Virginia Institute of Marine Science Shellfish Aquaculture Industry Advisory Committee (SAIAC)

February 2023

Karen Hudson & Lexy McCarty
VIMS Marine Advisory Program

VIMS | William & Mary
Virginia Institute of Marine Science

TABLE OF CONTENTS

SAIAC Background

The Virginia Institute of Marine Science (VIMS) established the Shellfish Aquaculture Industry Advisory Committee (SAIAC) in March 2018 to provide a forum for enhanced communication between VIMS and the Virginia's shellfish aquaculture industry. The committee was expanded from the Aquaculture Genetics and Breeding Technology Center's (ABC's) industry advisory committee made up of Virginia commercial hatcheries.

Mission

The SAIAC mission is to work with VIMS to advance sustainable shellfish aquaculture production in Virginia by providing an industry perspective on challenges and solutions related to growing shellfish. The committee advises VIMS on industry needs in the areas of research, education and outreach, and technical services.

Membership

The SAIAC shall be a 10-member committee with representation reflecting the diversity of the industry with respect to geography, species farmed, and stages in the production process. A minimum of four, and preferably five, members of the committee shall represent firms involved in hatchery production of shellfish. Representation will include individuals from large and small production operations. The Executive Director of the Shellfish Growers of Virginia will hold a permanent invitation to participate with the committee, while other members are appointed by the Dean and Director of VIMS to a three-year term that is renewable.

Operating Procedures

The Shellfish Aquaculture Specialist (SAS) with the Marine Advisory Program at VIMS will serve as the interface between SAIAC and VIMS, coordinating communications and organizing meetings. To facilitate efficient coordination of meeting agendas and communications, the SAIAC will elect among themselves two co-chairs, one that can represent the interests of operations with hatcheries and another that is involved in the grow-out phase only. There is an expectation that the co-chairs will work with the VIMS SAS to develop meeting agendas.

SAIAC Members 2022

Tommy Clark, Tom's Cove Aquafarms [C, O, H, G, P, ES]
Mike Congrove, Oyster Seed Holdings [O, H, WS]
AJ Erskine, KCB Oyster Holdings, Cowart Seafood Corp.,
Bevans Oyster Company [O, H, G, P, WS]
Ann Gallivan, JC Walker Brothers [C, O, H, G, ES]
Heather Lusk, H.M. Terry Company [C, O, G, H, ES]
Angelina Manyak filling in for Mike Manyak, Sapidus Farms [O, G, WS]
Patrick Oliver, Rappahannock River Oysters [O, G, WS]
Tom Perry, White Stone Oyster Company [O, G, WS]
Tim Rapine, Ballard Fish & Oyster Company [C, O, H, G, P, ES]
John Vigliotta, Ward Oyster Company [O, C, H, G, WS]

KEY:
Species cultivated: Clam (C), Oyster (O)
Business: Hatchery (H), Grower (G), Processor (P)
Location: Eastern Shore (ES), Western Shore (WS)

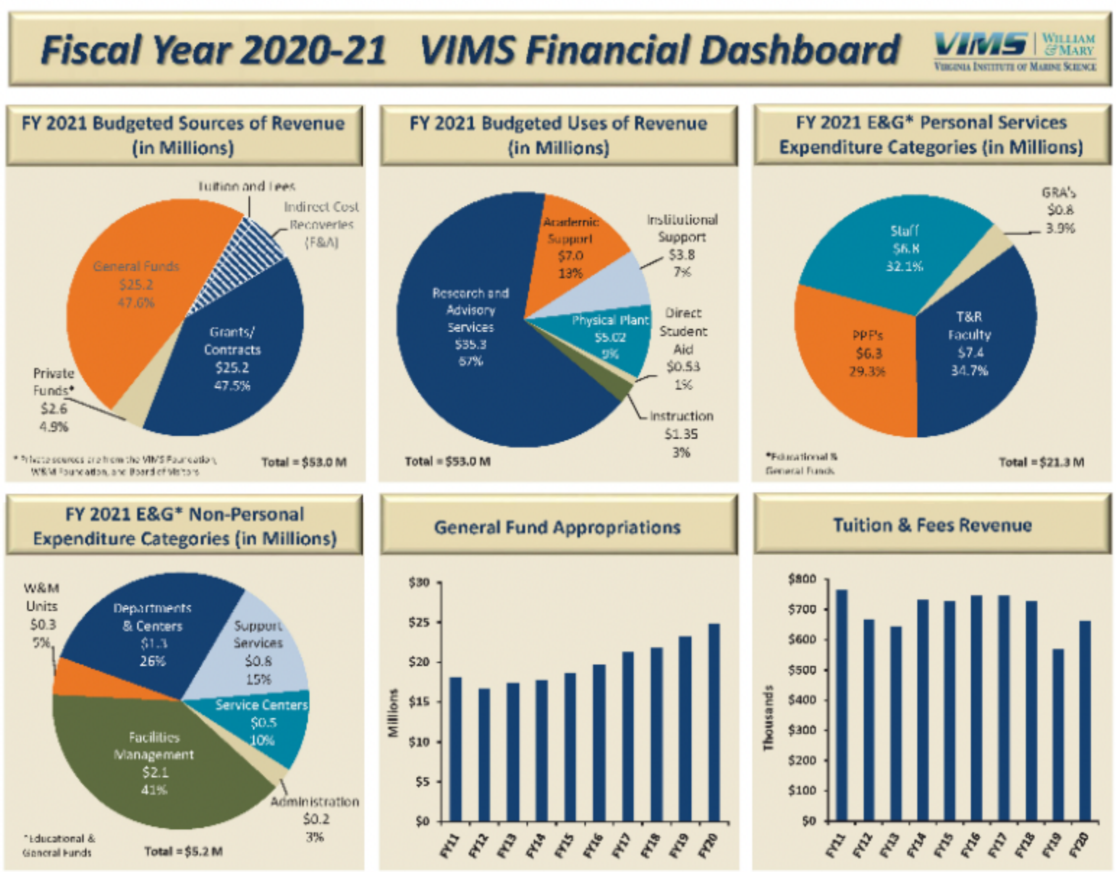
Invited Industry Participants:
Mike Oesterling, Executive Director of Shellfish Growers of Virginia
Kim Huskey, Legislative Affairs, Ballard Fish & Oyster Company

VIMS Leadership & Administration Introduction

Dr. Derek Aday has served as the Dean & Director of the William & Mary School of Marine Science at the Virginia Institute of Marine Science (VIMS) since September 2021. He oversees the Offices of Academic Studies, Research and Advisory Services, Finance and Administration, Advancement, and Operations.

As Dean and Director, Dr. Aday has administrative responsibilities at VIMS to provide the vision, strategic planning, and overall leadership for the Institute to be a center of excellence in marine research, education, and advisory service. This year, a new visionary strategic plan was developed for VIMS that emphasizes the commitment to: be global leaders in the development and maintenance of resilient systems by producing science for healthy ecosystems, stewardship of natural resources, and effective responses to global change; train the next generation of science leaders; remain the trusted science advisor to Virginia and coastal communities worldwide; expand our societal impact and engagement; and be leaders in sustainable action.

VIMS is currently among the largest marine research and education centers in the United States with an annual operating budget of $53 million.



Introduction of Select Programs/Units

Office of Research and Advisory Service (ORAS)

Mark Luckenbach, Associate Dean of Research and Advisory Services, luck@vims.edu
Lyle Varnell, Associate Director for Advisory Services
Emily Hein, Assistant Director for Advisory Services
Cecilia Lewis, Assistant to the Associate Dean

About our Office

The Virginia Institute of Marine Science (VIMS) is mandated by the Commonwealth of Virginia to conduct research and provide sound scientific advice concerning the often-contentious issues surrounding use and conservation of marine resources. The Office of Research and Advisory Services is the central administrative office charged with coordinating these state-mandated research and advisory activities.

As part of VIMS' broad legislative mission, the Institute serves as the Commonwealth's center of expertise in the marine environment, bringing together governmental, economic, regulatory, and scientific communities.

VIMS is a central partner in the state's environmental management infrastructure working with the Virginia Marine Resources Commission, the Department of Environmental Quality, the Department of Conservation and Recreation, and Department of Wildlife Resources, as well as the Department of Emergency Management, Virginia Department of Transportation, and the Virginia Department of Health. Additionally, VIMS is responsible for responding to requests from the General Assembly, the Governor's Office, and the Secretary of Natural and Historic Resources. VIMS works with local governments and management agencies such as Soil and Water Districts and Planning District Commissions, and researchers participate in and represent the state's interests on regional commissions such as the Potomac River Fisheries Commission, the Atlantic States Marine Fisheries Commission, and the New England Fisheries Management Council.

VIMS also serves as the state's Sea Grant Institution. Sea Grant is a Federal-University partnership program that works to create and maintain a healthy coastal environment and economy.

Marine Advisory Program (MAP)

Dave Rudders, Associate Director, Marine Advisory Program rudders@vims.edu
Karen Hudson, Shellfish Aquaculture
Lexy McCarty, Shellfish Aquaculture Research/Extension

Sarah Borsetti, Commercial Fisheries
Lisa Lawrence, Marine Education Program Leader
Susanna Musick, Marine Recreation
Dan Sennett, Marine Aquaculture/Aquarium

About our Program

In addition to research and education, advisory service represents one of the three components of the tripartite mission of VIMS. While all VIMS faculty and scientists are engaged to provide expert advice to the Commonwealth, the Marine Advisory Program (MAP) is unique in that it is home to scientists whose major focus is on advisory programs that benefit the citizenry, marine industries, and government of the Commonwealth.

The Marine Advisory Program at VIMS provides education, outreach, and technical assistance in the broad areas of aquaculture, commercial and sport fishing, marine education, marine business, and seafood technology and safety. The MAP also oversees the Fishery Resource Grant Program (FRGP) that was created by the Virginia Legislature and ratified House Bill 1634 in 1999. The objective of the FRGP is to: "Protect and enhance the state's coastal fishery resources through the awarding of grants". MAP also provides marine extension capacity on behalf of Virginia Sea Grant, a state/federal program administered through National Oceanic and Atmospheric Administration (NOAA).

MAP's Shellfish Aquaculture unit supports the sustainable growth of Virginia's molluscan shellfish culture industry. To accomplish this mission, specialists provide a point of contact to address stakeholder questions, technical support, research, educations, and liaison service. Highlighted deliverables include:

- Virginia Shellfish Growers Listserv
- Virginia Aquaculture Conference
- Virginia Shellfish Aquaculture Situation and Outlook Report

VIMS Shellfish Aquaculture Program & SALT-SI

William Walton, Program Director, walton@vims.edu, 804.684.7238

The VIMS Shellfish Aquaculture Program consists of dynamic team of faculty, staff and students who have identified shellfish aquaculture as a priority, engaging through research, education and advisory services. The program also includes several facilities (e.g. the Acuff Center for Aquaculture, the Eastern Shore Lab, the research farm at the Gloucester Point campus), other resources (e.g., vessels, equipment) and centers of excellence (the Aquaculture Genetics and Breeding Technology Center, the VIMS Shellfish Pathology Lab, the Marine Advisory Program, etc.).

The VIMS Shellfish Aquaculture Program is committed to conducting state-of-the-art research and offering world-class education in shellfish aquaculture science. Shellfish aquaculture is an essential element of the economy, environmental health, cultural heritage, and well-being of our coastal communities.

Mission

The overarching mission of VIMS' Shellfish Aquaculture Science Initiative (SALT-SI) is to advance and support a thriving sustainable shellfish aquaculture community in Virginia and the United States, through globally relevant shellfish aquaculture science, outreach, and education. The overall mission of VIMS' SALT-SI incorporates four integrated goals:

- Support prosperous and sustainable shellfish aquaculture production
- Produce highly capable individuals skilled in and knowledgeable about aquaculture production
- Engaged and empowered communities with the best available shellfish aquaculture science
- Increasing demand and market access for shellfish produced in the United States

VIMS' SALT-SI accomplishes these goals by:

- Encouraging active collaboration among the many research programs at VIMS and partnering organizations that are engaged in the multi-disciplinary science of shellfish aquaculture, regionally, nationally, and internationally;
- Engaging stakeholders and students in research to solve real-world problems and improve science-based decision making;
- Training a diverse, capable workforce through education and hands-on training, inclusive of underrepresented groups to meet the needs of the shellfish aquaculture industry today and into the future;
- Generating and testing innovative technologies that provide practical, actionable benefits to the shellfish aquaculture industry;
- Serving as a central source of knowledge concerning the practice of shellfish aquaculture and its role in our environment, society, and economy; and
- Responding to concerns, problems, and opportunities raised by the industry, regulatory agencies, and others, while also looking ahead to identify new challenges and opportunities.

Acuff Center for Aquaculture (ACA)

Bill Walton, ACA Director, walton@vims.edu, 804.684.7238

Lauren Gregg, Aquaculture Facility Manager
Haley Uliasz, Aquaculture Algologist

About

The Acuff Center for Aquaculture is a 22,000-square-foot shellfish hatchery that supports collaborative research, education, and advisory teams within VIMS' Shellfish Aquaculture Program. The building has an expansive, open floorplan allowing flexibility to meet the changing research and husbandry needs of many users, with capacity for shellfish spawning, larval culture and setting, as well as a specialized algae and broodstock rooms, 4 labs, 4 offices, and a workshop. There is ample space to accommodate hundreds of distinct shellfish cultures in their early life stages, from spawning through settlement. State-of-the-art seawater filtration and climate-control systems maintain optimal conditions for ripening broodstock, culturing shellfish larvae, and growing microalgae for feeding animals throughout the facility.

Updates

The facility is positioned for a full production season in 2023, serving as the base of operations for the Aquaculture Genetics and Breeding Technology Center (ABC) oyster breeding spawning, setting and nursery work. At least three other funded research projects intend to use the Center for conducting shellfish aquaculture research, with efforts to spawn additional species, such as soft-shell clams (*Mya arenaria*) and ribbed mussels (*Geukensia demissa*).

The facility will collect and share data on water parameters, larval performance, algal production, etc. Additionally, several questions are being explored including improvements in algal production.

In addition, several workshops are planned to share new techniques, demonstrate new technology or review useful procedures. Finally, efforts are being made to develop a workforce development program for the shellfish aquaculture industry.

Aquaculture Genetics and Breeding Technology Center (ABC)

Jessica Small, ABC Director, jamoss@vims.edu, 804.684.7955

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AQUACULTURE GENETICS & BREEDING TECHNOLOGY CENTER

Formed from a Legislative Initiative in 1997, ABC has grown into one of the world's leading oyster breeding programs. Our general operations can be divided into three major categories.

First and foremost, ABC serves as a **breeding program** in support of the Virginia industry, both growers and hatcheries. Our selection program focuses on breeding lines, both diploid and tetraploid, appropriate for the entire salinity range found in Chesapeake Bay (thus quickly growing to serve Virginia and Maryland). Furthermore, it turns out that some of the lines we produce have utility outside of the Chesapeake Bay area, so our hatcheries benefit from the program by widening their sales of seed and eyed larvae accordingly. The hallmark of ABC's activity is the distribution of fast-growing, disease resistant adult broodstock to hatcheries in Virginia and, now, several East Coast states.

Recent brood stock developments:

- Diploid family lines – Longstanding lines like "Deby" remain ever-popular, but are now accompanied by newer lines updated annually; "Lily" and "Hnry."
- Tetraploid lines – our original GEN line has been refreshed with new genes and hatcheries can now choose from GNL or NGN lines.
- Tetraploid family selection has allowed us to create another new 4N line for hatcheries – FYR.

Second, ABC serves as a **training and research** arm for the industry. Through our Oyster Aquaculture Training (OAT) program, we aspire to train young professionals in the commercial aspects of oyster aquaculture. We have dozens of former OAT trainees dispersed in companies locally and even nationally. Through the years, a number of PhD scientists, post-doctoral scientists, and graduate students have studied questions pertaining to advancing oyster aquaculture and emerging industry challenges. The infrastructure we have built in support of the breeding program increasingly serves as a foundation for collaborative studies in shellfish aquaculture with national partners. Results from all of these benefit our understanding of breeding better oysters.

Third, largely because of the success that ABC has had to enable the industry's growth, we have been increasingly sought as a source of **expertise** for other similar programs. We maintain collaborative relationships with the USDA Agricultural Research Service based at the University of Rhode Island, Taylor Shellfish in Washington State, as well as several private companies.

Eastern Shore Laboratory (ESL)

Richard Snyder, ESL Director, rsnyder@vims.edu, 757.787.5834

About

The Virginia Institute of Marine Science's Eastern Shore Laboratory (ESL) serves as both a field station in support of research and teaching and as a site for resident research in coastal ecology and aquaculture. By virtue of its access to unique coastal habitats, excellent water quality, and an extensive seawater laboratory, the ESL affords educational and research opportunities not available elsewhere within the region. Over its 40-year history, the laboratory has become internationally recognized for shellfish research, with important contributions to molluscan ecology and culture.

Updates

Water quality stations at Willis Wharf Cherrystone Aquafarms site and at the VIMS ESL pier continue to provide real time data, data summaries, and archived data are available. Data include: temperature, salinity, oxygen, algae chlorophyll, bacterial chlorophyll, turbidity, and pH. A request is pending for additional stations targeted for Chincoteague, Saxis, and a third bayside location between Silver Beach and Smith Beach.

New construction at the lab will be complete fall/winter 2022, including a modernized Castagna Research Hatchery and a Research Lab building with molecular biology, microbiology, and algae culture labs. Multiple classroom/conference rooms are available. New personnel for the lab include a custodial/maintenance worker, a marine tech for water quality stations, and a second faculty position (currently accepting applications).

Highlighted research:

1. Agriculture (field crop and poultry) effects on water quality are being addressed with the VT painter AREC building on the lab's recent assessment of 80 freshwater streams in Accomack County

    - Snyder, R.A., Ross, P.G. (2020) **Water quality in Accomack County freshwater streams 2020.** VIMS Eastern Shore Laboratory Technical Report No. 7. Virginia Insittue of Marine Science, William & Mary https://doi.org/10.25773/E73R-AM63

2. Initiated a long-term ecological monitoring program for Seaside, including annual assessment of oyster spat set and adult oyster population dynamics.

    - Ross, P. G., & Snyder, R. A. (2022) **Ecological Monitoring Program at VIMS ESL: Annual report 2021.** VIMS Eastern Shore Laboratory Technical Report No. 9. Virginia Institute of Marine Science, William & Mary. doi: 10.25773/evhr-a810

3. Continue to support the development and implementation of recirculating water treatment systems for hatcheries.

VIMS Shellfish Pathology Laboratory – The Carnegie Lab

PI: Ryan Carnegie, carnegie@vims.edu, 804.684.7713

The Carnegie Lab continues shellfish health research, education and advisory service that began under Jay Andrews in the 1950s and continued under Gene Burreson's direction from the 1980s-2000s. Central to lab activity is an annual monitoring program that has four elements. Three of these comprise a *targeted surveillance* program for endemic and emerging pathogens and diseases of aquaculture relevance, and include:

- An annual *Fall Survey* of oyster diseases on 32 Virginia oyster reefs;
- A *James Quarterly Survey* extending the Fall Survey to January, April and July for four James River reefs; and
- *Spring Imports* disease sentinel oysters deployed to the York River and monitored from May-November.

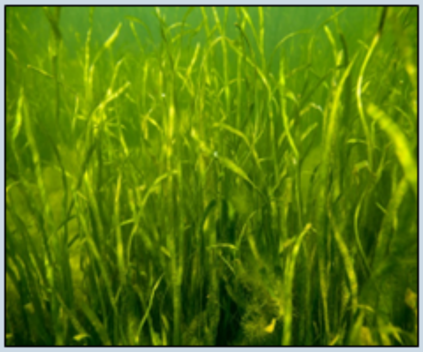
The fourth element is *samples from industry*, which now exceed 150 annually, that represent *passive surveillance* for known and potentially emerging clam and oyster pathogens. Among other benefits, these activities collectively provide a firm scientific foundation for informing decisions related to interstate transfers, typically to the benefit of Virginia producers.

Additional program activities include:

- Ongoing efforts to *streamline regulation of shellfish transfers* on the U.S. East Coast, with Karen Hudson and colleagues from Rutgers University, the ECSGA, and numerous collaborators from industry, regulation and the academic community;
- Research on the *ecology of shellfish diseases*, including disease interactions between aquaculture farms and wild shellfish populations. Our most recent paper, "Intensive oyster aquaculture can reduce disease impacts to sympatric wild oysters", highlights work in this area (*Aquaculture Environment Interactions*, in press; lead author Tal Ben-Horin);
- National and international efforts *to promote resilience of shellfish industries* to emerging pathogens, in collaboration with USDA and NOAA colleagues and through work on the ICES Working Group for Pathology and Diseases of Marine Organisms.

Current Shellfish Aquaculture Research Projects (By Topic)

Aquaculture & Environmental Interactions



Clam and oyster aquaculture involve direct interaction with the surrounding environment. Gear used in oyster aquaculture, specifically, provide structure that helps maintain important habitats, helps slow the rate of shoreline erosion, and provides substrate for many species to adhere to and seek refuge in, increasing biodiversity both within and surrounding an aquaculture operation. However, there is a growing need to better understand the ecological impacts of aquaculture to the surrounding ecosystem to help minimize or avoid potential negative effects as the industry expands.

i. Primary production monitoring to inform shellfish aquaculture

PI: Dr. Mark J. Brush, VIMS Biological Sciences
804.684.7402 | brush@vims.edu | www.vims.edu/people/brush_mj/
Collaborators at VIMS: Sara Blachman
Funding: VIMS (internal funds)

The expansion of shellfish aquaculture in Virginia has generated an important source of revenue and jobs, provided a local source of quality seafood, and supported the economy and culture of local communities. Sustaining the current industry and enabling future expansion are dependent on an adequate food supply for the shellfish. This supply comes primarily in the form of phytoplankton primary production, but local measurements of this vital rate are scarce.

We have instituted a monitoring program to measure phytoplankton primary production in shallow tributaries around the lower Chesapeake Bay to enable calculation of food availability for cultured bivalves. Work is currently focused in Cherrystone Inlet where samples were collected monthly from January to December 2021, and are again being collected monthly from April to October 2022. Measurements are being combined with modeled rates of bivalve feeding to estimate the number of hard clams and oysters that can be supported by local phytoplankton productivity in Cherrystone. Sampling is funded to continue in 2023 in a new site (to be determined), and we intend to continue monitoring annually across a variety of locations.

ii. Modeling shellfish production capacity and ecosystem services

PI: Dr. Mark J. Brush, VIMS Biological Sciences
804.684.7402 | brush@vims.edu | www.vims.edu/people/brush_mj/
Collaborators at VIMS: Chris Patrick and Sara Blachman
Funding: Commonwealth of Virginia

The expansion of shellfish aquaculture in Virginia has created unique "agro-ecosystems" in which cultured bivalves are an integral part of the local marine environment. These bivalves play an important role in ecosystem processes in these systems and provide a number of ecosystem services (e.g., water filtration, nutrient removal), and are simultaneously influenced by the surrounding system (e.g., water quality, long-term change). We are utilizing our predictive ecosystem modeling capabilities to explore these interactions at the ecosystem level, focused primarily on the Virginia Eastern Shore (VAES):

i. Cherrystone Inlet Ecosystem Model: Previous VIMS graduate student Michael Kuschner developed an ecosystem model including hard clam aquaculture in Cherrystone Inlet in 2015. The model was used to quantify clam growth and harvest biomass as a function of stocking density, food sources supporting clam growth, the influence of cultured clams on water quality, and potential impacts of climate change. We have recently updated the model to include cultured oysters and are simulating growth and harvest biomass of both species in the system.

ii. SAV-Clam Aquaculture Interactions (with Patrick and Blachman): VIMS received funding from the Commonwealth of Virginia in 2021 to explore the interactions between hard clam aquaculture and submerged aquatic vegetation (SAV) on the seaside of the VAES. We are combining existing and new data with our previously developed models of VAES watershed loading, lagoon ecosystem response, hard clams, and eelgrass to quantify the ecosystem services provided by both habitats.

Genetic Improvement

The Virginia oyster aquaculture industry prefers genetically improved triploid seed because they are viewed as more viable from a commercial standpoint (i.e. disease-resistant, fast growth, can be harvested year-round). The clam industry in Virginia is reaching a saturation point as available habitat is constrained by suitable water and sediment conditions. Thus, there is a continued need to develop high performing shellfish stocks that thrive in regional environments to ensure continued success and sustain Virginia's position as an aquaculture leader.

i. Initial development of a genetic testing service to support industry hard clam breeding

PI: Alexandra McCarty, VIMS Marine Advisory Program

804.684.7810 | ajmccarty@vims.edu | www.vims.edu/about/directory/staff/mccarty

Collaborators at VIMS: Karen Hudson, Jan McDowell, Richard Snyder, Kimberly S. Reece, William Walton

Funding: Virginia Sea Grant

Virginia is home to a mature hard clam aquaculture industry that is the largest in the United States. In 2021, hard clam landings accounted for 43.4% of all fishery landings in Virginia and were valued at $57.8 million. While the clam culture industry is considered mature, industry leaders have requested help assessing the genetic diversity of their broodstock for future sustainability and resilience, especially considering the potential dangers from climate and disease-related threats. Previous work at VIMS has characterized the geographic genetic variation in hard clams along the east coast of the United States and identified a subset of informative genetic makers for differentiating between wild stocks, but the genetic diversity of VA cultured clams remains unknown.

Utilizing previous genomic data, new sequence data from VA cultured clams will be analyzed and tools will be developed to assess broodstock genetic diversity. These tools will be presented to industry in the form of a service center, where industry can submit samples, which will remain confidential, to have the diversity of their broodstock assessed. If useful, this framework can be modified and expanded to best support the industry moving forward.

ii. Sea Grant Hard Clam Selective Breeding Collaborative

VIMS PIs: Jan McDowell, Kimberley Reece, Karen Hudson

The goal of this 3-year funded project was to establish a regional aquaculture hub using genomic technology to conduct selective breeding for the hard clam (*Mercenaria mercenaria*) to benefit the aquaculture industry along the Atlantic coast.
Visit the website for updates and more information: https://arcg.is/fXXrm

iii. Comparing gene expression in response to low salinity among hard clam, *Mercenaria mercenaria*, lines

PI: Leslie S. Youtsey
804.684.7605 | lgspeight@vims.edu
Advisors: Jan McDowell& Kimberly S. Reece
Funding: VIMS and VAC

The hard clam is an important ecological and economic resource along the U.S. Eastern Seaboard. In Virginia alone, farm gate sales were estimated at $57.8 million in 2021, making Virginia the largest producer of hard clams in the U.S. This industry is primarily limited to higher salinity habitats on the seaside of the Eastern Shore of Virginia or lower Chesapeake Bay. Although the hard clam can be found in lower salinity habitats, they do not grow or survive at rates that are practical for productive aquaculture. In the spring of 2019 and 2021, clam lines were created at the VIMS Eastern Shore Laboratory. Salinity exposures were conducted in the summer of 2021 with eight clam lines at four different salinities (35, 20, 15 and 12 ppt). RNA sequencing (RNA-Seq) data from either the gill (2019) or pooled whole bodies (2021) of exposed clams at 35 and 15 ppt were used to assess the transcriptomic response to low salinity stress. Transcriptomic analysis is a powerful tool for that occur under osmotic stress.

This study found 545 genes in the gills of adult hard clams and 465 genes in the whole bodies of juvenile hard clams that were significantly differentially expressed between 15 and 35 ppt. Some of the top genes included those in the categories of heat shock proteins, apoptosis, and cellular polarity. The amino acid sequences of these differentially expressed genes (DEGs) were assigned to key pathways like protein processing in the endoplasmic reticulum and Rap1 signaling. Some clam lines from the same population had large differences in which genes were expressed in response to low salinity (15 ppt) and some clam lines from different populations showed minimal differences. Some of the genes differentially expressed by different clam lines included heat shock proteins, inorganic ion regulators, and free amino acid isomerase enzymes. The observed difference between clam lines could indicate different tolerance to low salinity and adapted molecular approaches to combat osmotic stress, which could benefit the aquaculture industry and lead to strategic breeding programs for enhanced low salinity tolerance in hard clams.

iv. From Sequence to Consequence: Genomic selection to expand and improve selective breeding for the eastern oyster

PI: Jessica Small, VIMS Aquaculture Genetics & Breeding Technology Center
804.684.7955 | jamoss@vims.edu | www.vims.edu/about/directory/faculty/small_jm.php
Other key personnel: Ximing Guo (Rutgers University), Bassam Allam (Stony Brook University), Standish K Allen (retired VIMS), Marta Gomez-Chiarri (University of Rhode Island), Matthew Hare (Cornell University), Ming Liu (Morgan State University), Katie Lotterhos (Northeastern University), Louis Plough (UMCES Horn Point Laboratory), Dina Proestou (USDA ARS National Coldwater Marine Aquaculture Center), Jonathan Puritz (URI), Paul Rawson (University of Maine), Gary Wikfors (NOAA NEFSC Milford Laboatroy), Ami Wilbur (UNC Wilmington)
Funding: NOAA/Atlantic States Marine Fisheries Commission for the Eastern Oyster Breeding Consortium

The goal of this research is to accelerate and expand selective breeding of *C. virginica* for all growing regions on the East Coast by developing, testing, and verifying genome-based breeding. This research will provide proof-of-principle for the application of genome-based tools to existing Eastern oyster breeding programs and will outline standard procedures for the expansion of breeding to fulfill the needs of a diverse industry. This project will:

1. Develop an efficient genotyping platform for *C. virginica*.
   - Ximing Guo *, Jonathan Puritz , Zhenwei Wang, Dina Proestou, Standish Allen, Jr., Jessica Small, Klara Verbyla, Honggang Zhao, Jaime Haggard, Noah Chriss, Dan Zeng, Kathryn Lundgren, Bassem Allam, David Bushek, Marta Gomez-Chiarri, Matthew Hare, Christopher Hollenbeck, Jerome La Peyre, Ming Liu, Katie E. Lotterhos , Louis Plough, Paul Rawson, Scott Rikard, Eric Saillant, Robin Varney, Gary Wikfors, Ami Wilbur. **Development and evaluation of high-density SNP arrays for the eastern oyster Crassostrea virginica**. *Marine Biotechnology* doi.org/10.1007/s10126-022-10191-3
2. Use the genotyping platform to characterize selected lines and establish associations between phenotype and genotype for important production traits.
3. Apply estimated genotype/phenotype associations to breeding through genomic selection (GS).
4. Evaluate traditional and genomic-based breeding and identify high performance lines across growing regions (ME, RI, NY, NJ, MD, VA, NC).

ABC's intent with this research is to examine the potential for using genomic selection (GS) in eastern oysters and then applying it to ABC's family breeding program. To investigate the potential of GS to improve breeding at ABC, over 2500 brood stock and progeny have been genotyped utilizing the high-density SNP array (SNP stands for single nucleotide polymorphism), with an additional 800 to be completed by mid-November. Measurements of growth, survival, meat yield, shell shape, and Dermo resistance were recorded for each progeny as traits of interest. ABC, along with collaborators at the Center for Aquaculture Technologies in San Diego California, have been analyzing the genotype data to assess the improvement in prediction accuracy when using genotype data opposed to pedigree-based data (current method of breeding at ABC). If genomic-based models produce higher prediction accuracies, candidate brood stock for subsequent year classes will be genotyped and genotypes applied to this reference model to predict genomic breeding values (or genetic merit) for the series of traits. It is anticipated that GS will provide higher predictive accuracies, thus increasing the capacity for genetic gains in traits of interest. In addition, ABC is developing a lower-cost genotype array to be used in routine analysis of progeny going forward.

v. Production of pedigree families for research on disease resistance and correlation of resistance with field traits

PI: Jessica Small, VIMS Aquaculture Genetics & Breeding Technology Center
804.684.7955 | jamoss@vims.edu | www.vims.edu/about/directory/faculty/small_jm.php
Other PIs: Dina Proestou (USDA ARS National Coldwater Marine Aquaculture Center)

Researchers at ABC and USDA ARS at URI have been examining the genetic parameters associated with Dermo resistance over the past three years. This has included more than 3 large lab challenges performed at URI using ABC families. It is the intention to incorporate the lab challenge as a trait into the family-selection breeding models at ABC.

Environmental Challenges

The Chesapeake Bay has been identified as a vulnerable region due to the emergence of multiple environmental stressors. In Virginia, the occurrence and frequency of HABs have increased over the past several years. The shellfish aquaculture industry is most concerned about the effect of HABs on larvae and small seed, but an emerging concern is the potential for human illness when toxins from HABs are concentrated in shellfish that are consumed. Ocean acidification poses another threat to shellfish aquaculture, as a more acidic ocean may impact the ability of shellfish to properly form and maintain their shells, grow, and ultimately survive. Heavy precipitation events are predicted with global climate change scenarios, which can result in eutrophication, dead zones, and decreased salinity levels in coastal areas. Success of the Virginia shellfish aquaculture industry relies, in part, to understanding how these cultured species react to these emerging environmental challenges.

i. Modeling the influence of multiple stressors on shellfish aquaculture

PI: Dr. Mark J. Brush, VIMS Biological Sciences
804.684.7402 | brush@vims.edu | www.vims.edu/people/brush_mj/
Collaborators at VIMS: Emily Rivest, Marjorie Friedrichs, Pierre St-Laurent, Karen Hudson, and Sara Blachman
Funding: NOAA

Cultured oysters in Chesapeake Bay and on the Virginia Eastern Shore are subject to a myriad of stressors including fluctuations in temperature and salinity, elevated total suspended solids, low dissolved oxygen, and declining pH due to ocean acidification (OA). These stressors will likely become increasingly detrimental with ongoing climate change. To address the impact of these stressors on Eastern oysters, we are utilizing our model of oyster growth and energetics, *EcoOyster*, in the following grant-funded projects. *EcoOyster* simulates the growth of oyster tissue and shell over daily, seasonal, annual, and multi-annual time scales as a function of local environmental conditions.

- NOAA OA Thresholds Project (with Rivest and Blachman): We used results from a controlled OA experiment to expand *EcoOyster* to include the impacts of OA on oysters, particularly on shell growth. The model is being used to predict thresholds of OA and other stressors that will result in negative growth and thus be detrimental to the industry. We are also exploring the potential for seagrasses to offset the negative impacts of OA on oysters by coupling *EcoOyster* to the seagrass model *GrassLight*.

- NOAA Regional Vulnerability Assessment Project (with Friedrichs, Rivest, St-Laurent, Hudson, and Blachman): We are applying *EcoOyster* around the entire Chesapeake Bay to identify hot spots for oyster growth. Model output is being compared to observed growth rates from the scientific literature. *EcoOyster* has also been implemented within the high resolution ChesROMS-ECM model to explore the impact of current and future OA on oyster growth.

ii. Understanding spring mortality events in mid-Atlantic oysters

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Funding: North Carolina Commercial Fishing Resource Fund

Recent years have seen recurring spring mortality events impact oyster fisheries and aquaculture throughout North Carolina and locations all across the Southeast. Mortality approaching 30% is common, but in some years has exceeded 85% of oysters planted at numerous sites across North Carolina's Sounds and the lower Chesapeake Bay. Most reports are from adult, sub-market sized triploid oysters, but wild and cultured diploids, as well as smaller seed oysters, have also seen similarly timed mortality events. These events do not seem to be associated with known oyster pathogens and disease but do seem to follow large rain events in the spring. The only unusual sign of pathology is increased inflammation in the gills, and the timing of these mortality events corresponds with seasonal peaks in gametogenic development in diploids. These observations, taken together, suggest that oyster physiology and energetics interact with water quality changes and environmental stress to drive pathology.

Our objective is to test how metabolic changes in oysters through the spring and summer drive oyster microbiota and pathology. We will quantify these changes across eight sites, six in North Carolina and two in Virginia, using hatchery-produced diploid and triploid oyster lines, testing whether triploid oysters are more sensitive to physiological changes and spring mortality events as compared to diploids. Our goal is to identify metabolic processes and microbes associated with spring mortality events, and whether these vary with ploidy, which will allow us to better identify water quality risks to North Carolina oyster fisheries and aquaculture while assessing how to move industries forward in the face of continued spring mortality.

iii. A transcriptomic study of the differential stress response between diploid and triploid eastern oyster *Crassostrea virginica*, and its potential involvement in triploid mortality

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Industry partners: Cherrystone Aqua-Farms, Big Island Aquaculture

The eastern oyster, *Crassostrea virginica,* aquaculture industry (New England to Gulf of Mexico) is primarily based on the use of hatchery-derived, selectively-bred oysters, which are often triploid in ploidy. Triploid oysters are favored by growers because they have higher growth rates than diploids and maintain higher meat quality during the spawning season. However, aquaculturists along the east coast face significant unexplained mortalities of near market-sized oysters in late spring and early summer months (average ~30%, but has approached 50-85% in some cases), resulting in significant economic impacts to growers. Triploid oyster mortality represents a direct threat to the viability and long-term profitability of oyster aquaculture along the East and Gulf coasts. We will conduct a series of side-by-side field experiments and concurrent laboratory-based exposure studies utilizing diploid and triploid *C. virginica* to better understand the differential gene expression responses of diploid and triploid oysters to stressors (ex. elevated water temperature and food limitation). The proposed studies will help better define this nebulous condition, and the pathways involved in responses to stress. If a relationship between mortality, gene expression and ploidy is uncovered, these data could be incorporated into the well-established eastern oyster breeding program at the Virginia Institute of Marine Science Aquaculture Technology and Breeding Center (VIMS ABC) and thereby used to produce lines of oysters for industry that have a better resistance to this phenomenon.

iv. Vulnerability of oyster aquaculture and restoration to ocean acidification and other co-stressors in the Chesapeake Bay

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Funding: NOAA

Coastal acidification and its associated co-stressors present a serious and credible threat to the success of both oyster aquaculture and restoration in the Chesapeake Bay. Recent research provides a clearer understanding of the physiological sensitivity of different economically and culturally valuable shellfish species to ocean acidification (OA), but we still lack a basic understanding of how vulnerability differs across the range of shellfish-reliant stakeholders, specifically participants in oyster aquaculture, the growers, watermen and coastal restoration managers. This basic knowledge gap motivates this proposed Regional Vulnerability Assessment (RVA) in the Chesapeake Bay, which aims to: **(1) assess the vulnerability of the oyster aquaculture industry and oyster restoration to OA and other co-stressors,** and **(2) produce the information required by regional communities to aid in adaptation to these stressors.** In achieving these goals, we this project will help us better understand which shellfish stakeholders will be able to successfully adapt, which will seek alternative livelihoods, and what specifically causes the difference between these two disparate outcomes. Specifically, this proposal frames OA as a problem of social-ecological resilience. Our five project objectives are:

1. Identify locations of oyster aquaculture leases, public harvest sites, and sites of existing and future restoration activities ***where*** critical OA thresholds for oysters will be routinely exceeded.
2. Identify the approximate year ***when*** critical OA thresholds will be routinely exceeded at oyster aquaculture leases, public harvest sites, and sites of existing and future restoration activities.
3. Characterize effects of OA on the resilience of different types of aquaculture stakeholders.
4. Characterize ***where*** and ***at what threshold of OA*** do stakeholders *abandon* oyster reliance.
5. Identify assets available to enable adaptation to the impacts of OA for the most vulnerable.

Improving Aquaculture Production

Virginia leads the nation in hard clam production and leads the east coast in eastern oyster production, and the eastern oyster is the most rapidly developing sector of Virginia's shellfish aquaculture industry. For the industry to continue expanding, it is important to identify methods that increase fertilization success and survival in the hatchery, to optimize methods of larval rearing, setting, and field grow-out, and to better understand the effect of domestication and genotype-by-environment interactions. Additionally, there is an opportunity to improve efficiency and profitability through innovative techniques and new technology, positioning the industry to thrive and be a national leader. Co-culture of multiple species, multi-trophic aquaculture, and the culture of new species will further expand the Virginia aquaculture industry.

i. Effect of biofouling and stocking density on microclimate in off-bottom oyster culture grow-out bags

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Oyster farmers growing C*rassostrea virginica* are increasingly utilizing off-bottom culture practices as there is a potential to improve growth, survival, shell shape, meat quality, and product consistency relative to traditional methods. Despite this potential, sudden spring/summer mortality events impacting both on and off-bottom farms, occurring without apparent connection to disease or harmful algal blooms, have been observed along the East and Gulf Coasts, with mortality reaching 85% in 2014 at some Virginia farms. These mortality events could not be explained by changes in ambient water parameters (e.g., a sudden drop in salinity) and typically, the mortalities do not affect every farm in a given waterbody. This pattern suggests that the seed stock and/or the farming practices may be key factors in these mortality events. For the latter, farming practices can lead to substantially different grow-out conditions for oysters.

Conditions inside an oyster bag (dissolved oxygen, pH, turbidity, and chlorophyll-a) may vary from ambient conditions due to a spatio-temporal lag induced by reduced water exchange rates which may be exacerbated by biofouling and oyster stocking density. This ongoing project addresses three questions: 1) Do biofouling control (air-dried vs. not air-dried) and oyster stocking density (high, normal, and empty) decisions affect the microclimate (water quality parameters) inside grow-out bags; 2) Do any observed differences in the water parameters correlate with *C. virginica* performance, including oyster health and disease prevalence; and 3) What combination of farm practices can growers employ to maximize oyster performance while minimizing costs?

Results from this ongoing study suggest that farmers can influence the water parameters within their floating bags through various husbandry decisions, which could correlate to oyster performance. We hope to provide producers with better data so farmers can make more informed husbandry decisions while also identifying factors that could be driving these spring/summer mortality events.

ii. Comparison of bottom and floating cage oyster production

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Intensive aquaculture is the practice of raising oysters in containers through their life cycle to market. In Virginia, intensive aquaculture is dominated by two basic practices, bottom cages and surface floats. There are costs and tradeoffs in both practices, and the challenge for any new farmer is to determine which practice is cost effective and fits their business model best. The purpose of the project is to compare a series of metrics between oysters raised in floating cages versus those raised in bottom cages.

Oysters are being raised to market size and undergo normal husbandry practices that include grading and sorting through the life cycle. At the same time, oysters will be measured for specific metrics at regular intervals. These metrics can be loosely divided into three categories: 1) Yield (growth and survival); 2) 'Quality' (e.g., shell shape, meatiness, etc.); 3) Consumer Satisfaction. Metrics include, but are not limited to, attributes such as: growth and survival, shell shape, meat fullness, color, cleanliness, shelf life, and willingness to pay; with the latter being assessed after oysters have reached market size later in the study.

The study team will compile an information pamphlet/newsletter that will describe the study and the results. This literature will be distributed to various industry organizations and resources for distribution to the aquaculture community of practice. Examples may include sites such as the Shellfish Growers of Virginia, the VIMS Marine Advisory Services, the East Coast Shellfish Growers Association, etc.

iii. Co-culture of grazers and oysters: a nature-based solution approach for biofouling control

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Oyster culture is a longstanding traditional business in the Chesapeake Bay where environmental conditions support the production of shellfish that can be marketed under different grades (size, shape), and taste profiles, including from sweet to briny, with an increasing emphasis on aspects of quality. The control of biofouling, which is exacerbated in high salinity areas, is still an issue for farming on the Virginia Eastern Shore, especially in traditional bottom cages (which tend to reduce social conflicts). Physical methods for biofouling control are time and staff-consuming and can be a considerable expense for the farmer, while types of anti-fouling paints are either prohibited or under development (natural base). Recognizing these limitations, growers have sought alternatives with an interest in more sustainable forms of aquaculture seeking natural and most sustainable solutions is the best strategy.

This work explores if the co-culture of suspension-feeding Eastern oysters (*Crassostrea virginica*) and native grazers, Atlantic Purple Sea urchins (*Arbacia punctulata*), and periwinkles (*Littorina littorea*), representing a possible effective natural solution to control biofouling on farming bags without compromising - and instead potentially improving – the main product, oysters. Two different species' sizes and stocking densities are being tested for comparing potential effects on the cleanliness of cages and species performance while maintaining available space inside the farming gear and species survival, which will be assessed for both species. Broadly, we expect the advantage of deploying urchins with oysters to improve the bivalve quality and bring a secondary additional marketable product, and income, for the farmer.

iv. Predicting growth rates of cage-cultured oysters to help growers manage their crop

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My project focuses on predicting the growth rates of cage-cultured oysters to help growers manage their crop proactively rather than reactively. As oysters grow, they are graded into similar size classes and then split into containers at lower densities for additional room to grow. Experienced growers are in tune with the relative growth rates year-round – pick up in the spring, take off in the summer, and slowdown in the winter - and have a general sense of when to process. I'm investigating how different predictive quantitative models of growth can aid in fine-tuning the planning and management aspect so that oysters are processed on time to limit the extra labor associated with processing too soon or too late. I'm comparing three predictive growth models based on different inputs and mechanisms but focused on the same output – oyster growth rate.

v. Characterizing the role of toxic phytoplankton byproducts in shellfish hatchery failures

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Collaborating Partners: Oyster Seed Holdings, Mook Sea Farm, Fishers Island Oyster Farm
Funding: NOAA-NMFS Saltonstall-Kennedy

This project is expected to promote the sustainability of oyster seed production through mitigation of an emerging disease syndrome on the US East Coast. A troublesome new syndrome presented in young oyster larvae at several regional hatcheries in 2020, resulting in production failures decreasing seed output by over 40%. Moribund, dwarfed, and delayed in development, affected larvae clearly displayed pale digestive glands suggesting failure of digestion despite a stomach full of microalgal food. The signs reappeared in 2021, demonstrating the disease's persistence. Similar signs presented across a wide geography, with reports of seed not digesting their food, and therefore not growing, at hatcheries in Maine, New York, and Virginia. Preliminary work conducted by one of these hatcheries, Mook Sea Farm (Maine), demonstrated a link between the signs and toxic phytoplankton byproducts in the hatchery water. This timely project now builds on this information, expanding the study to include three East Coast hatcheries and experts in disease and lipidomics. Through partnerships between industry and academia, this project strives to understand this new but persistent disease, increase awareness along the East Coast, and identify mitigation technology to avoid or minimize symptoms with the overall goal of improving product yield.

This work addresses three objectives expected to benefit the eastern oyster *Crassostrea virginica* aquaculture industry in the US: (1) Characterize byproducts and their algal producer(s) in incoming seawater and within hatchery-treated water at three East Coast hatcheries across seasons; (2) Correlate hatchery performance (yield) with pathology and byproduct abundance; and (3) Determine the relative potency of the most abundant byproducts, and identify a water-treatment solution to remove these byproducts. An informative brochure, webpage, listserve, and presentation at an industry-focused conference will be used to communicate results and application to end users in shellfish aquaculture. Outcomes expected from the following activities include an increased awareness of empty-gut syndrome among East Coast hatcheries, and adoption of optimized water treatment step(s).

Shellfish Health and Biosecurity

Historically, disease has had devastating effects on shellfish stocks and aquaculture farms along the east coast of the United States. Growth and expansion of the shellfish aquaculture industry has largely relied on transfer of hatchery seed between states, and historic disease spread was often associated with the transfer of shellfish. It is important to regulate and streamline the transfer of seed between locations to control for disease. Today, reduced and/or failed clam and oyster hatchery production has been reported in our region. Hatchery production is critical to the shellfish aquaculture industry in Virginia and effective management of shellfish pathogens, both endemic and emerging, remains key to sustainable aquaculture development.

i. Impact of OsHv-1 Microvariants on *Crassostrea virginica* Family Lines

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Funding: United States Department of Agriculture

Continued growth of the eastern oyster aquaculture industry relies on consistent and increasing hatchery production, availability of disease resilient stocks and the ability to move oysters among leases (following state regulatory structure). The Ostreid herpesvirus 1 (OsHV-1) and its variants, in particular the more pathogenic OsHV-1 microvariants (OsHV-1 μvars), are emerging infectious disease agents of global concern. Though OsHV-1 μvars are primarily known to affect Pacific oysters, there is concern over the potential impacts of the OsHV-1 μvars to the Eastern oyster and hard clam with the possibility of the spread of the virus to the US East and Gulf Coasts. Therefore, the goal of this research is a proactive approach focused on applied solutions, such as selective breeding, in order to limit impact of OsHV-1 μvars on the rapidly growing eastern oyster aquaculture industry.

We will perform laboratory challenges with diploid eastern oyster families and DEBY and LOLA lines created by the Aquaculture Genetics and Breeding Technology Center (ABC) at VIMS in addition to diploid *Crassostrea gigas*, known to be susceptible to the OsHV-1. All laboratory trials have been and will continue to occur in a quarantine laboratory in Arizona.

The objectives of our research are as follows: 1) Conduct laboratory trials to examine differential survival and viral loads of spat and juveniles from 30 eastern oyster families and two lines exposed to OsHV-1 μvar, 2) Perform quantitative genetic analysis based on survival and OsHV-1 viral loads in order to assess the heritability of resistance/tolerance to OsHV-1 in ABC families, and 3) Develop tools for industry preparedness in case of an OsHV-1 μvar introduction.

ii. Influence of selective breeding on human pathogenic *Vibrio* spp. in eastern oysters

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Funding: NOAA-NMFS Saltonstall-Kennedy

The production of oysters safe for human consumption is one key factor for promoting a sustainable oyster aquaculture industry. The threat of human pathogenic *Vibrio* bacteria naturally associated with oysters is managed through broad measures that fail to account for the wide range of *Vibrio* spp. concentrations observed among individual oysters within a population. Building upon results from our previous studies, we propose to evaluate the effects of oyster lines and associated oyster health on variations in levels of these pathogens among individual oysters.

We will deploy eastern oysters from three genetically-distinct lines at a polyhaline and at a mesohaline salinity site and we will determine 1) concentrations of total *V. vulnificus* and total and pathogenic *V. parahaemolyticus* and 2) general oyster health through expression profiling of seven genes involved in stress response as well as histopathological analyses. Potential influence of oyster line and health status will be analyzed through generalized linear mixed-effects models. This project will determine whether inclusion of *Vibrio* spp. levels should be an additional trait controlled through oyster selective breeding programs, and whether using lines that are selected for specific grow-out environments may bring potential benefits with regard to *Vibrio* spp. abundance and thus control.

iii. Virginia coast bay scallops, *Argopecten irradians:* aquaculture and wild restoration

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This project is testing the potential of using bay scallops (*Argopecten irradians*) for both ecological restoration and aquaculture. Restoring the wild population of bay scallops to seaside Eastern Shore of Virginia (ESVA) is focused on increasing genetic diversity. Aquaculture efforts for bays scallops are focusing genetics on traits suitable for culture (fast growth, size, shell color). Both of these goals are using genetic stocks maintained at VIMS ESL from Florida, North Carolina, and New York. Aquaculture efforts also include working with local growers on different techniques, and replicating those at ESL at smaller scale, and developing a plan for a private commercial hatchery.

iv. Chesapeake Bay Environmental Forecasting System: Accelerating the transition of HAB and pathogen models from research to operations

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Funding: NOAA

For many years our existing Chesapeake Bay Environmental Forecasting System (CBEFS; www.vims.edu/cbefs) has been providing real-time nowcasts and forecasts of salinity, temperature, hypoxia, and acidification metrics such as pH. Input from our Stakeholder Focus Group meetings has revealed that these forecasts have proven very useful to end-users whose livelihoods depend on the Chesapeake Bay: information on hypoxia helps guide anglers to productive fishing grounds and information on pH can help aquaculturists and oyster hatchery operators decide when to delay spawning and avoid supplying spawning tanks with Bay intake water when water quality is poor. However, feedback from these stakeholders and local managers indicates that they require additional information including nowcasts and forecasts of harmful biotic events, so they can assess health threats and make decisions on beach closures or avoid harvesting shellfish when HABs are present. Based on this feedback, we are currently adding additional forecasts to CBEFS. We now include nowcasts/forecasts of *Vibrio* as well as *Prorocentrum minimum*, a harmful algal bloom (HAB) that is often found in Bay waters. Forecasts of additional HABs such as *Microcystis* and *Karlodinium* are also under development. As part of this project we are also establishing a prototype alert system that will send automated alerts when thresholds for the probable occurrence of these noxious organisms are present. To ensure end users are inherently involved in the continuing development and design of our operational management tools, we are working in partnership with extension specialists to continue conducting Stakeholder Focus Group meetings to receive feedback on the format and content of the forecasts directly from our end users.

Socioeconomics

It is important to understand how society perceives and interacts with the shellfish aquaculture industry for the industry to grow and expand. Societal changes and/or changes in the economic climate may make it difficult for the industry to succeed and action may be necessary to help overcome these obstacles. Furthermore, with increasing production, there may be a need to expand markets and increase consumer demand. Understanding marketing opportunities, consumer attitudes and distribution channels may increase in importance.

i. Virginia Oyster Productivity Information Tool

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Outside Collaborators: James Wesson (retired VMRC), Marcia Berman (retired VIMS).

Funding: Virginia Coastal Zone Management Program, Virginia Department of Environmental Quality

The most rapid expansion of the aquaculture industry in Virginia has been in hatchery-based production of cage-cultured oysters on private grounds. Conflicts have arisen between new user groups residing along the Chesapeake Bay shore, and a growing industry that works primarily in nearshore waters. Additionally, as the shoreline has been developed, there are very limited access points along the waterfront where commercial activity can occur. This study characterized the oyster industry as it exists today, examined the regulatory framework, and closely examined major issues that impact future expansion.

The majority of this study was conducted within the framework of GIS and used geospatial data from mapping and monitoring databases collected over many years. These data were used to map, model, and assess environmental condition, productivity on public grounds, management boundaries, ecological conflicts, and the spatial distribution of cultured oyster productivity as indicated by harvest productivity. The purpose of these assessments was to determine if there was opportunity for aquaculture expansion in Virginia within the current boundaries constrained by public and private grounds without generating new or added conflict. The maps can be used to examine current Baylor Ground and private lease productivity as well potential future aquaculture sites within Baylor Grounds based on restoration potential and environmental constraints. A policy and regulation review examining the use of public Baylor Grounds and the private leasing system conducted by the William and Mary Law School is also included.

The tool can be found here: https://cmap2.vims.edu/OysterInfoToolVa/

ii. Assessing the need and opportunity for workforce development in shellfish aquaculture at the high school level in Virginia

PIs: (VIMS Marine Advisory Program) Celia Cackowski, Karen Hudson, Lisa Ayers Lawrence, Alexandra McCarty

Funding: Virginia Sea Grant 2022-2024 Fisheries and Aquaculture Omnibus

Labor supply is one of the top issues facing the aquaculture industry nation-wide. VIMS provides ongoing support for industry labor needs through The Oyster Aquaculture Training Program (OAT), an annual paid 6-month internship program with special focus on hatchery-skill training run by the Aquaculture Genetics and Breeding Technology Center (ABC), and a summer internship program for local students at the Eastern Shore Laboratory. Despite these current opportunities, Virginia's shellfish aquaculture industry has expressed a need for additional labor pool options.

The MAP educators will assess the current non-college track aquaculture-related opportunities available to high school students in Virginia. MAP educators will also survey these schools to gauge level of interest for enhancing/creating brief shellfish culture "exposure" opportunities. Simultaneously, the MAP shellfish aquaculture specialists will survey the Virginia shellfish aquaculture industry to determine how the industry recruits labor, their labor needs, if they have a relationship with their local high school career programs, and gauge interest in a more structured program and their desired level of involvement.

Results from these communications and surveys will give MAP an understanding of current aquaculture-related career paths in high schools, the desire to create/enhance these programs, and how these programs should be designed according to industry input and needs. Depending on results, next steps would include the development of shellfish aquaculture exposure opportunities along with information on shellfish aquaculture careers, engaging local technical centers and/or regional education centers to determine interest in developing shellfish culture training/certification programs, and considerations of enhancing/expanding established internship programs.

iii. Improving the messaging from servers to patrons regarding half-shell oysters at restaurants

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Despite the recent expansion of oyster aquaculture in the southern US, lack of knowledge about the quality of these farm-raised oysters and even negative perceptions about southern farm-raised oysters continue to persist, especially in regions outside of the southern US. Over two years, as part of a regional effort in a project led by Oyster South, we are providing trainings to servers in seafood restaurants that serve oysters on the half shell to allow servers to better 'tell the story' of the oysters that are served. We will also conduct formal quantitative and qualitative assessments of the effectiveness of this training program to estimate the causal effect of training on knowledge of and, ultimately, sales of farm-raised oysters.

Specifically, we are pursuing the following objectives:

1. Conduct at least 6 trainings in Virginia restaurants and 6 trainings in regional markets (e.g., Washington, DC, Baltimore, New York) with a total of at least 120 seafood food service professionals trained,
2. Quantitatively assess impacts of the training in terms of changes in knowledge and, critically, changes in sales at both the server level (before and after training) and the restaurant level allowing evaluation of differences among restaurants and regions in terms of impacts.
3. Share the specific results of this work with the Virginia oyster aquaculture community about how trainings of seafood service professionals might increase sales.

Potential commercial benefits to the fishing community of this project include a substantial, measurable increase in sales of farm-raised oysters in near (i.e., during the project period) to mid-term. The project design includes an explicit quantification of these increased sales, which could be accomplished through a combination of restaurants beginning sales of southern farm-raised oysters as well as increased sales at restaurants currently offering these oysters. In addition, this project will also serve as the proof of concept of the value of training programs targeting seafood server industry professionals as a means of increasing sales of domestic seafood products in the mid- to long-term.

iv. Feasibility of Offshore Wind Farm Areas as Multi-Use Platforms for Lower-Trophic Aquaculture

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Funding: Atlantic States Marine Fisheries Commission, Regional Pilot Projects in Support of Sustainable Aquaculture

Over two million acres are presently leased for offshore wind energy development in federal waters of the US Northeast and Mid-Atlantic. The public and federal managers have expressed an interest in maintaining seafood production within these areas. This study investigates the biological, economic, and regulatory feasibility of co-locating lower-trophic aquaculture within offshore wind farms along the US Atlantic coast. Opportunities and barriers for co-location will be identified for a suite of potentially feasible cultured species.

v. Economic and environmental feasibility of soft-shell clam aquaculture in Virginia

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Collaborators outside of VIMS: Brian Beal (University of Maine), Mike Congrove (Oyster Seed Holdings, LLC)
Funding: Saltonstall-Kennedy Grant, NOAA Fisheries

In Virginia, shellfish aquaculture is dominated by the eastern oyster (*Crassostrea virginica*) and hard clam (*Mercenaria mercenaria*). The soft-shell clam *(Mya arenaraia)* has been successfully cultured in Maine, but aquaculture of the soft-shell clam in Virginia has not been explicitly explored. This study will determine the clam's ability to grow and reproduce under conditions found farther south, in Virginia. A team of researchers from the Virginia Institute of Marine Science, the University of Maine, and the aquaculture industry are studying the economic and environmental feasibility of soft-shell clam aquaculture in Virginia. Previous field experiments in Virginia have shown the soft-shell clam can grow from less than one inch to a two-inch market size in six months, but this study will further determine how factors such as temperature, salinity, and predator-exclusion equipment can affect optimal soft-shell clam production in Virginia. Additionally, the study will test optimal hatchery conditions and estimate costs for starting and maintaining a soft-shell clam aquaculture operation. Lastly, the team will organize focus groups and conduct interviews with seafood dealers and processors to determine the viability of a commercial soft-shell clam market in Virginia.

Results from this study will be used to create instructional manuals and workshops to help growers start a new soft-shell clam aquaculture operation. Soft-shell clam aquaculture will give growers in Virginia's already successful oyster and hard-shell clam industries a new option to expand their operations.

vi. Testing sugar kelp as a winter crop

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Collaborators: Scott Lindell (Woods Hole Oceanographic Institution)
Funding: Department of Energy's ARPAE MARINER Program

The purpose of this project is to test the commercial potential and temperature tolerance of sugar kelp in Virginia coastal waters. Kelp farming is a young, fast growing, million-dollar industry in Maine and Alaska. A seedstring was set out November 2022, harvest will be in April 2023. Targeting our cooler temperatures, this has potential for an annual winter crop for ESVA Aquaculture. Utilizing other alage species is on the horizon for ESL research.

Appendix: Research updates from 2018

i. Submerged aquatic vegetation and floating aquaculture

The effect of floating aquaculture gear on submerged aquatic vegetation (SAV) was investigated at two commercial aquaculture sites in Virginia starting in the summer of 2019:

- Eastern shore site: At this site, lines of floating wire mesh cages containing six bags of oysters each (15 cages per ~165' line) were deployed over an SAV bed dominated by eelgrass. From the start of the study in the summer of 2019 through the summer of 2022, farm-scale impacts of aquaculture on the underlying SAV bed were minor or nonexistent but some minor impacts at the scale of individual cages were observed. Surveys in the fall of 2022 indicated the possibility of farm-scale impacts, but the severity of these impacts is unclear at present. Ongoing studies will assess persistence and scale of the observed impacts by sampling throughout the 2023 SAV growing season.
- Western shore site: At this site, lines of floating bags (~100 bags per 150' line) were deployed over an SAV bed historically dominated by widgeon grass. Starting in July 2021, significant farm-scale impacts on widgeon grass cover were observed at this site. These effects persisted for the remainder of the 2021 growing season and throughout the 2022 growing season. Unfortunately, because the farm was poorly maintained and bags were allowed to drag on the bottom, it is unclear whether the impacts on the underlying grass bed were due primarily to the presence of aquaculture gear or to poor farm management practices. Studies at this site have been terminated. ASelection of a new site that will allow us to further our understanding of the impacts of floating aquaculture on widgeon grass is underway.

View the recorded PowerPoint presentation from the 2022 Virginia Aquaculture Conference: **Submerged Aquatic Vegetation (SAV) and Shellfish Aquaculture:** *Quantifying the impacts of oyster aquaculture on submerged aquatic vegetation*

- o https://vaaquacultureconference.com/program-schedule/

ii. Genetic Basis of Triploid Mortality

◆ Matt, Joseph L., Guévélou, Eric, Small, Jessica Moss, Allen, Standish K (2020) **A field test investigating the influence of brood stock origin and ploidy on the susceptibility of *Crassostrea virginica* to "triploid mortality" in the Chesapeake Bay**. Aquaculture 526:735375 https://doi.org/10.1016/j.aquaculture.2020.735375

In 2016, a mortality event occurred in late spring at only one testing site and only affected the triploid crosses. There was no evidence of substantial disease pressure from *Haplosporidium nelsoni* or *Perkinsus marinus,* or of stressful environmental conditions based on temperature, salinity, pH, and dissolved oxygen. At 18 months, shell height was similar in the diploids and triploids with the most similar genetic origin. Triploids maintained meat weight through the summer, while meat weight in diploids dropped sharply. Triploids may be especially susceptible to late spring mortality events in the Chesapeake Bay.

- Guévélou, Eric; Carnegie, Ryan; Moss, JA; Hudson, Karen; Reece, Kimberly S.; Rybovich, Molly M.; and Allen, Standish K. Jr. (2019) **Tracking triploid mortalities of eastern oysters *Crassostrea virginica* in the Virginia portion of the Chesapeake Bay**. VIMS Articles. 1333. https://scholarworks.wm.edu/vimsarticles/1333

Mortality peaked among triploid oysters at most experimental sites in the Chesapeake Bay during June, with no evidence of the major oyster pathogens causing Dermo and MSX disease. Harmful algal blooms, abnormal salinities, and high temperatures were not associated with the mortalities. There was variability in mortality between oyster lines and between oyster lines at specific sites, with no clear relationship of genetic heritage and mortality. At some locations, triploid oysters seemed to be more susceptible to mortality than diploids and mortality in triploids coincidence with the timing of peak gametogenic development in diploids.

iii. Chromosome Stability in Tetraploids

- de Sousa, JT; Allen, Standish K. Jr.; Wolfe, BM; and Moss, JA. (2018) **Mitotic instability in triploid and tetraploid one-year-old eastern oyster, *Crassostrea virginica*, assessed by cytogenetic and flow cytometry techniques**. *Genome*, 61(2), 79-89. https://scholarworks.wm.edu/vimsarticles/1294

This worked investigated the unique observation of chromosome loss in polyploidy oysters, especially tetraploids – a process called reversion. Results demonstrated that reversion is not problematic for the oyster aquaculture industry but does warrant consideration in ABC's tetraploid breeding program. Results from this study have changed the way ABC interprets genetic testing during tetraploid spawns.

iv. Physiology of Commercial Breeding Traits

- Guévélou, Eric; Matt, Joseph L.; and Allen, Standish K. Jr. (2017) **Glycogen concentration in freeze-dried tissues of eastern oyster (*Crassostrea virginica*) using near infrared reflectance spectroscopy to determine the relationship between concentrations of the tissues excised for histological sampling and the remaining tissues**. *Journal Of Shellfish Research*, 36(2), 325-333. https://scholarworks.wm.edu/vimsarticles/302/

This study determined that near-infrared reflectance spectrometry of freeze-dried oyster tissue is highly efficient at determining glycogen content of whole oyster meat. Ploidy and size (shell height) we found to affect glycogen content.

v. Triploid Oyster Fecundity

◆ Ritter, Kate (2019) **Fecundity of triploid eastern oyster (*Crassostrea virginica*) as a function of tetraploid lineage**. *Dissertations, Theses, and Masters Projects*. Paper 1582642221. http://dx.doi.org/10.21220/m2-9tyh-qe83

Oyster growers in Virginia reported significant mortality events of triploid oysters in spring and summer months, in some cases as high as 50-85% of the crop. Surviving oysters from some of these mortality events were sent to VIMS, and 38% of the triploid oysters examined were females, most with significant quantities of eggs. Fecundity in triploids was an unusual finding because, historically, triploid *C. virginica* appeared effectively sterile. This raised an important question: is there a possible association between triploid mortality and triploid fecundity?

Several lines of tetraploids were crossed with diploid oysters to generate multiple triploid lines. Fecundity was estimated for triploid females after field exposure for 2 years. Results suggest that the site location had more to do with fecundity and mortality than the type of triploid produced, and the fecundity trait is not likely problematic because it occurred in significantly healthier oysters. Therefore, it does not seem likely that ABC inadvertently selected for fecundity in their tetraploid oysters.

vi. Clam Genetic Diversity

◆ Ropp, Ann Janette (2020) **Population Structure Of The Hard Clam, *Mercenaria Mercenaria*, Along The East Coast Of North America**. *Dissertations, Theses, and Masters Projects*. William & Mary. Paper 1616444311. http://dx.doi.org/10.25773/v5-wa9q-8c19

This study used genotyping-by-sequencing to delineate the genetic stock structure of wild clams sampled from 15 locations along the East Coast of North America (Prince Edward Island, Canada, to South Carolina, USA). Data provided evidence of five genetic breaks separating six genetically distinct populations: Canada, Maine, Massachusetts, Mid-Atlantic, Chesapeake Bay and the Carolinas. Data were used to identify a subset of SNP markers capable of geographic discrimination and population assignment with 75–93% accuracy, which will be helpful for future testing and allow for this technology to be economically-feasible for the industry.

vii. ECOHAB 2017: Toxicity and potential food-web impacts of *Alexandrium monilatum* and its toxins

Harmful effects of *Alexandrium monilatum* and a toxin it produces goniodomin A (GDA) on aquatic organisms were demonstrated by laboratory and field studies. GDA was detected in oysters that were deployed during *A. monilatum* blooms with the highest concentrations in those just after the bloom peak. Laboratory exposure studies also demonstrated the uptake and depuration of GDA in oysters even at exposure to very low concentrations of *A. monilatum* cells. Bioassays with *A. monilatum*, *Margalefidinium polykrikoides* and *Karlodinium veneficum* (VA and MD isolates) indicated that mortality was higher and more rapid with exposure to *A. monilatum* assays than to the other two HAB species. Histopathological analyses demonstrate that exposure causes tissue pathology, most notably gill erosion. Oyster field studies done in collaboration with aquaculturists in the York River region indicated that oyster mortality was generally slightly higher at the bloom impacted sites. Models indicated that growth rate was lower when there were bloom concentrations of *M. polykrikoides* or *A. monilatum*. Publications from this work include:

- Fortin, SG, Song, B, Anderson, IC, Reece, KS (2022). **Blooms of the harmful algae *Margalefidinium polykrikoides* and *Alexandrium monilatum* alter the York River Estuary microbiome.** *Harmful Algae 114(102216)* doi:10.1016/j.hal.2022.102216

Blooms of two HAB species, *Margalefidinium* (formerly *Cocholodinium*) *polykrikoides* (Marge) and *Alexandrium monilatum* (Alex), impacted the estuarine microbiome in different ways, likely leading to shifts in estuarine carbon and nutrient cycling.

- Harris, C.M., Reece, K.S., Stec, D.F., Scott, G.P., Jones, W.M., Hobbs, P.L.M. & T.M. Harris. (2020a). **The toxin goniodomin, produced by *Alexandrium* spp., is identical to goniodomin A.** *Harmful Algae* 92(101707) doi: 10.1016/j.hal.2019.101707

This study provides compelling evidence that goniodomin (isolated from an unidentified Puerto Rican dinoflagellate) is identical to goniodomin A, a metabolite of *Alexandrium hiranoi* originally isolated from Japan). Morphological characterization of the dinoflagellate suggests that it was the genus *Alexandrium* and this is the only report of goniodomin in the Caribbean region.

- Harris, C.M., Reece, K.S. and Harris, T.M. (2020b) **34-Desmethyl Goniodomin A: Structure Revision of a Truncated Congener.** *Toxicon* 188:122-126. doi.org/10.1016/j.toxicon.2020.09.013

- Harris, C.M., Krock, B., Tillmann, U., Tainter, C.J., Stec, D.F., Andersen, A.J.C., Larsen, T.O., Reece, K.S. and T.M. Harris (2021) **Alkali metal-and acid-catalyzed interconversion of goniodomin a with congeners B and C**. *J. Nat. Prod.* 2554-2567. doi.org/10.1021/acs.jnatprod.1c00586

- Tainter, C.J., Schley, N.D., Harris, C.M., Stec, D.F., Song, A.K., Balinski, A., Mary, J.C., Mclean, J.A., Reece, K.S. and T.M. Harris 2020. **Algal toxin goniodomin A binds potassium ion selectively to yield a conformationally altered complex with potential biological consequences.** *J. Nat. Products.* 83:1068-1081. https://dx.doi.org/10.1021/acs.jnatprod.9b01094

This study provides insight into the structural features and chemistry of GDA that may be responsible for significant ecological damage association with the GDA-producing algal blooms.

- Wolney, J.L., Tomlinson, M.C., Uz, S.S., Egerton, T.A., McKay, J.R., Meredith, A., Reece, K.S., Scott, G.P. and R.P. Stumpf. (2020) **Current and Future Remote Sensing of Harmful Algal Blooms in the Chesapeake Bay to Support the Shellfish Industry.** 7: article 337 https://doi.org/10.3389/fmars.2020.00337

Here we present a summary of common marine and estuarine HAB species found in the Chesapeake Bay, *Alexandrium monilatum*, *Karlodinium veneficum*, *Margalefidinium polykrikoides*, and *Prorocentrum minimum*, that have been detected from space using multispectral data products from the Ocean and Land Colour Imager (OLCI) sensor on the Sentinel-3 satellites and identified based on *in situ* phytoplankton data and ecological associations. We review how future hyperspectral instruments will improve discrimination of potentially harmful species from other phytoplankton communities and present a framework in which satellite data products could aid Chesapeake Bay resource managers with monitoring water quality and protecting shellfish resources.

viii. CBTOX – HAB toxins in the Chesapeake Bay

- Onofrio, Michelle D.; Egerton, Todd A.; Reece, Kimberly S.; Pease, Sarah K.D.; Sanderson, Marta P.; Jones, William III; Yeargan, Evan; Roach, Amanda; DeMent, Caroline; Reay, William G.; Place, Allen R.; and Smith, Juliette L. (2021). **Spatiotemporal distribution of phycotoxins and their co-occurrence within nearshore waters.** *Harmful Algae* 103:101993 doi:10.1016/j.hal.2021.101993

This study characterized and mapped the spatial and temporal distribution of eight algal toxins across the Chesapeake Bay and identified which of these biotoxins are a current threat to shellfish health and seafood safety in the Lower Chesapeake Bay.

- Pease, Sarah K.D., Reece, Kimberly S., O'Brien, Jeffery, Hobbs, Patrice L.M., Smith, Juliette L (2021). **Oyster hatchery breakthrough of two HABs and potential effects on larval eastern oysters (*Crassostrea virginica*).** *Harmful Algae* 101(101965) doi:10.1016/j.hal.2020.101965

Dinoflagellate species *Karlodinium veneficum* and *Prorocentrum cordatum* (prev. *P. minimum*) were detected in water samples from a local Virginia oyster hatchery collected during the oyster spawning season, meaning the species were not degraded or removed by the hatchery's water treatment process. Results from laboratory experiments suggest that low concentrations of both *K. veneficym* and *P.cordatum* are harmful to larval oysters and *K. veneficym* seems to be more harmful than *P. cordatum.* This research shows that certain water filtration techniques are not successful at removing harmful algal species from ambient water, which could cause reductions in oyster productivity during the spawning season.

- Sanderson, Marta Pilar, Hudson, Karen L., Gregg, Lauren S., Chelser-Poole, Amanda B., Small, Jessica M., Reece, Kimberly S., Carnegie, Ryan B., Smith, Juliette L. (2022). **Breakthrough of toxins and HAB cells into shellfish hatcheries and efforts towards removal.** *Aquaculture* 562:738714 doi:10.1016/j.aquaculture.2022.738714

Several types of toxins were detected is post-treatment water from six different hatcheries in the Chesapeake Bay, but the HAB species themselves were not identifiable. An investigation into the water treatment process found two steps, 24-hour circulation through sand filters and activated charcoal filtration, that were successful in removing a substantial portion of toxins from incoming water. Future experiments are needed to further investigate toxin breakthrough potential and water treatment-techniques during more intense blooms, as these detected toxin breakthroughs occurred during non-bloom conditions.

ix. Ocean Acidification and Data Portal

- Bever, Aaron J., Friedrichs, Majorie A.M., St-Laurent, Pierre. (2021) **Real-time environmental forecasts of the Chesapeake Bay: Model setup, improvements, and online visualization**. *Environmental Modeling & Software* 140:105036 doi:10.1016/j.envsoft.2021.105036

To view real-time visualizations of salinity, temperature, oxygen, and acidification metrics in the Chesapeake Bay, please visit: www.vims.edu/cbefs

- Da, F., Friedrichs, M. A. M., St-Laurent, P., Shadwick, E. H., Najjar, R. G., & Hinson, K. E. (2021) **Mechanisms driving decadal changes in the carbonate system of a coastal plain estuary**. *Journal of Geophysical Research: Oceans* 126

The primary drivers of acidification in the Chesapeake Bay over the past three decades are increased atmospheric CO_2 concentrations and decreased terrestrial nutrient inputs. The pH reductions resulting from decreased nutrient loads indicate that the system is reverting back to more natural conditions when human-induced nutrient inputs to the Bay were lower. As nutrient reduction efforts to improve coastal water quality continue in the future, controlling the emissions of anthropogenic CO2 globally becomes increasingly important for the shellfish industry and the ecosystem services it provides.

x. Can carryover effects improve oyster aquaculture production?

VIMS PIs: Emily Rivest, Jan McDowell

Carry-over effects (COE) were evaluated in a variety of hatchery-relevant scenarios: (a) relatively small differences in salinity relative to variation over an operation season (COE-S), (b) OA conditions during setting (COE-OA), and (c) multiple stressors (OA and high temperature, COE-OAT) throughout the larvae, setting, and early juvenile periods. A general theme emerged from this body of work: although we observed direct effects of the various water treatments on the larvae, setting individuals, and early juveniles, once oysters were returned to ambient (i.e., uncontrolled) conditions, no carryover effects were detected. Overall, survival and growth of juvenile oysters outplanted under different in situ environmental conditions did not correlate with hatchery conditions of those individuals as larvae, though differences between grow-out sites were observed.

Results of the genetic analyses from COE-S and COE-OAT showed no differences in genetic diversity among treatments, suggesting that selective mortality did not occur. Rather, samples kept under control conditions appear to have similar levels of allelic diversity as treatment samples, with few exceptions.

There were many significant differences in analyses of genetic differentiation in COE-S and very few in COE-OAT, which may be due to the use of triploid samples in COE-S (i.e. genotypes had to be imputed). COE-S broodstock was found to be significantly different from all offspring samples, while COA-OAT broodstock were highly similar to all offspring groups. This could be due to 1) differences in ploidy between the broodstock and offspring or 2) to the fact that the non-contributing broodstock were identified during analysis and ultimately removed.

xi. Impact of parasitism on levels of human-pathogenic *Vibrio* species

- Bienlien, LM, Audemard, C, Reece, KS, Carnegie, RB. (2022) **Impact of parasitism on levels of human-pathogenic *Vibrio* species in eastern oysters.** *Journal of Applied Microbiology* 132(2): 760-771. DOI: 10.1111/jam.15287

Histology revealed that oysters infected with *Perkinsus marinus,* the parasite that causes Dermo disease in oysters, displayed increased levels of *Vibrio vulnificus* (*Vv*), but there was no correlation between *P. marinus* infection and *Vibrio* spp. levels. These results suggest that factors other than *P. marinus* presence influence *Vibrio* spp. levels in oysters, which could be explored further to improve risk management within a hatchery.

xii. New Techniques for Quantifying Dermo Disease

- Guevelou, E., Carnegie, R.B., Whitefleet-Smith, L., Small, J.M., Allen, S.K., Jr. (2021) **Near infrared reflectance spectroscopy to quantify *Perkinsus marinus* infecting Crassostrea virginica**. *Aquaculture. 533*

Near infrared reflectance (NIR) spectroscopy technology was used to test the feasibility of an alternative means of quantitative detection of *P. marinus* in the eastern oyster. Results indicate that *P. marinus* cells present a quantifiable spectral signature against the background of *C. virginica* oyster tissues (wet and freeze-dried), which implies that NIR spectroscopy is a promising technology to detect protozoan infection in animal tissues. This technology seems unlikely to replace qPCR or other classical methods like RFTM, primarily because of the amount of preparation needed to effect NIR sampling. However, if NIR spectroscopy is used to assess other facets of oyster condition, such as glycogen and lipid, reasonable estimates of *P. marinus* infection could be obtained simultaneously when more traditional methods are not used.

xiii. Polyploid Oyster Health

- Audemard, C., Reece, K. S., Latour, R. J., Bienlien, L. M., Carnegie, R. (2023) **Influence of oyster genetic background on human-pathogenic *Vibrio* spp.** *Aquaculture* 562 https://doi.org/10.1016/j.aquaculture.2022.738763

Human-pathogenic *Vibrio* bacteria are common inhabitants of oyster tissues, but our understanding of factors driving the wide range of concentrations found in individual oysters is extremely limited. We examined the influence of oyster sex and parasitism in light of their profound effects on oyster tissues against a backdrop of eastern oysters, *Crassostrea virginica*, from two diploid and two triploid aquacultured lines. A key outcome of these analyses was the consistent inclusion of oyster line as a predictor variable across *Vibrio* targets. A potential effect of *Perkinsus marinus* infections and/or oyster sex was also suggested, although the combination of variables varied with *Vibrio* target. This study suggests that the influence of oyster genetic background should be further investigated, and that the dynamics of human-pathogenic *Vibrio* spp. in oysters is likely driven by multiple, interacting factors, some of which may be under oyster host genetic control.

xiv. Human-Pathogenic *Vibrio* Species in Shellfish

- Ben-Horin, T., Audemard, C., Calvo, L., Reece, K.S., Bushek, D. (2022) **Pathogenic *Vibrio parahaemolyticus* increase in intertidal-farmed oysters in the mid-Atlantic region, but only at low tide.** *North American Journal of Aquaculture* 84(1):95-104. DOI: 10.1002/naaq.10218

Another concern to the shellfish industry is *Vibrio* concentrations in intertidal cultured oysters, as *Vibrio* spp. proliferate rapidly in shellfish tissues exposed to warm ambient conditions. Results reveal slight evidence for increased *Vp* concentrations in oysters grown with exposure to ambient air at low tide (intertidal) compared to subtidal oysters, but these differences disappeared with the incoming tide. These results do not suggest restricting intertidal harvest, which is important as many oysters are grown intertidally, but support the current time-to-cooling protocols put in place by many state *Vibrio* control plans detailing the amount of time harvested shellfish can be held before they are cooled or refrigerated.

- Audemard, C., Ben-Horin, T., Kator, H. I., Reece, K.S. (2022) ***Vibrio vulnificus* and *Vibrio parahaemolyticus* in oysters under low tidal range conditions: Is seawater analysis useful for risk assessment?** *Foods* 11(24) https://doi.org/10.3390/foods11244065

Human-pathogenic *Vibrio* bacteria are acquired by oysters through filtering seawater, however, the relationships between levels of these bacteria in measured oysters and overlying waters are inconsistent across regions. The reasons for these discrepancies are unclear hindering our ability to assess if -or when- seawater samples can be used as a proxy for oysters to assess risk. We investigated whether concentrations of total and human pathogenic *Vibrio vulnificus* (*vvhA* and *pilF* genes) and *Vibrio parahaemolyticus* (*tlh*, *tdh* and *trh* genes) measured in seawater reflect concentrations of these bacteria in oysters (*Crassostrea virginica*) cultured within the US lower Chesapeake Bay region. We found seawater concentrations of these bacteria to predictably respond to temperature and salinity over chlorophyll *a*, pheophytin or turbidity. We also inferred from the SEM results that *Vibrio* concentrations in seawater strongly predict their respective concentrations in oysters. We hypothesize that such seawater-oyster coupling can be observed in regions of low tidal range.

xv. Microbiome of oyster larvae

- Arfken, A, Song, Bongkeun, Allen, SK, Carnegie, RB. (2021) **Comparing larval microbiomes of the eastern oyster (*Crassostrea virginica*) raised in different hatcheries.** Aquaculture 531:735955 doi:10.1016/j.aquaculture.2020.735955

Differences were detected in the microbiome of oyster larvae collected from separate spawning events and from four different hatcheries in the Chesapeake Bay, and the larval microbiome composition differed compared to the microbiome of the hatchery water they were grown in. In addition, the species richness of the larval microbiome decreased as the larvae grew, suggesting a shift towards a more selective microbiome as the larvae developed. These results highlight potentially differences in larvae microbiome composition that may be related to oyster health and disease resistance.

xvi. Oyster lease use and associated conflicts in Virginia

- Beckensteiner, J., Kaplan, D.M., Scheld, A.M. (2020) **Barriers to Eastern Oyster Aquaculture Expansion in Virginia.** *Frontiers in Marine Science* 7:53. doi: 10.3389/fmars.2020.00053

- Beckensteiner, J., Scheld, A.M., St-Laurent, P., Friedrichs, M.A.M., Kaplan, D.M. (2021) **Environmentally-determined production frontiers and lease utilization in Virginia's eastern oyster aquaculture industry.** *Aquaculture* 542. doi: 10.1016/j.aquaculture.2021.736883

Non-productive use of shellfish leases has been a long-standing concern in Virginia's aquaculture industry. Beckensteiner et al. (2020) found that from 2006 to 2016 only a third of commercial leases reported production, and that over 60% of leaseholders had no history of commercial harvests. Non-used leases tended to be smaller and located in densely populated areas. Beckensteiner et al. (2021) used econometric production frontier models to evaluate production efficiency for leases growing oysters using intensive culture methods. Findings indicated that given the available area and environmental conditions, production could double or triple in many leases. Additionally, leaseholders holding multiple leases were seen to be more efficient, possibly benefiting from economies of scale, while leases in densely populated areas tended to be less efficiently used. This research highlights the importance of socioeconomic drivers and interactions affecting the oyster aquaculture industry in Virginia.