 297 kb, which is similar to values calculated for zebrafish and medaka TILLING libraries.
Conclusions: These results demonstrate that the TILLING method in fugu was established. We anticipate that this TILLING approach can be used to generate a wide range of mutant alleles, and be applicable to many farmed fish that can be chemically mutagenized.

Jiang X-Y, Sun C-F, Zhang Q-G, Zou S-M (2011) ENU-Induced Mutagenesis in Grass Carp (*Ctenopharyngodon idellus*) by Treating Mature Sperm. *PLoS ONE* 6(10): e26475. doi:10.1371/journal.pone.0026475
N-ethyl-N-nitrosourea (ENU) mutagenesis is a useful approach for genetic improvement of plants, as well as for inducing functional mutants in animal models including mice and zebrafish. In the present study, mature sperm of grass carp (Ctenopharyngodon idellus) were treated with a

range of ENU concentrations for 45 min, and then wild-type eggs were fertilized. The results indicated that the proportion of embryos with morphological abnormalities at segmentation stage or dead fry at hatching stage increased with increasing ENU dose up to 10 mM. Choosing a dose that was mutagenic, but provided adequate numbers of viable fry, an F1 population was generated from 1 mM ENU-treated sperm for screening purposes. The ENU-treated F1 population showed large variations in growth during the first year. A few bigger mutants with morphologically normal were generated, as compared to the controls. Analysis of DNA from 15 F1 ENU-treated individuals for mutations in partial coding regions of igf-2a, igf-2b, mstn-1, mstn-2, fst-1and fst-2 loci revealed that most ENU-treated point mutations were GC to AT or AT to GC substitution, which led to nonsense, nonsynonymous and synonymous mutations. The average mutation rate at the examined loci was 0.41%. These results indicate that ENU treatment of mature sperm can efficiently induce point mutations in grass carp, which is a potentially useful approach for genetic improvement of these fish.

W. L. Russell, P. R. Hunsicker, D. A. Carpenter, C. V. Cornett and G. M. Guinn (1989) Effect of dose fractionation on the ethylnitrosourea induction of specific-locus mutations in mouse spermatogonia, *Proc. Nat'l Acad. Sci. USA* Vol. 79, pp. 3592-3593

As measured by' specific-locus mutations in mouse spermatogonia, fractionating a dose of 100 mg of ethylnitrosourea per kg of body weight into doses of 10 mg/kg injected intraperitoneally at weekly intervals greatly reduces the mutation frequency compared with that from a single dose of 100 mg/kg. Because there is independent evidence that the doses of 10 and 100 mg/kg reach the germ cells in amounts directly proportional to the injected dose, the lower mutational response with the fractionated dose is attributed to repair. The induced mutation rate expected from a single 10-mg/kg dose (on the assumption that this would be 1/10th the rate observed after 10 such doses) would be only 75% of the spontaneous mutation rate. Mouse spermatogonia apparently have an efficient repair system that is effective even against a potent mutagen.

W. L. Russell, Liane Brauch Russell, Elizabeth M. Kelly (1958) Radiation Dose Rate and Mutation Frequency, *Science*, Vol. 128, No. 3338 (Dec. 19, 1958), pp. 1546-1550

New data have clearly confirmed the earlier finding that specific locus mutation rates obtained with chronic gamma irradiation of spermatogonia are lower than those obtained with acute x-rays. Since this result is in contrast to classical findings for Drosophila spermatozoa, and apparently contradicts one of the basic tenets of radiation genetics, it was important to determine what factors were responsible for it. Experiments undertaken for this purpose reveal the following: (i) the lower mutation frequency is due mainly to difference in dose rate of radiation, rather than quality; (ii) a dose-rate effect is not obtained in experiments with mouse spermatozoa, confirming classical findings for spermatozoa, and indicating that the ex-planation for intensity dependence in spermatogonia resides in some characteristic of gametogenic stage; and (iii) a dose-rate effect is found not only in spermatogonia but also in oocytes, where cell selection is improbable, indicating that the radiation intensity effect is on the mutation process itself. A threshold response for all mutations in spermatogonia and oocytes is not a necessary consequence of the findings. Plausible hypotheses consistent with the present results can lead to other predictions. From a practical point of view, the results indicate that the genetic hazards, at least under some radiation conditions, may not be as great as those estimated from the mutation rates obtained with acute irradiation. However, it should not be forgotten that even the lower mutation rates obtained with the present intensity levels are still appreciable (16).

8. *Genome-enabled selection doubles the accuracy of predicted breeding values for bacterial cold water disease resistance compared to traditional family-based selection in rainbow trout aquaculture*

Roger L. Vallejo[1*], Timothy D. Leeds[1], Guangtu Gao[1], James E. Parsons[2], Kyle E. Martin[2], Jason P. Evenhuis[1], Breno O. Fragomeni[3], Gregory D. Wiens[1] and Yniv Palti[1]

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Abstract
We have shown previously that bacterial cold water disease (BCWD) resistance in rainbow trout can be improved using traditional family-based selection, but progress has been limited to exploiting only between-family genetic variation. Genomic selection (GS) is a new alternative enabling exploitation of within-family genetic variation. We compared three GS models to predict genomic-enabled breeding values (GEBVs) for BCWD resistance in a commercial rainbow trout population, and compared the accuracy of GEBVs to traditional breeding values (EBVs) estimated with a pedigree-based BLUP model. For these comparisons, we used BCWD survival phenotypes recorded on 7893 training fish from 102 families, from which 1473 fish from 50 families had genotypes (57K SNP array). Naïve siblings of the training fish (n = 930 testing fish) were genotyped to predict their GEBVs, of which 193 were mated to produce 138 progeny testing families (PTFs). In the following generation, 9968 progeny from the PTFs were BCWD phenotyped to empirically assess the accuracy of GEBV predictions made on their non-phenotyped parents. The accuracy of GEBVs from all three GS models were substantially higher than the BLUP model EBVs, with the increase in accuracy ranging from 83.3% to 108.8% depending on the GS model and survival phenotype used. Reducing the training sample size to n = ~1000 had no negative impact on the accuracy (0.67−0.72), but with n = ~500 the accuracy dropped to 0.53−0.61 if the training and testing fish were full-sibs, and even substantially lower to 0.22−0.25 when they were not full-sibs. Thus using progeny performance data, we have shown that the accuracy of genomic predictions with GS models (0.63−0.71) is substantially higher than the traditional pedigree-based BLUP model (0.34−0.36). We also found that using a much smaller training sample size compared to similar studies in livestock, GS can substantially improve the selection accuracy and genetic gains for BCWD resistance in commercial rainbow trout aquaculture.

Annotated Bibliography of Key Works
Meuwissen, T. H. E., B. J. Hayes and M. E. Goddard. 2001. Prediction of total genetic value using genome-wide dense marker maps. Genetics 157: 1819-1829.
This is a landmark publication on whole genome-enabled selection. Using computer simulated data, the authors proposed the method of genomic selection (GS) when high density SNP chips were not yet available for any plant and livestock species. The authors attempted to estimate the effects of ~50000 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 =100), the marker haplotypes were in linkage disequilibrium with the QTL located between the markers. Then 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. Next, with best linear unbiased prediction of haplotype effects assuming equal variances associated to each 1-cM chromosomal segment, the accuracy of genomic predictions increased to 0.73. They also used Bayesian methods that assumed a prior distribution of the variance associated with each chromosome segment and increased the accuracy to 0.85. The authors concluded that selection on genetic values predicted from high density marker maps could substantially increase the rate of genetic gain in animals and plants. With this publication, the authors proposed a GS method that today has revolutionized traditional animal and plant breeding programs. The authors laid out concepts and methods that are used in our GS research in rainbow trout aquaculture.

Odegard, J., T. Moen, N. Santi, S. A. Korsvoll, S. Kjoglum and T. H. Meuwissen. 2014. Genomic prediction in an admixed population of Atlantic salmon (*Salmo salar*), Frontiers in Genetics 5: 402.
The authors for the first time tested the reliability of genomic selection (GS) models in an admixed population of Atlantic salmon. The models included ordinary genomic BLUP models (GBLUP), using genome-wide SNP markers of varying densities(1–220K), a genomic identity-by-descent model (IBD-GS), using linkage analysis of sparse genome-wide markers, as well as a classical pedigree-based model. The authors compared the reliability of the models using 5-fold cross-validation, and the studied traits were salmon lice resistance (LR) and fillet color (FC) with heritability of 0.14 and 0.43, respectively. Overall, the authors found that all genomic models outperformed the classical pedigree-based model, for both traits and at all marker densities. However, the relative improvement differed considerably between traits, models and marker densities. For the highly heritable FC, the IBD-GS had similar reliability as GBLUP at high marker densities (>22K). In contrast, for the lowly heritable LR, IBD-GS was clearly inferior to GBLUP, irrespective of marker density. Hence, GBLUP was robust to marker density for the lowly heritable LR, but sensitive to marker density for the highly heritable FC. The authors hypothesized that this phenomenon may be explained by historical admixture of different founder populations, expected to reduce short-range linkage disequilibrium (LD) and induce long-range LD. The authors also highlighted that using ordinary GBLUP, the typical long-range LD of an admixed population may be effectively captured by sparse marker density, while efficient utilization of relationship information may require denser markers (e.g., >22K).

Vallejo, R. L., T. D. Leeds, B. O. Fragomeni, G. Gao, A. G. Hernandez, I. Misztal, T. J. Welch, G. D. Wiens and Y. Palti. 2016. Evaluation of Genome-Enabled Selection for Bacterial Cold Water Disease Resistance Using Progeny Performance Data in RainbowTrout: Insights on Genotyping Methods and Genomic Prediction Models, Frontiers in Genetics 7: 96.
The authors evaluated the potential utility of implementing GS in traditional family-based selective breeding programs using training and testing fish sampled from the first generation of the NCCCWA's BCWD resistance breeding line. The authors used GS models to predict genomic breeding values (GEBVs) for BCWD resistance, compared the predictive ability (PA)

of GEBVs to pedigree-based estimated breeding values (EBVs), and compared the impact of two SNP genotyping methods on the accuracy of GEBV predictions. The best GEBV predictions were similar to EBV with PA values of 0.49 and 0.46 vs. 0.50 and 0.41 for DAYS and STATUS, respectively. Among the GEBV prediction models, ssGBLUP consistently had the highest PA. The RAD genotyping platform had GEBVs with similar PA to those of GEBVs from the Chip platform. The overall GEBV accuracy in this study was low to moderate, likely due to the small training sample used. In this study, the authors explored the potential of GS for improving resistance to BCWD in rainbow trout using, for the first time, progeny testing data to assess the accuracy of GEBVs, and it provided the basis for further investigation on the implementation of GS in commercial rainbow trout populations.

9. *Genetic improvement of Atlantic salmon (Salmo salar L.) and Eastern oyster (Crassostrea virginica Gmelin 1791) at the USDA-ARS National Cold Water Marine Aquaculture Center*

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Abstract:
The USDA-ARS National Cold Water Marine Aquaculture Center (NCWMAC) focuses on the coldwater marine aquaculture industry's highest priority research needs including development of improved genetic stocks. Coldwater aquaculture production has potential for expansion, and both Atlantic salmon and Eastern oysters are widely accepted by American consumers. Commercial salmon and oyster producers predominantly utilize stocks that are not many generations removed from wild, unselected strains. North America salmon producers are legally required to culture certified stocks of North American salmon. The NCWMAC is the USDA-ARS facility that has been supporting the U.S. coldwater marine aquaculture industry by developing genetically improved salmon stocks for the past thirteen years. Aquaculture of the Eastern oyster is a large segment of shellfish aquaculture in the U.S. and minimal selective breeding has been accomplished. In both species, there is a need to improve the performance of existing stocks. Our research meets this need through the following objectives: 1) define phenotypes, estimate genetic and phenotypic parameters, and develop a selection index in Atlantic salmon for important traits such as carcass weight, cold tolerance, fillet color, fat content, and sea lice resistance; 2) evaluate and validate the usefulness of incorporating genomic information into the salmon breeding program; and 3) establish links between disease susceptible and resistant phenotypes and genotype for the Eastern oyster. Identification of genes associated with oyster disease will provide markers that can be used to enhance and accelerate the development of high-performing oyster lines through selective breeding and will support the East Coast shellfish aquaculture industry. Research accomplished with salmon will result in the development of genetically improved Atlantic salmon for release to U.S. producers and consumers.

Annotated Bibliography of Key Works

Gjedrem, T. (ed). 2005. Selection and breeding programs in Aquaculture. Springer. Dordecht, The Netherlands. 364 pp.

T. Gjedrem and M. Baranski. 2009. Selective breeding in Aquaculture: An Introduction. Reviews: Methods and Technologies in Fish. Biology and Fisheries 10, Springer.

The books referenced above describes selective breeding programs for a wide range of cultured species. The editor and many authors are from Norway which has the longest running Atlantic salmon selective breeding program in the world.

Gjerde, B., Odegard, J. & Thorland, I. 2011. Estimates of genetic variation in the susceptibility of Atlantic salmon (Salmo salar) to the salmon louse Lepeophtheirus salmonis. Aquaculture, 314, 66-72.

The paper above provides a brief overview of previous research looking at the heritability of sea lice resistance, while importantly demonstrating the need to normalize lice infection counts to a factor, such as lice density, that accounts for fish size at the time of infection.

Proestou, D.A., Vinyard, B.T., Corbett, R.J., Piesz, J., Allen, S.K., Small, J.M., Li, C., Liu, M., DeBrosse, G., Guo, X. and Rawson, P., 2016. Performance of selectively-bred lines of eastern oyster, Crassostrea virginica, across eastern US estuaries. Aquaculture. 464:17-27.

The article referenced above looked at the performance of selectively-bred lines of eastern oysters in five representative grow-out sites and detected significant effects of selection and large and significant genotype by environment interactions. This information will be useful in evaluating current breeding strategies for the eastern oyster.

Ermgassen, P., Spalding, M., Blake, B. et al. 2012. Historical ecology with real numbers: past and present extent and biomass of an imperiled estuarine habitat. Proceedings of the Royal Society of London B: Biological Sciences. 279:3393-3400.

The article referenced above analyzed historical shellfish landing and biomass data along the Eastern U.S. coast and correlated it to various anthropogenic stressors including overexploitation, habitat degradation, climate change and the resultant spread of disease.

10. *The Molluscan Breeding Program: 20 years of selective breeding of the Pacific oyster, Crassostrea gigas, on the West Coast of the US*

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Abstract:

The Molluscan Broodstock Program (MBP) was initiated in 1996 by Oregon State University (OSU) and the US Dept. of Agriculture with the goal of increasing the performance of Pacific oysters (*Crassostrea gigas*) for use in aquaculture on the West Coast, USA. MBP utilizes a family-based selection breeding design and was founded by broodstock collected from wild populations in Washington State and British Columbia, Canada. Breeding design and hatchery production of larvae and spat is undertaken by OSU at the Hatfield Marine Science Center in Newport, Oregon and families are planted out for field trait estimates at industry sponsored field sites along the West Coast. The selection process utilizes an index based on family performances for three traits of interest: family yield, average individual harvest weight and survival. Results of five generations of selective breeding show that substantial genetic gains have been attained. Realized gains are lower than expected, however, and we suspect this might be caused by competition for food in dense culture conditions and a changing climate, including intensified upwelling events which bring deep, acidified and nutrient-rich water to the surface. Heritability estimates have declined in the last two generations of the program, which corroborate these results and further suggest a changing environment. Significant genetic by environment (GxE) interactions have been detected between Puget Sound and coastal estuarine plant-out sites, indicating major environmental differences between the two types of sites. Taken together, these results are being used to develop modified breeding approaches for MBP, including a more careful indexing approach to take into account GxE interactions. Further considerations for MBP include breeding for new traits in response to emerging issues such as ocean acidification and diseases.

Annotated Bibliography of Key Works

Langdon, C., Evans, F., Jacobson, D., & Blouin, M. (2003). Yields of cultured Pacific oysters *Crassostrea gigas* Thunberg improved after one generation of selection. *Aquaculture*, *220*(1), 227-244.
This publication marks the first evaluation of the genetic gain obtained by the Molluscan Broodstock Program, after one generation of selection. The authors demonstrate significant improvement in yields of selected lines compared to unselected controls. However, they found only weak correlation between family performances in intertidal and subtidal environments, which indicated possible interactions between family performance and environment.

Evans, F., Matson, S., Brake, J., & Langdon, C. (2004). The effects of inbreeding on performance traits of adult Pacific oysters (*Crassostrea gigas*).*Aquaculture*, *230*(1), 89-98
The effect of inbreeding was evaluated in the context of a commercial growing operation cby measuring survival, family yield and individual growth after one or two growing season. Results showed that families with a high (F=0.2) inbreeding coefficient performed worse than families with a low (F=0.006) inbreeding coefficient, and this effect was stronger after 2 growing seasons. The result called for a strict monitoring of inbreeding in a selective broodstock program such as the MBP.

Melo, C. M. R., Durland, E., & Langdon, C. (2016). Improvements in desirable traits of the Pacific oyster, *Crassostrea gigas*, as a result of five generations of selection on the West Coast, USA. *Aquaculture*, *460*, 105-115.
Authors evaluated the heritability, genetic correlations and genetic gain for survival, individual weight and yield over 5 generations of selective breeding in the MBP. Substantial genetic gain

was observed throughout generations except for individual weight, where gains were limited after the second generation. Realized gains were less than expected however given the genetic gain, perhaps due to poor control over the selection of broodstock for controlled crosses. Heritabilities seemed to decline in the 4th and 5th generations, and the authors suspect this was caused by changing environmental conditions such as the intensification of upwelling events due to climate change.

11. *Seriola genomics and the knowledge repository Serioladb.org*

Andrew Severin[2*], Catherine Purcell[1], Margaret Carter[2], Rick Masonbrink[2], Arun Seetharam[2], John Hyde[1]

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Abstract:
Due to the high value of *Seriola* in the seafood industry, *Seriola* aquaculture is a great interest in the US and Japan. Hatchery production of *Seriola* species is accelerating 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.

To advance *Seriola* aquaculture, we are developing genomic resources for *Seriola dorsalis* and *Seriola rivoliana*. The starting point for this resource includes a draft genome assembly with gene annotations for *S. dorsalis.* In addition, we have resequenced 90 fish from three locations (Cedros Island, La Paz, and San Diego) and generated an F2 population to explore the genetic component in malformations in juvenile fish (swim bladder inflation, several head and jaw deformities and malformations in the operculum and branchiostegal structures).

To consolidate this data into a single knowledge repository, we are creating an international web resource Serioladb.org. As yellowtail culture grows, the genetic tools on the website will be made available to producers and other groups working to improve culture for this and other *Seriola* species. This research may also serve as a model for genetic resource development for aquaculture species or conservation dependent species with limited genomic resources, and may help reveal genomic regions and genes contributing to trait variation in wild populations.

Annotated Bibliography of Key Works
Martinez-Takeshita, N, Purcell CM, Chabot CL, Craig MT, Paterson CN, Hyde JR, and Allen LG. 2015. A Tale of Three Tails: Cryptic Speciation in a Globally Distributed Marine Fish of the Genus *Seriola.* Copeia: Vol. 103, No. 2, pp. 357-368.
Authors sampled 42 *Seriola lalandi* from South Africa, Japan, New Zealand, California, Mexico Pacific, Mexico Gulf and Chile to better understand regional populations and evolutionary patterns. Two mitochondrial genes (CR and COI) and four nuclear genes (RAG2, EHHADH, UBE3A, and MLL) were used to generate a phylogenetic tree with *Seriola dumerili* as an outgroup. Based on this data, it was concluded that fish currently named as *Seriola lalandi* in fact

comprise three species. The authors propose that *Seriola lalandi* in the Northwest Pacific revert to the name *Seriola aureovittata* and the Northeast pacific fish should revert to the name *Seriola dorsalis* while the fish in the southern hemisphere should remain as *Seriola lalandi*.

Westesson O, Skinner M, and Holmes I. 2012. Visualizing next-generation sequencing data with JBrowse. Briefings in Bioinformatics: Vol. 14, No. 2, pp. 172-177.
The authors describe a web-based browser for displaying genetic sequencing data at the nucleotide level. JBrowse is the successor to the very successful GBrowse genome browser. The most significant advance is the user-side processing of tracks for visualization, which allows for greater scalability without significant investment on the server side. Other improvements include Google maps-style browser interface with smooth transitions. JBrowse is actively maintained and can be downloaded from https://github.com/GMOD/jbrowse. JBrowse is an important tool for the visualization of high throughput sequencing data and should be included in any aquaculture genomic resources toolkit.

Ficklin SP, Sanderson LA, Cheng CH, Staton ME, Lee T, Cho IH, Jung S, Bett KE and Main D. 2011. Tripal: a construction toolkit for online genome databases. Database: Vol 2011, doi:10.1093/database/bar044.
In this work, the authors describe web based content management system named Tripal. Once a research group starts to accumulate high throughput sequencing data such as a genome assembly, annotation, SNPs, a genetic map, and epigenetic data etc., Tripal provides a web interface that organizes this data in a database for easy display on web pages. It is widely used by model organisms (FlyBase, WormBase, Genome Database for Rosaceae, TreeGenes and so on). The Chado database that serves to store and distribute the data to the webpages can also be used by JBrowse to visualize genomic data.

12. *The first meeting of Seriola genomics and breeding consortium*

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Abstract:
The first *Seriola* Genomics Symposium was held at the NOAA Fisheries, Southwest Fisheries Science Center in La Jolla, California on January 7th and 8th of 2016. 35 people attended this symposium from six countries (USA, Japan, Mexico, Brazil, Chile, and Australia), and the list of

participants included both scientists and *Seriola* producers. Specifically, the participants represented eight universities, five government agencies, four *Seriola* producers, and two biotechnology companies. During the two-day symposium, presenters described both the nature of their research and the progress of the research being conducted; these presentations outlined development of genetic/genomic resources to improve aquaculture for five different *Seriola* species (*S. quinqueradiata, S. dumerili, S. lalandi, S. dorsalis, and S. rivoliana*). Presenters were also asked to identify research needs and collaborative potential they could identify from their work. Discussions on the second day described the process of making a web-based *Seriola* genetic/genomic resource and addressed concerns in collaborative projects in terms of intellectual property. Notably, there was a long discussion with the *Seriola* producers identifying key needs and how to address those needs. By the end of the workshop, plans were formed for future workshops to occur at regular intervals (every other year) and steps were generally outlined for individual research groups to contribute to the genetic/genomic resources for *Seriola*. Participants agreed that this meeting would serve to identify them as part of a more formal *Seriola* Consortium. Several collaborations have already been formed from the *Seriola* Symposium, including with the NOAA Fisheries, SWFSC and Iowa State University to share genomic sequencing data, and with the University of Chile for the development of a *S. lalandi* SNP array. The National Research Institute of Aquaculture, FRA Japan, agreed to supply the SNP data of *S. quinqueradiata and S. dumerili*, to development of a *Seriol*a genotyping array.

Keywords: Yellowtail Seriola species (*S. quinqueradiata, S. dumerili, S. lalandi, S. dorsalis, and S. rivoliana*).

Annotated Bibliography of Key Works

Akiyuki Ozaki., Kazunori Yoshida., Kanako Fuji., Satoshi Kubota., Wataru Ka.i, Jun-ya Aoki., Yumi Kawabata., Junpei Suzuki., Kazuki Akita., Takashi Koyam.a, Masahiro Nakagawa., Takurou Hott., 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.

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.

Paul Whatmore., Nguyen Hong Nguyen., Adam Miller., Rob Lamont., Dan Powell., Trent D'Antignana., Erin Bubner., Abigail Elizur., Wayne Knibb. (2013) Genetic parameters for economically important traits in yellowtail. Kingfish Seriola lalandi., Auaculture 400-401 77-84
The aim of the present study was to estimate genetic parameters for body and carcass traits, visual condition score, and deformity in yellowtail kingfish Seriola lalandi, an emerging aquaculture species in Australia. These novel data and genetic parameters are required to solve the problem of how to conduct efficient selection in this and related species. Analyses were performed on a total of 400 data records collected from a yellowtail kingfish breeding population. These results suggest that selection for high growth would result in concomitant increase in fillet weight, a carcass trait of paramount importance. It is concluded that there is substantial potential for genetic improvement of economically important traits especially growth performance and fillet weight in the current population of yellowtail kingfish.

Catherine M., Chris L. Chabot., Matthew T. Craig., Natalie Martinez-Takeshita., Larry G. Allen., John R. Hyde. (2015) Developing a genetic baseline for the yellowtail amberjack species complex, Seriola lalandi sensu lato, to assess and preserve variation in wild populations of these globally important aquaculture species. Conservation Genetics 16-6,1475–1488
Recent study suggest the globally distributed yellowtail amberjack, Seriola lalandi sensu lato, is a complex of three closely related species. Together, these and three other species of Seriola comprise an important component of global aquaculture production with an estimated annual value of $1.3 billion. As yellowtail aquaculture grows, the impact of unintentional releases on wild populations has become an increasingly important issue, particularly in light of international trade of hatchery seed. To create a genetic baseline, we examined spatial genetic structure in 260 specimens collected from seven locations over a wide geographical range using 15 nuclear microsatellites and mitochondrial control region sequences. Overall genetic differentiation among locations, as revealed by microsatellite data, was highly significant (F_{ST} = 0.085, D_{EST} = 0.382, $P < 0.001$), and pairwise estimates of divergence derived from mitochondrial and microsatellite data support the presence of four significantly differentiated populations corresponding to the N.E. Pacific, N.W. Pacific, S. Pacific, and South Atlantic. Based on the genetic differentiation detected in this study, and recently published sequence data, these populations more accurately reflect the presence of at least three cryptic species of Seriola. Especially strong genetic differentiation between hemispheres indicates that the equatorial region is a significant dispersal barrier for yellowtail. This study represents the broadest geographic investigation of genetic population structure conducted, to date, for specimens of the S. lalandi complex.

A. Patel., P. Dettleff., E. Hernandez., V. Martinez (2015) A comprehensive transcriptome of early development in yellowtail kingfish (Seriola lalandi) Molecular Ecology Resources 16-1, 364-376
Seriola lalandi is an ecologically and economically important species that is globally distributed in temperate and subtropical marine waters. The aim of this study was to identify large numbers of genic single nucleotide polymorphisms (SNPs) and differential gene expression (DGE) related to the early development of normal and deformed S. lalandi larvae using high-throughput RNA-seq data. A de novo assembly of reads generated 40 066 genes ranging from 300 bases to 64 799 bases with an N90 of 788 bases. Homology search and protein signature recognition assigned gene ontology (GO) terms to a total of 15 744 (39.34%) genes. A search against the Kyoto

Encyclopedia of Genes and Genomes Pathway database (KEGG) retrieved 6808 KEGG orthology (KO) identifiers for 10 520 genes (26.25%), and mapping of KO identifiers generated 337 KEGG pathways. Comparisons of annotated genes revealed that 1262 genes were downregulated and 1047 genes were upregulated in the deformed larvae group compared to the normal group of larvae. Additionally, we identified 6989 high-quality SNPs from the assembled transcriptome. These putative SNPs contain 4415 transitions and 2574 transversions, which will be useful for further ecological studies of S. lalandi. This is the first study to use a global transcriptomic approach in S. lalandi, and the resources generated can be used further for investigation of gene expression of marine teleosts to investigate larval developmental biology. The results of the GO enrichment analysis highlight the crucial role of the extracellular matrix in normal skeleton development, which could be important for future studies of skeletal deformities in S. lalandi and other marine species.

13. *Uses of Genetic Parentage Analysis in Cultured California Yellowtail (Seriola dorsalis)*

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Abstract
The genus *Seriola* is an important group of cultured species representing a more than USD$1.5 million industry. With the demand for seafood continually rising, this popular group of fishes is a prime candidate for culture thus supplementing supply and relieving harvest pressure on wild populations. In a culture setting, we have the ability to obtain a complete set of genetic markers for the brood stock, allowing for determination of the parental contribution to each spawning event. In order to maximize output and quality of offspring, we explored the various usages of genetic parentage data in a cultured population of the California Yellowtail, *Seriola dorsalis*. It was determined that parentage was informative in not only allowing for an estimate of parental contribution numerically, but was also able to provide linkages to egg quality and may provide linkages to the presence/absence of deformities in offspring.

Annotated Bibliography of Key Works
Purcell, C., Chabot, C., Craig, M., Martinez-Takeshita, N., Allen, L., Hyde. J. 2015. Developing a genetic baseline for the yellowtail amberjack species complex, *Seriola lalandi sensu lato*, to assess and preserve variation in wild populations of these globally important aquaculture species. Con. Gen. 16(6):1475-1488
The authors present a global description of genetic variation in the *Seriola lalandi* species complex. Overall genetic differentiation among locations, as revealed by microsatellite data, was highly significant (FST = 0.085, DEST = 0.382, $P < 0.001$), and pairwise estimates of divergence derived from mitochondrial and microsatellite data support the presence of four significantly differentiated populations corresponding to the N.E. Pacific, N.W. Pacific, S. Pacific, and South Atlantic. Based on the genetic differentiation detected in this study, and recently published sequence data, these populations more accurately reflect the presence of at least three cryptic species of Seriola. Especially strong genetic differentiation between hemispheres indicates that

the equatorial region is a significant dispersal barrier for yellowtail. This study represents the broadest geographic investigation of genetic population structure conducted, to date, for specimens of the S. lalandi complex.

Baskett, M., Waples, R. 2013. Evaluating alternative strategies for minimizing unintended fitness consequences of cultured individuals on wild populations. Con. Biol. 27(1):83-94
The authors discuss important considerations for the maintenance of adaptively positive traits in cultured populations in order to minimize impact on wild populations due to accidental or intentional release into the wild. The authors quantitatively assessed two strategies: 1. Reduce selection in captivity, or 2. Maintaining a second population to minimize captive/wild interactions. They concluded that the appropriate approach depends on the goals of the culture activity.

Martinez-Takashita, N., Purcell, N., Chabot, C., Craig, M., Paterson, C., Hyde, J., Allen, L. 2015. A Tale of three tails: Cryptic speciation in a globally distributed marine fish of the genus *Seriola*. Copeia 103(2):357-368.
This paper demonstrates that the nominal species *Seriola lalandi* is actually comprised of three distinct species. The authors present compelling evidence for genetic separation of populations. This information is critical in culture applications for this species in that what were once considered populations of the same species will now be separated in new broodstock groups.

14. *Past, present and future research on Ostreid herpesvirus 1 infections of the Pacific oyster in Tomales Bay, California*

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Abstract
Large-scale sporadic mortalities of juvenile Pacific oyster, *Crassostrea gigas* have occurred in Tomales Bay, California for 20 years. The Ostreid herpesvirus 1 (OsHV-1) is the identified causative agent consistently associated with mortalities, with a confirmed infectious etiology. OsHV-1 is a global pathogen of bivalve molluscs, although detection in the United States is limited to Tomales Bay and nearby Drakes Bay. OsHV-1 is a member of the Order *Herpesvirales*, sharing common morphological criteria with vertebrate herpesviruses, although sequence data indicates a tenuous relationship. OsHV-1 was classified as the first member of an invertebrate herpesvirus family, Family *Malacoherpesviridae.* Sequence data indicate that multiple global variants of OsHV-1 exist, and the virus detected in Tomales Bay is not identical to any one variant. Elevated water temperatures are consistently associated with oyster mortalities in Tomales Bay, and may trigger viral replication and/or transmission of OsHV-1 to naïve juvenile oysters. Laboratory trials indicate qPCR and RT qPCR can be used to demonstrate virus replication and gene expression. Survival of young Pacific oysters in Tomales Bay is

dependent on outplant time, size, and oyster stock indicating genetic improvement and development of biomarkers for improved survival of Pacific oysters infected with OsHV-1 is possible. Since 2008, an economically devastating increase in *C. gigas* mortality in France has been associated with a new genetic variant OsHV-1 µvar, which is lethal to all life history stages. OsHV-1 µvar continues to spread in Europe and a similar variant causes losses in Australia, New Zealand, and Asia. OsHV-1 µvar's ability to kill seed and adults heightens concern over this variant relative to its progenitor, OsHV-1, which is lethal to larvae and seed only. OsHV-1 resistance has been shown to confer resistance to µvar. Recent studies demonstrated the ability to select for resistance to OsHV-1 and we propose studies to evaluate selection of resistance to both OsHV-1 µvars and their progenitor in US oyster lines.

15. *Type 1 Ostreid Herpesvirus (OsHV-1) variants in Japan*

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Abstract:
Ostreid herpesvirus 1 (OsHV-1) µVar is a variant of OsHV-1 newly reported from France (GenBank accession no. HQ842610), and it is suspected of being the causative agent of acute mass mortality events of Pacific oysters *Crassostrea gigas* during summers in Europe since 2008. OsHV-1 µVar differed from reference OsHV-1 by nucleotide mutations in the C2/C6 fragment including ORF4, and in the IA2/IA1 fragment including ORF42/43 (Segarra *et al*., 2010). Japan is one of the major producers of Pacific oyster. Our previous study indicated that, 23 types of OsHV-1 variant, showing 96% to 99% similarity to the reference OsHV-1, were obtained from Pacific oyster, kumamoto oyster, *C. sikamea* and suminoe oyster, *C. ariakensis* collected in 2007 and from Pacific oyster in 2011 in Japan (Shimahara *et al*., 2012). Although 18 variants among the obtained 23 possessed a microsatellite deletion in C2/C6 unique to OsHV-1 µVar, the nucleotide sequence was not identical to the OsHV-1 µVar. In this study, further surveillance of OsHV-1 variants was conducted for Pacific oysters in 2012. Nine hundreds of spats, or juveniles of Pacific oyster collected in the 4 main oyster producing areas were subjected to PCR assay using the primer pairs C2 and C6 (Renault and Arzul 2001). PCR products were amplified from 40 out of 900 oysters, and 13 different nucleotide sequences, showing 96% to 99% similarity to the reference OsHV-1, were obtained. Although 11 sequences among the obtained 13 possessed

a microsatellite deletion unique to OsHV-1 µVar, all PCR products contained 2 conserved nucleotides that were shared with the reference OsHV-1 and not with OsHV-1 µVar. Here, we found variable types of OsHV-1 in oysters in Japan, which had different nucleotide sequences from that of OsHV-1 µVar in France (HQ842610).

Keywords: Ostreid herpesvirus, OsHV-1, *Crassostrea gigas*, Japan, Pacific oyster

Annotated Bibliography of Key Works

Shimahara, Y., Kurita, J., Kiryu, I., Nishioka, T., Yuasa, K., Kawana, M., Kamaishi, T., and N. Oseko. 2012. Surveillance of Type 1 Ostreid Herpesvirus (OsHV-1) variants in Japan. Fish Pathology, Vol: 47: 4. Pp 129-136

Ostreid herpesvirus 1 (OsHV-1) µVar is a variant of OsHV-1 that is suspected of being the causative agent of acute mass mortality events of Pacific oysters during summers in Europe since 2008. The authors investigated a distribution of OsHV-1 variants in six main oyster-producing areas in Japan by PCR to determine whether OsHV-1 µVar was present in Japan. PCR products were amplified from 123 out of 1,714 oysters, and 23 different nucleotide sequences were obtained. Although 18 sequences among the 23 obtained possessed a microsatellite deletion unique to OsHV-1 µVar, all PCR products contained two conserved nucleotides that were shared with reference OsHV-1 and not OsHV-1 µVar in ORF4. It was found that variable types of OsHV-1 distribute throughout Japan, but their nucleotide sequences were not completely identical to OsHV-1 µVar.

16. Characterization of genetic and epigenetic variation in hatchery and natural-origin steelhead, Oncorhynchus mykiss

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Abstract:
Relative reproductive success studies have documented substantial fitness loss for wild steelhead after a single generation of rearing in the hatchery, but the relative contribution of genetic selection and/or environmentally-induced heritable epigenetic changes passed through the germline are unknown. Here, as a step toward understanding mechanisms of fitness loss, we describe genetic and epigenetic variation in adult hatchery and natural-origin steelhead from the Methow River, Washington USA. Our main objectives were to determine if hatchery and natural-origin fish from this stock could be distinguished genetically by examining SNPs across the entire genome, and whether differences in epigenetic programming (DNA methylation) in somatic and germ cells could be detected between the two groups. Although genetic analysis using RAD-Seq did not reveal differences between the hatchery and natural fish, we found significant differences in epigenetic programming in both somatic (red blood cells) and germ cells (sperm). Because hatchery fish experience similar environmental conditions as their wild

conspecifics once they leave the hatchery, our results raise the possibility that these DNA methylation changes occurred during the first year in the hatchery and persisted into adulthood in the form of an 'epigenetic memory' of the hatchery environment.

Annotated Bibliography of Key Works

Baerwald M. R., Meek M. H., Stephens M. R., Nagarajan, R. P., Goodbla, et al. 2015 Migration-related phenotypic divergence is associated with epigenetic modifications in rainbow trout. *Mol. Ecol.* **25**: 1785-1800.

This study investigates the role of epigenetics (DNA methylation) in migration-related life history traits in *Oncorhynchus mykiss*. The authors used reduced representation bisulfite sequencing to perform comparative DNA methylation analysis between juvenile resident and smolt F2 siblings generated from a cross between steelhead (migratory) and rainbow trout (nonmigratory). Fifty-seven differentially methylated regions, many of which were in gene regulatory regions, were identified between residents and smolts, suggesting a relationship between epigenetic variation and variation in migration-related phenotypes.

Gavery MR and Roberts SB. 2013 Predominant intragenic methylation is associated with gene expression characteristics in a bivalve mollusc. *PeerJ.* 1:e215.

This dataset, generated using methylation-enriched high-throughput bisulfite sequencing, represents the first high-resolution methylome in any mollusc. DNA methylation data were compared to gene expression datasets and a positive relationship between intragenic methylation and gene expression levels was identified. These data suggest that DNA methylation patterns may play a role in regulating gene expression in molluscs.

Navarro-Martín L., Viñas J., Ribas L., Díaz N., Gutiérrez A., et al. 2011 DNA methylation of the gonadal aromatase (*cyp19a*) promoter is involved in temperature-dependent sex ratio shifts in the European sea bass. *PLoS Genet.* 7: e1002447

The authors report that in European sea bass (*Dicentrarchus labrax*), which exhibits temperature-dependent sex determination, exposure to high temperature in early development was associated with increased DNA methylation in the promoter of the aromatase gene (*cyp19a1a*) and a higher proportion of phenotypic males. Furthermore, *in vitro* methylation of the aromatase promoter was sufficient to suppress transcription of the gene, supporting a role for DNA methylation as a mechanistic link between temperature and sex ratios in species exhibiting temperature-dependent sex determination.

Potok M. E., Nix D. A., Parnell T. J., Cairns B. R., 2013 Reprogramming the maternal zebrafish genome after fertilization to match the paternal methylation pattern. *Cell* 153:759-72.

Genome-wide DNA methylation patterns in zebrafish gametes, various stages of embryos and a somatic tissue (muscle) were analyzed using whole genome bisulfite sequencing. This high-resolution approach identified dynamic and unique patterns of DNA methylation during development in zebrafish. Results suggest that the functional significance of sperm DNA methylation patterns in fish is to provide transcriptional competency to the early embryo, which 'inherits' the DNA methylation pattern in the sperm.

Shao C., Li Q., Chen S., Zhang P., Lian J., et al., 2014 Epigenetic modification and inheritance in sexual reversal of fish. *Genome Res.* 24: 604–615.

The half-smooth tongue sole (*Cynoglossus semilaevis*) was used as a model to investigate the role of epigenetic regulation in species with environmental sex determination. Using genome-wide bisulfite sequencing of normal male, female and pseudomale fish (generated by exposing genetic females to high

temperature during a sensitive developmental window), the authors showed that sex-reversed genetic females exhibit methylation patterns consistent with genetic males, both of which differ from the methylome of normal females. Furthermore, it was reported that global methylation patterns are inherited by F1 pseudomale offspring generated by crosses between temperature-induced sex-reversed pseudomales and normal females, suggesting transgenerational epigenetic inheritance of sex reversal in this species.

17. *Towards a functional understanding of DNA methylation in shellfish and implications for aquaculture.*

Mackenzie Gavery, Claire Olson, Steven Roberts*

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Abstract:
There is an amazing amount of diversity incorporated into the genome of oysters and other marine invertebrates including vastly expanded gene families, high mutation rates, and numerous mobile elements. These are certainly a benefit to broadcast spawners living in fluctuating environments. Recent work examining DNA methylation is revealing new insight into similar diversity at the epigenetic level. The function of DNA methylation in species such as bivalves where the limited amount of DNA methylation is predominantly found in gene bodies is not completely understood. One emerging possible explanation is that the role of gene body DNA methylation is dependent on gene function, a potential phenomenon that has arisen from selective pressure on lineage-specific life history traits.

With respect to commercially important traits, we know that in other taxa epigenetic marks are associated with phenotypes independent of genetic variation, the environment can influence DNA methylation, and epigenetic marks can be inherited. In shellfish, we are still learning about role of epigenetic processes such as DNA methylation in controlling the phenome. However it does appear that epigenetic processes will be important to consider in future efforts to advance aquaculture, particularly in changing environments.

Annotated Bibliography of Key Works

Gavery MR and Roberts SB. (2014) A context specific role for DNA methylation in bivalves Briefings in Functional Genomics. doi:10.1093/bfgp/elt054 https://goo.gl/YrnzXi
A review of current knowledge of DNA methylation in bivalves. A primary conclusion is that the functional role of the gene could influence the role of DNA methylation in influencing expression.

Claire E. Olson, Steven B. Roberts. Indication of family-specific DNA methylation patterns in developing oysters bioRxiv doi: http://dx.doi.org/10.1101/012831
This study provides the first single-base pair resolution DNA methylomes for both oyster sperm and larval samples from multiple crosses. While sample sizes are very low, this work suggests DNA methylation patterns could be inherited.

Roberts, Steven (2015): Compilation of DNA Methylation Genome Feature Tracks (*Crassostrea gigas*). figshare. https://dx.doi.org/10.6084/m9.figshare.1456226.v2
Genome feature tracks and accompanying IGV session file to visualize DNA methylation data for the Pacific oyster (*Crassostrea gigas*).