**HKD Stuttgart National Aquaculture Research Center**
**USDA-ARS Stuttgart, Arkansas**

**NOV – FEB 2024**

# QUARTERLY RESEARCH HIGHLIGHTS

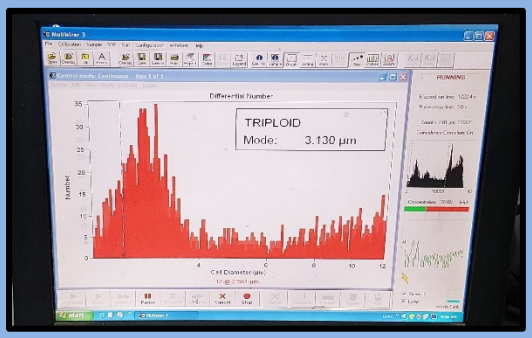
**For More Information: Dr. Yulin Jia, Acting Research Leader/Center Director**
yulin.jia@usda.gov

- **Recent Scientific Publications**

  *This addresses USDA-ARS Research Goal:* Enhance Hybrid Striped Bass Aquaculture Production.

  **Straus, D.L.,** Abernathy, J., Kelly, A.M., Quintero, H.E., Freeze, T.M., and, Williams, R.S. 2024. Initial investigations into the production of triploid sunshine bass using temperature shock. North American Journal of Aquaculture, 10329. https://doi.org/10.1002/naaq.10329

  This study was intended to develop a protocol to produce sterile (aka triploid) sunshine bass. Sterile fish are desired because egg production by females can cause economic losses in commercial aquaculture. Temperature shock (3 warm and 3 cold temperatures at several times after fertilization) on freshly fertilized eggs was used to do this in a small-scale study. We found the best cold and warm combination and then did a full-scale, commercial production of larvae and grew them in ponds for 30 days. We only found 15 fish in the pond from the cold-treated eggs, and none of them were triploid. There were more survivors in the warm-treated eggs and testing 50 survivors indicated 14% were triploid. This study demonstrated that warm temperature shock was better than cold shock to induce a greater percentage triploid sunshine bass.



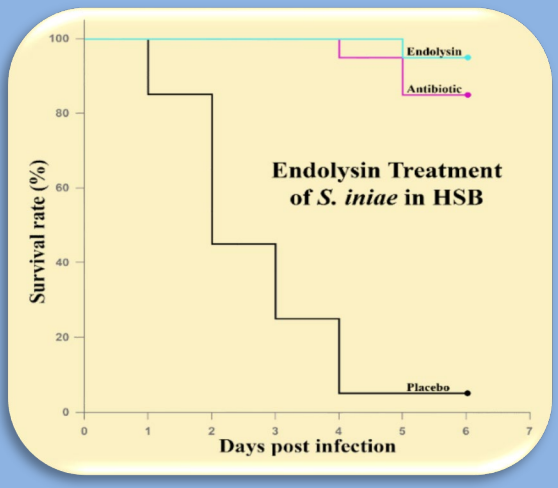

Applying temperature shock to fertilized eggs.

Coulter Counter readout for a triploid fish.

*This addresses USDA-ARS Research Goal:* Disease Control in Aquaculture

**Deshotel, M. B**., Dave, U., **Farmer, B. D**., Kemboi, D., Nelson, D. (2024). Bacteriophage endolysin treatment for systemic infection of *Streptococcus iniae* in hybrid striped bass. Fish Shellfish Immunology. 2024 Feb:145:109296.

Aquaculture outbreaks of *Streptococcus iniae* bacteria cause approximately $150 million loss annually within the industry. There is only one approved antibiotic within the U.S. for treating these infections; however, there have already been 7 resistance genes found. Thus, a new method of treating these bacteria is sorely needed. We developed a new non-antibiotic antimicrobial protein that kills these bacteria upon contact and demonstrated a 95% survival rate in infected hybrid striped bass.




Survival of fish treated with endolysin, antibiotic or a placebo.

*This addresses USDA-ARS Research Goal:* Enhance Hybrid Striped Bass Aquaculture Production.

**Fuller, S.A.**, Abernathy J.W., Sankappa, N.M., Beck, B.H., **Rawles, S.D.**, **Green, B.W.**, Rosentrater, K.A., **McEntire, M.E.**, **Huskey, G.**, **Webster, C.W.** (2024) Hepatic transcriptome analyses of juvenile white bass (*Morone chrysops*) when fed diets where fish meal is partially or totally replaced by alternative protein sources. *Frontiers in Physiology* 14: 1308690.

White bass (*Morone chrysops*; WB) are a popular sportfish throughout the southern United States, and one parent of the commercially successful hybrid striped bass (*M. chrysops x M. saxatilis*; HSB). Commercial production of WB does not currently exist in the U.S. partially due to a lack of information regarding nutritional requirements and cost-effective diets. Currently, WB are cultured using diets formulated for other carnivorous fish, such as largemouth bass (*Micropterus salmoides*) or HSB and contain a significant percentage of marine fish meal (FM). The effects of diet formulation on growth and body composition of WB are largely unknown. Since diets may exert a strong influence on gene expression of an

organism, we conducted a gene expression analysis on WB which had been fed diets containing various percentages of marine fish meal. We found a total of 1563 genes expressed differently (DEGs) between all the comparisons, with most of these differences (95.9%) found in comparisons between the FM control and the no FM test diet or high soybean meal except for the test diet containing high levels of poultry byproduct meal. Important metabolic genes involved in the regulation of glucose and fatty acid metabolism were differentially expressed with generally higher metabolic activity in fish reared on the FM control. These data are impactful in determining the effects of diet formulation on WB so that an optimal diet can be fed to optimize health and growth of the fish.

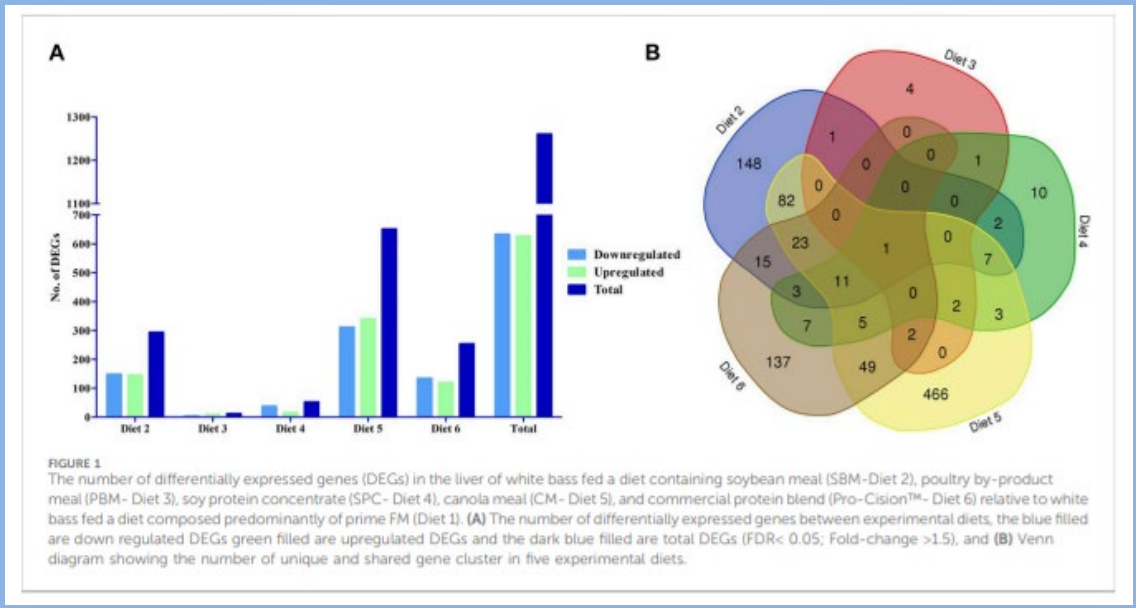



FIGURE 1
The number of differentially expressed genes (DEGs) in the liver of white bass fed a diet containing soybean meal (SBM-Diet 2), poultry by-product meal (PBM- Diet 3), soy protein concentrate (SPC- Diet 4), canola meal (CM- Diet 5), and commercial protein blend (Pro-Cision™- Diet 6) relative to white bass fed a diet composed predominantly of prime FM (Diet 1). **(A)** The number of differentially expressed genes between experimental diets, the blue filled are down regulated DEGs green filled are upregulated DEGs and the dark blue filled are total DEGs (FDR< 0.05; Fold-change >1.5), and **(B)** Venn diagram showing the number of unique and shared gene cluster in five experimental diets.

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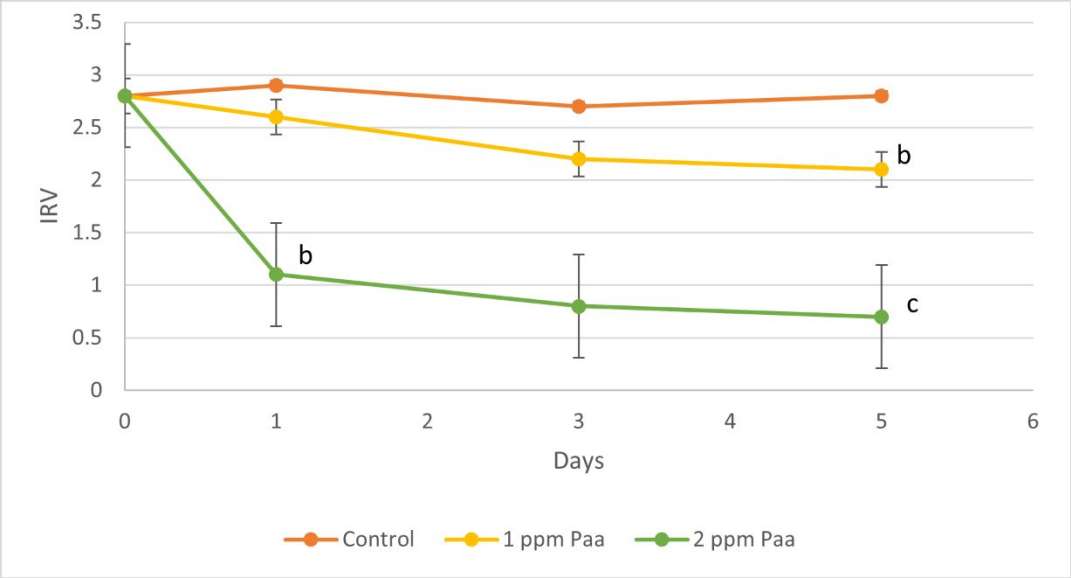
**Farmer, B.D.**, **Straus, D.L.**, **Deshotel, M.**, **Fuller, S.A.**, Reading, B.J., Meinelt, T. (2024) Antiparasitic effects of peracetic acid on striped bass infested with *Trichodina* spp. *North American Journal of Aquaculture*, DOI:10.1002/naaq.10332.

To improve fish health and preserving economic value the antiparasitic effects of the Environmental Protection Agency labeled aquaculture disinfectant peracetic acid (PAA) was evaluated against an infestation of the protozoan *Trichodina* spp. in striped bass, *Morone saxatilis*. Concentrations of 0, 1 and 2 mg/L (or ppm) PAA were applied every other day for 3 treatments (30 min static exposure). Infection intensity was assessed 20-24 h after each treatment cycle by counting the number of parasites present in a wet mount of gill tissue. Neither treatment rate was able to completely eradicate the parasite, however the 2mg/L PAA

resulted in a statistically significant reduction which equated to 75% reduction of observed parasites. The 2 mg/L PAA treatment regimen in the present study is proposed as a safe and environmentally friendly effective method for reducing the intensity of *Trichodina* infections in striped bass.



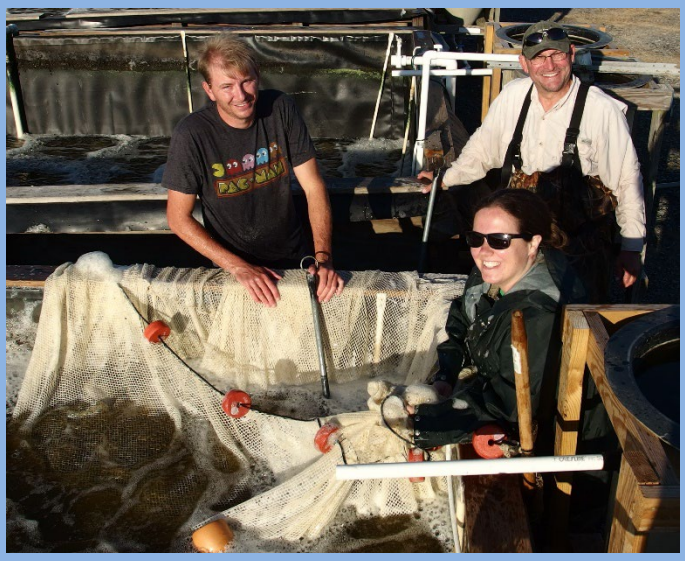

Mean infestation intensity of *Trichodina* spp. on gill samples of Striped Bass after treatment with 1 or 2 mg/L PAA every other day for 3 treatments. Error bars depict standard errors and means.

*This addresses USDA-ARS Research Goal:* Improve the performance of hybrid striped bass fingerlings in intensive production systems.

**Ray, C.L., Abernathy, J.W., Green, B.W**., Rivers, A.R., Schrader, K., **Rawles, S.D., McEntire, M.E.,** Lange, M.D., **Webster, C.D**. 2024. Effect of dietary phytase on water and fecal prokaryotic and eukaryotic microbiomes in a hybrid tilapia (*Oreochromis aureus* x *O. niloticus*) mixotrophic biofloc production system. Aquaculture. 581:740433. https://doi.org/10.1016/j.aquaculture.2023.740433. (Published 15 Jan 2024)

Fish feed typically is supplemented with inorganic phosphorus to ensure adequate dietary phosphorus availability, especially in diets that contain high percentages of plant feedstuffs, which is the case for diets formulated for tilapia. Phosphorus is present in plant feedstuffs as phytate, which is not bioavailable to fish because their digestive system lacks sufficient phytase enzyme. Thus, phytate is excreted unmetabolized and accumulates to high concentrations in the environment. Including phytase in the feed formulation increases phosphorus bioavailability but affects the microbial communities in the culture water and tilapia gastrointestinal tract. Optimal utilization of supplemental dietary phytase requires an understanding of its influence on microbial community dynamics in biofloc water and the tilapia gastrointestinal tract during growth to market size. Hybrid tilapia were grown for five months to market size in outdoor biofloc production system tanks and fed either a phosphorus-free practical diet treated with phytase or a positive and negative control

practical diets. The bacterial and eukaryotic populations in the biofloc tank water and hybrid tilapia gastrointestinal tract were sampled and characterized at four time points throughout the experiment. Overall abundance of Fusobacteria (namely Cetobacterium) and the protist Capsaspora predominated in the tilapia gut, regardless of test diet, indicating that the main driver of the gut microbiome is the biofloc rather than the manufactured diet. Compared to gut content samples, planktonic bacterial and eukaryotic communities in biofloc water exhibited greater diversity. This study demonstrated that dietary sources with variation in bioavailable P can significantly impact prokaryotic and eukaryotic communities in tilapia biofloc aquaculture and increases our understanding of synergistic fish-microbial interactions in response to dietary phytase supplementation.



Dr. Steve Rawles, Candis Ray, and Paxton Harper harvesting biofloc tanks at the end of the study.

- **Technology Transfer**

  ✓ **<u>Interactions with the Research Community</u>**

  On November 13, 2024, Dr. Adam Fuller and Mr. Matt McEntire visited the USDA-ARS Warmwater Aquaculture Research Unit, to meet with Dr. Brian Bosworth to discuss aquaculture breeding strategies and improvement of white bass select lines.

  On February 5-7, 2024, Dr. Dave Straus attended the Midcontinent Warmwater Fish Culture Workshop held in Hot Springs, AR. Dr. Straus gave two presentations on 1) producing triploid hybrid striped bass with temperature shock and 2) compounds to prevent adhesiveness on hybrid striped bass eggs.

  On February 6, 2024, Dr. Adam Fuller consulted with Dr. Moira Sheehan from Breeding Insight and Cornell University to discuss leveraging resources from both facilities towards enhancing white bass selective improvement through genomic selection methods.

On February 8, 2024, Dr. Yulin Jia, Dr. Dave Straus, Bradley Farmer, and Jason Brown attended the Arkansas Bait and Ornamental Fish Grower's Association meeting with about 40 fish farmers and investigators from nearby states. Dr. Straus gave a presentation on upcoming research on unintentional shipment of non-target species (hitchhikers) with baitfish. Subsequently, Dr. Jia and Straus visited nearby fish farms.

On February 9, 2024, Dr. Adam Fuller consulted with Dr. Benjamin Reading from North Carolina State University to discuss genetic improvement strategies for hybrid striped bass, closer alignment of implemented strategies for improved parental lines of white bass and striped bass, sharing of germplasm between facilities, and long-term goals of each selection program.

On February 14, 2024, Dr. Dave Straus had research scientists Drs. Brad Richardson and Cailin Older from the USDA/ARS Warmwater Aquaculture Research Unit (Stoneville, MS) tour SNARC and discuss collaborative research on biofilter microbiome for an upcoming project on raising largemouth bass in recirculating aquaculture systems (RAS). They also met with Dr. Bart Green and Candis Ray to discuss collaboration on analysis of microbiome data from a SNARC production experiment. During the visit, they discussed collaboration on phytoplankton research as part of the upcoming project plan.


On February 14, 2024, Dr. Adam Fuller met with Dr. Yathish Ramena from the University of Arkansas-Pine Bluff School of Aquaculture and Fisheries, Pine Bluff, AR, to discuss areas of future collaborative research. Among topics discussed were potential funding opportunities, undergraduate and graduate student research opportunities at SNARC, and leveraging resources of both facilities to meet research goals.

On February 15, 2024, Dr. Adam Fuller consulted with Dr. Harvey Blackburn from the USDA-ARS National Animal Germplasm Program to discuss the development of

cryopreservation methods in *Morone* and archiving genetically improved germplasm from SNARC white bass into the National Germplasm Program.

February 18-21, 2024, Drs. Dave Straus, Mike Deshotel, and Bart Green attended the Aquaculture America conference in San Antonio, TX. Dr. Straus was a co-author on two presentations and co-organized/co-moderated (with Dr. Deshotel) an industry-oriented special session on hitchhikers in the baitfish industry. Dr. Bart Green presented, "Production of hybrid striped bass and water quality in split ponds compared to traditional earthen ponds," in the Striperhub: Striped Bass and Hybrid Striped Bass session and discussed with Dr. Ken Semmens, Kentucky State University, the role of calcium in reducing hybrid striped bass handling stress and provided literature and a spreadsheet to calculate calcium dose (as calcium chloride) based on water calcium hardness and tank volume.

On February 26, 2024, Dr. Bart Green provided Dr. Beth Cleveland, ARS National Center for Cool and Cold-Water Aquaculture (Leetown, WV), design and operation details for the tank system he used for his hybrid striped bass hypoxia studies.

- **Stakeholder Interactions**

On February 13, 2024, Dr. Adam Fuller met with Mr. Mike Freeze, Mr. Ric Williams, and Mr. Seth Summerside about the creation of an industry control line of hybrid striped bass to better evaluate the progress of genetic improvement in SNARC white bass and NCSU striped bass.

Dr. Bart Green consulted by phone/Teams with extension aquaculture personnel and hybrid striped bass farmers in January (11 & 18) and met with hybrid striped bass farmers at the Aquaculture America 2024 conference (February 18-21) to discuss production issues that could be addressed as part of the project plan in development.

Dr. Dave Straus visited with several individual fish farmers (Keo Fish Farm, I.F. Anderson Minnow Farm, Aquatec Fish Farms, Harry Saul Minnow Farm, Dunns Fish Farm) to discuss research ideas for the upcoming project plan. Dr. Straus had many stakeholder interactions at the Catfish Farmers of Arkansas meeting (January 26), the Arkansas Bait and Ornamental Fish Grower's Association meeting (Feb 8), the Midcontinent Warmwater Fish Culture Workshop (February 5-7), and the Aquaculture America conference (February 18-21).


Sandra Dorman helping with registration at the Catfish Farmers of Arkansas Meeting

- **Education and Outreach**

  Dr. Steve Rawles retired on January 26, 2024. Matt McEntire, Rebecca Roberts, Candis Ray, and Teresa Lazenby organized a reception and slide show to celebrate his more than 25 years of service.

On February 28, 2024, Dr. Dave Straus met with a graduate student and professor from UAPB to plan upcoming research to produce triploid hybrid striped bass at Keo Fish Farm during their spawning season.

Science Day at Stuttgart Public Library

On February 29, Paxton Harper, Cindy Ledbetter, Rebecca Roberts, and Candis Ray presented work done at SNARC at the 2nd Annual Science Day at the Stuttgart Public Library. This event was such a success in its inaugural year, the Stuttgart Public Library decided to make it an annual event to introduce local students to science in their hometown. More than 100 students ranging from 3rd through 6th grades from various Stuttgart schools participated. They learned about SNARC fish production systems, nutrition, and disease management research and the rice-fish experiment conducted in the summer of 2023 by both units. The students were told about ongoing research and plans for more rice-fish studies.

- **International Research Collaborations**

Dr. Mike Deshotel completed a successful bacteriophage study in collaboration with Nitte University and Central Agricultural University in India.