

## **Research Priorities to address productivity of shellfish aquaculture on the East and Gulf Coasts**

East Coast Shellfish Breeding Consortium (ECSBC)

### **Elements of common concern**

In 2008 the East Coast Shellfish Growers Association assembled a diverse team of shellfish geneticists from institutions along the East coast and has now grown to include members from Gulf institutions. Our goal was to develop tools and perform research to improve production traits for the shellfish aquaculture industry. Our initial vision, based on possibilities of long-term funding from ARS, involved a core of ARS scientists with expertise in key areas of genetics and breeding, such as quantitative genetics, genomics, and bioinformatics to contribute to regional breeding centers along the coast. This vision has been partly realized with the establishment of an ARS position at the University of Rhode Island and several regionally based programs that are actively engaged in the genetic improvement of lines for industry.

While development of genetically improved lines for *C. virginica*, the East coast oyster, necessarily has to be regionally based, there are some overarching priorities that can benefit genetic improvement writ large. These were recently identified in a conference call among geneticists and breeders from the ECSBC.

Exploiting the recently sequenced genome of *C. virginica*. Due to recent advances in sequencing the genome of *C. virginica*, the Consortium recognizes the opportunity to now incorporate genomic breeding into our developing arsenal of approaches. We believe the first step in this approach is the development and testing of high-density SNP arrays with 200k base pairs and associations with phenotypes. Development of this technology benefits the entire region as it provides a common platform for regional programs.

Tackling contemporary issues facing commercial producers. Triploid oysters have gained in popularity widely along the East Coast and represent an important added value, especially for summer marketing. Their increasing usage have come with recent observations of what has been called triploid mortality, ranging at least from Rhode Island to the Gulf. Early investigations into the etiology of triploid mortality have failed to demonstrate a specific pathogen or overt mechanism for the mortalities that occur in late Spring, an otherwise favorable time for oyster health. It seems clear that further investigations into the physiological differences between susceptible triploids and resistant diploids is warranted and would benefit the entire region accordingly. Understanding the mechanism responsible for triploid mortality is the first step in defining the trait that will be used to breed for triploid survival.

Coordinating and communicating among programs. Constituents of the Consortium comprise a diverse array of talent, approaches, and expertise. To help coordinate the efforts of the consortium, we recognize the need to convene a Breeder's Roundtable. The Roundtable will allow shellfish geneticists to learn about recent developments, highlight

current challenges, and identify fruitful avenues of research to focus our efforts on the most promising approaches.

## **Scope of Work**

### **SNP array for Genotype-Phenotype association and breeding**

A high-density SNP array is an initial goal to begin the process of integrating genomics and practical breeding. Oyster resequencing data at 30X genome coverage is available for 92 individuals collected from multiple regions and ecotypes within the species range through the eastern oyster sequencing project led by Dr. Marta Gómez-Chiarri. Additional samples will be resequenced to capture broader geographic variation for array design. All resequencing data will be used to develop a SNP array with 200,000 features including 195,000 high-quality oyster SNPs and 5,000 markers for key pathogens. We envision the use of the arrays on two levels.

#### Association studies

Initially we would endeavor to genotype up to 2000 samples for genome-wide association studies to identify markers associated with disease and physiological stressors. Examples of the type of genotyping required for this approach are as follows.

- Samples from 3 mapping families to construct genetic linkage map and correct assembly errors (n ~ 300)
- Before- and after-mortality samples from up to 5 populations, where the mortality is primarily caused by MSX, Dermo and JOD (n ~ 600)
- Before- and after-mortality samples from up to 5 populations, where the mortality is primarily caused by cold stress (n ~ 500)
- Survivors and deceased samples from up to 3 Dermo challenge experiments (n ~ 300)
- Survivors and deceased samples from up to 3 JOD challenge experiments (n ~ 300)
- Survivors and deceased samples from up to 3 physiological challenge experiments, such as cold, OA, and hypoxia (n ~ 300)
- 100 population samples including quality control of the resequenced individuals

#### Genomic selection

Genomic selection within the framework of family-based breeding is a powerful new tool for the enhancement of genetic improvement in aquaculture species. It extends traditional approaches for generating breeding values based on phenotypes among related individuals by including genotype information and is particularly useful for selecting traits that are costly or difficult to measure, such as, disease resistance and tolerance to environmental stress. Genomic selection can accelerate performance gains and increase aquaculture production by increasing selection intensity, Improving accuracy in brood stock identification, and minimizing generation intervals.

To incorporate genomic selection in existing eastern oyster breeding programs, we must develop training and test sets of individuals for traits of interest and conduct progeny testing of the offspring generated by the test set. A training set is comprised of individuals from several families that have been extensively phenotyped and genotyped, while the test set consists of animals where only genotype information is available. The opportunity to advance to this level of selection is available from the legacy of family selection ongoing at ABC VIMS and therefore the infrastructure to associate phenotypes with markers already exists, and does not have to be supported *de novo*. Requirements for this approach are as follows:

- Training set- 1000 individuals (25 families, 40 individuals/family) phenotyped and genotyped
- Test set- 1000 individuals from the same 25 families genotyped only
- Progeny test set- phenotyped only

Approximate costs: Association – \$700,000; GS – \$700,000

### **Triploid physiology**

Surprisingly little work has been done on comparative physiology of diploid and triploid *C. virginica*, probably because of its recent development compared to *C. gigas* and also because the practical value of triploids has been demonstrated by widespread commercial adoption, obviating the need for such research. With the advent (or simply, realization) of selective mortality of triploids, compared to diploid counterparts, it is clear that a more comparative approach is required. This work should evaluate fundamental metabolic processes (oxygen uptake, food assimilation efficiency, various enzymatic and metabolic processes, scope for growth, reproduction, etc.) in an attempt to identify causative factors for triploid mortality. Deployment of specific genetic constructs across regions experiencing mortality followed by comparative physiology, possible through “omics” approaches, is needed.

Approximate costs: \$500,000

### **Breeders Roundtable**

Over the last few decades, selective breeding of the eastern oyster has resulted in significant improvement of growth and survival and had a positive economic impact at the commercial level. However, industry priorities vary greatly across geographic regions and they change over time. As yet, extant breeding programs do not address many of the challenges faced by shellfish growers throughout the species range. In other regions, breeding programs are only incipient. Furthermore, advances in genetic and genomic resources available for the eastern oyster now can be exploited to enhance existing breeding practices. The purpose of the Breeders Roundtable is to gather interested parties from regions in the US where the eastern oyster is grown to discuss 1) overlapping breeding priorities across regions and specific breeding priorities within regions, 2)

identify geographic regions where more selective breeding is needed, and 3) identify how advances in technology can be leveraged to improve practical breeding approaches.

Approximate costs (3 yrs): \$50,000

### **Consortium Members**

**Bassem Allam**, Marinetics Endowed Professor and Director of the Marine Animal Disease Laboratory, Stony Brook University. [Bassem.Allam@stonybrook.edu](mailto:Bassem.Allam@stonybrook.edu)  
Allam's research centers on the physiology and host-microbe interactions in shellfish, and how environmental conditions affect the resistance of these animals to infectious diseases. These activities use a diverse range of approaches ranging from ecological physiology and immunobiology to modern "omics" tools and methods. His current collaborative research focuses on developing marker-assisted selection for disease resistance in the hard clam, and identification of molecular features (single nucleotide polymorphism and other features) associated with resilience to ocean acidification and microbial pathogens in eastern oysters. His group has also been developing functional genomics methods (e.g. gene silencing) to link oyster traits to particular genomic features.

**Standish K. Allen, Jr.**, Professor of Marine Science and Director (Founder) of the Aquaculture Genetics and Breeding Technology Center (ABC), Virginia Institute of Marine Science, the College of William and Mary. [ska@vims.edu](mailto:ska@vims.edu)  
Allen's primary area of interest is the development of triploid and tetraploid technology and its commercialization. ABC supports oyster aquaculture through selective breeding focusing on disease resistance, market traits, and reproductive sterility through a tetraploid breeding program. Broodstock are distributed to over a dozen hatcheries on the east coast.

**Brian Callam** Research Associate and Breeding Research Manager, Louisiana Sea Grant College Program, Louisiana State University. [bcalla3@lsu.edu](mailto:bcalla3@lsu.edu)  
Callam directs research at the La Sea Grant Oyster Research Laboratory. His research focuses on the improvement of eastern oysters using genetic techniques and improving husbandry practices. These activities support oyster aquaculture in the northern Gulf of Mexico and southeastern Atlantic coasts with broodstock distribution through our breeding programs.

**Chris Davis . . .**

**Julie Davis**, South Carolina Sea Grant Consortium, Living Marine Resources Extension Specialist. [julie.davis@scseagrant.org](mailto:julie.davis@scseagrant.org)  
Davis' work focuses on equipping shellfish growers with information based on rigorous scientific research such that they can confidently make business decisions. Her background is in the private sector shellfish aquaculture and coastal engineering realms. In recent years, Davis has guided efforts in establishing South Carolina's first oyster hatchery, including application of triploid technology in the state. Davis' current research efforts

include leading a USDA-SRAC funded project to improve production of cultured oysters by addressing biofouling using floating gear in seven Southern US states.

**Marta Gomez-Chiarri**, Chair, Department of Fisheries, Animal and Veterinary Science, University of Rhode Island. [gomezchi@uri.edu](mailto:gomezchi@uri.edu)

My research interests include the use of multidisciplinary approaches to the prevention and management of infectious diseases in cultured and wild shellfish. These approaches include studying the distribution of disease using traditional and molecular tools, evaluating the effect of environmental parameters, and investigating host-parasite interactions using genomic tools. I am also developing probiotics to control bacterial diseases in shellfish hatcheries. I am a member of the International Oyster Genome Consortium, and the East Coast Shellfish Breeding Consortium.

**Ximing Guo**, Professor of Marine Science, Haskin Shellfish Research Laboratory, Rutgers University, [xguo@hsrl.rutgers.edu](mailto:xguo@hsrl.rutgers.edu)

Guo's research is on genetics and reproduction of marine molluscs. He is interested in genetic bases of molluscan development and adaptation, and their applications in the genetic improvement of cultured molluscs. Guo is the co-inventor (with Allen) of tetraploid oysters that have led to worldwide commercial production of triploids. He participated in the sequencing of several bivalve genomes. Guo directs the shellfish genetics and breeding program at Rutgers University. His current research includes genetics of genomic stability in tetraploid oysters, genetics of disease/stress resistance in oysters, and marker-assisted selection for disease resistance in oysters and hard clam.

**Louis Plough**, Assistant Professor, University of Maryland Center for Environmental Science, Horn Point Laboratory. [lplough@umces.edu](mailto:lplough@umces.edu).

My research generally focuses on the ecology and population genetics of marine animals with special interest in oyster/shellfish aquaculture and the use of genomic tools to advance selective breeding. Current projects include examination of the genetic basis of salinity tolerance in eastern oysters and domestication selection in larval oyster traits.

**Dina Proestou**, Lead Scientist for shellfish genetics, USDA ARS National Coldwater Marine Aquaculture Center, Kingston, RI. [Dina.Proestou@ARS.USDA.GOV](mailto:Dina.Proestou@ARS.USDA.GOV)

Research at the NCWMAC focuses on genetic improvement of Atlantic salmon and eastern oysters. The goal of the shellfish program is to identify links between phenotype and genotype for commercially important traits in eastern oysters. Thus far, her team has developed experimental disease challenge methods to identify traits associated with disease resistance and quantify genetic variation in disease-resistant phenotypes among selectively-bred eastern oyster families. They have also contributed to the sequencing, assembly, and annotation of a high quality reference genome for the eastern oyster. Currently they are utilizing the tools they've developed to characterize gene expression patterns of disease-resistant and -susceptible oysters in response to pathogen exposure using RNAseq and differential gene expression analysis.

**Jonathan B. Puritz**, Assistant Professor, Department of Biological Sciences, University of Rhode Island. [jpuritz@uri.edu](mailto:jpuritz@uri.edu)

Puritz's lab focusing on developing cutting-edge population genomic methods, bioinformatic tools, and on understanding how human populations impact the evolution of marine populations. Puritz has recently started investigating how natural and anthropogenic stressors affect the genomic composition of larval cohorts of the eastern oyster. He also has contributed to the eastern oyster genome project.

**Hollie Putnam**, Assistant Professor, Department of Biological Sciences, University of Rhode Island. [hputnam@uri.edu](mailto:hputnam@uri.edu)

Putnam's research is focused on understanding acclimatization at the ecological to molecular levels using a molecular eco-physiology approach. Putnam assesses the link between epigenetic mechanisms (such as DNA methylation) and transcriptomic and organismal responses that can produce phenotypic plasticity and thus influence growth and production. Putnam works on assessing the potential for environmental conditioning, or environmental hardening, to contribute to positive traits.

**Paul Rawson**, Ecological Genetics of Marine Bivalves, School of Marine Sciences, University of Maine, [prawson@maine.edu](mailto:prawson@maine.edu)

The Rawson lab's research is centered on the development of hatchery methodologies and selective breeding to support the increased aquaculture-based production of marine bivalves in the northeastern U.S. Recent projects have focused on feeding physiology in oysters to provide a deeper understanding of how nutrient utilization and growth respond to spatial and temporal variation in the types of nutrition (e.g., algal and detrital materials) available in coastal habitats where oysters are cultured. The results of this research will contribute to improved models of carrying capacity and site selection to allow for informed expansion of the shellfish culture industry and help shape selective breeding programs targeting not only disease-resistance but also improved growth for the long-term health and development of the industry. Other recent projects have sought to identify and refine hatchery methodologies for the production of razor clam and mussel seed to support industry diversification.

**Robert Rheault**, Executive Director, East Coast Shellfish Growers Association, Wakefield, RI. [bob@ECSGA.org](mailto:bob@ECSGA.org)

As a former oyster farmer and hatchery operator, Rheault is now well positioned to serve as an industry liaison, to identify industry priorities for research and trait selection and to assist in extending the results of any successful work to growers and hatcheries on the east coast.

**Jessica Small**, Assistant Director at the Aquaculture Genetics and Breeding Technology Center (ABC), Virginia Institute of Marine Science, the College of William and Mary.

[jamoss@vims.edu](mailto:jamoss@vims.edu)

Small directs the breeding program for ABC. ABC focuses on *C. virginica* breeding and polyploid technology, utilizing primarily quantitative genetic approaches. Selected brood stock from ABC have become the basis for the notable increase in aquaculture production both within the state of Virginia and throughout the East Coast.

**William C. Walton**, Associate Professor & Extension Specialist, Auburn University & Alabama Cooperative Extension System, Auburn University Shellfish Laboratory.

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Walton has conducted research along the US Gulf Coast, evaluating differences in triploid and diploid oysters to better understand the late spring/summer mortality events observed in triploids. In addition, working with colleagues at Auburn University and Louisiana State University, he has evaluated a variety of selected lines of oysters for genetic improvement of cultured oyster stocks.

**Gary H. Wikfors**, Director, NOAA NEFSC Milford Laboratory, Milford, CT.

[Gary.Wikfors@noaa.gov](mailto:Gary.Wikfors@noaa.gov)

The Milford Laboratory has many decades of engagement in science support of shellfish aquaculture development. The current staff has expertise in microbiology, nutrition, physiology, ecology, and genetics. As importantly, the Laboratory has underutilized facility resources, including a functional shellfish hatchery and an expansive raceway tank farm that has been, and can be again, used as a nursery system in support of oyster breeding. Seawater quality at the Milford site is excellent and supports robust growth of oysters and other shellfish.

**Ami Wilbur**, Professor, Department of Biology and Marine Biology & Director, Shellfish Research Hatchery, University of North Carolina Wilmington, Wilmington, NC.

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Wilbur's research group uses molecular genetic techniques to resolve issues in the design, implementation and evaluation of shellfish aquaculture, restoration efforts and other fisheries management practices. Research foci at the Shellfish Research Hatchery include the selective breeding of oysters, impact of polyploidy on performance in oysters and crop diversification.