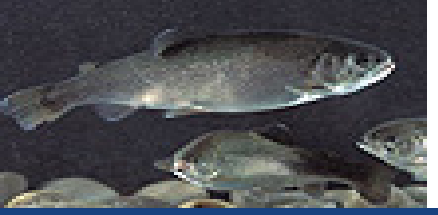
National Program 106 Aquaculture

Retrospective Review

Agenda

- Panel Introductions
- NPL Presentation
  - Program Information
  - Summary Information
  - Questions?
  - Sample Accomplishments
  - Questions for NPL, Scientists
- Break
- Panel Only Discussion
- Panel Debrief ONP
- Panel Written Report (to follow)



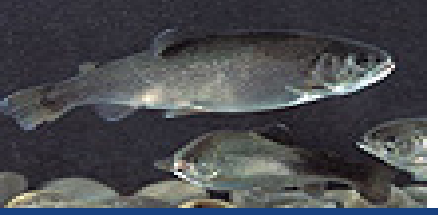
MISSION

ARS conducts research to develop and transfer solutions to agricultural problems of high national priority and provide information access and dissemination to:

- Ensure high-quality, safe food, and other agricultural products;
- Assess the nutritional needs of Americans;
- Sustain a competitive agricultural economy;
- Enhance the natural resource base and the environment;
- Provide economic opportunities for rural citizens, communities, and society as a whole; and
- Provide the infrastructure necessary to create and maintain a diversified workplace.

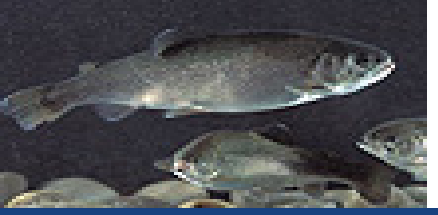
CORE PRINCIPLES

- ✓ Food and Fiber
- ✓ Problem solving agency
- ✓ Conduct long term research for existing industries
- ✓ Assemble teams, including multidisciplinary expertise when appropriate and including critical partnerships, to address problems facing American agriculture
- ✓ Integrate with short term funding from other agencies/Organizations
- ✓ Take higher risks than industry, but not short term funding
- ✓ Programs balance basic and applied research, include some developmental



ARS ROLE

- Inherently Federal
- Work with stakeholders to identify constraints to improving production, production efficiency, product quality, healthfulness, sustainability and/or animal welfare that we have the resources and expertise to address
- Develop science based approaches that complement industry efforts and capacity for problem solving
- Conduct Research and Technology Transfer
- Focus is pre-competitive research that can be facilitated through partnering, including public-private partnerships
- Accountability for taxpayer funds spent on projects

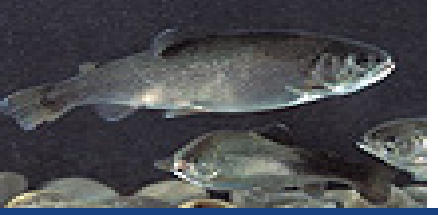


RESEARCH PRIORITIES

- **Presidential and Secretary Initiatives**

- **Congress**

- **Customers/Stakeholders**
  - **Producers**
  - **Support Industries**
  - **Allied Organizations**



ARS National Program Cycle

Nutrition, Food Safety/Quality

- Human Nutrition (NP #107)
- Food Safety (animal and plant products) (NP #108)
- Product Quality and New Uses (NP #306)

Animal Production and Protection

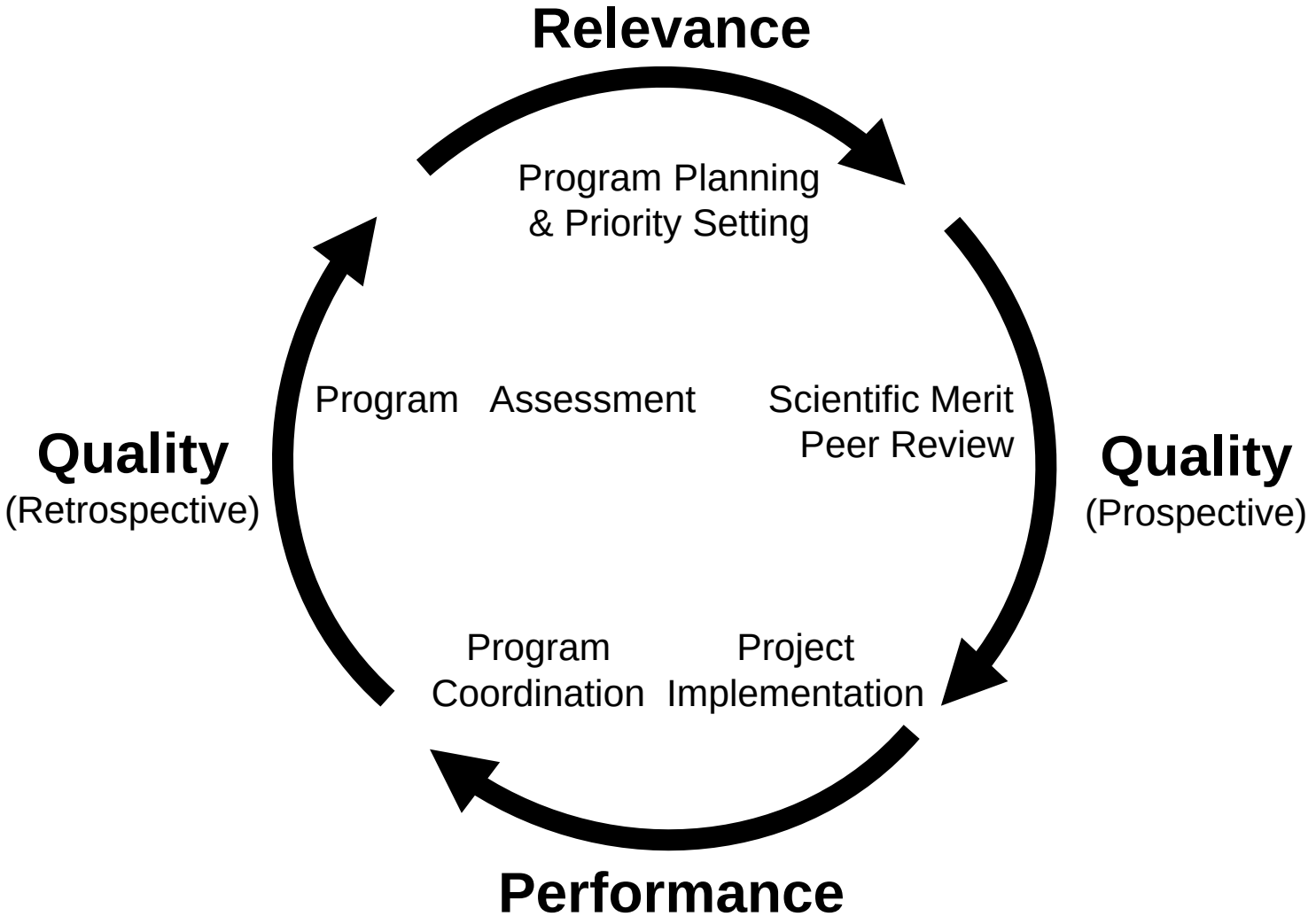
- Food Animal Production (NP #101)
- Animal Health (NP #103)
- Veterinary, Medical, and Urban Entomology (NP #104)
- Aquaculture (NP #106)

Crop Production and Protection

- Plant Genetic Resources, Genomics and Genetic Improvement (NP #301)
- Plant Diseases (NP #303)
- Crop Protection and Quarantine (NP #304)
- Crop Production (NP #305)

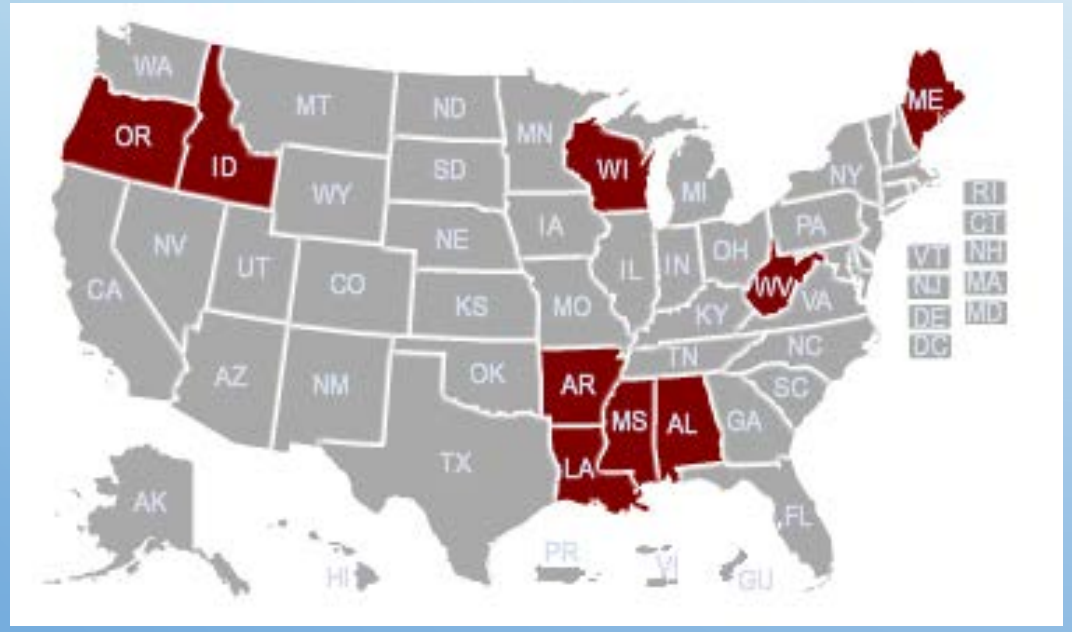
Natural Resources and Sustainable Agricultural Systems

- Water Availability and Watershed Management (NP #211)
- Soil and Air (NP #212)
- Biorefining (NP #213)
- Grass, Forage, and Rangeland Agroecosystems (NP #215)
- Sustainable Agricultural Systems Research (NP #216)



ARS AQUACULTURE

Mission: To conduct high quality, relevant, fundamental, and applied aquaculture research, to improve the systems for raising domesticated aquaculture species, and to transfer technology to enhance the productivity and efficiency of U.S. producers and the quality of seafood and other aquatic animal products.

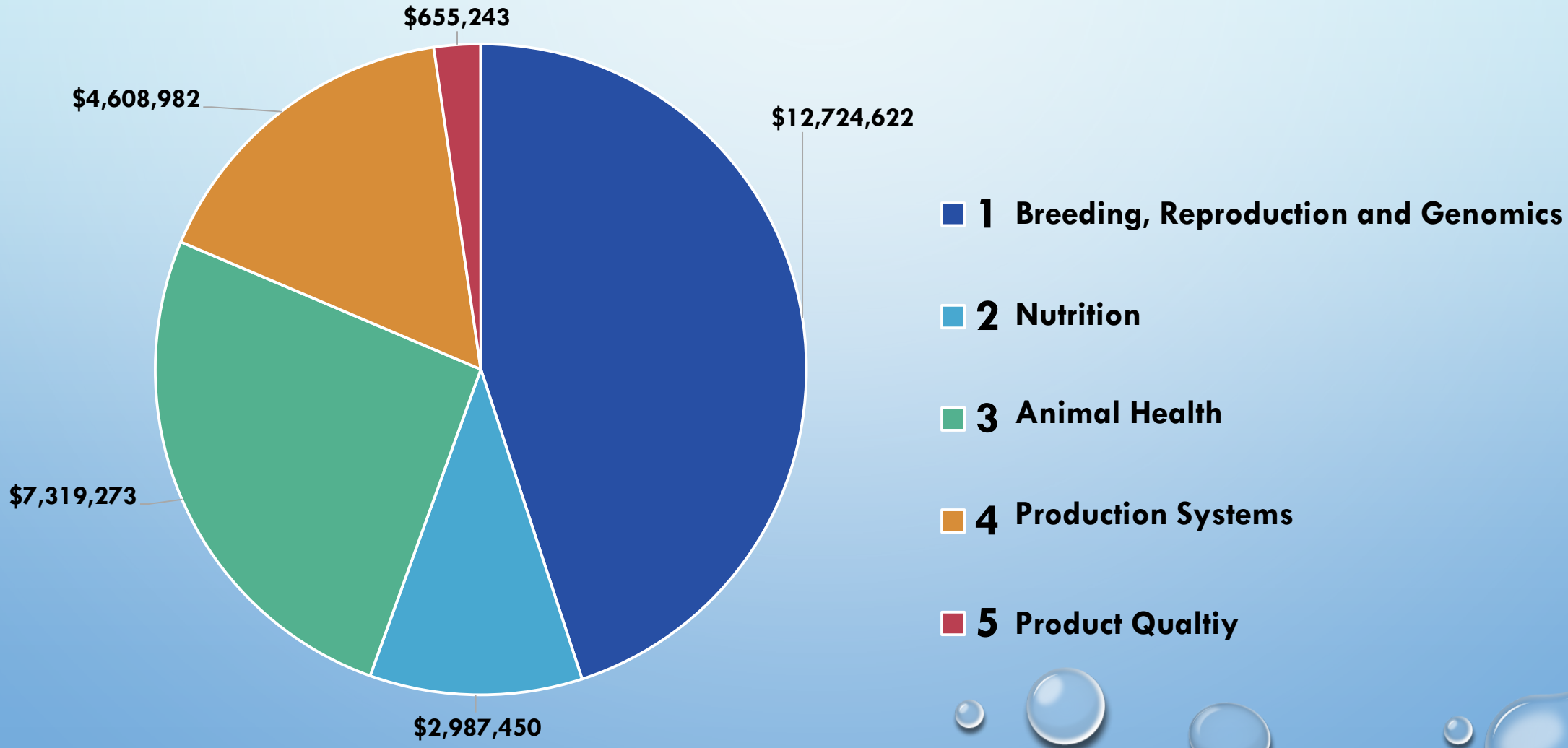


- 1/16 NATIONAL PROGRAMS
- 17 “PERMANENT” PROJECTS
- 41 SCIENTISTS
- ~9 FUNDED COLLABORATORS
- 10 LABORATORY SITES
- BUDGET: ~$28.3 MILLION INTRAMURAL
  ~$.4 MILLION/YR EXTRAMURAL
- FRESHWATER AND MARINE SYSTEMS

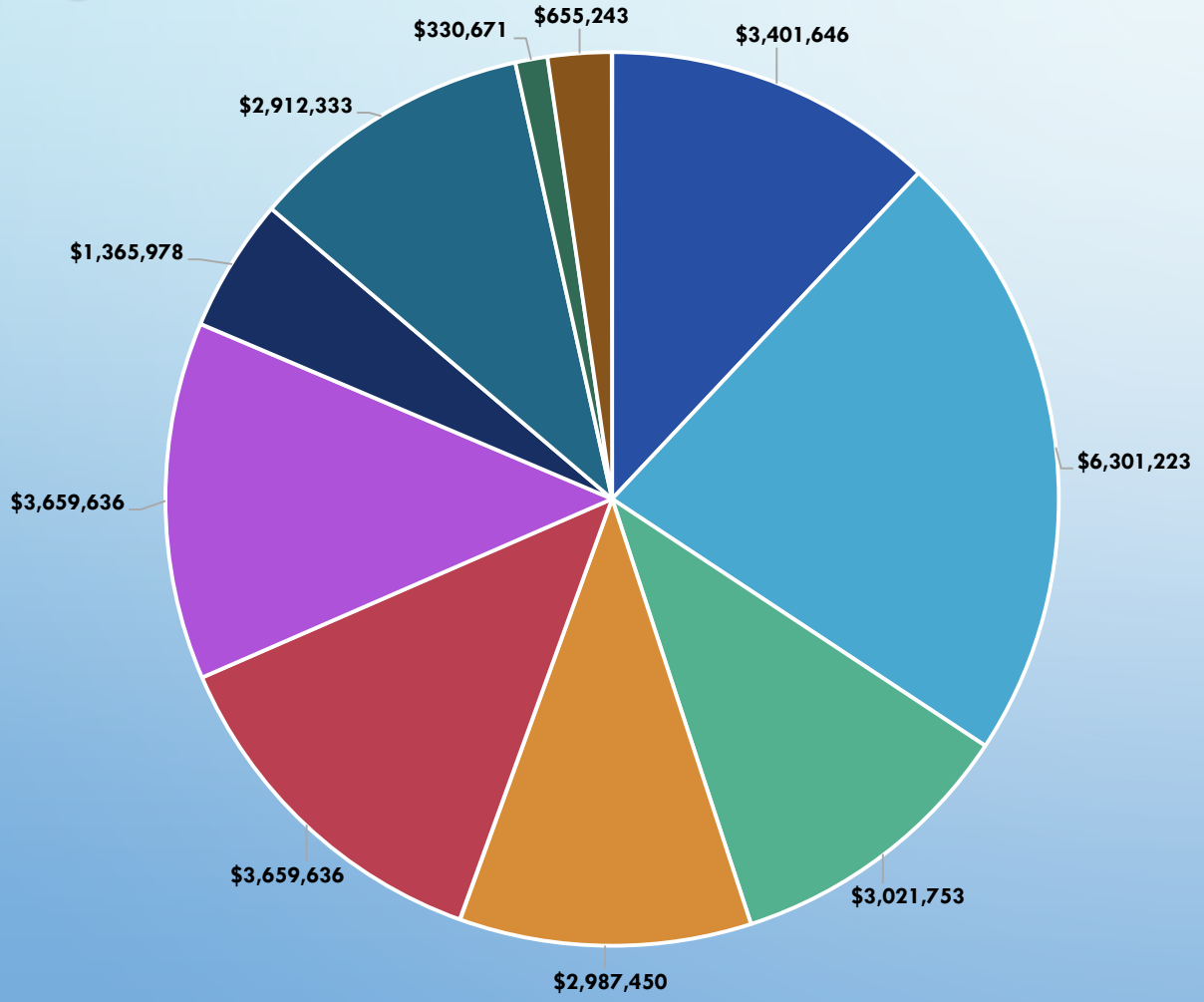
NATIONAL ACTION PLAN COMPONENTS

- SELECTIVE BREEDING, DIRECTED REPRODUCTION, AND DEVELOPMENT OF GENOMIC TOOLS
- NUTRIENT REQUIREMENTS AND ALTERNATIVE SOURCES OF PROTEIN AND LIPID
- HEALTH OF AQUATIC ANIMALS
- SUSTAINABLE PRODUCTION SYSTEMS
- PRODUCT QUALITY AND NEW PRODUCTS

Estimated ARS Aquaculture Research Annual Investment by Component

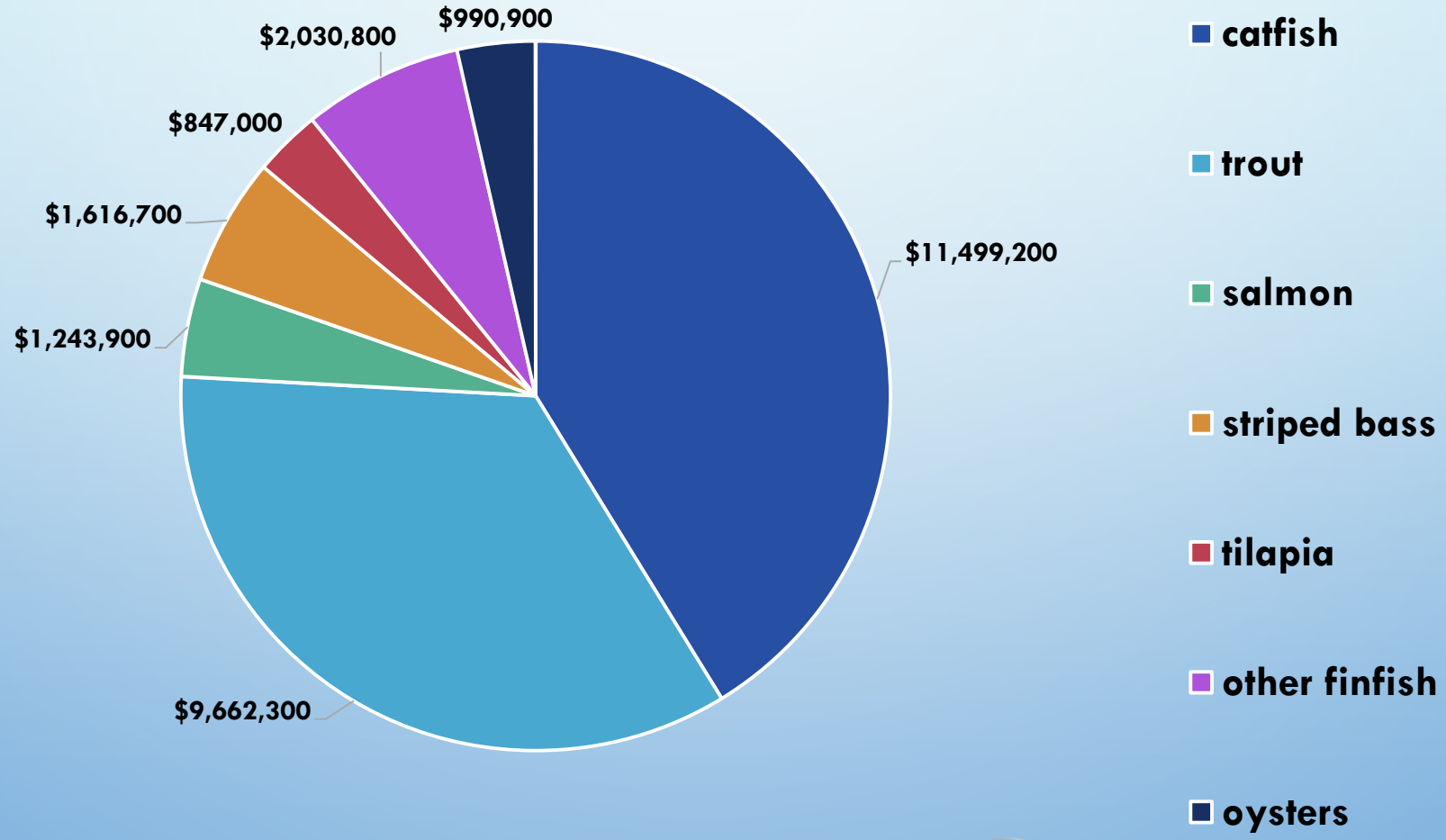


Estimated ARS Aquaculture Research Annual Investment by Problem Statement



- PS 1a Genomic Tools and Genotype to Phenotype
- PS 1b Define Phenotypes and Develop Genetic Improvement Programs
- PS 1c Enhance Aquatic Animal Reproduction
- PS 2 Determine Nutrient Requirements and Evaluate the Nutritional Value of Alternative Sources of Protein and Lipid
- PS 3a Improve Understanding of Host Immunity, Immune System Evasion by Pathogens, and Disease-Resistant Phenotypes.
- PS 3b Control of Pathogens and Prevention of Disease
- PS 4a Improve Technologies for Recirculating and Flow-through Production Systems.
- PS 4b Enhance Control of Pond-Based Ecosystems to Maximize Production and Product Quality
- PS 4c Develop Shellfish Systems to Maximize Productivity and Environmental Compatibility
- PS 5 Product Quality and New Products

Estimated ARS Aquaculture Research Annual Investment by Species

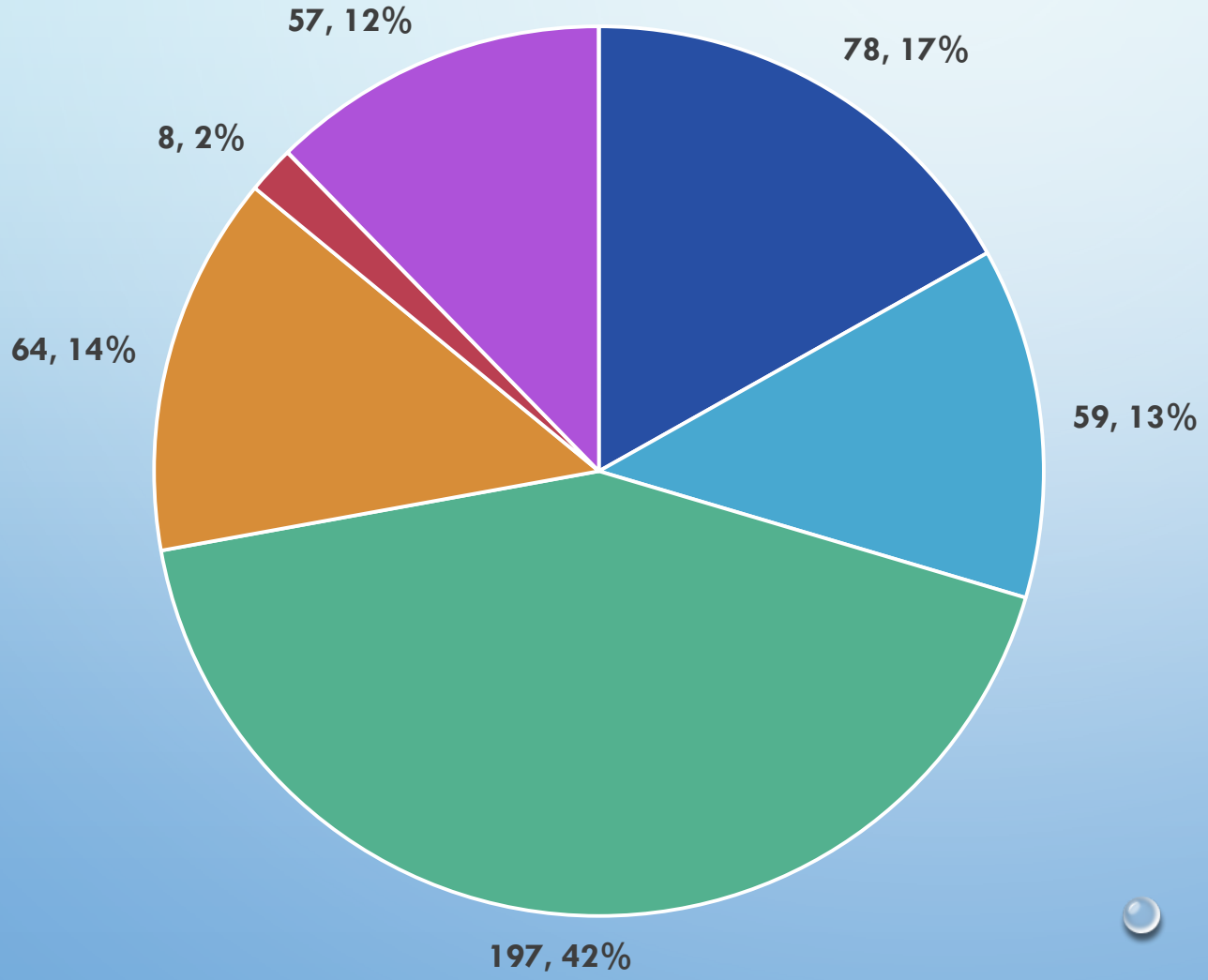


ARS AQUACULTURE NATIONAL PROGRAM SUMMARY INFORMATION 2013 - 2017

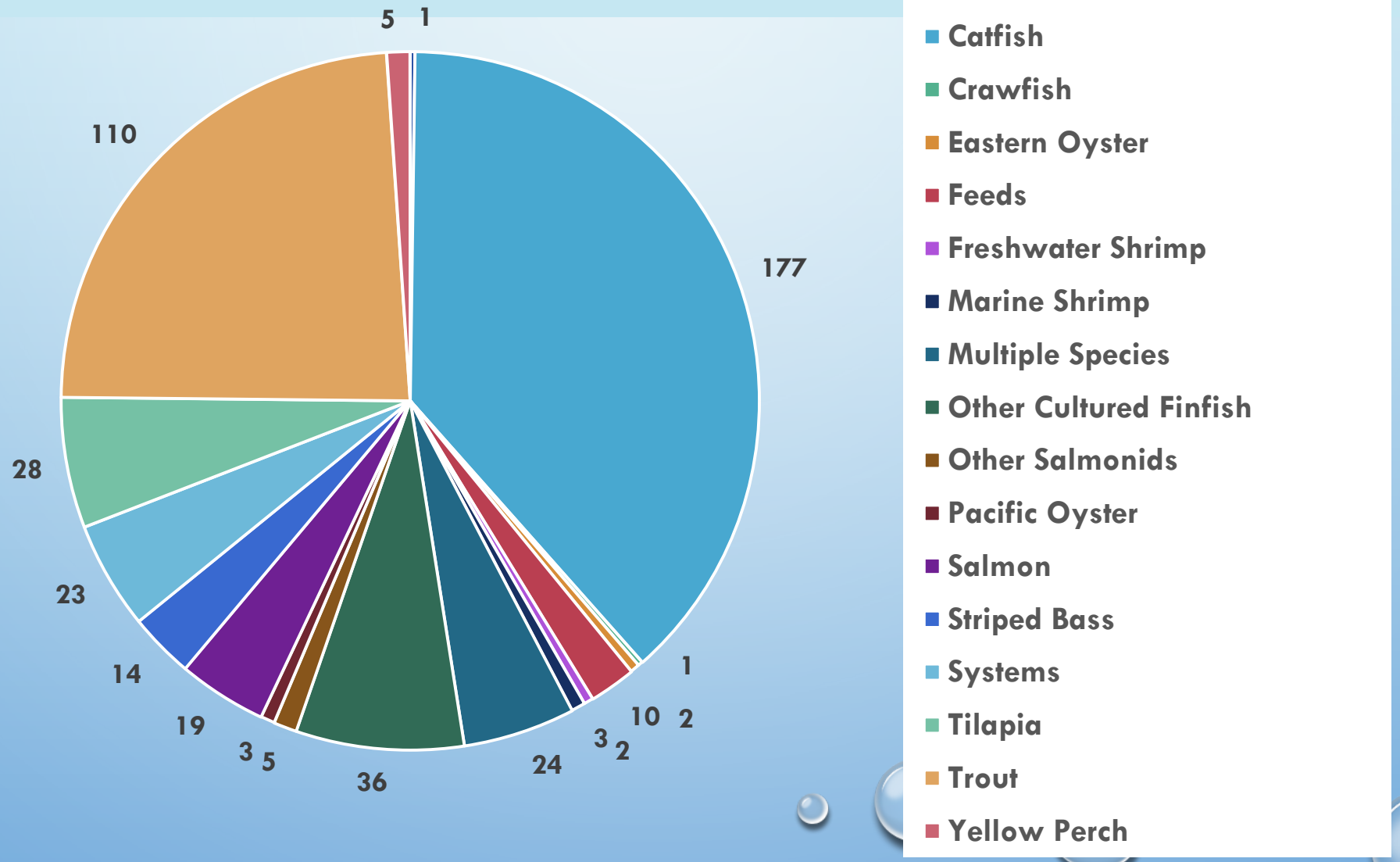
ARS AQUACULTURE PUBLICATIONS 2013 - 2017

Publication Type	Number
Abstracts	302
Book Chapters	25
Other	10
Patent Applications	3
Peer Reviewed Articles	463
Popular Publications	20
Proceedings	35
Reviews	6
Trade Publications	32

PEER REVIEWED PUBLICATIONS BY COMPONENT 2013 - 2017



PEER REVIEWED PUBLICATIONS BY SPECIES/SUBJECT 2013 - 2017



Other Technology Transfer

Fiscal Year	Number of Patents Filed	Number of Inventions Patented	Number of Active Cooperative Research and Development Agreements	Number of Material Transfer Research Agreements	Number of publications published
2013	0	0	1	0	191
2014	4	0	2	28	219
2015	0	2	2	24	187
2016	0	0	1	13	180
2017	0	1	1	10	138

External Funding Received

~$2 Million

Sources of External Funding to NP 106 Scientists 2013 - 2017				
INDUSTRY	**FEDERAL GOVERNMENT**	**UNIVERSITY**	**INTERNATIONAL**	**STATE**
42	1	27	3	2

Mentoring and Editorship

	Postdoctoral Fellowships	Graduate Students	Undergrad Students	Visiting Scientists	High School Students	Editorships
Total 2013-2017	12	22	59	105	3	19

Society and Professional Organization Memberships

American Association of the Advancement of Science
American Association of Cereal Chemists (International)
American Fisheries Society
American Meat Science Association
American Oil Chemists Society
American Society of Nutrition
American Society of Agricultural & Biological Engineers
American Society of Agronomy
Aquacultural Engineering Society
Association of Fish & Wildlife Agencies
Institute of Food Technologists
Maine Aquaculture Genomics
Maryland Society of Professional Engineers
National Restaurant Association
USDA NIFA National Research Support Project 8 (NRSP8)
Striped Bass Growers Association
US Trout Farmers Association
US Aquaculture Society
Northeastern Regional Aquaculture Center
Western Regional Aquaculture Center Technical Advisory Board
World Aquaculture Society

International Collaborations

86 collaborations

20 countries

Country	Number of Research Collaborations
BELGIUM	5
BRAZIL	7
CANADA	10
CHINA	14
DEMARK	5
DOMINICA	2
FRANCE	5
GERMANY	5
GREECE	3
ITALY	1
JAPAN	1
NIGERIA	1
NORWAY	16
PAKISTAN	1
RUSSIA	1
SCOTLAND	1
SPAIN	1
SYRIA	1
THAILAND	5
UNITED KINGDOM	1

Sample Accomplishments

COMPONENT 1: SELECTIVE BREEDING, DIRECTED REPRODUCTION, AND DEVELOPMENT OF GENOMIC TOOLS

PROBLEM STATEMENT 1A: GENOMIC TOOLS AND GENOTYPE TO PHENOTYPE

PROBLEM STATEMENT 1B: DEFINE PHENOTYPES AND DEVELOP GENETIC IMPROVEMENT PROGRAMS

PROBLEM STATEMENT 1C: ENHANCE AQUATIC ANIMAL REPRODUCTION

Problem: Despite rapid expansion of eastern oyster aquaculture, genomic tools to characterize genetic resources and apply state of the art breeding strategies lag significantly behind other livestock species.

Solution: Sequence, assemble, and annotate the eastern oyster genome

- Established a key partnership with the Eastern Oyster Genome Consortium led by Drs. Marta Gómez-Chiarri, Wesley Warren, Ximing Guo, and Dina Proestou
- Produced a high-quality sequence from a single gynogen oyster derived from an inbred line
- Generated transcriptome data from multiple tissues of the sequenced oyster to enable gene annotation using the automated NCBI pipeline.
    - ~40,000 protein-coding and ~4,000 non-coding genes predicted

Impact: This resource will facilitate the rapid discovery of commercially important genetic variants and enabling accelerated genetic improvement of the eastern oyster.

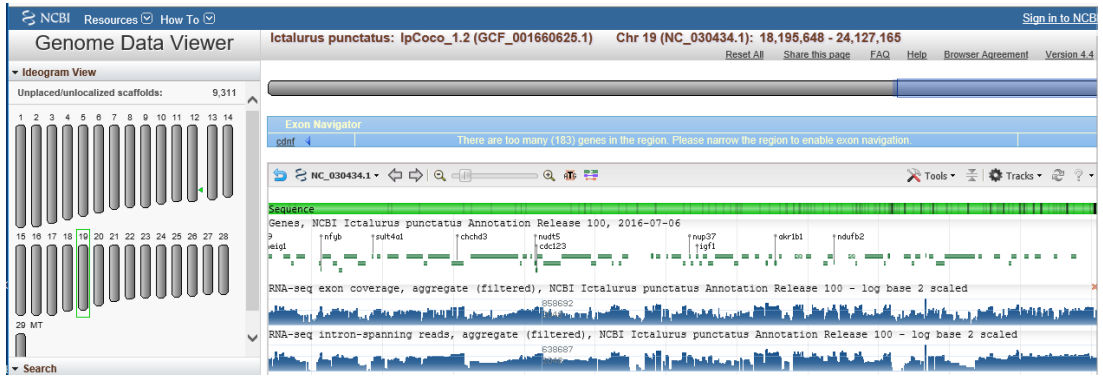
Outcomes:

- Gómez-Chiarri, Marta, et al. "Developing tools for the study of molluscan immunity: the sequencing of the genome of the eastern oyster, *Crassostrea virginica*." *Fish & shellfish immunology* 46.1 (2015): 2-4.
- Co-organized a Shellfish Comparative Genomics Workshop to introduce the draft eastern oyster genome assembly at the National Shellfisheries Association meeting in March 2017.

Molecular tools for catfish genomic selection and parentage analyses

Channel catfish genome assembly
  55K SNP array for genomic selection

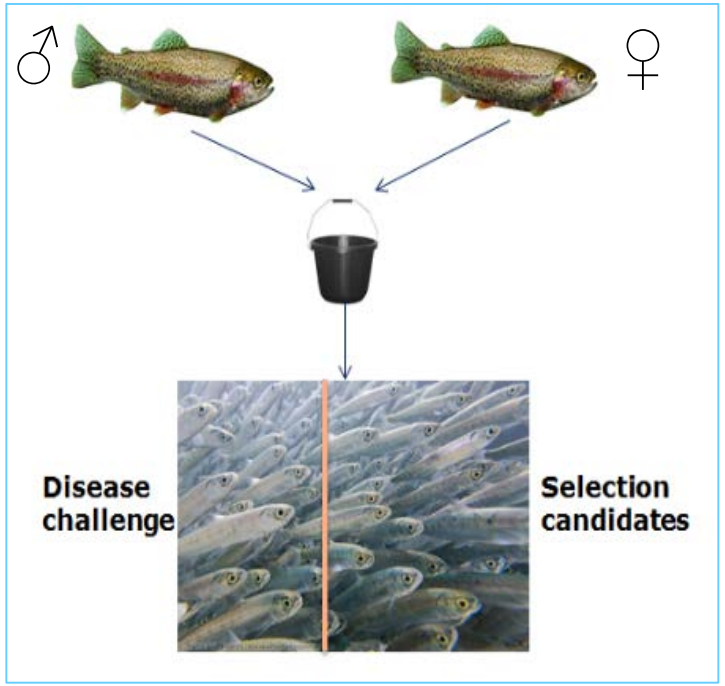
Blue catfish draft assembly
  SNP discovery



https://www.ncbi.nlm.nih.gov/genome/gdv/browser/?acc=GCF_001660625.1&context=genome

High-throughput DNA fingerprinting
  Parentage determination
  Family identification

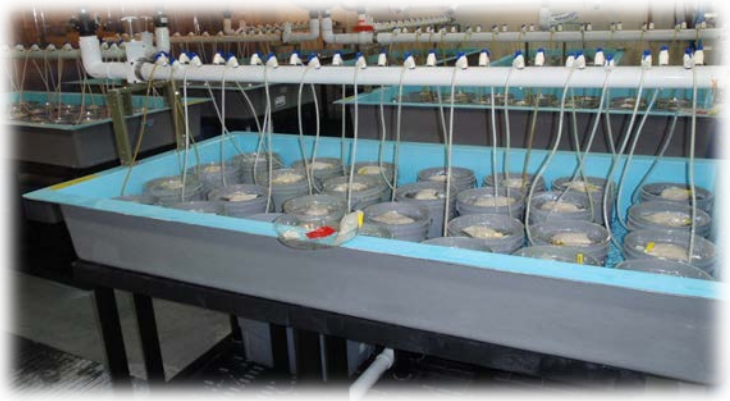
Genomic Selection for BCWD Resistance in a Commercial Rainbow Trout Population



Generation 1

TRAINING
Genotyping and Phenotyping

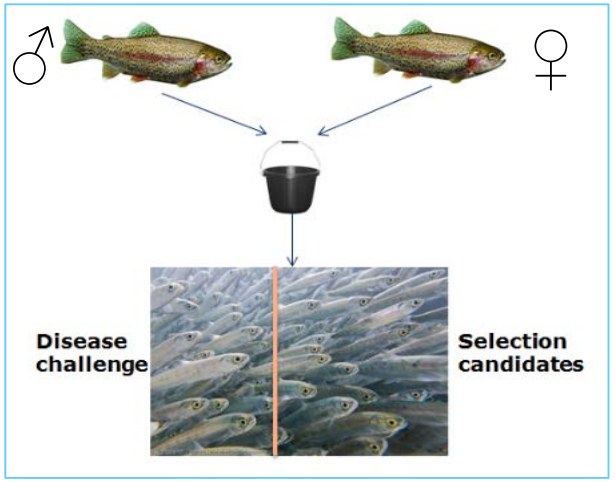
BCWD Survival Challenge



Individual breeding values are estimated for the Testing Fish based on their Genotype similarity to the Training fish.

TESTING
Genotyping Only

Parents Are Selected

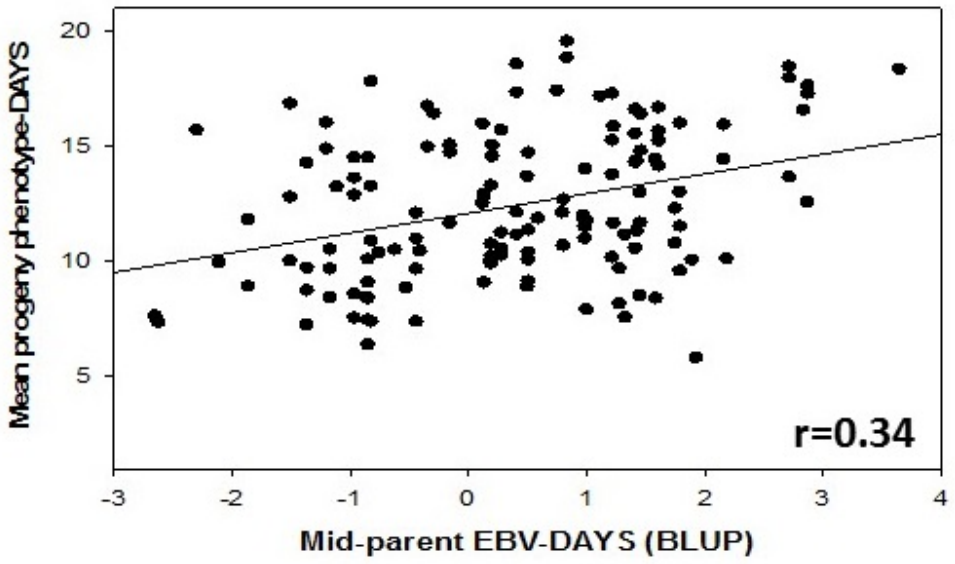


Generation 2

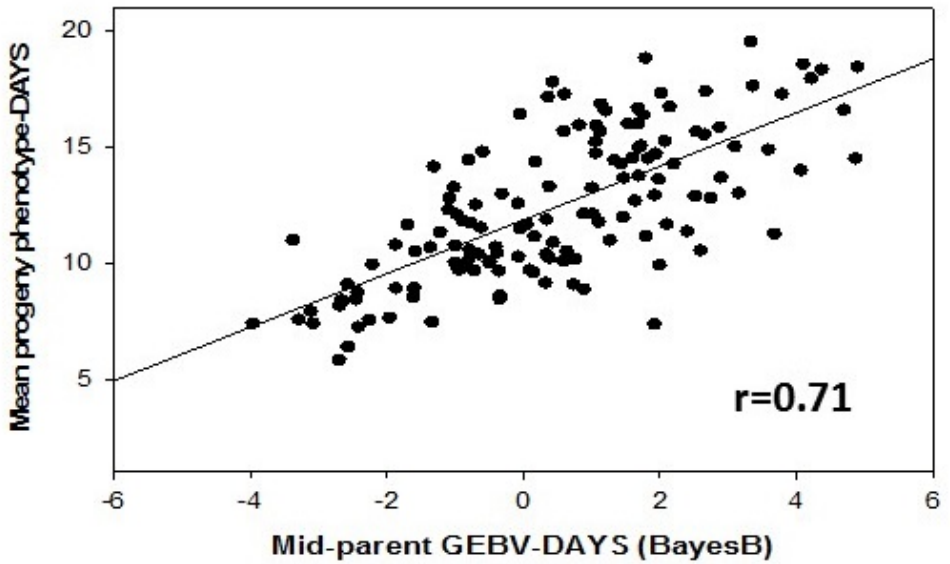
Progeny Testing

Correlation of parental genetic predictions with disease survival performance of their progeny

Pedigree Only EBVs



Genomic Predictions

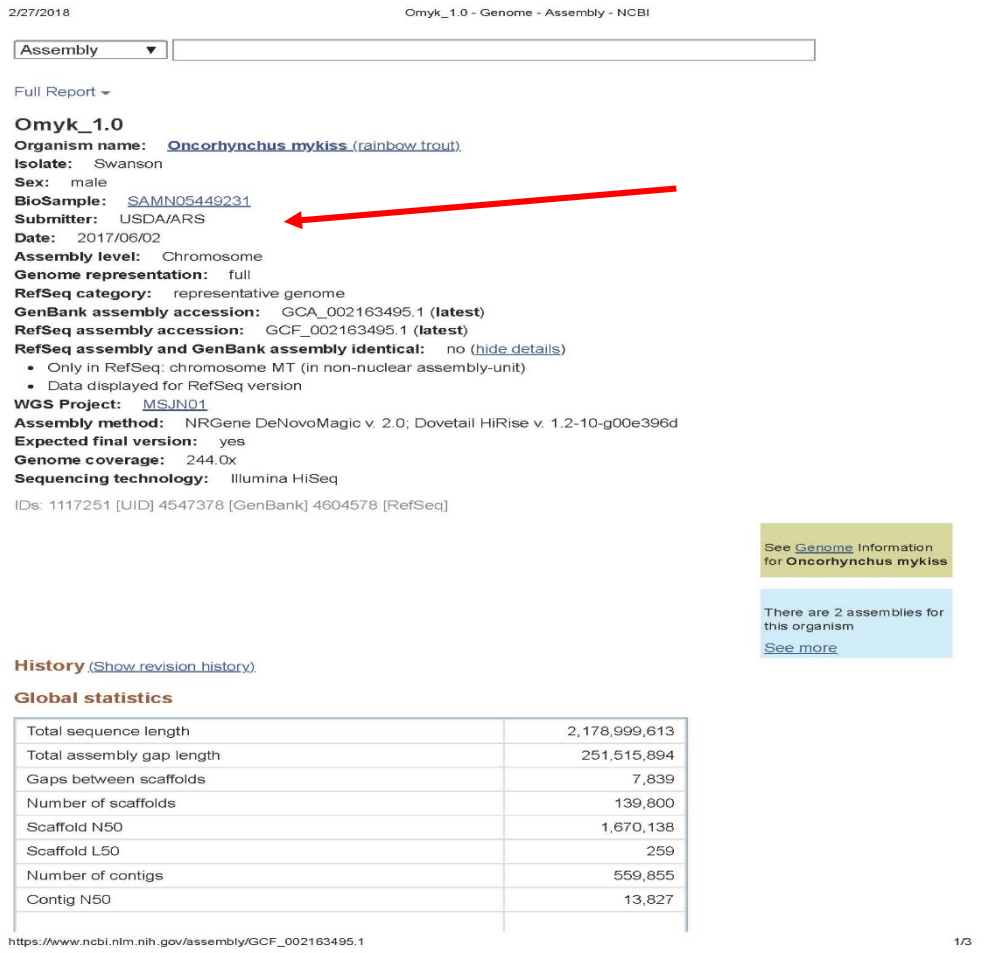


*** Genomic selection doubles the accuracy compared with traditional pedigree-based predictions!**

Vallejo *et al. Genet Sel Evol (2017) 49:17*

A new reference genome assembly for rainbow trout

- Generated a new reference genome assembly for rainbow trout.
- Approximately 88% of the new assembly sequences are aligned within chromosomes to generate contiguous chromosome sequences.
- An assurance for the high quality of this genome resource was provided by the NIH National Center for Biotechnology Information (NCBI).
- The new reference genome and the annotation of protein coding genes are now available for browsing and analyses through the NCBI online interactive databases.



2/27/2018 Omyk_1.0 - Genome - Assembly - NCBI

Assembly

Full Report

Omyk_1.0

Organism name: Oncorhynchus mykiss (rainbow trout)
Isolate: Swanson
Sex: male
BioSample: SAMN05449231
Submitter: USDA/ARS
Date: 2017/06/02
Assembly level: Chromosome
Genome representation: full
RefSeq category: representative genome
GenBank assembly accession: GCA_002163495.1 (**latest**)
RefSeq assembly accession: GCF_002163495.1 (**latest**)
RefSeq assembly and GenBank assembly identical: no (hide details)
- Only in RefSeq: chromosome MT (in non-nuclear assembly-unit)
- Data displayed for RefSeq version

WGS Project: MSJN01
Assembly method: NRGene DeNovoMagic v. 2.0; Dovetail HiRise v. 1.2-10-g00e396d
Expected final version: yes
Genome coverage: 244.0x
Sequencing technology: Illumina HiSeq

IDs: 1117251 [UID] 4547378 [GenBank] 4604578 [RefSeq]

See Genome Information for **Oncorhynchus mykiss**

There are 2 assemblies for this organism
See more

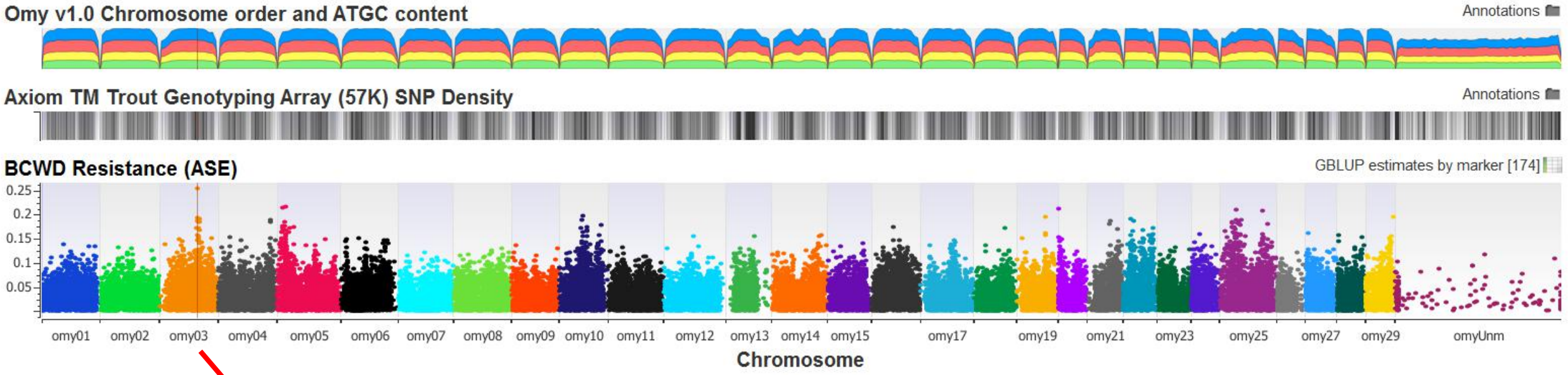
History (Show revision history)

Global statistics

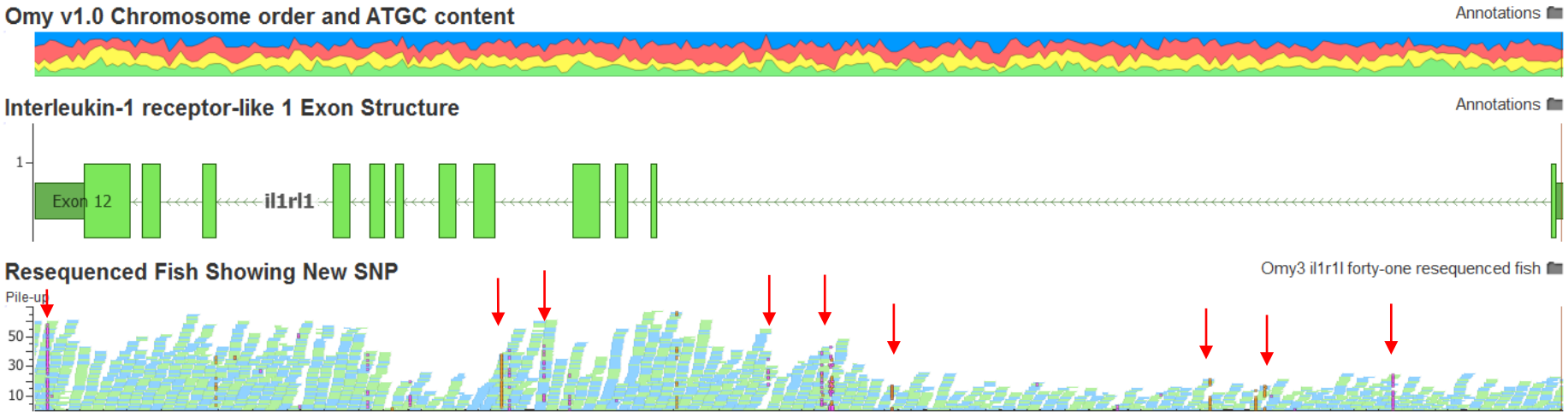
Total sequence length	2,178,999,613
Total assembly gap length	251,515,894
Gaps between scaffolds	7,839
Number of scaffolds	139,800
Scaffold N50	1,670,138
Scaffold L50	259
Number of contigs	559,855
Contig N50	13,827

https://www.ncbi.nlm.nih.gov/assembly/GCF_002163495.1 1/3

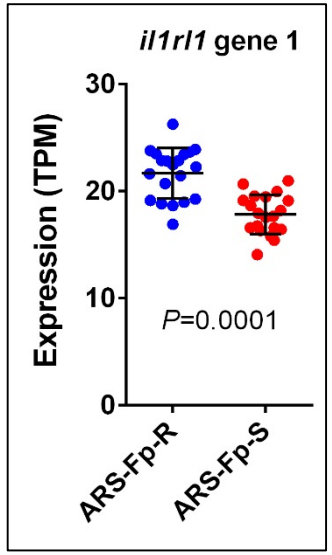
Rainbow Trout Genome (Omyk v1.0): Integration of SNP, QTL and Gene Expression Data.



Chr. Omy03 blown-up view with a candidate gene (QTL)

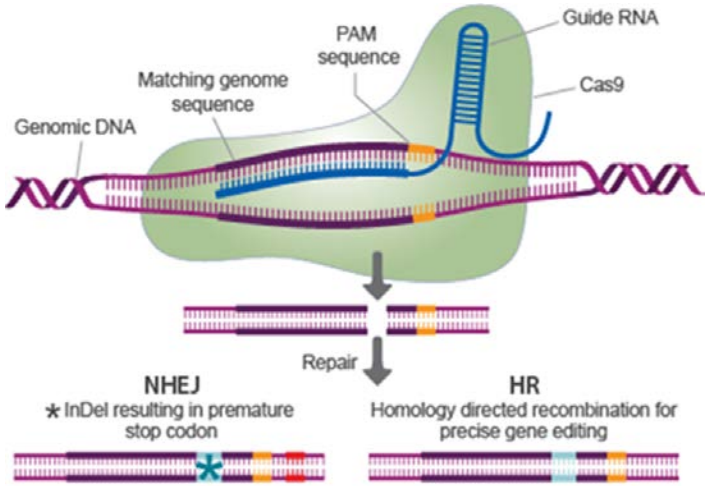


Gene Expression



Established Ability to Edit Genes in Rainbow Trout

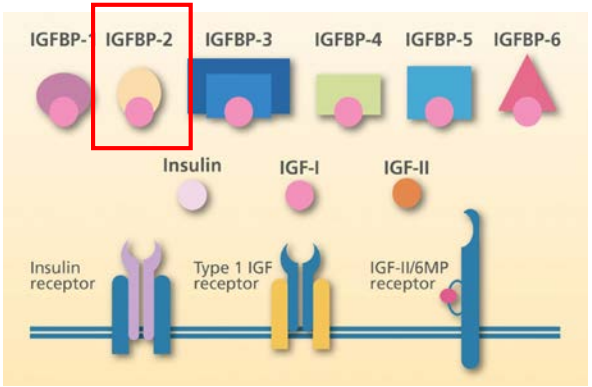
Gene Editing via CRISPR-Cas9



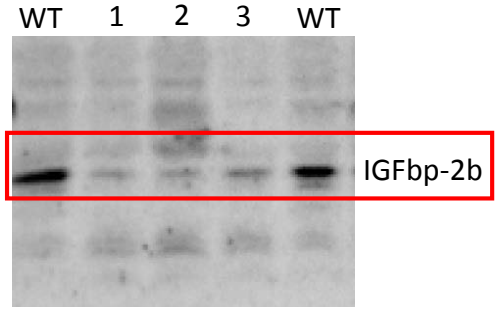
Phase I: Proof of Concept with Albino Phenotype

```
Fish # 10 tyr2 locus
AGCAGTGCTGCCCGGTGTGGGAGGGGGACGGGTCGGCCTGTGGA          Reference
AGCAGCGCTGCCCGG--------GGGGACGGGTCGGCCTGTGGA          -8 [x3]
AGCAGTGCTGCCCGGTGTGGGCAGGGGGACGGGTCGGCCTGTGGA         +1 [x3]
AGCAGTGCTGCCCGGTGTGGGGACGGGCGGGGACGGGTCGGCCTGTGGA     +5(-1, +6) [x3]
AGCAGTGCTGCCCGG---GGGGGGGGGTCGGGCCGGCCTGTGCA          -3
AGCAGTGCTGCCCGGTGTG----GGGGACGGGTCGGCCTGTGGA          -4
```

Phase II: Edit and Identify Genes Important for Production Traits



IGF ligand binding assay



Potential Outcomes/Impact

- Identify markers for traits
- Precision breeding for faster genetic gain
- Understand polygenic traits
- Induce sterility

COMPONENT 1: SELECTIVE BREEDING, DIRECTED REPRODUCTION, AND DEVELOPMENT OF GENOMIC TOOLS

PROBLEM STATEMENT 1A: GENOMIC TOOLS AND GENOTYPE TO PHENOTYPE

PROBLEM STATEMENT 1B: DEFINE PHENOTYPES AND DEVELOP GENETIC IMPROVEMENT PROGRAMS

PROBLEM STATEMENT 1C: ENHANCE AQUATIC ANIMAL REPRODUCTION

Problem: Few commercial eastern oyster lines are available, their performance across production environments has not been characterized.

Solution:

- Generated seed from 6 selected lines under common hatchery conditions.
- Evaluated performance at 5 sites that varied in temperature, salinity, and disease.
- Significant differences in performance detected among lines within some sites.

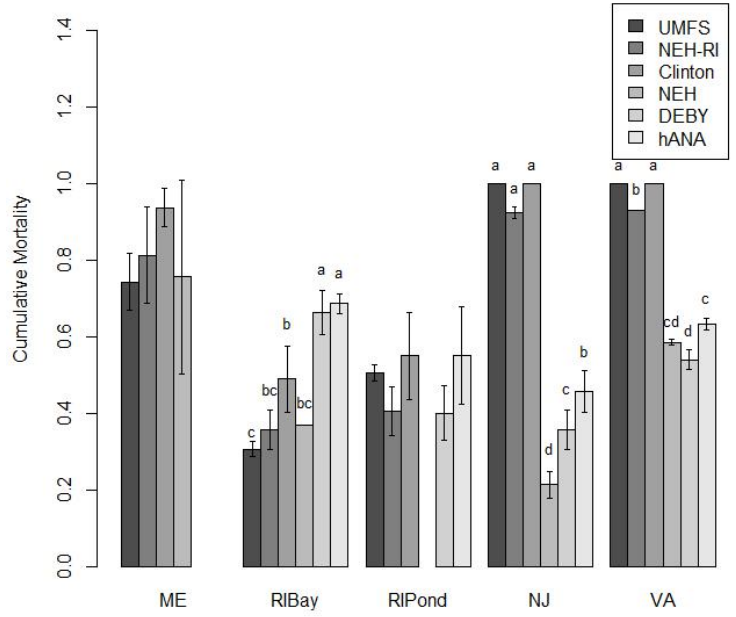
Impact:

- Demonstrated superior performance of lines at their native sites.
- Large, significant line x site interaction effects observed for mortality and yield.
- Identified need for more aggressive selection of Northern stocks.

Outcomes:

- Proestou, Dina A., et al. "Performance of selectively-bred lines of eastern oyster, *Crassostrea virginica*, across eastern US estuaries." *Aquaculture* 464 (2016): 17-27..
- Key partnerships with U Maine, URI, Rutgers University, and VIMS

Line	Site of Selection	Generations of Selection	Environmental Conditions
UMFS	Damariscotta River, ME	2	Cold, high salinity, ROD
NEH-RI	Wickford, RI	NA	Warm, high salinity, Dermo, MSX, SSO
Clinton	Long Island Sound, CT	NA	Warm, low-high salinity, MSX
NEH	Cape May, NJ	15	Warm, low salinity, Dermo, MSX
DEBY	York River, VA	13	Warm, moderate-high salinity, Dermo, MSX
hANA	York River, VA	3	Warm, moderate-high salinity, Dermo, MSX



Genetic Improvement in Catfish - Delta Selects

Selection since 2008 based on estimated breeding values (BLUP) for increased growth rate and carcass yield.

Pedigree information on 36,365 fish

25% increased growth rate, 1% increased carcass yield

2015 year class selected based on genomic breeding values
- -2911 fish genotyped with 54k SNPs
- -Estimated 25% increase in accuracy of breeding values

Problem: North American strain Atlantic salmon are not many generations removed from wild, unselected stocks.

Solution:

- Selectively breed Atlantic salmon for important traits such as carcass weight, fillet color, fat content, and sea lice resistance.
- Include genotypic information into selective breeding program.

Impact:

- Yearly germplasm release of more than 600,000 eggs to Cooke Aquaculture.
- Improved carcass weight of more than 100% over control line for last 3 years.
- Breeding values for fillet color, fat content, and sea lice resistance calculated.
- F1 offspring for improved sea lice resistance.
- 288 SNP panel developed.

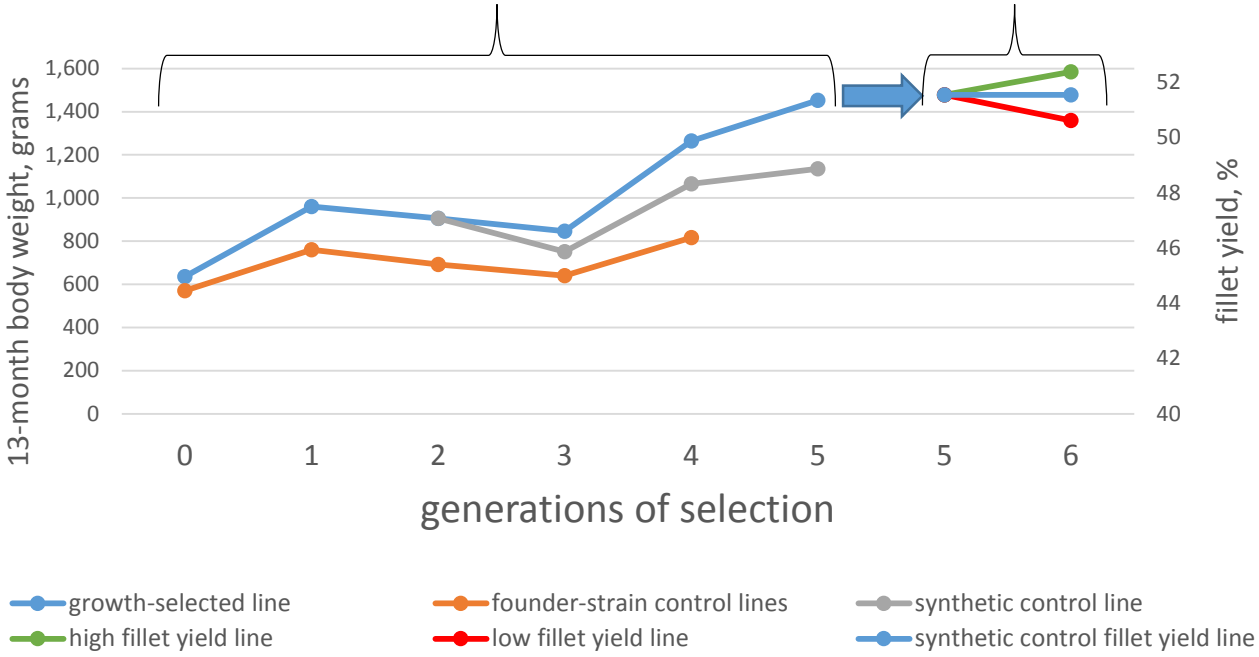
Outcomes:

- Key partnerships with the Maine Aquaculture Association, Cooke Aquaculture, National Center for Cool and Coldwater Aquaculture, University of Maine.
- Invited presentations to Seafood Expo North America, Hendrix Genetics Academy, Aquaculture Canada, Aquaculture America.
- Burr, GS, Pietrak, MR, Peterson, BC, Proestou, DA, Wolters, WW. 2017. Atlantic salmon and Eastern oyster breeding programs at the National Cold Water Marine Aquaculture Center. Proceedings of the 44th United States-Japan Aquaculture Panel Symposium. Seattle, WA November 1-2, 2016. p. 44-48.
- Pietrak, MR, Wolters, WR, Rexroad III, CE, Peterson, BC. 2016. Selective breeding program for sea lice, Lepeophtheirus salmonis (Krøyer 1838), resistance at the USDA's National Cold Water Marine Aquaculture Center. Bulletin of the Aquaculture Association of Canada. 2016-2 p. 46-52.

Selective Breeding Rainbow Trout For Faster Growth and Higher Fillet Yield

5 generations of growth selection: 80-100 grams of body weight gain/generation (~12% gain per gen.)

1 generation of divergent fillet yield selection using growth-selected line: high fillet yield line = +0.8 percentage points low fillet yield line = -0.9 percentage points



representative high fillet yield fish

1,750 =	BW, grams	= 1,750
52.3 =	fillet yield, %	= 45.9
152.4 =	head weight, g	= 176.3
157.6 =	viscera weight, g	= 229.9
26.3 =	fillet thickness, mm	= 23.9

representative low fillet yield fish

COMPONENT 1: SELECTIVE BREEDING, DIRECTED REPRODUCTION, AND DEVELOPMENT OF GENOMIC TOOLS

PROBLEM STATEMENT 1A: GENOMIC TOOLS AND GENOTYPE TO PHENOTYPE

PROBLEM STATEMENT 1B: DEFINE PHENOTYPES AND DEVELOP GENETIC IMPROVEMENT PROGRAMS

PROBLEM STATEMENT 1C: ENHANCE AQUATIC ANIMAL REPRODUCTION

Developed a Systematic Method (algorithm) for Non-Lethal Gender Identification in Yellow Perch

- Shepherd, B.S., Rees, Christopher, B., Sepulveda-Villet, O.J., Palmquist, D.E., Binkowski, F.P. (2013) “Identification of Gender in Yellow Perch (*Perca flavescens*) by External Morphology: Validation in Four Geographic Strains and Effects of Estradiol”. *North American Journal of Aquaculture* 75:361-372.

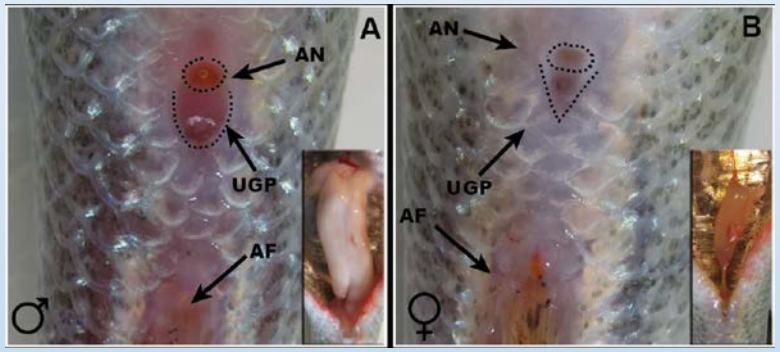
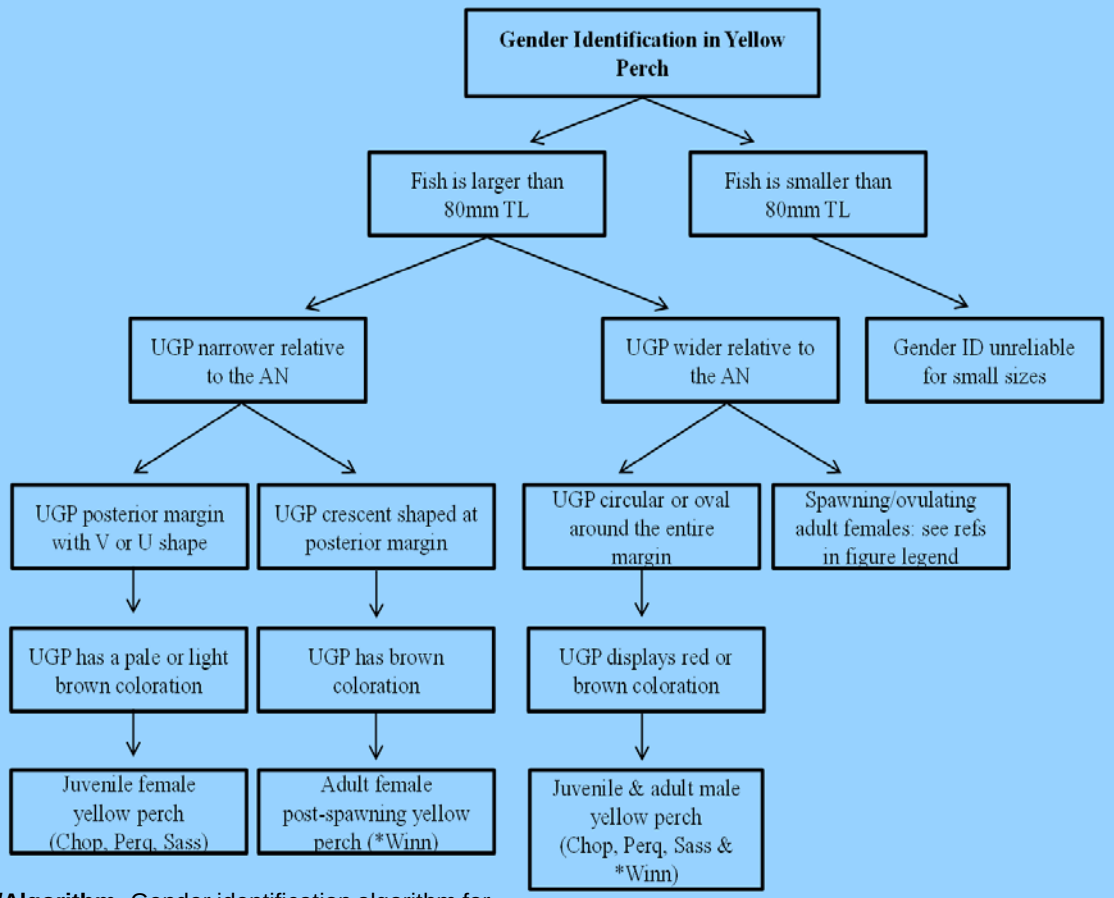


Figure: External morphology of the urogenital papilla in untreated male and female Yellow Perch from three distinct size-classes. An example of sex confirmation is included in each panel as an inlay. Representative examples were all externally identified correctly. (**A**) Male (♂) Sassafras strain Yellow Perch in size category 1 (80–170 mm). (**B**) Female (♀) Sassafras strain Yellow Perch in size category 1 (80–170 mm).



Table/Algorithm: Gender identification algorithm for domesticated strains of Yellow Perch. Abbreviations: AN = anus, UGP = urogenital papilla.

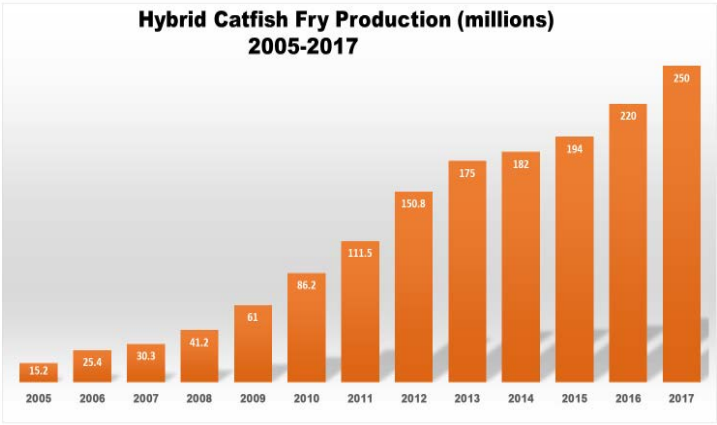
Channel x blue hybrid catfish production

Evaluation of blue catfish broodstock for hybrid performance

Cryopreservation of blue catfish sperm

Evaluation of salmon and chicken gonadotropin releasing hormone for industry

Development and transfer of new methods for commercial hybrid production
- Reduced parental stress
- Increased embryo survival in hatching jars



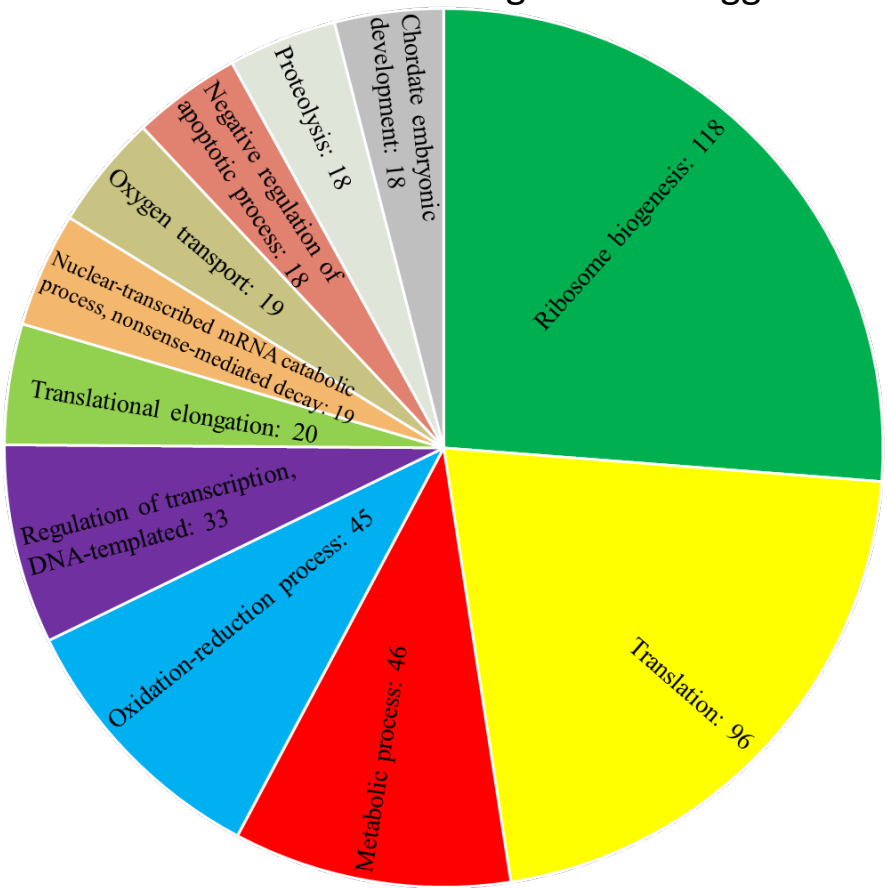
Transcriptome Analysis of Egg Quality in Rainbow Trout

Differentially expressed transcripts (DETs) between unfertilized eggs of varying quality.
Survival at eyeing: Low, 0-5%; Medium, 30-50%; High, 80-100%

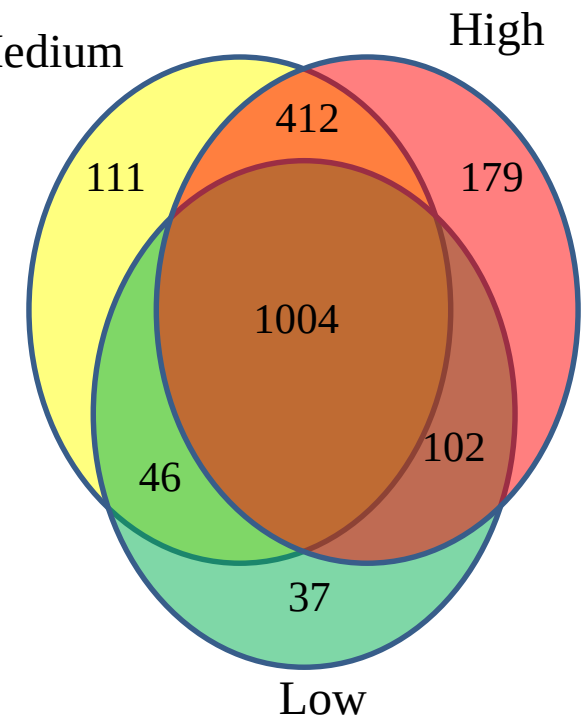
	Low vs Medium	Low vs High	Medium vs High
R-RNA removal	1	1	0
Poly(A) retention	1012	944	2

612 shared

Gene ontology analysis of polyadenylated DETs between Low and High survival eggs



Distribution of miRNAs among Low, Medium, and High survival eggs



COMPONENT 2: NUTRIENT REQUIREMENTS AND ALTERNATIVE PROTEIN AND LIPID INGREDIENTS.

PROBLEM STATEMENT: DETERMINE NUTRIENT REQUIREMENTS AND VALUATE THE NUTRITIONAL VALUE OF ALTERNATIVE SOURCES OF PROTEIN AND LIPID

Improving the sustainability and production efficiency of rainbow trout aquaculture

- **Problem**: Fish meal and oil are limited resources, expanding aquaculture to sustainably meet future seafood demands requires developing alternative ingredients for fish feeds

- **Approach:** Focus on alternative ingredients, feed processing technologies, defining nutrient requirements, optimizing production systems and genetic improvement of fish for performance on alternative diets

Alternative Feed Ingredients

- Tested and evaluated more than 100 ingredients and entered this information into the ingredient evaluation program and digestibility database.
- Evaluated algal sources potential to replace fish oil as a source of omega-3 fatty acids in aquaculture feeds.

Nutrient Requirements

- Determined optimum vitamin and mineral levels necessary for maintaining optimum growth and health for fish reared on plant based aquaculture feeds.

Feed Processing

- Developed low cost method to produce feed grade soy protein concentrate for trout feeds.
- Developed a new analytical method developed for determining degree of starch gelatinization.
- Determined the effects of feed processing method (extrusion and expansion-compression pelleting) on water quality and growth of rainbow trout in a commercial setting
- Developed improved method for extrusion processing of plant material for aquaculture feeds.

Genetic Improvement

- Continued improvement in rainbow trout for the ability to utilize and grow on a plant protein based feed. Increased soybean meal from 15 to 25% and average family weight from 175g to >350g at 5 months.
- Demonstrated that genetic variation exists in rainbow trout to biosynthesize and convert plant oils to healthy omega-3 fatty acids and store in the muscle.
- Released germplasm to nine commercial stakeholders

Production Systems

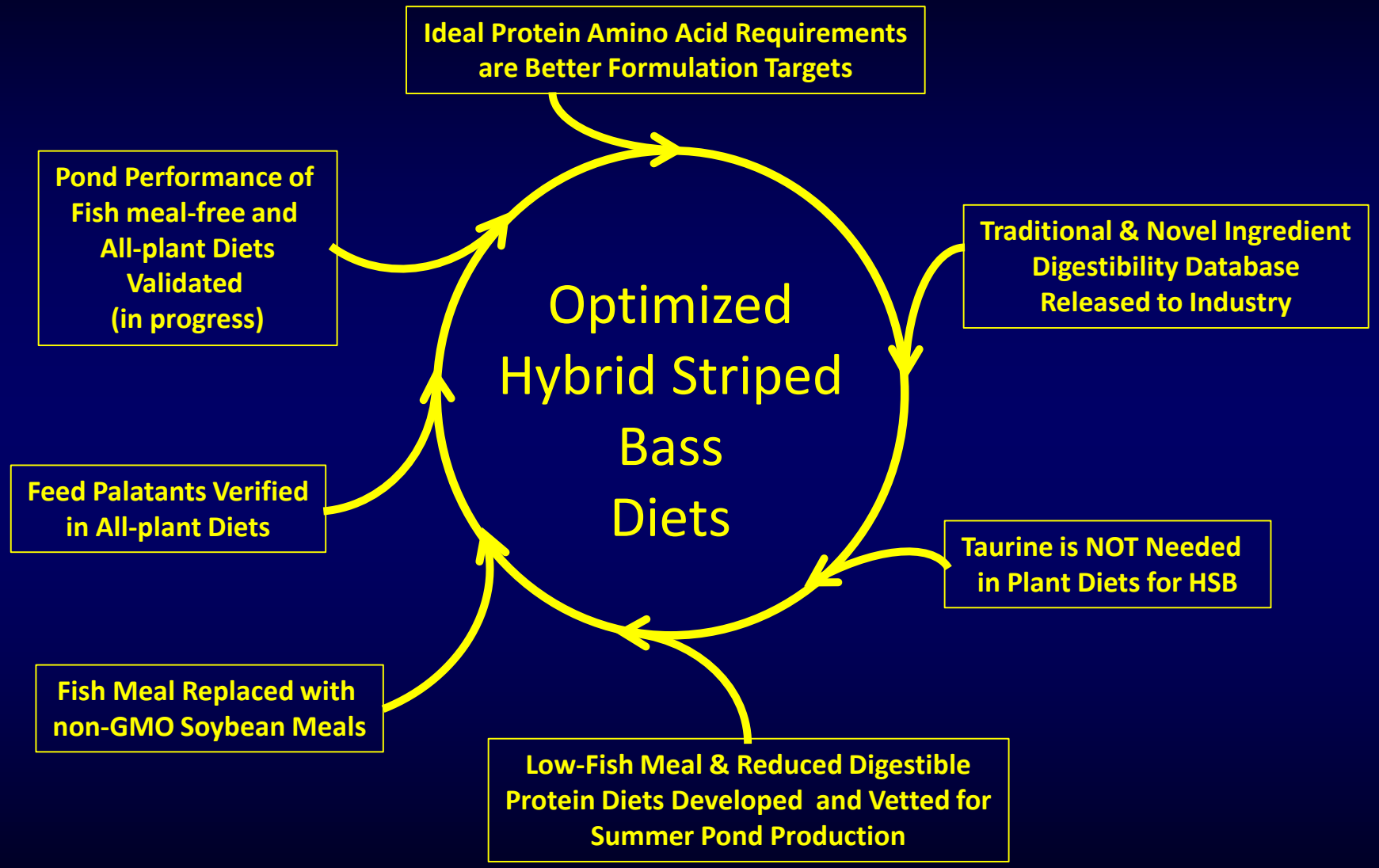
- Effect of short-term feeding cessation prior to harvest on fillet yield of rainbow trout
- Developed dietary formulations and processing methods to improve water quality in aquaculture production.
- Use of aeration and oxygen supplementation on growth of rainbow trout at a commercial hatchery

Refining nutritional requirements & developing diets that conserve fish meal in warmwater finfish.

Industry Problems & Research Questions

- *Uncertain supply & volatile pricing of fish meal…*
- *Gaps in composition & digestibility data for substitute ingredients…*
- *Gaps in nutrient requirement data for hybrid striped bass…*
- *Diets based on published requirements don't always perform as expected…*
- *Alternate diets have to be vetted in production style conditions to typical market size to encourage industry adoption…*

Solutions


Ideal Protein Amino Acid Requirements are Better Formulation Targets
Traditional & Novel Ingredient Digestibility Database Released to Industry
Taurine is NOT Needed in Plant Diets for HSB
Low-Fish Meal & Reduced Digestible Protein Diets Developed and Vetted for Summer Pond Production
Fish Meal Replaced with non-GMO Soybean Meals
Feed Palatants Verified in All-plant Diets
Pond Performance of Fish meal-free and All-plant Diets Validated (in progress)
Optimized Hybrid Striped Bass Diets

Tech Transfer & Key Partnerships

- *AB Vista*
- *BioOregon*
- *Cargill Nutrition*
- *Evonik*
- *Rangen Feeds*
- *Simmons Proteins*
- *Skretting North America*
- *Zeigler Feeds*

- ✓ Refined nutrient requirements for diet development
- ✓ Ingredient Digestibility Database for industry
- ✓ Commercially viable low fish meal diets
- ✓ Commercially viable fish meal-free diets
- ✓ Commercially viable all-plant protein diets

- ➢ *ARS Trout Grains Project – Rick Barrows, Jason Frost*
- ➢ *USFWS Bozeman Fish Tech. Lab – Gibson Gaylord, Wendy Sealey*
- ➢ *University of Arkansas at Pine Bluff – Rebecca Lochmann*
- ➢ *Texas A&M University – Delbert Gatlin, III*

COMPONENT 3: HEALTH OF AQUATIC ANIMALS

PROBLEM STATEMENT 3A: IMPROVE UNDERSTANDING OF HOST IMMUNITY, IMMUNE SYSTEM EVASION BY PATHOGENS, AND DISEASE-RESISTANT PHENOTYPES.

PROBLEM STATEMENT 3B: CONTROL OF PATHOGENS AND PREVENTION OF DISEASE

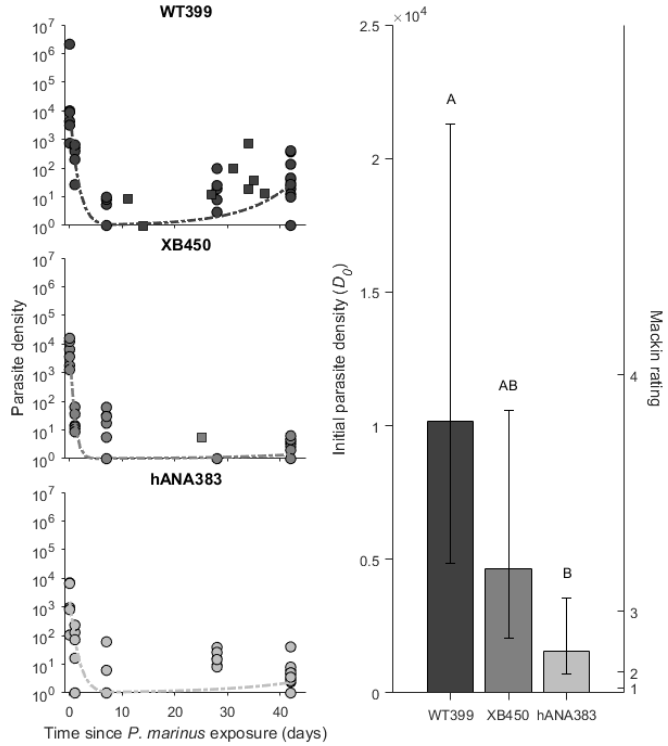
Problem: Resistance to Dermo disease is a commercially important trait.

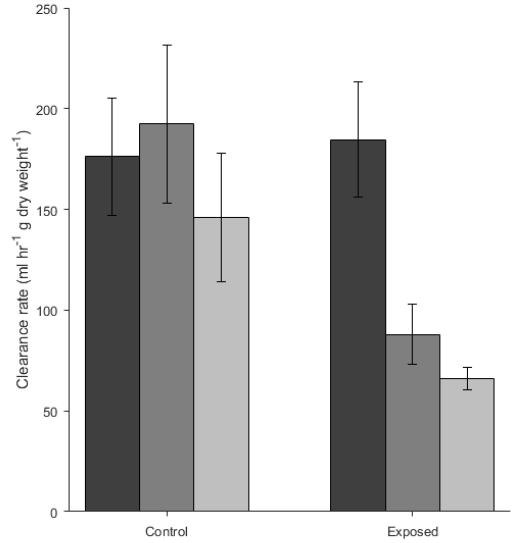
Solution:

- Performed disease challenge in the laboratory and compared response to Dermo exposure among 3 selectively-bred oyster families.
  - Survival and the trajectories of parasite density post-exposure varied by family.
  - Immediate parasite densities (6hr post-exposure) also varied by family.

- Performed feeding experiment to examine the effect of Dermo exposure on feeding behavior.
  - In the presence of Dermo, family differences detected for clearance rate and the proportion of oysters with open valves during the experiment.

- The ability to alter feeding behavior in the presence of Dermo correlates with measured resistance.





Impact:

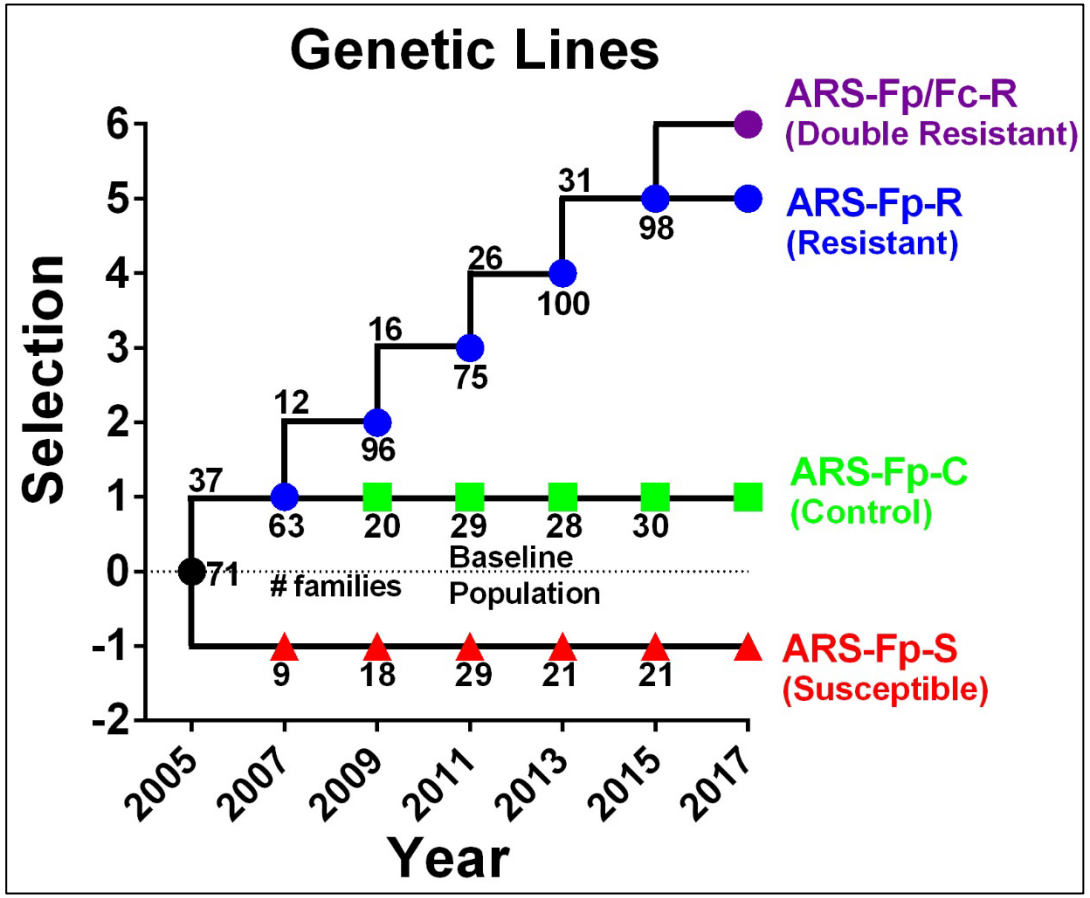
- Demonstrated that parasite avoidance behavior is a mechanism of Dermo resistance and that it varies among oyster families, therefore selective breeding can improve resistance to Dermo.

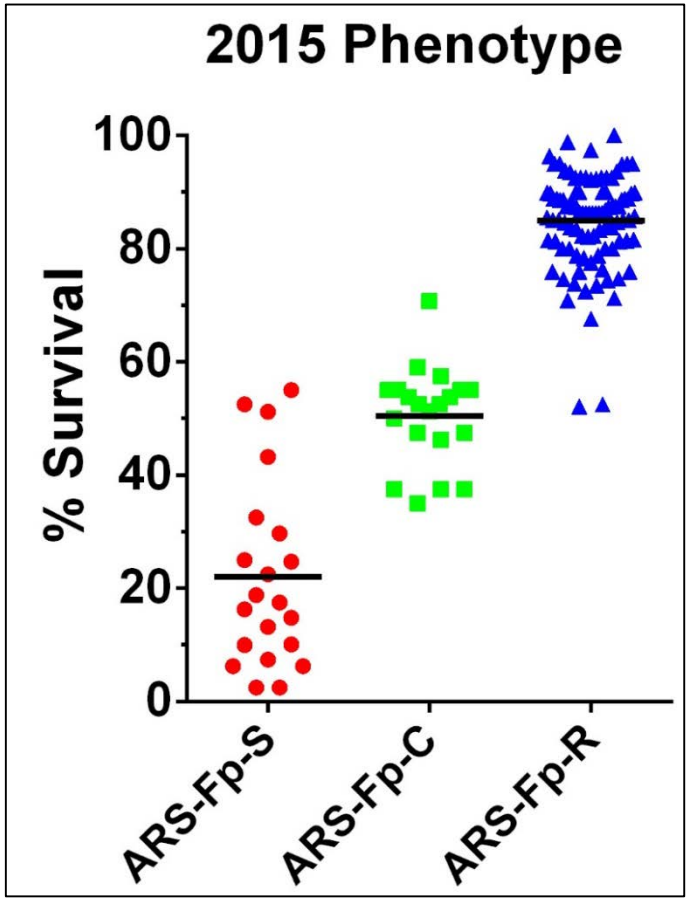
Outcomes:

- Ben-Horin, T., et al. “Genetic variation in anti-parasite behavior in oysters." *Marine Ecology Progress Series* In press: https://doi.org/10.3354/meps12511
- Key partnership with the Aquaculture Genetics, Breeding, and Technology Center at VIMS

Genetic improvement of disease resistance: host variation, response to selection, and farm improvement.

Bacterial cold water disease: widespread, no licensed vaccine, frequent cause of antimicrobial use.





Summary:

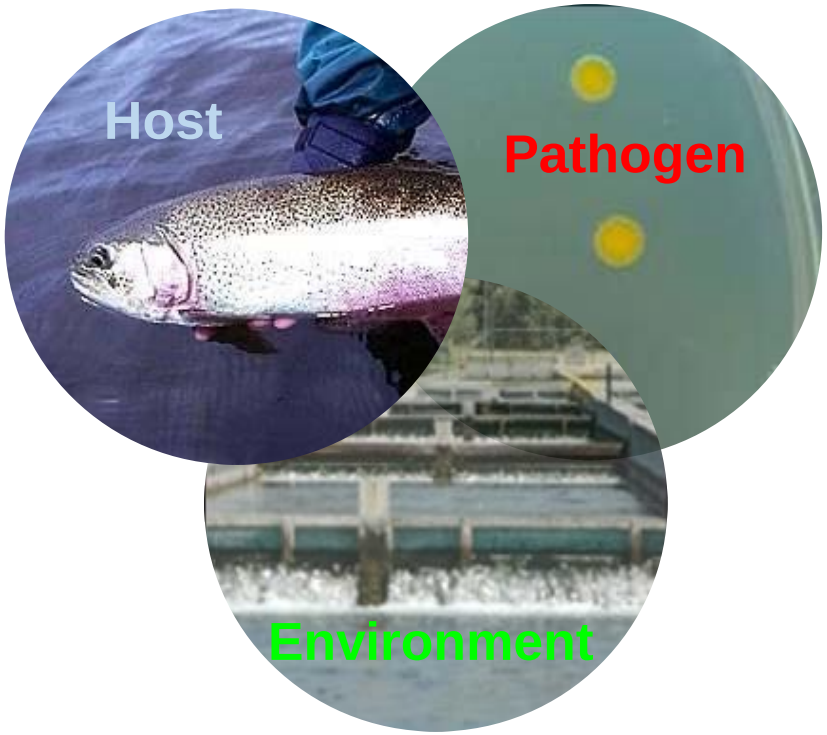
1. Response to selection for BCWD resistance ~11 percentage points per gen.
2. Stable trait during growth (0.2 g through 800g)
3. Positive genetic correlation between BCWD and CD.
4. Inbreeding <8%/line.
5. **Increased farm survival (15 ARS trials completed, no BCWD outbreak in resistant line).**
6. Pathogen strain specificity.
7. IHNV susceptibility.
8. ERM vaccine response unaffected by breeding.

Germplasm Release: >2 million eggs; Troutlodge, Pacific Aquaculture, Clear Springs Foods Inc , Utah Division of Wildlife Resources, California Dept. of Fish and Wildlife, Idaho Department of Fish and Game, and numerous academic collaborators interested in phenotype.

Increased understanding of genetic basis of BCWD resistance and host-pathogen and environmental interaction.

Host Immune Characterization:

1. RNA-seq of whole-body transcripts identified 1884 genes regulated by infection and/or differentially expressed between genetic lines.
2. BCWD QTL on Omy03 candidate gene: interleukin-1 receptor-like 1.
3. Applied high-throughput disease resistance phenotyping to commercial lines to validate genomic selection.



Pathogen Characterization:

1. Published complete genome sequences of Fp and Fc strains used in selective breeding program.
2. Published qPCR and typing assays – widespread usage.
3. Typed and/or draft genome sequenced large collection of farm isolates.

Environmental monitoring:

1. Published reference plasma biochemistry values and implemented multiprobe sensor during farm trials
2. Univ of Conn. subaward - analyzed microbiome diversity of two farm sites.

Pathogen Detection and Characterization – Columnare

- **Characterize effects of stress and pathogen virulence on host endocrine/immune response to improve production and control Rhabdoviral and Flavobacterial diseases in percid/salmonid aquaculture.**

  - Bartelme, R.P., R.J. Newton, Y. Zhu, N. Li, B.R. LaFrentz, and M.J. McBride (2016) “Complete genome sequence of the fish pathogen *Flavobacterium columnare* strain C#2”. *Genome Announcements* 4:1-2. doi.org/10.1128/genomeA.00624-16.

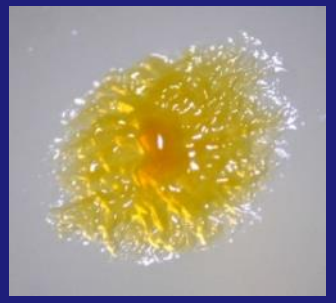
  - Ke, Q., Weaver, W., Pore, A., Gorgoglione, B., Wildschutte, J., Xiao, P., Shepherd, B., Spear, A., Malathi, K., Stepien, C.A., Vakharia, V., Leaman, D.W. (2017). “Role of viral hemorrhagic septicemia virus matrix (M) protein in suppressing host transcription”. *Journal of Virology* 91:e00279-17. https://doi.org/10.1128/JVI.00279-17.

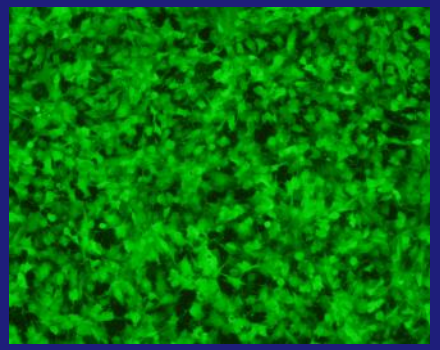
  - Li, N., Y Zhu, B.R. LaFrentz, J..P Evenhuis, D.W. Hunnicutt, R.A. Conrad, P. Barbier, C.W. Gullstrand, J.E. Roets, J.L. Powers, S.S. Kulkarni, D.H. Erbes, J.C. García, P. Nie, M.J. McBride (2017) ‘The type IX secretion system is required for virulence of the fish pathogen *Flavobacterium columnare”*. *Applied and Environmental Microbiology* 83:e01769-17. doi.org/10.1128/AEM.01769-17.

  - Shepherd, B.S., Spear, A.R., Philip, A.M., Leaman, D.W., Stepien, C.A., Sepulveda-Villet, O.J., Palmquist, D.E. and M.M.Vijayan (2018) “Effects of Cortisol on Expression of Select Growth-, Stress-, and Immune-related Genes in Rainbow Trout Liver”. *Fish and Shellfish Immunology* 74C: 410-418. https://doi.org/10.1016/j.fsi.2018.01.003.

Bridging knowledge gaps between host and pathogen

- Columnaris disease (*Flavobacterium columnare*)
  - Pioneered genetic tools to identify 4 different players in this disease
    - Analysis of over 100 archived isolates from industry indicate that 3 of these have caused disease on catfish farms; guiding next generation vaccine development
  - Identified columnaris susceptible and resistant families of fish and described the role of host-derived receptors for pathogen attachment and colonization
  - Two CRADA's were implemented with Merck Animal Health (MAH) focused on columnaris vaccine development
  - Partnered with Auburn University and Kennebec River Biosciences to conduct lab and field trials with an autogenous columnaris vaccine
- Parasites
  - Characterized the suite of immune genes responding to vaccination against the protozoan parasite *Ichthyophthirius* ("Ich")
  - Discovered new relationships between mixed bacteria and parasite infections
  - Extended a CRADA with MAH to test a new vaccine platform (nucleic acid particle vaccines)





Streptococcus spp. are a leading cause of disease related mortality in tilapia production

- **Approach**: selectively breed tilapia for disease resistance as an additional tool for reducing impact
  - Partnered with Spring Genetics and Akvaforsk Genetics Center AS in Norway through MTA and MTA-CRADA.
  - Demonstrated that resistance to *S. iniae* and *S. agalactiae* is heritable.
  - Demonstrated via assortive mating that gains in resistance to both pathogens is possible.
- Spring Genetics has incorporated selective breeding for disease resistance into their breeding program
  - The improved tilapia are being sold throughout the Americas and abroad.
  - Based on current production statistics and models, representative gains from growing the improved tilapia on an average sized farm are US $635,000 in additional revenue assuming a conservative 5% increase in survival, which research trials indicate will be substantially higher.

An emerging hypervirulent *Aeromonas hydrophila* is threatening the US catfish industry

- **Approach**: developed reproducible challenge models to reliably induce the disease and evaluate preventative and therapeutic strategies
  - Determined key host:pathogen interactions including route of pathogen entry, distribution and growth kinetics within the host, the role of dietary status in host susceptibility, and the genes and their protein products governing virulence
- These fundamental studies paved the way for novel interventions
  - Partnered with Merck Animal Health and Auburn University through an MTRA and NACA to test new vaccine candidates and delivery strategies in laboratory and field conditions. Lab studies revealed excellent protection, field trials and new invention disclosure for oral delivery are underway
  - Research on alternatives to antibiotics including clay minerals, feed additives, and chemical therapeutants showed safety and efficacy in combatting *Aeromonas* infections

COMPONENT 3: HEALTH OF AQUATIC ANIMALS

PROBLEM STATEMENT 3A: IMPROVE UNDERSTANDING OF HOST IMMUNITY, IMMUNE SYSTEM EVASION BY PATHOGENS, AND DISEASE-RESISTANT PHENOTYPES.

PROBLEM STATEMENT 3B: CONTROL OF PATHOGENS AND PREVENTION OF DISEASE

Pathogen Detection and Characterization – VHS

- **Developed and validated a standardized reverse transcriptase polymerase chain reaction (StaRT-PCR) test to accurately and rapidly quantify live, replicating VHS virus in aquaculture species:**
  - Pierce, L.R., Willey, J.C., Crawford, E.L., Palsule, V.V., Leaman, D.W., Faisal, M., Kim, R.K., Shepherd, B.S., Stanoszek, L.M. and Stepien, C.A (2013) "A new StaRT-PCR approach to detect and quantify fish Viral Hemorrhagic Septicemia virus (VHSV): Enhanced quality control with internal standards". *Journal of Virological Methods* 189: 129-142.

- **Converted the StaRT-PCR test to an easier-to-use real-time polymerase chain reaction platform using a two-color detection system.**
  - Pierce, L.R., Willey, J.C., Palsule, V.V., Yeo, J., Crawford, E.L., Shepherd, B.S. and Stepien, C.A. (2013) "Accurate detection and quantification of the fish Viral Hemorrhagic Septicemia virus (VHSV) with a two-color fluorometric real-time PCR assay". *PLoS One* 8(8): e71851. *doi:10.1371/journal.pone.0071851.*

- **Characterized the evolution of the VHSV pathogen in Great Lakes finfish species as a means to predict pathogen movement and virulence.**
  - Pierce, L.R. and Stepien, C.A. (2012) "Evolution and biogeography of emerging quasispecies: Diversity patterns of the fish Viral Hemorrhagic Septicemia virus (VHSV)". *Molecular Phylogenetics and Evolution,* 63:327-341.
  - Stepien, C.A., Pierce, L.R., Leaman, D.W. and Shepherd, B.S. (2015) "Gene Diversification of an Emerging Pathogen: A Decade of Mutation in a novel Fish Viral Hemorrhagic Septicemia (VHS) substrain since its first appearance in the Laurentian Great Lakes". *PLoS One* 10(8): e0135146.

Development of vaccines for control of *Weissella ceti* and *Lactococcus garvieae*

- ***Weissella ceti*** **in North Carolina and** ***Lactococcus garvieae*** **in Washington State, both emerging pathogens of farmed Rainbow trout and both have never before been reported in the United States**

- **In both cases disease losses were substantial and the disease agents were identified soon after outbreaks started-This early identification provided a unique opportunity to develop and implement a control strategy quickly before these diseases became a serious industry problem**

- **Rapid vaccine development and deployment required a team effort involving ARS staff, farmers, extension staff, and vaccine companies**

- **Vaccines developed and validated as safe and effective in laboratory tests done at the NCCCWA**
- **Vaccines produced for farm application by commercial companies**
- ***On-farm* vaccine-challenge experiments showed high efficacy when applied under field conditions**

Outcomes

Weissella vaccine use resulted in a complete cessation of losses due to this pathogen

Lactococcus vaccine use has reduced disease to negligible levels

Rapid vaccine development and deployment effectively controlled outbreaks and likely reduced the dissemination of these emerging pathogens

USDA Agricultural Research Service

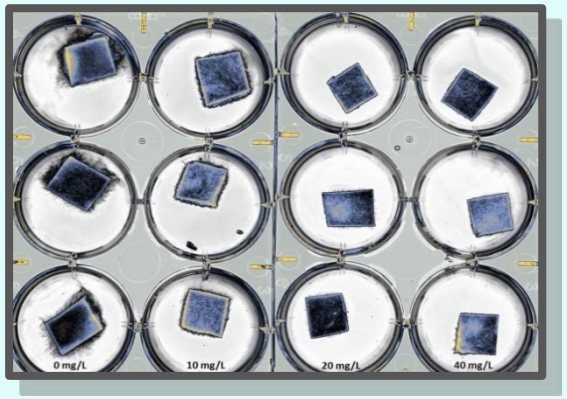
Problem

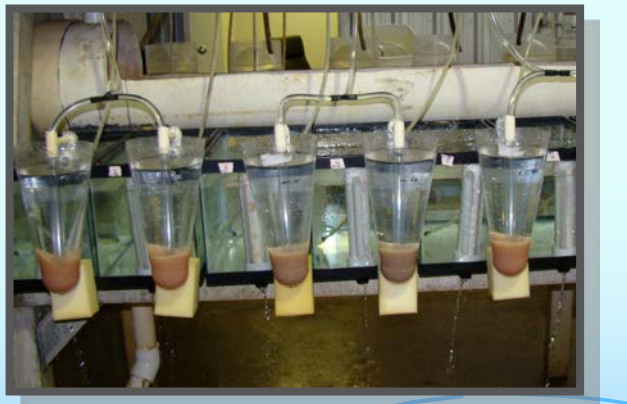
- Producing hybrid striped bass and large-mouth bass for the food-fish industry is inherently difficult due to recurrent and costly outbreaks of fungus (*Saprolegnia* spp.) when hatching eggs.
- These species have different egg incubation times and are hatched using drastically different techniques.
- Industry wanted a simple solution to this problem.

USDA Agricultural Research Service

Solution

- ARS scientists spoke to industry leaders about using copper sulfate under their specific hatching requirements.
- Worked with a team to develop an innovative *in vitro* bioassay to test copper sulfate on *Saprolegnia* spp.
- Conducted on-farm research studies to determine optimum copper sulfate treatment rates under each scenario; additionally, distinctive water chemistry at the hatcheries demanded special attention.



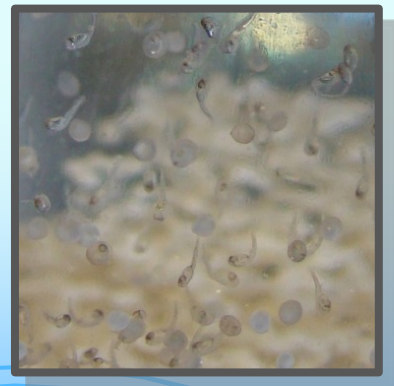


USDA Agricultural Research Service

Impact

- From this research, copper sulfate is used exclusively for treating fungus that attacks newly fertilized eggs by Keo Fish Farm, the largest hybrid striped bass fingerling producer in the world, and Dunn's Fish Farm, the largest U.S. largemouth bass food-fish producer.
- This research has increased fry production 10 – 15% and farms no longer experience catastrophic losses of entire batches of eggs as was commonplace in the past.

Largemouth Bass



Eggs and larvae

Hybrid Striped Bass

COMPONENT 4: SUSTAINABLE PRODUCTION SYSTEMS

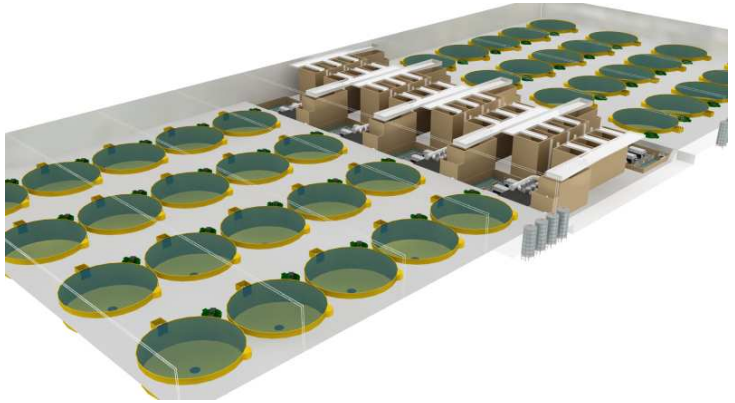
PROBLEM STATEMENT 4A: IMPROVE TECHNOLOGIES FOR RECIRCULATING AND FLOW-THROUGH PRODUCTION SYSTEMS.

PROBLEM STATEMENT 4B: ENHANCE CONTROL OF POND-BASED ECOSYSTEMS TO MAXIMIZE PRODUCTION AND PRODUCT QUALITY

PROBLEM STATEMENT 4C: DEVELOP SHELLFISH SYSTEMS TO MAXIMIZE PRODUCTIVITY AND ENVIRONMENTAL COMPATIBILITY

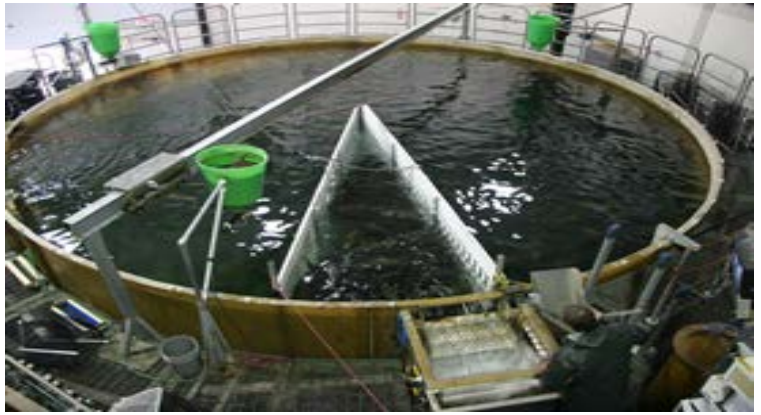
Developing and Refining Technologies for Sustainable Fish Production in Closed Containment Systems

Establishing safe limits for environmental parameters in RAS for Atlantic salmon & rainbow trout



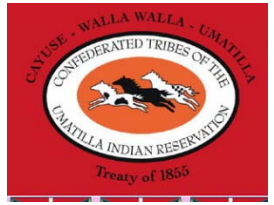
- Optimal environmental parameters must be determined for raising Atlantic salmon in RAS to support growth of land-based salmon and trout production in the US.
  - ✓ARS funded researchers defined the safe upper limit to produce the best performance:
    - Nitrate
    - Oxygen
    - Carbon dioxide
    - Swimming speeds
  - ✓Findings define the acceptable ranges for water quality that are critical for US producers in the nascent RAS Atlantic salmon and rainbow trout industry.

Comparing the economic performance and carbon footprint of Atlantic salmon production systems



- ✓ ARS funded researchers worked with collaborators to use performance data from growout trials along with engineering design experience to compare production of Atlantic salmon in RAS to ocean net pen systems.
- ✓ The cost of producing salmon in land-based closed containment RAS is roughly the same as that of traditional open net pen salmon systems.
- ✓ The return on investment for traditional open net pen salmon farming is twice that of RAS when RAS salmon is sold at a premium price.
- ✓ The carbon footprint of salmon produced in RAS delivered fresh to market in the U.S. is less than half that of open net pen salmon delivered from Norway to the U.S. by air freight.
- ✓ The benefits that are being realized by producing fish in RAS domestically, including reduced shipping costs, improved fish growth, feed conversion, and survival rates, as well as reduced vaccination and treatment costs, have made RAS an attractive technology option for increasing production of market size Atlantic salmon in the US.

Technologies & Practices Benefit Farmers, Consultants, Suppliers , Public Agencies and Tribes



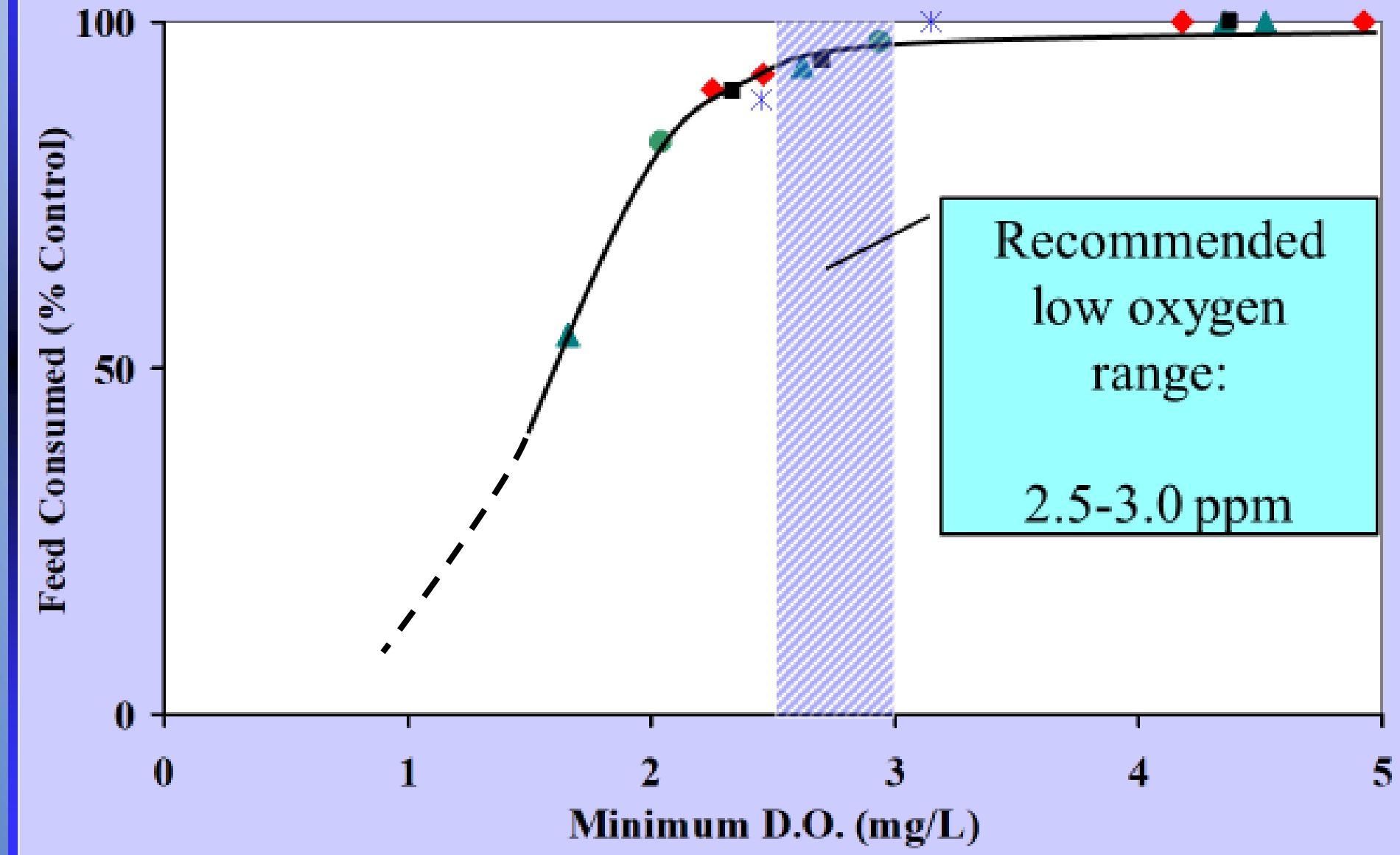
COMPONENT 4: SUSTAINABLE PRODUCTION SYSTEMS

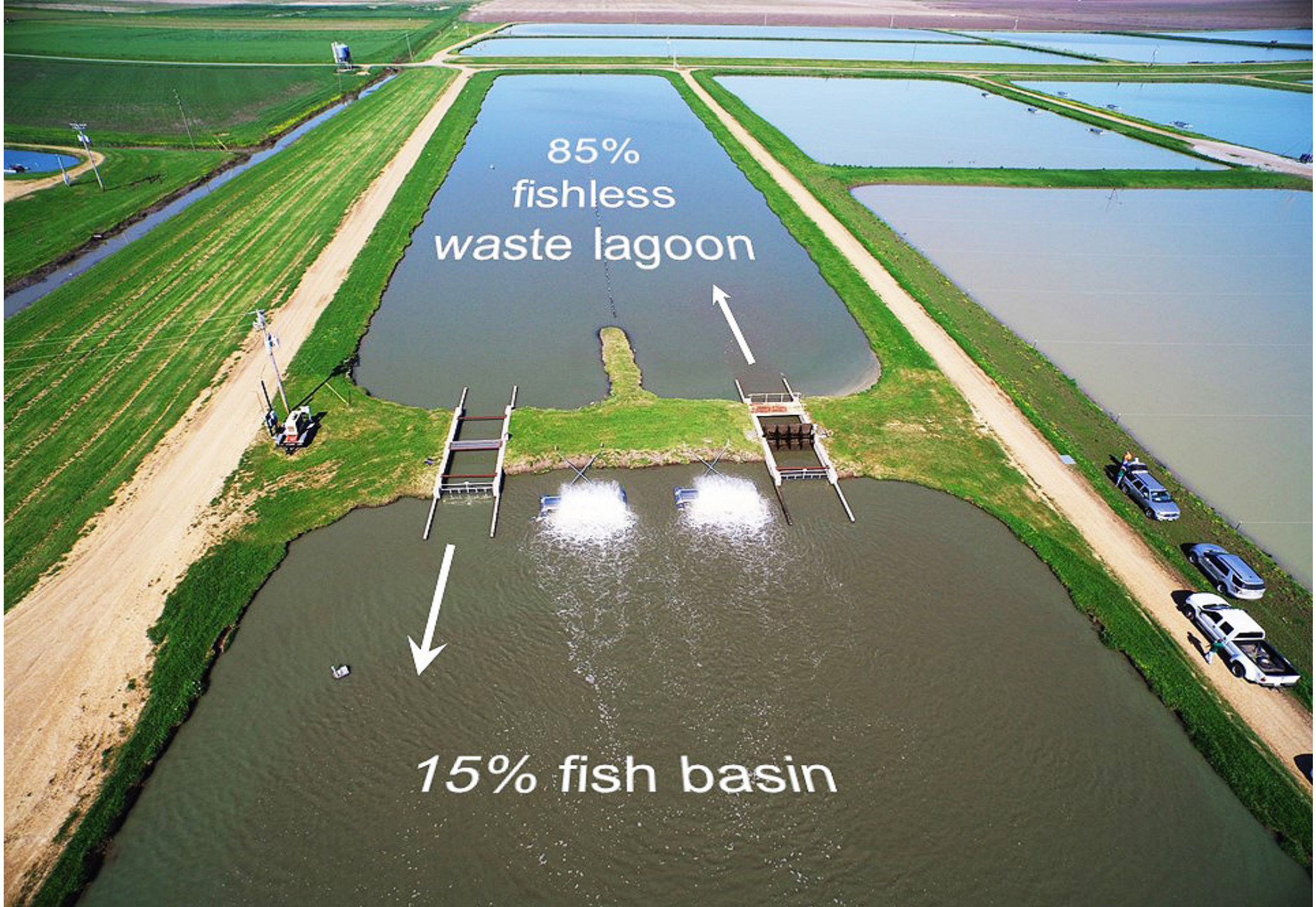
PROBLEM STATEMENT 4A: IMPROVE TECHNOLOGIES FOR RECIRCULATING AND FLOW-THROUGH PRODUCTION SYSTEMS.

PROBLEM STATEMENT 4B: ENHANCE CONTROL OF POND-BASED ECOSYSTEMS TO MAXIMIZE PRODUCTION AND PRODUCT QUALITY

PROBLEM STATEMENT 4C: DEVELOP SHELLFISH SYSTEMS TO MAXIMIZE PRODUCTIVITY AND ENVIRONMENTAL COMPATIBILITY

Catfish Oxygen Studies




85%
fishless
waste lagoon
15% fish basin

COMPONENT 4: SUSTAINABLE PRODUCTION SYSTEMS

PROBLEM STATEMENT 4A: IMPROVE TECHNOLOGIES FOR RECIRCULATING AND FLOW-THROUGH PRODUCTION SYSTEMS.

PROBLEM STATEMENT 4B: ENHANCE CONTROL OF POND-BASED ECOSYSTEMS TO MAXIMIZE PRODUCTION AND PRODUCT QUALITY

PROBLEM STATEMENT 4C: DEVELOP SHELLFISH SYSTEMS TO MAXIMIZE PRODUCTIVITY AND ENVIRONMENTAL COMPATIBILITY

Developing Methods to Improve Survival and Maximize Productivity and Sustainability of Pacific Shellfish Aquaculture

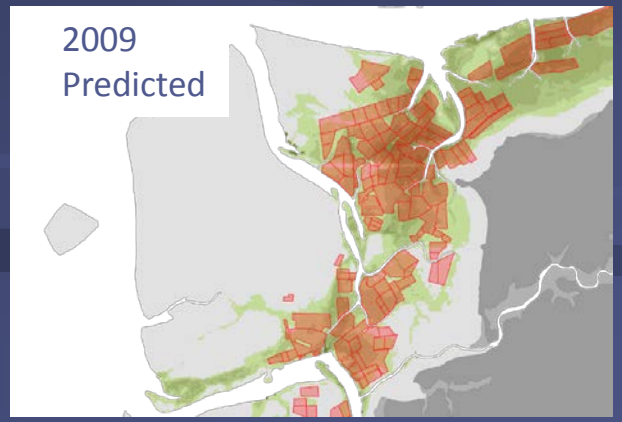
Brett Dumbauld, Newport, Oregon

Accomplishment: Demonstrated minimal impacts of oyster aquaculture to eelgrass at the estuarine landscape scale.

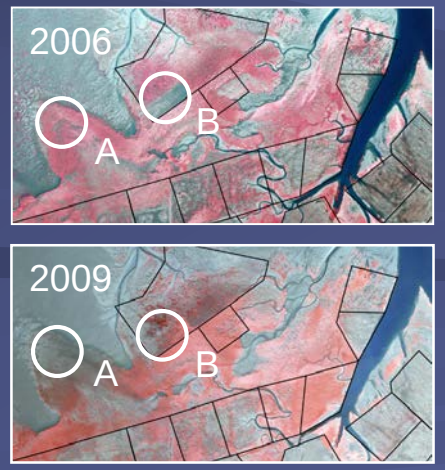
- Submerged aquatic vegetation like eelgrass provides valuable habitat for estuarine fish and invertebrates. Recent Endangered Species Act listings of some of these species like juvenile salmon on the US west coast and updates to regulatory measures to protect eelgrass as essential fish habitat under the Magnuson Stevens Act have resulted in renewed interest to understand the influence of aquaculture on eelgrass, since it is also declining in many areas.

- ARS researchers created GIS spatial layers for various factors that influence eelgrass (tidal elevation, wave stress, salinity, distance to estuary mouth and distance to nearby channels) and used them to model and quantify the influence of bivalve aquaculture on eelgrass at the landscape scale in Willapa Bay, Washington. Impacts for each culture bed were also quantified over three non-consecutive years to determine whether impacts were chronic or transitory.

- Results demonstrated that while significant small scale and short term temporal effects due mostly to harvest method occurred, oyster aquaculture only reduced eelgrass presence in Willapa Bay by less than 1.5% at the landscape scale and more eelgrass was present than predicted in many aquaculture areas. This research has directly influenced policies being adopted at both federal and state levels to regulate and permit shellfish aquaculture practices. Research is now underway to document the function of eelgrass and both on-bottom culture and alternative off-bottom culture structures for these fish and invertebrates at this scale.



GIS spatial data layers showing predicted eelgrass presence (green) and shellfish culture beds (red overlay) in a portion of Willapa Bay, Washington.



Aerial photographs of a portions of the tide flat with culture beds outlined (black) and eelgrass (red) showing change over time from 2006 to 2009. Note A) the decrease in eelgrass outside the cultured areas and yet apparent increase over this time inside one of the cultured beds as eelgrass recovered from apparent disturbance.

On- bottom oyster culture and eelgrass (*Zostera marina*)

Off-bottom longline oyster culture and eelgrass

Snapshot from underwater video of fish using longline oyster culture

Developing Methods to Improve Survival and Maximize Productivity and Sustainability of Pacific Shellfish Aquaculture

Brett Dumbauld, Newport, Oregon

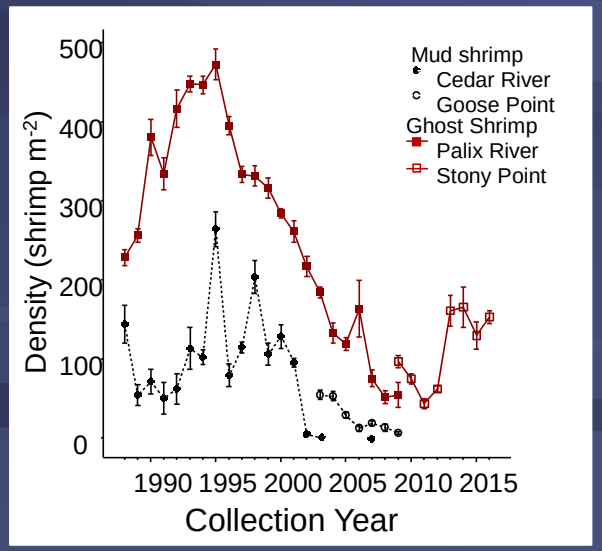
Accomplishment: Demonstrated that models of burrowing shrimp population dynamics can be used to advance integrated pest management and identified shrimp recruitment as the most significant factor to monitor in planning for pest control on aquaculture beds.

- Burrowing shrimp are a problem for the U.S. West Coast shellfish aquaculture industry because they cause oysters to sink under the surface of the sediment and die.
- ARS scientists monitored shrimp populations, quantified annual patterns of shrimp recruitment to West Coast estuaries and built an age based population dynamics model for these shrimp by quantifying the amount of lipofuscin, a pigment in their brains. Recruitment of small young- of- the year shrimp varies widely from year to year and from estuary to estuary, but is directly related to the abundance of older shrimp that are present thereafter.
- The age based model suggested that there was also consistent and relatively high natural mortality of older shrimp after recruitment, but monitoring the abundance of these small newly recruited shrimp allows a window of opportunity for control. These small recruits may also be more vulnerable to control due to their shallow burrows and potential susceptibility to predation and other factors at this size.

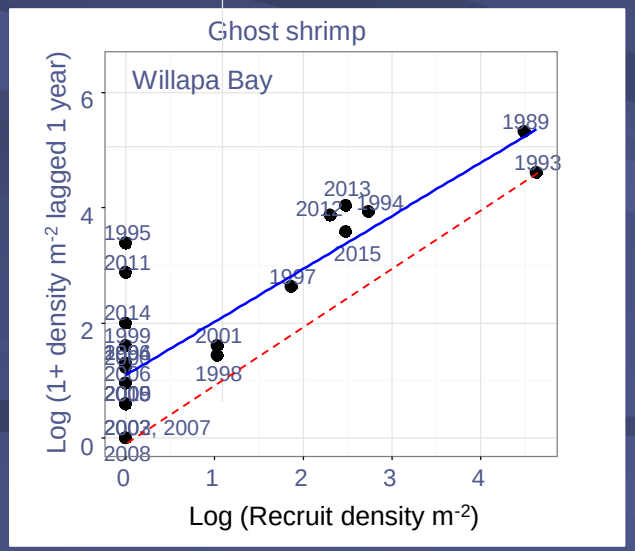
Oyster seed disappearing under sediment surface due to shrimp bioturbation

Adult shrimp in burrow

Recruit in burrow



Long term record of shrimp abundance at monitoring sites in Willapa Bay showing declines from about 1996-2010 followed by a recent increase in the ghost shrimp population.



Relationship between the density of ghost shrimp recruits during the year that they settle and the density of 1 year old shrimp a year later. Exceptions where no recruitment was observed, but older shrimp were found suggests that they recruited later or moved in as small juveniles.

Fiberglass resin casts of adult and recruit burrows with 1m stick for scale

COMPONENT 5: PRODUCT QUALITY AND NEW PRODUCTS

- Problem: Catfish processing byproducts are underutilized and have little value.

- Need: The chemical and nutritional composition of commercially produced byproduct from channel and hybrid catfish was needed for developing new uses for catfish byproducts.

- Results: Catfish byproducts including heads, frames, viscera, trim and skin were obtained from commercial processing plants and composition determined.

	Channel						Hybrid				
	Frame	Head	Skin	Trim	Viscera		Frame	Head	Skin	Trim	Viscera
% Moisture	59.50	68.29	65.65	64.68	67.80		61.62	68.23	70.40	63.01	66.17
% Ash	5.09	6.70	0.59	2.25	1.04		4.58	6.99	0.57	1.93	0.76
% Lipid	20.03	9.74	13.62	19.22	17.65		19.13	9.33	10.00	21.58	21.40
% Protein	16.35	15.80	22.82	15.41	13.35		15.72	15.99	19.89	14.45	13.77

Characterization of Off-flavors and Color in Catfish

Problem: Some catfish fillets have off-flavors which are objectionable to consumers. Catfish processing plants have trained “flavor checkers” test each batch of fish prior to processing. Each processing plant has their unique procedure for checking the presence of off-flavor. Need to evaluate different procedures used to detect off-flavor in catfish fillets.

Study: Surveyed all large catfish processors to learn of procedures used to detected off-flavor. Using a trained sensory panel to detect off-flavors using different procedures identified in the survey.

Results: The trained sensory panel detected differences in off flavor intensities using procedures from different processing plants.

Dissemination: Results from the survey were presented to a well attended workshop for flavor checkers on March 19, 2015 in Indianola MS. The completed sensory evaluation is being disseminated to the catfish processing plants in March 2018. this project and line of research has the potential to have a high impact on the industry.

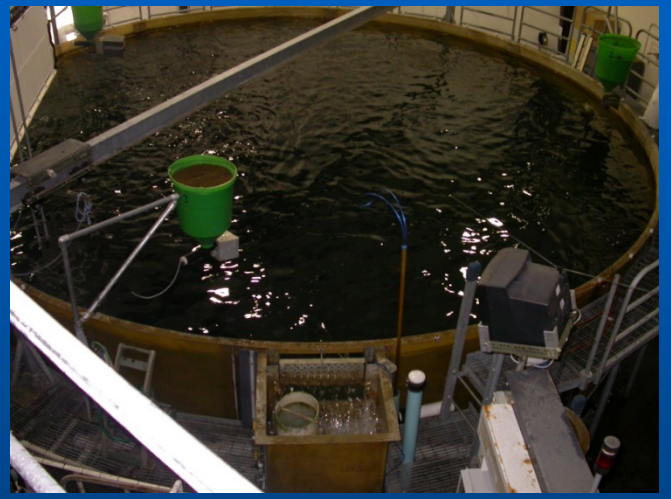
Evaluate and identify the impact of split-pond production practices in reducing or enhancing the incidences and intensities of common pre-harvest off-flavors in channel catfish.

- Two-year field study determined that occurrences and intensities of earthy and musty off-flavor problems in split-pond systems are very similar to those that can occur in conventional (non-partitioned) catfish ponds.
- Therefore, catfish farmers can utilize the same management practices for pond water quality and undesirable species of cyanobacteria (blue-green algae) as those used in conventional ponds.
- This conclusion is of critical importance to commercial culturists who are considering adopting split-pond technology and have assumed that fish off-flavor incidence might be reduced in split-ponds due to previous research on partitioned aquaculture systems.

Develop management strategies to mitigate pre-harvest microbial-derived off-flavors in fish cultured in recirculating aquaculture systems (RAS)

- Discovered that hydrogen peroxide disinfection of purging systems and removal of water aeration media from these systems will enhance the reduction of the common off-flavor compounds geosmin and 2-methylisoborneol from the flesh of fish raised in RAS.

- Aquaculturists using RAS to produce fish were provided with improved standard operating procedures for depuration systems in order to optimize the depuration processes for salmonids cultured in RAS.



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National Program 106 Aquaculture

Retrospective Review

Agenda

- Panel Introductions
- NPL Presentation
  - Program Information
  - Summary Information
  - Questions?
  - Sample Accomplishments
  - Questions for NPL, Scientists
- Break
- Panel Only Discussion
- Panel Debrief ONP
- Panel Written Report (to follow)