

NRSP-8 Aquaculture 2021 Full Annual Report

Leadership

Coordinator: Benjamin J. Reading, North Carolina State University

Co-coordinators: Steven Roberts, University of Washington
Moh Salem, University of Maryland
Eric Peatman, Auburn University

Species Leaders:

Catfish: Sylvie Quiniou, ARS Stoneville, Mississippi

Oyster/shellfish: Dina Proestou, ARS University of Rhode Island, Rhode Island

Salmonids: Yniv Palti, ARS Leetown, West Virginia

Striped Bass: Benjamin Reading, North Carolina State University, North Carolina

2020 Aquaculture Workshop Report:

Workshop Chair-elect 2022-2023: Rafet Al-Tobasei (Rafet.Al-tobasei@mtsu.edu)

Workshop Chair 2021-2022: Moh Salem (mosalem@umd.edu)

Workshop Chair 2020-2021 (cancelled): Moh Salem (mosalem@umd.edu)

Workshop Chair 2019-2020: Louis Plough (lplough@umces.edu)

Workshop Chair 2018-2019: Catherine Purcell (catherine.purcell@noaa.gov)

Theme

The Aquaculture Genomics Workshop 2021 event was cancelled due to COVID-19 related travel restrictions for state and federal employees including academic and government participants.

Aquaculture Genomics Workshop 2022: The group resumed the workshop at the International Plant and Animal Genome (PAG) Conference in 2022, all in person travel was restricted; the Workshop was held virtually.

Attendees 2022

Number: **66** (80 in 2020) (average of 35-45 participants tuned into each presentation)

Number of institutes/organizations represented: **41** (49 in 2020)

Number of Countries represented: **11** (13 in 2020)

Invited Presentations (Plenary Speakers; \$1,000 travel awards) 2022

There were no plenary travel awards in 2021 as the Aquaculture Workshop was conducted virtually due to COVID-19 pandemic. **\$0** 2022.

Other Contributed Presentations: 13 (15 abstracts, 2 cancellations) (14 in 2020)

Poster Session Participants: 0 in person activities were cancelled (15 in 2020)

Aquaculture Reception Attendees: 0 in person activities were cancelled (100 in 2020)

Business Meeting Minutes

Time: 3:10-3:40 PM January 8, 2022

Place: Virtual (Zoom)

Number of Attendees: 18 (19 in 2020)

NRSP – 8 Aquaculture Business Meeting Saturday January 8, 2022 3:10 – 3:40 p.m. Virtual Zoom

AGENDA

Business Meeting Attendance: (1) Moh Salem, (2) Benjamin Reading, (3) Linnea Andersen, (4) Rafet Al-Tobasei, (5) Louis Plough, (6) Mary Duke, (7) Ridwan Ahmen, (8) Ping Wangq, (9) Adhar, (10) John Buchanan, (11) Steven Roberts, (12) Yaamini R. Venkataraman, (13) Jasmine Richman, (14) Yniv Palti, (15) Marta Gomez-Chiarri, (16) Ali R. Ali, (17) Zhang XM, (18) James Koltes

Coordinators

Coordinator: Benjamin J. Reading, North Carolina State University

Co-coordinators: Steven Roberts, University of Washington
Moh Salem, University of Maryland
Eric Peatman, Auburn University

Species Leaders

Catfish: Sylvie Quiniou, ARS Stoneville, Mississippi

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Striped Bass: Benjamin Reading, NC State University, North Carolina

Aquaculture Workshop

Workshop Chair-elect 2023-2024: Yniv Paltu yniv.palti@usda.gov)

Workshop Chair 2022-2023: Rafet Al-Tobasei (Rafet.Al-tobasei@mtsu.edu)

1. Welcome and introductions – Benjamin Reading

Linnea Andersen (scribe); minutes to be included in final annual report

2. James Koltes NRSP-8 Bioinformatics Coordinator

-1 of 3 NRSP-8 Bioinformatics Coordinators → Fiona McCarthy and James Reecy being the other two

> Overview of Bioinformatics Team focus:

-data sharing

- supporting best practices to curate and integrate data
- supporting training for the next generation of bioinformatics scientists and those just getting started
- supporting user needs
- focus on genotype to phenotype and FAANG initiatives

> James and Jim (Reecy) are writing a proposal.

-James has heard approaches from NRSP-8 folks democratizing genomics and/or 20% of current rate smaller proposal (experiment station director mentioned support of this)

> Bioinformatics is writing their own proposal and has looked for other funds

-Congressional appropriations → was not successful last year

-eQTLdb funding was approved for more text mining tools and automation of this process), big ticket items are keeping funding for databases and data visualization tools (Animal Genome, FAANG, etc.)

> Establishment and continuation of longer term back-ups Bioinformatics has been in contact with BioAg Library and they can retain these data but only static (Open Science Framework, OSF, is another store of data and is the primary static backup of data)

-CyVerse is also a store of some of this data

3. NRSP-8 Leadership. Select 2022-23 Chair from Poultry group; Select Chair-elect from Aquaculture group for 2023-2024 term. Chair Rotation (changes after annual meeting at PAG 2022):

Sheep/Small Ruminants – 2020-21

Swine – 2021-22

Poultry – 2022-23

Aquaculture – 2023-24

Equine – 2024-25

Cattle – 2025-26

Propose for nominations:

Chair-elect:

Benjamin J. Reading, North Carolina State University

Aquaculture Coordinator:

Steven Roberts, University of Washington

Co-coordinators:

Moh Salem, University of Maryland

Eric Peatman, Auburn University

Benjamin J. Reading, North Carolina State University

Who will be the next Aquaculture Coordinator? Moh and Ben supports Steven Roberts as the Aquaculture Coordinator

Who will be the Aquaculture Chair? Moh supports Ben as the NRSP-8 Chair (Current Aquaculture Coordinator for NRSP-8)

4. Overview of plans for NRSP8 Sunday January 9, 2022—CANCELED. May be rescheduled for April 2022 at the inaugural AGBT (Animal Discussion Advances in Genome Biology and Technology) meeting. Please stay tuned...

5. Project Chairs and Coordinators comments (for the future of NRSP-8 April 2022):

“To begin with, as much as other species groups are in a position to "move on" from the NRSP-8, the Aquaculture Group still has several species that require substantial work to complete the genome resources. In this regard, genomic information of most aquatic species is still greatly lacking behind that of terrestrial species. We are not one species but many diverse animals ranging from invertebrates to vertebrates; also complexity as many species have recent polyploid ancestry. Some members of this group are able to move forward with annotation or other upstream elements that this new NRSP-8 may support, but many still need to finish developing these resources (the old NRSP-8 mission) before these next steps can be taken.

We would be glad to participate in this effort and some groups are prepared to move forward with it, however we also will need to pursue our own line of funding to form an Aquaculture Genomics Community/Consortium to help finish these genome projects over the next few years and bring everybody in the group up to speed to catch up with the new NRSP-8.”

5. Proposal for NRSP-8 Writing

Committee Structure – Brenda Murdoch and Jack Dekkers. Any volunteers to help with the writing assignments from Aquaculture? AG2PI Grant (an NRSP-8 community needs assessment).

Who wants to be on the Writing Team for the new NRSP-8 project? (this has been opened to the entire forum). Ben will volunteer if there are no others. Steven may also help although this is not a commitment.

6. Annual reports

- a. Include at least one impact statement from the project’s work since Oct 1, 2020.
- b. Include any grants awarded since Oct 1, 2020 (granting agency and amount) that

contributed to the objectives of NRSP 8.

c. Include workshops' attendance numbers and institutions represented (if possible).

d. Need to get annual reports to Jack Dekkers and the new chair (Poultry) by mid-February.

A draft of the annual report will be sent to all NRSP-8 members for final comments and then it will be distributed 1) through the list serv, 2) posted online at the Aquaculture NRSP-8 site, and 3) sent to Jack Dekkers and Archie Clutter and Steve Lommel.

7. Follow up writing workshop for the white paper / grant proposal in 2022-2023. We will aim to hold a further discussion of this at a Workshop at NC State University in June 2022.

8. Other business (open). None mentioned.

ADJOURN

Travel Support & Opportunities for Trainings

In person travel to the 2022 PAG meeting and Aquaculture Workshop was cancelled due to the COVID-19 pandemic. The travel of four students/postdocs was funded to cover the registration fee for PAG 2022 (\$1,500 each). The purpose of the travel award program is to help graduate students and postdoctoral fellows to travel to the annual PAG meetings and present their research. **\$6,000** 2022.

4. Yaamini R. Venkataraman, *Ployploidy and Environmental Stress Have Distinct Impacts on Pacific Oyster (Crassostrea gigas) Ctenidia Methylomes*. Woods Hole Oceanographic Institution.

3. Jasmine Alia Richman, *Investigating the Activin Receptor Signaling Pathway as a Key Regulator of Muscle Growth Following Whole Genome Duplication Events*. Washington State University.

2. Homere Jacques Alves Monteiro, *How Many Parents You Got? Parental Contribution in Flat Oyster's Hatchery*. Cornell University / Technical University of Denmark.

1. Ali Reda Eid Ali. University of Maryland.

Machine Learning Workshop

A wide range of high-throughput technologies enable us to evaluate biological systems at various levels—at the genome, epigenome, transcriptome, proteome, and metabolome. These technologies are now being used to generate data to answer an ever-increasingly diverse set of questions. The next great challenge is integrating data analysis in a systems biology approach that utilizes novel supervised machine learning methods, which accommodate heterogeneity of data, are robust to biological variation, and provide mechanistic insight. The workshop will not focus on detailed mathematical models, but instead on how these machine learning tools may be used to analyze biological data, in particular gene and protein expression. The Workshop was led by Benjamin J. Reading (North Carolina State University).

A motion was made to continue these virtual trainings and walk-through data analyses monthly or every other month via a Zoom (2022-2023) and effort will be made to extend these offerings to graduate students and post docs as well.

Participants: 16

NRSP-8 Aquaculture 2021 Progress: Species Leaders indicated in **BOLD**.

Members of the NRSP-8 Aquaculture Group contributed content matter expertise to advise US Department of Homeland Security and the Office of the Director of National Intelligence on national food security issues, particularly related to seafood.

Contributions were published in the following 2021 report:

Threats to Food and Agricultural Resources. United States Department of Homeland Security, Office of Intelligence and Analysis, Analytic Exchange Program. 95 pp.
https://www.dhs.gov/sites/default/files/publications/threats_to_food_and_agriculture_resources.pdf

Objective 1: Advance the quality of reference genomes for all agri-animal species through providing high contiguity assemblies, deep functional annotations of these assemblies, and comparison across species to understand structure and function of animal genomes.

Generally, genomic information of most aquatic species is still lacking and greatly lagging behind that of terrestrial species.

Catfish (Quiniou, Liu)

The complete germline repertoires of the channel catfish, *Ictalurus punctatus*, T cell receptor (TR) loci, TRAD, TRB, and TRG were obtained by analyzing genomic data from PacBio sequencing.

Oyster/Shellfish (Roberts, Gómez-Chiarri, Putnam, Puritz, Guo, Warren, **Proestou**, Eastern Oyster breeding Consortium)

Re-sequencing of an additional 179 wild and selected individuals from 16 eastern oyster populations situated along the US east Coast >20X coverage.

Improved eastern oyster (*Crassostrea virginica*) reference genome assembly using updated assembly pipeline, additional Hi-C data, and high-density genetic linkage map.

The recent availability of a high-quality reference genome for the eastern oyster, and whole-genome sequence data of samples from across the species range in the USA, provides an opportunity to explore structural variation across the genome of this species. Our analysis shows significantly greater individual-level duplications of regions across the genome than that of most model vertebrate species. The eastern oyster shows a large interindividual variation in duplications as well as particular chromosomal regions with a higher density of duplications.

Salmonids (Salem, **Palti**, Al-Tobasei)

The first *De-novo* genome assembly for the North American Atlantic salmon lineage was submit to NCBI and is in final review.

A new transcriptome assembly using PacBio long-read sequencing improved the rainbow trout genome annotation and identified alternative splicing associated with economically important phenotypes in rainbow trout.

Striped Bass (*National Breeding Program for the Hybrid Striped Bass Industry*, Fuller, Abernathy, Borski, Berlinsky, **Reading**)

The second (2.0), updated draft of the striped bass genome was uploaded to NCBI GenBank (NCSU_SB_2.0, GenBank Accession no. GCA_004916995.1). This version of the genome consists of 629 scaffolds with a final size of 598.11 Mb. Striped bass transcriptome data was used by NCBI for annotation and the fully annotated striped bass genome 2.0 is publicly available.

The Dovetail Genomics Hi-Rise pipeline was employed to create a white bass genome assembly (645.14 Mb in size), by scaffolding Illumina short read sequences with Illumina sequencing data generated by Chicago and Dovetail Hi-C pipelines.

The genome has been uploaded to NCBI GenBank (DOM_MoChry_2.0, GenBank Accession no. GCA_019097615.1) is publicly available. This genome has not yet been fully annotated.

Objective 2: Advance genome-to-phenome prediction by implementing strategies to identify and validate genes and allelic variants predictive of biologically and economically important phenotypes and traits.

Catfish (Quiniou, Liu)

The bacterium *Edwardsiella piscicida* causes significant losses in global aquaculture, particularly channel (*Ictalurus punctatus*) × blue (*I. furcatus*) hybrid catfish cultured in the south-eastern United States. Intraspecific genetic variability of *E. piscicida* isolates recovered from diseased channel and hybrid catfish were assessed and virulence associations among genetic variants was determined revealing 5 discrete phylogroups that correlated with virulence gene profiles.

Exogenous estrogen 17 β -estradiol (E2) was shown to effectively induce feminization in catfish. Global DNA methylation and gene expression profiles of channel catfish during early sex differentiation after E2 treatment using Bisulfate sequencing and RNA-Seq. Levels of global DNA methylation after E2 treatment were not significantly different from those of controls. However, a specific set of genes were differentially demethylated, which included many sex differentiation-related pathways, such as MARK signaling, adrenergic signaling, Wnt signaling, GnRH signaling, ErbB signaling and ECM-receptor interactions. Many genes involved in these pathways were also differentially expressed after E2 treatment. Specifically, E2 treatments resulted in upregulation of female-related genes and downregulation of male-related genes in genetic males during sex reversal. However, E2-induced sex reversal did not cause sex-specific changes in methylation profiles or gene expression within the sex determination region (SDR) on chromosome 4, suggesting that E2-induced sex reversal was a downstream process independent of the sex determination process that was regulated by sex-specific methylation within the SDR.

Oyster/Shellfish (Eastern Oyster Breeding Consortium, Roberts, Gomez-Chiarri, Putnam, Lotterhoos, Puritz, Johnson, Eirin-Lopez, Allen, Zhang, Plough, Proestou) Differences in DNA methylation patterns for 50 families of Pacific oyster (*Crassostrea gigas*) were characterized based on heat-tolerance. Hyper-methylated regions were more abundant than hypo-methylated regions among heat-phenotypes. Epigenetic marks associated to heat-resistant phenotypes occurred mainly in genes involved in catalytic activity and binding function among Pacific oyster families.

Complementary methods of metagenomics and metaproteomics were applied to more fully characterize bacterial taxa in shellfish hatchery water at high (pH 8.2) and low (pH 7.1) pH. The complementary methods of metagenomics and metaproteomics contribute to a more complete characterization of bacterial taxa that are potentially

active versus truly metabolically active and thus impact water quality and inter-trophic relationships.

A seascape genomics approach was used to investigate population structure, local adaptation, and the extent to which environmental gradients influence genetic variation among natural and restored populations of Chesapeake Bay eastern oysters (*Crassostrea virginica*). Environmental factors, namely salinity, pH, and temperature, were found to play a major role in the distribution of neutral and adaptive genetic variation. This is one of a few studies to demonstrate empirically that hatchery practices have a major impact on the retention of genetic diversity.

A selectively bred Eastern oyster (*Crassostrea virginica*) family was exposed to *Perkinsus marinus* (Dermo disease) via either suspension feeding or direct injection and gene expression and GWAS was evaluated. Oysters exposed via feeding minimize infection through controlled cell death and limited inflammation responses, whereas oysters exposed via injection mount a prolonged, inflammatory immune response resulting in self harm and lower survival.

Completion of Affymetrix 60K SNP chip for U.S. East Coast eastern oyster populations. SNP array is available through ThermoFisher SKU #551377. Led by X. Guo, D. Proestou, J. Puritz of the *Eastern Oyster Breeding Consortium*.

Genomic Selection initiated in an eastern oyster breeding program. Led by J.M. Small (VIMS).

Salmonids (Salem, **Palti**, Al-Tobasei)

In a study of retrospective evaluation of genetic predictions accuracy with a training population size of 2,000 animals, genomic selection models increased the predictions accuracy compared to the traditional pedigree-based model by up to 50% for Fillet Yield and 28% for Body Weight, which suggests that using genomic selection can substantially enhance genetic improvement for the trait of fillet yield in rainbow trout aquaculture.

The genetic architecture of resistance to infectious hematopoietic necrosis virus (IHNV) was found to be oligogenic with a few moderate effects QTL and many loci with small effects in two commercial aquaculture breeding populations that were not previously exposed to the pathogen.

An improved genomic prediction for fillet yield and firmness in rainbow trout was achieved using reduced-density SNP panels.

A study revealed that sRNAs enriched in outer membrane vesicles of pathogenic *Flavobacterium psychrophilum* interact with immune genes of rainbow trout

Markers associated with adult migration timing were validated for natural and hatchery origin populations of Chinook salmon and steelhead, with genotypes explaining between 50-80% of phenotypic variation.

Markers associated with age-at-maturity in steelhead were validated for natural and hatchery origin populations, with genotypes explaining 10-17% of phenotypic variation depending on sex.

Whole genome resequencing in coho salmon identified a sex determining region that was validated with amplicon assays, along with candidates for differing phenotypes of adult migration.

Whole genome resequencing in ecotypes of redband trout identified a candidate gene (ceramide kinase; *cerk*) associated with thermal adaptation phenotypes.

Whole genome resequencing and landscape genomics analyses of Chinook salmon populations revealed candidate regions of the genome associated with environmental factors responsible for local adaptation.

Striped Bass (*National Breeding Program for the Hybrid Striped Bass Industry*, Berlinsky, Fuller, Abernathy, Woods, McGinty, Borski, **Reading**)

A machine learning pipeline was developed to analyze a dataset of 15,000 single nucleotide (SNP) markers (expressed quantitative trait loci, eQTL) that were identified among muscle transcriptome data generated from sunshine hybrid striped bass produced from striped bass males of varying geographic strains (Texas, Florida, South Carolina, Virginia, and North Carolina aquaculture domestic strain). The pipeline reduced these data to 500 unique SNPs that were considered important to the classification hybrid fish into groups based on size and sire strain (heterosis effects). When further condensed by the removal of redundant orthologs / paralogs, these 500 SNPs annotated to 33 genes, the primary functions of which were largely glucose and lipid metabolism, ATP synthesis, and structural support of muscle fibers. The novel machine learning pipeline also enabled alleotyping of these genes. Specifically, we found that 9 of the 33 genes were inherited exclusively from the white bass (maternal) parent. All of these genes have a known role in the electron transport chain and generation of ATP. The remaining 24 genes were potentially inherited from either the striped bass or white bass parent. A deeper pathway analysis is currently being

conducted to examine the relationship of these gene expressions in white muscle tissue for predictive phenomics.

Adult, male, F6 domestic striped bass (n=60) from our selective breeding program were disseminated to major aquaculture producers in the U.S. for hybrid striped bass fry and fingerling production (directly contributing to the \$50 million farm gate per year industry). Additionally, fingerlings (n=40,000) and larvae (n=750,000) representing the F7 generation captive bred striped bass with 25% better growth were also disseminated to commercial aquaculture producers.

Piscidin antimicrobial peptides of the innate immune system were first described in hybrid striped bass (*Morone chrysops* x *Morone saxatilis*). Previous work demonstrated broad antimicrobial activity of class I and II piscidins against Gram-negative and Gram-positive bacterial species. The class I and II piscidins demonstrate potent activity against *Escherichia coli* and *Flavobacterium columnare* biofilms. The class II piscidins showed more activity against *E. coli* and *F. columnare* isolates than did the class I piscidins. The piscidins in general were much less effective against inhibiting *Aeromonas hydrophila* and *A. veronii* biofilm growth; only the class I piscidins showed significant growth inhibition among the *Aeromonas* spp. examined.

Objective 3: Advance analysis, curation, storage, application, and reuse of heterogeneous big data to facilitate genome-to-phenome research in agricultural animal species of agricultural interest.

Catfish (Quiniou, Liu)

The rapid advances of second-generation sequencing (SGS) and third generation sequencing (TGS) is causing a paradigm shift from gene-based to genome-wide research in aquaculture; SGS and TGS could aid in establishing proper breeding strategies for sustainable aquaculture

Genomic selection plays an increasing role in selective breeding of the major aquaculture species.

The potential of genomic selection to increase precision of estimated breeding value of candidates is now well established.

The main challenge for further implementation of genomic selection is associated with the costs and benefits of genotyping; Methods of low-cost genotyping hold potential and will help transfer the benefits to aquaculture industries.

Oyster/Shellfish (*Eastern Oyster Breeding Consortium*, Gómez-Chiarri, Roberts, **Proestou**)

Eastern Oyster Genome Workshop, held during the *National Shellfisheries Association Annual Meeting*, March 22-25, virtual.

Salmonids (Salem, **Palti**, Al-Tobasei)

None from salmonids this year.

Striped Bass (USDA *National Breeding Program for the Hybrid Striped Bass Industry*, Abernathy, Borski, **Reading**, NOAA Sea Grant *StriperHub*)

Twelve (12) students (graduate and post doc) were trained in machine learning approaches in biological sciences including application of pattern recognition to evaluate gene and protein expression to predict traits and phenotypes in a variety of agriculturally important animals including fishes as well as poultry.

Digitization of machine learning training modules are being produced for the public and a CRISPR/Cas9 Guide to RNA design was published for student training on *CourseSource*: <https://doi.org/10.24918/cs.2020.46>.

Follow Striped Bass Genome Community and *StriperHub* research on Facebook: <https://www.facebook.com/stripedbassgenome/>

Research support mini-grants (Coordinator Small Research Project Grants)

Four (4) mini-grants (\$10,000 each; \$40,000 total) supported projects that fall under all three primary NRSP-8 objectives and include a variety of species as already reported in 2020-2021. Decisions regarding funding these projects were made in 2020, just prior to COVID-19 pandemic closures (in many states “shelter in place” executive orders), which made disbursement of funds challenging at best. These funds were dispersed in the 2021 calendar year due to relaxation of research and other work-related restrictions that had been implemented by various state and federal agencies across the country.

1. Shelly Trigg and Steven Roberts “**Comparative Epigenomic Analyses Across Bivalve Genome Resources (CEABiGR)**”, University of Washington.
2. Russell Borski and Benjamin Reading “**From Genotype to Phenotype: A Gene Editing Tool for Any Life History Stage using Adeno-Associated Viral Vectors for Application of CRISPR/Cas9 in Farmed Finfishes**”, North Carolina State University.
3. Refet Al-Tobesi and Moh Salem “**FAASG Functional Annotation of the Rainbow Trout Genome: Role of DNA Methylation in Gene Expression**”, Middle Tennessee State.

4. Kevin Johnson, Morgan Kelly, and Jerome La Peyre “**Transcriptome sequencing to describe the genomic basis for hypoxia tolerance in the Eastern oyster**”, Louisiana State University.

Another round of mini-grants were offered in 2021 and funded for 2022 (two grants, \$10,000 each; \$20,000 total):

1. Matthew George, Mackenzie Gavery, and Steven Roberts “**Identifying genomic architecture features that contribute to critical phenotypes in shellfish**”, University of Washington.
2. Mohamed Salem “**FAASG Functional Annotation of the Rainbow Trout Genome: Development of atlas of the RNA editome**”, University of Maryland.

Leveraged Funds and Stakeholder Use of Project Outputs

NRSP-8 2021-2022 Seed Funding: **\$20,000** (\$40,000 in 2019-2020 delayed until 2020-2021 due to COVID-19; \$30,000 in 2018-2019)

Total Leveraged 2021 Funding: **\$ 2,218,362**

Leveraged funds from diverse projects exceed \$ 2,000,000 from federal sources, which is about a 1:34 return on investment of the \$65,000 Aquaculture Coordinators funds for 2020-2021. Selected grants are highlighted below:

1. **National Oceanic and Atmospheric Administration (NOAA), Saltonstall-Kennedy Program**, Prime US Department of Commerce GRANT #573694-02785, *Collaboration with Local Fish Processing Industry to Convert Fish Trimmings and Skins into Value Added Fish Meal and Fish Oil to Promote Sustainability*. (PIs M.V. Joseph, A.V. Chouljenko, S.G. Hall, M.O. Frinsko, and B.J. Reading) **\$265,625** (09/01/2021 through 08/31/2023).
2. **Rhode Island Sea Grant**. *Does breeding disease-tolerant oysters increase disease in coastal marine ecosystems?* (PIs M. Gomez-Chiarri, D. Proestou, R. Hudson, T. Ben-Horin) **\$250,000** (2020 through 2023).
3. **Northeast Regional Aquaculture Center (NRAC)**, US Department of Agriculture National Institute of Food and Agriculture (USDA NIFA). *Comparing the performance of diploid and triploid eastern oysters in the Northeast*. **\$250,000** (2021-2023).
4. **Washington Sea Grant**, GRANT#: R/SFA-11. *Enhancing sustainability of shellfish aquaculture through streamlined maturation control*. (PIs S. Roberts, A. Luckenbach, C. Trapnell, B. Vadopalas, T.-T. Wong) **\$175,947** (2020 through 2023).

5. **Foundation for Food and Agriculture Research (FFAR)**. *Development of environmental conditioning practices to decrease impacts of climate change on shellfish aquaculture*. (PI S. Roberts) **\$753,716** (2018 through 2021).
6. **National Science Foundation (NSF)**, GRANT #1634167. Collaborative Research: Does ocean acidification induce a methylation response that affects the fitness of the next generation in oysters? (PI S. Roberts) **\$429,674** (2017 through 2021).
7. **Pacific States Marine Fisheries Commission**. *Applying cutting-edge technology for reproductive control in emerging bivalve species*. (PI S. Roberts) **\$93,400** (2019 through 2022).

Other Active Research Projects and Total Leveraged Funds (Previously Reported)

Total Leveraged 2020 Funding: **\$ 3,419,110** (1:53 return on investment)

Leveraged funds from diverse projects exceed \$ 3.4 million from federal sources, which is about a 1:53 return on investment of the \$65,000 Aquaculture Coordinators funds for 2019-2020. Selected grants are highlighted below:

1. **US Department of Agriculture (USDA)**, USDA-NIFA special research grants program aquaculture research, GRANT # 2018-70007-28828, *Underlying mechanisms for selected disease resistance and enhanced non-specific resistance in rainbow trout*. (PI T. Welker) **\$309,489** (10/01/2018 through 09/30/2021).
2. **US Department of Agriculture (USDA)**, USDA-NIFA-AFRI Foundational, Diseases of Agricultural Animals program area, GRANT # 2020-06096, *Seed Grant: Phage endolysins, Alternative antimicrobials for Streptococcus iniae*. (PI G. Ramena) **\$200,000** (02/01/2021 through 01/31/2022).
3. **US Department of Agriculture (USDA)**, Agricultural and Food Research Initiative (AFRI), *FACT: AquaMine - A High Performance Genomic Data Mining System for Species of Importance to US Aquaculture*. (PI C. Elsik) **\$500,000** (4/1/2021-3/31/2025).
4. **NOAA**, *Leveraging transformative 'omics technologies to alleviate barriers to US shellfish production*. (PI S. Roberts) **\$233,135** (07/01/20 through 06/30/25).
5. **NOAA**, *Development of 'omics and bioinformatics approaches for marine organisms in support of research in aquaculture, ocean acidification, and fisheries assessments*. (PI S. Roberts) **\$285,153** (07/01/20 through 06/30/25).
6. **NOAA, Washington Sea Grant**, *Enhancing sustainability of shellfish aquaculture through streamlined maturation control*. (PI S. Roberts) **\$200,000** (02/01/20 through 01/31/23).
7. **US Department of Agriculture (USDA)**, National Institute of Food and Agriculture (NIFA) GRANT # 2021-67015-33388, *Whole-Genome Analyses/Selection to*

Increase Muscle Yield and Reduce Fillet Downgrading In Rainbow Trout. (PIs M. Salem, Leeds, T.I., Kumar, V.I., Smith, B.R., Cleveland, B.E., and Al-Tobesi, R.A.) **\$500,000** (2021 through 2025).

8. **National Oceanic and Atmospheric Administration (NOAA)**, National Sea Grant Aquaculture Program, Advanced Aquaculture Collaborative Programs. *Establishing the Sea Grant Striped Bass Aquaculture Hub (StriperHub): Commercialization, Economics, and Marketing.* (PIs North Carolina Sea Grant, B.J. Reading--StriperHub Coordinator, R.J. Borski, D.L. Berlinsky) **\$1,191,333** (2/1/2020 through 01/30/2023).

Total Leveraged 2019 Funding: **\$ 6,340,999** (1:98 return on investment)

1. **Southern Regional Aquaculture Center**, US Department of Agriculture National Institute of Food and Agriculture (USDA NIFA). *Evaluation of Probiotics in Finfish Hatcheries to Improve Larval Production.* (PIs M.O. Frinsko, S.G. Hall, B.J. Reading) **\$150,000** (09/01/2018 through 08/31/2021).
2. **US Department of Agriculture (USDA)**, Agricultural and Food Research Initiative (AFRI), *Molecular basis of sex determination and differentiation in catfish.* (PIs Z.J. Liu, R. Dunham) **\$500,000** (6/1/2019 through 5/31/2022).
3. **NOAA**, *Developing new oyster sterilization technology to avoid triploid summer mortality.* (PI L. Plough) **\$100,000**.
4. **Ratcliffe Foundation**, *Shellfish Aquaculture Innovation Laboratory (SAIL): Using Science and New Technologies to Assist Shellfish Aquaculture Businesses in Maryland.* (PI L. Plough) **\$391,000**.
5. **NOAA Regional Shellfish Aquaculture Consortium Grants**, States Marine Fisheries Commissions, *From sequence to consequence: genomic selection to expand and improve selective breeding for the eastern oyster.* (PIs Eastern Oyster Breeding Consortium, X. Guo - Coordinator) **\$4,400,000** (8/1/2019 through 7/31/2024); similar grants were also awarded to the Gulf of Mexico and Pacific States.
6. **US Department of Agriculture (USDA)**, National Institute of Food and Agriculture (NIFA) Special Research Grants Program Aquaculture, *Modifying microbiomes to mitigate infectious diseases in aquaculture facilities.* (PI M. Gomez-Chiarri) **\$299,999** (10/1/2019 through 9/30/2021).
7. **US Department of Agriculture (USDA)**, National Institute of Food and Agriculture (NIFA) GRANT # 2018-06539, *High-Quality Reference Assembly and Annotation of the Rainbow Trout Genome.* (PIs M. Salem, Y. Palti, G. Gao, H. Zhou) **\$500,000** (2019 through 2022).

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