Alabama Fish Farming Center *Fish Farming News, 2023(1):1-24*

EXTENSION NEWSLETTER

Quantifying the Age Structure of Carry-over Hybrid Catfish Following Commercial Harvest: Preliminary Results

Julia Palmer, Jesse James, Anita Kelly, and Luke Roy, AFFC

Figure 1. Market-size fish (left) compared to five over-market-size fish (right) from the same pond.

In west Alabama, hybrid catfish producers routinely face the challenge of fish that exceed market size (aka "big fish") in their commercial ponds (Fig. 1). These fish are evading harvest and can increase in size significantly before the next harvest occurs. This is problematic because processing plants prefer catfish in the 1.25 - 4 lb. range, and farmers are paid a premium price for this size. Farmers are typically compensated evenly for price per pound up until the weight of the live fish exceeds 8 lbs. Once over this weight, processing plants are forced to hand-fillet fish versus automated methods that are more cost-effective and have the additional task of finding a market for more oversized fillet products. This leaves the farmer receiving a lower price per pound or, little, if any, financial return for fish above 8 lbs. The cost of resources that went into feeding and caring for that fish for an additional time must also be factored in. Harvesting inefficiencies and ponds with uneven bottoms that allow catfish to escape seines are significant drivers of the big fish problem. Due to their larger size and growth potential, hybrid catfish tend to be a more significant big fish issue than channel catfish. Little is known regarding the age structure and growth rates of hybrid catfish that repeatedly evade capture and remain in commercial ponds for extended periods.

The objective of our study is to quantify the age structure and growth of hybrid catfish that evade

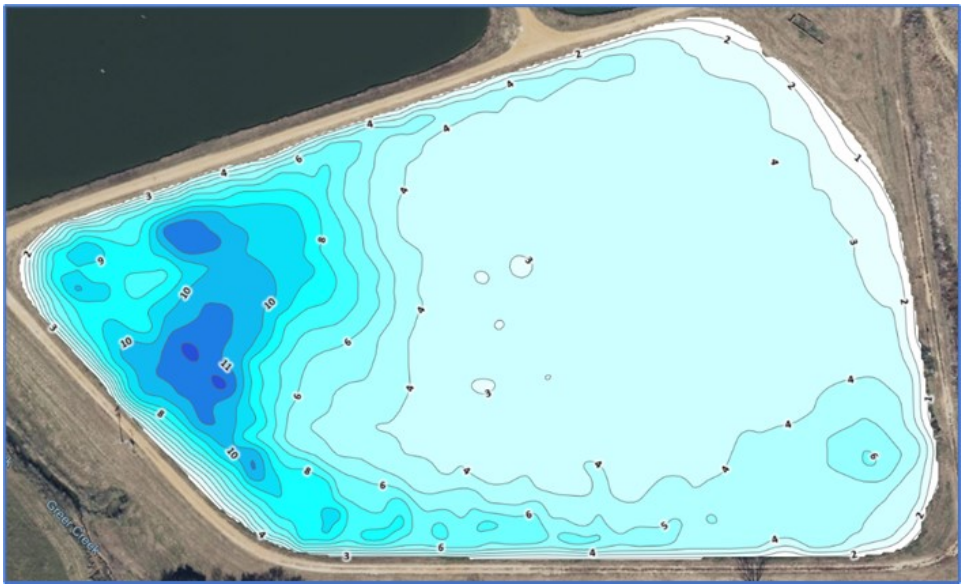

Figure 2. Sonar survey taken by Jesse James using a Lowrance Fish Finder. Numbers represent depth (feet).

capture and remain in ponds following commercial harvest. Twelve recently harvested hybrid catfish ponds (3 - 4 harvests per pond) were sampled (n = 1,006 total fish) using an electrofishing boat. The team sampled study ponds before they were restocked with a subsequent crop of hybrid fingerlings to assess the size of carryover fish. Following collection, fish were numbered, and the total length, weight, and sex of fish were recorded. Otoliths, a bone structure with annual growth rings, were extracted from the brain cavity, and the cut method was used to estimate fish age. This method involved cleaning the otolith of any brain matter, embedding the otolith in a clear epoxy resin, and cutting into it with an IsoMet low-speed precision saw to reveal the core and annuli (annular growth "rings"). All 1,006 otoliths have been mounted at this stage, and we are beginning the aging process. Fish age will be accessed using high-quality image analysis software connected to a microscope to count the rings surrounding the core. Counting will be conducted by two readers independently and compared. If there is any dispute regarding age estimation, a third reader will be brought in to resolve the disagreement. Data collected on fish age, growth, and longevity of hybrid catfish found in ponds following harvest will help producers and researchers better understand the big fish problem. Additionally, looking at the seining frequency and pond characteristics, like area and average depth (Fig. 2), will contribute to solutions to the big fish issue. Data analysis will be performed in the next few weeks, and the study's final results will be summarized in a future issue of *Fish Farming News*. Preliminary results have revealed that around half of the catfish caught in this study fell into the 1-4 lb. weight range, around 45% of the fish fell above that premium size category, with around 25% of those catfish caught falling into the "Big Fish" category (Fig. 3). Because of the hybrid's growing potential, all these fish have the potential to reach "Big Fish" size by the next production season (Creel et al. 2020).

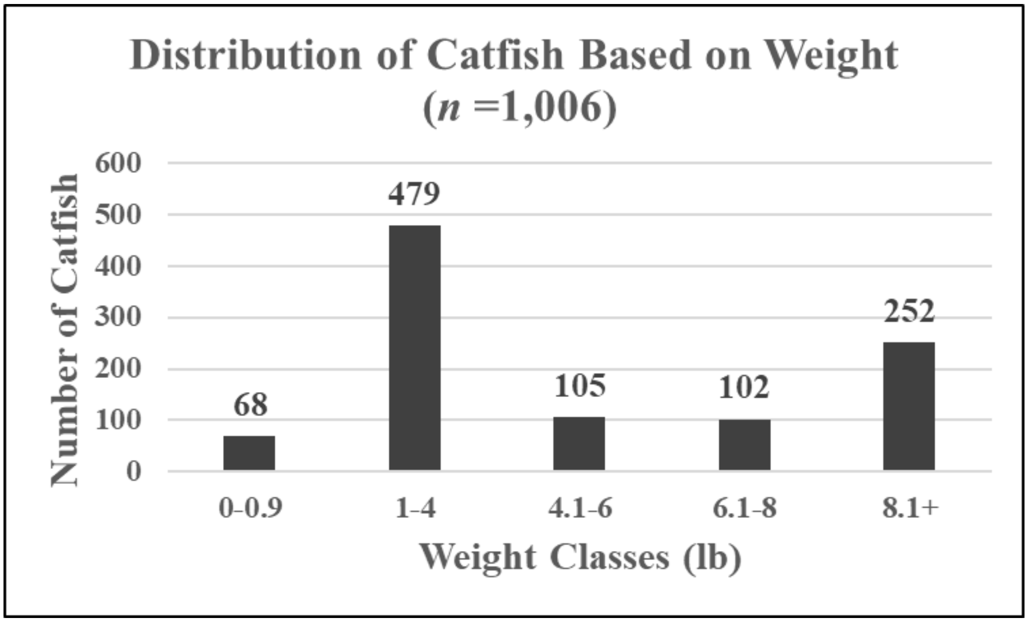

Figure 3. Distribution of all sampled catfish by weight.

Repeated Low-dose Copper Sulfate Applications May be a Safe and Effective Way to Manage Catfish Trematode Infections

Charles C. Mischke[1], Bradley M. Richardson[2] Matt J. Griffin[1], and David J. Wise[1]
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Figure 1. Rams-horn snails (top) and ghost rams-horn snails (bottom) are intermediate hosts to the trematode *Bolbophorus damnificus*.

In the catfish industry, the trematode *Bolbophorus damnificus* has been associated with catfish mortality and reduced economic returns since the 1990s. The life cycle of the trematode involves the American white pelican, either the rams-horn snail or the ghost rams-horn snail (Fig. 1), and catfish. There are no treatments available for parasitized fish. As such, management strategies have focused on disrupting the trematode life cycle. Since pelicans are protected, eradicating, or at least reducing snail numbers in ponds is the only practical method to break the life cycle and minimize trematode infestations.

Copper sulfate has been the main chemical used to reduce snail populations. Initial investigations evaluated applying copper sulfate along the edge of ponds where snails are concentrated in aquatic vegetation. Dissolved copper sulfate applied at 1.3 lbs/33 foot of shoreline killed most snails when water temperatures exceeded 70° F. This method of snail control is effective for killing snails along the pond margin and should be safe in large ponds (>10 ac) where the ratio of shoreline to pond volume is small.

While margin treatments are effective in killing snails located in vegetation along the pond levee, snails can be distributed throughout the pond and significant infestations have been observed in ponds with limited snail numbers along the pond bank, suggesting snails can be present outside the treatment zone along the pond margins. Additional studies were conducted to evaluate the safety and efficacy of whole pond treatment at 2.5 and 5.0 ppm copper sulfate (water temperature ranged from 68-74° F). Both doses killed nearly all snails throughout the entire pond, however one pond treated with 5 mg/L copper sulfate had significant fish mortality (33% loss). Comparably, no fish mortality was observed in ponds treated with 2.5 mg/L copper sulfate. Whole pond treatments with 2.5 mg/L copper sulfate are effective in killing snails throughout the entire pond although this treatment may be risky in hot weather when copper is more toxic to fish. Further, copper applications are hazardous if there is a dense phytoplankton bloom as copper can kill the bloom leading to dissolved oxygen issues. Additional studies conducted in June, also demonstrated fish toxicity issues when granular copper sulfate was evenly applied at a rate of 2 ppm along the pond margin. As such, extreme caution should be taken when applying copper sulfate to ponds at high pond water temperatures.

In efforts to reduce the risk of these snail treatments, scientists at NWAC evaluated multiple, low-dose copper sulfate treatments. Adult snails were exposed to four weekly applications of copper sulfate. For rams-horn snails, four doses of 0.75 mg/L copper sulfate killed about 60% of the snails (Fig. 2), whereas only two doses of 0.75 mg/L copper sulfate killed 100% of ghost rams-horn snails (Fig. 3).

In addition to adult snails, the ability of copper to reduce reproductive success of these snail pests was also evaluated. Both rams-horn and ghost rams-horn snail eggs hatch in 10 days. Therefore, eggs were exposed to copper on day 0 and day 7 post-spawn. Hatching success was then determined on day 10. Rams-horn snail eggs were sensitive to copper with doses as low as 0.09 mg/L copper sulfate, which prevented about 50% of the eggs from hatching (Fig. 4). Ghost rams-horn snail eggs were not as sensitive, requiring 0.75 mg/L copper sulfate to prevent eggs from hatching (Fig. 5).

When immediate snail control is needed, a single application of copper sulfate to the shoreline or a single whole-pond treatment may be effective. However, a benefit to risk analysis should be considered before applying copper sulfate to ponds at high pond water temperatures. Typically, single whole pond treatments (2.5-3.0 ppm) should only be applied during the summer months in instances of severe infestations where feeding activity of resident fish is significantly reduced – assuming decreased feeding activity is associated with trematodes and not other fish health issues which could be exacerbated by copper treatments. Repeated copper sulfate applications between 0.5 and 0.75 mg/L have the potential to be effective while being much safer for the fish and are likely more appropriate for control of light to moderate infestation where fish are still actively feeding. At present, some operations use weekly applications of copper sulfate at these lower dose to control off-flavor issues caused by blue-green algae.

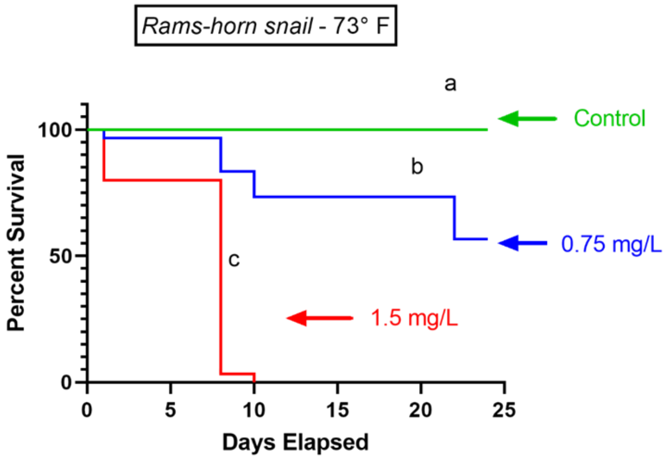


Figure 2. Survival of rams-horn snails exposed to four weekly treatments (day 0, day 7, day 14, and day 21) of different amounts of copper sulfate.

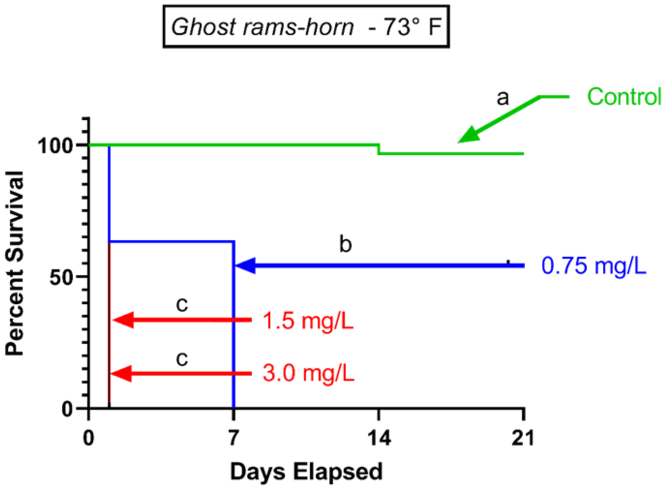


Figure 3. Survival of ghost rams-horn snails exposed to four weekly treatments (day 0, day 7, day 14, and day 21) of different amounts of copper sulfate.

Snail control in catfish ponds using copper sulfate is complicated as the toxicity of copper is affected by several environmental variables (temperature, pH, total alkalinity, hardness, salinity, and dissolved organic matter) and the practicality of this approach is situationally dependent. Also, the optimal timing for copper sulfate application is still being determined. Therefore, the data reported here may not have universal applicability and producers must determine which approach to trematode control works best for their individual operations.

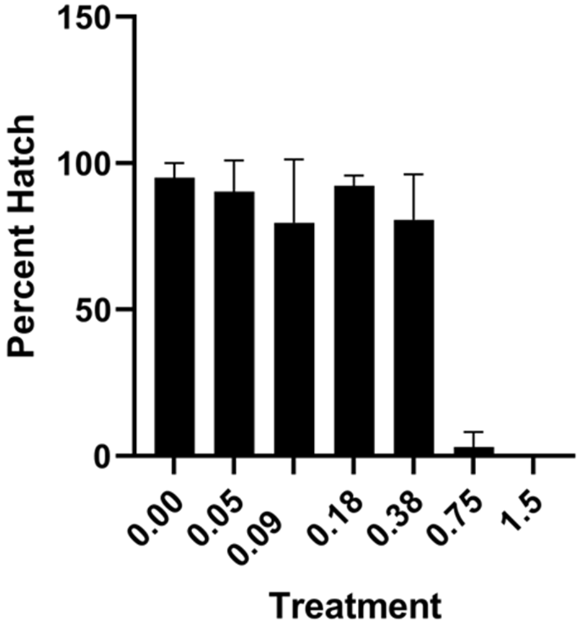


Figure 4. Percent hatch of rams-horn snail eggs exposed to two doses of copper sulfate on day 0 and day 7.

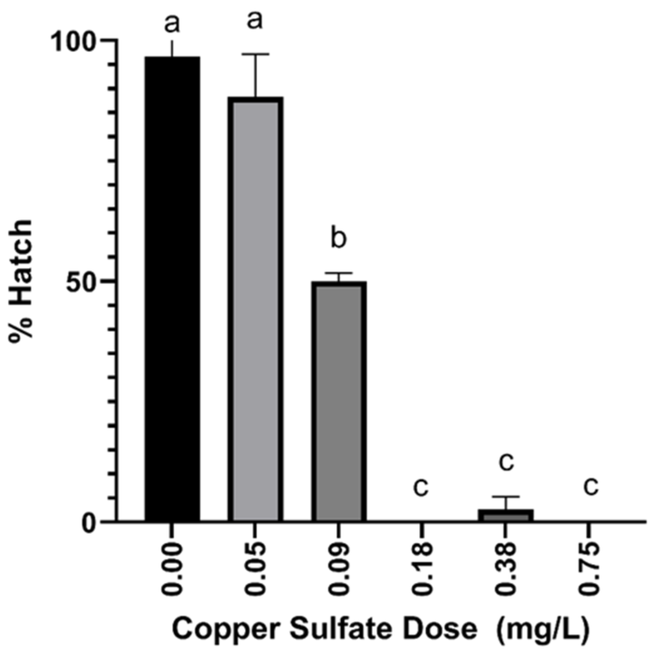


Figure 5. Percent hatch of ghost rams-horn snails eggs exposed to two doses of copper sulfate on day 0 and day 7.

An upcoming Channel Catfish Study Seeks to Examine the "Big Fish" Issue in Multi-batch Production Systems in West Alabama

Julia Palmer, Jesse James, Anita Kelly, and Luke Roy, AFFC

As we enter the spring season and wrap up our big hybrid catfish project, the AFFC is preparing for a second project to investigate the age and growth of Channel Catfish that exceed desired market size (> 4 lbs) in west Alabama commercial ponds. While the "Big Fish" problem has most often been associated with hybrid catfish, channel catfish can also grow above market size. Processors have reported that ponds with larger channel catfish, while not as big of an issue as hybrid catfish in terms of weigh backs, can still be problematic, particularly in larger irregularly shaped ponds which can be difficult to seine efficiently and often yield large numbers of channel catfish in the 4 – 12 lb range. Though Channel Catfish do not have as great of a growing potential as their hybrid counterparts, which can exceed 50 lbs, farmers still have challenges with catfish evading seines and growing fish beyond a profitable size. The objective of this project will be to quantify the age structure of big channel catfish (4+ lbs) in multi-batch systems to acquire a better understanding of seining efficiency, particularly in watershed ponds.

The methods of this project will be somewhat different from the hybrid catfish project, although we will still be using an electrofishing boat to sample catfish from commercial ponds. This is due to the lesser growth potential of channel catfish, and farmers raise these fish in a multiple-batch system compared to a single-batch system used to raise hybrids. With a multiple batch system, we expect to see multiple-year classes throughout the pond, and will need to impose a catch limit for market-size and under-market-size fish. If any farmers in the west Alabama region are interested in participating in this study, please call the Alabama Fish Farming Center to speak to Jesse James (334-624-4016).

Proof of Concept: Frozen Sperm Shows Promise for Catfish Hatchery Production

Helen R. Montague, V. MacKenzie Tackett, Larry L. Lawson, Luke A. Roy, Rex A. Dunham, Ian A.E. Butts
SFAAS

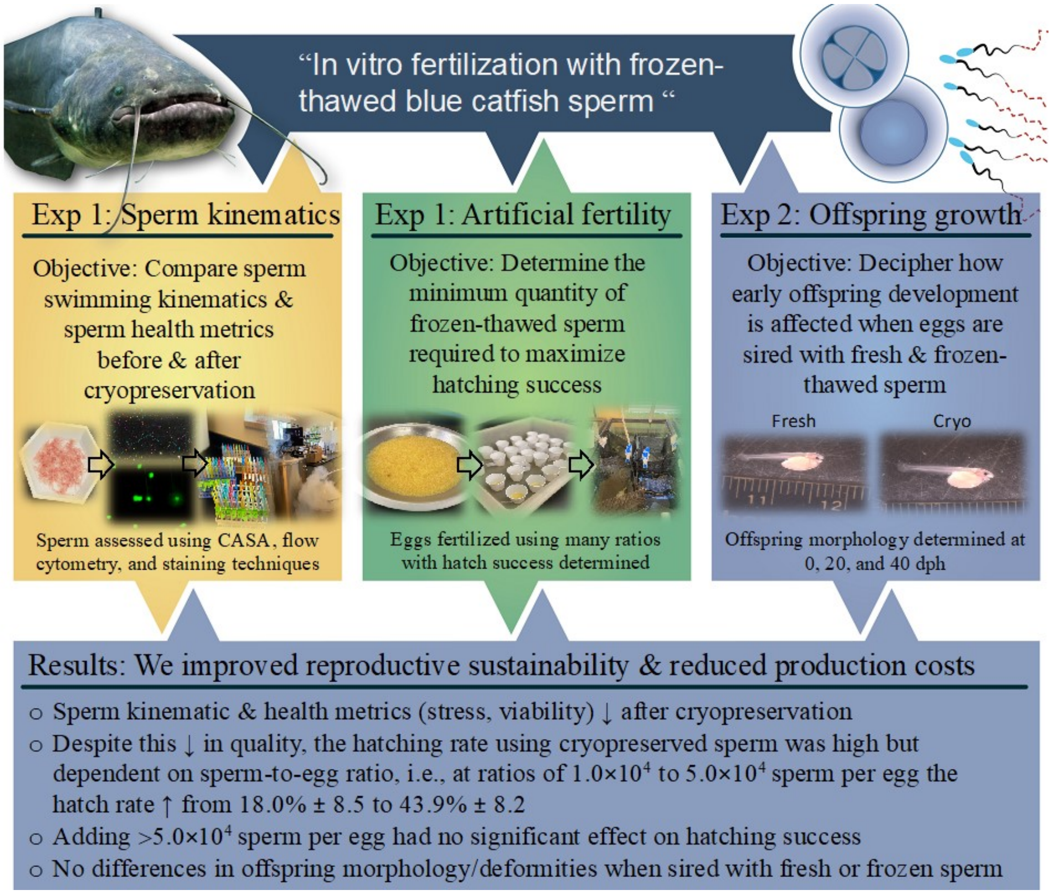
Catfish farming constitutes around 60% of total U.S. freshwater aquaculture, in which the channel catfish, blue catfish hybrid accounts for greater than 60% of the harvest. Although the catfish industry has experienced sustained growth, there are still challenges with reproduction between the parent species. Blue catfish males mature after 4-7 years, and their sperm is not readily expressed and collected by stripping. This makes gamete collection a lethal procedure which makes sperm a substantial investment as it can only be collected once per male. Cryopreservation techniques have been developed to alleviate problems related to male readiness for spawning, as gamete availability can be synchronized between parent species and genetic repositories can be created; however, this technology is new to the catfish industry and there has been high variability reported between male quality after cryopreservation. Thus, our goal was to improve understanding of cryopreservation using blue catfish gametes and identify impacts on offspring to advance hybrid hatchery production.

Our objectives in this study were to i) compare sperm swimming kinematics and health metrics both before and after cryopreservation where we hypothesized that sperm quality would decline following cryopreservation, ii) determine the minimum quantity of frozen-thawed sperm required to maximize hatching success, and iii) decipher how early offspring development is affected when eggs are sired with fresh and frozen-thawed sperm (Fig. 1). Combined, the results from these objectives will allow us to identify the impacts of cryopreservation on male gametes and the offspring created from cryopreserved cells.

In brief, all experiments were conducted at Auburn University's E.W. Shell Fisheries Center in Auburn, Alabama. All broodstock were fed a 32% protein diet until the month leading up to spawning when protein was increased to 36%. We collected broodstock from 0.1-acre ponds and processed the males to retrieve the testes.

Starting with objective one, all broodstock were fed a 32% protein diet until the month leading up to spawning when protein was increased to 36%. We collected broodstock from 0.1-acre ponds and processed the males to retrieve the testes. In 2021 we processed 43 males and in 2022, we processed 40 males. We extracted the sperm from the testes and ran sperm assays such as computer assisted sperm analysis focusing on curvilinear velocity, progressive velocity, progressive motility, and percent motility. We then used flow cytometry to measure cell viability, and oxidative stress and fluorescent microscopy to measure DNA fragmentation. Our samples were then cryopreserved at a concentration of 1×10^9 cells/mL with HBSS plus 10% methanol as a cryoprotectant following methods from Hu et al 2011 and 2014. Sperm solutions were pipetted into 0.5 cc straws. Straws were frozen at a rate of -5ºC/min, starting at 5ºC, until they reached -80ºC. The same analysis (CASA, flow cytometry, fluorescent microscopy) was done on the cells post-thaw.

For objective two, each female received two intraperitoneal injections of luteinizing hormone-releasing hormone analogue. Ovulation was checked every 4 hours until eggs adhered to bags, and then eggs were collected. We crossed eggs from 18 females with 2 males, on average. Cryopreserved milt was then thawed and diluted to five ratios ranging from 9.0×10^4 sperm per egg to 9.0×10^5 sperm per egg in 2021 and five ratios ranging from 1.0×10^4 sperm per egg to 1.0×10^5 sperm



"In vitro fertilization with frozen-thawed blue catfish sperm "

Exp 1: Sperm kinematics

Objective: Compare sperm swimming kinematics & sperm health metrics before & after cryopreservation

Sperm assessed using CASA, flow cytometry, and staining techniques

Exp 1: Artificial fertility

Objective: Determine the minimum quantity of frozen-thawed sperm required to maximize hatching success

Eggs fertilized using many ratios with hatch success determined

Exp 2: Offspring growth

Objective: Decipher how early offspring development is affected when eggs are sired with fresh & frozen-thawed sperm

Offspring morphology determined at 0, 20, and 40 dph

Results: We improved reproductive sustainability & reduced production costs

- Sperm kinematic & health metrics (stress, viability) ↓ after cryopreservation
- Despite this ↓ in quality, the hatching rate using cryopreserved sperm was high but dependent on sperm-to-egg ratio, i.e., at ratios of 1.0×10^4 to 5.0×10^4 sperm per egg the hatch rate ↑ from 18.0% ± 8.5 to 43.9% ± 8.2
- Adding >5.0×10^4 sperm per egg had no significant effect on hatching success
- No differences in offspring morphology/deformities when sired with fresh or frozen sperm

Figure 1. Graphical abstract highlighting the work completed in this study [Montague, H.R., Hess, H.N., Tackett, V.M., Britt, S.L., Lawson, L.L., Aguilar, G.L., Jenkins, L.E., Roy, L.A., Holland, L.W., Dunham, R.A., Litvak, M.K., Butts, I.A.E. In vitro fertilization with frozen-thawed blue catfish (*Ictalurus furcatus*) sperm and implications for gene banking. *Aquaculture* (2023), https://doi.org/10.1016/j.aquaculture.2023.739611

per egg in 2022. This allowed us to test a wide spectrum of ratios to see if adding more sperm inhibited hatch success through polyspermy, and to find the lowest amount of sperm required for fertilization, allowing conservation of cells frozen in genetic repositories. Our embryos were then incubated in baskets suspended in RAS tanks and hatch success was determined.

For our third objective, we looked at offspring sired from frozen-thawed cells. Fresh and cryopreserved cells from the same males were used for fertilization. Males 1-3 were paired with female 1, males 4-6 were paired with female 2, and males 7-9 were paired with female 3. For each fertilization event, an aliquot of fresh and cryopreserved sperm from each male was pooled equally to ensure every male had an equal chance of fertilizing an egg. The fresh and cryopreserved cells were kept separate to form our treatments. All eggs were fertilized using a conservative ratio of 100,000 sperm per egg and there were 6 replicate fertilization events for each treatment. Offspring were then assessed for morphology and deformities at, 0-, 20-, and 40- days post hatch. Morphological measurements included total length (distance from the tip of the snout to the fork of the tail), notochord length (distance from the tip of the snout to the end of the notochord), yolk-sac area, eye diameter, jaw length, myotome height (body height immediately posterior to anus), and body area (excluding fin fold area and yolk sac). Weights and survival were also taken at the conclusion of experimentation.

In conclusion, we found that sperm kinematics and health metrics declined after cryopreservation, however frozen-thawed sperm is still able to fertilize just at a higher concentration than the fresh control. Thus, adding greater than 50,000 frozen-thawed sperm per egg had no impact on hatching success. Lastly, there was generally no decline in offspring performance when sired with frozen-thawed sperm. Overall, cryopreservation is a viable option for catfish hatcheries to improve production of hybrid catfish and allow for more synchronous spawning, but more work is needed to look at epigenetics and gene expression of offspring created with frozen-thawed cells.

Simple, Low-cost Traps May Help Manage Snails and Reduce Trematode Risk in Catfish Ponds

Bradley M. Richardson[1], Charles C. Mischke[2], Matthew J. Griffin[2], and David J. Wise[2]
[1]USDA/ARS Aquatic Animal Research Unit [2]National Warmwater Aquaculture Center, Stoneville, MS

Snails are one of the most insidious threats to commercial catfish production. Their slow movement and relatively small size make them easy to overlook in the grand scheme of production. However, their propensity for transmitting problem parasites makes them an important target for management. The primary parasite of concern, with respect to snails in catfish aquaculture, is the trematode *Bolbophorus damnificus*.

The trematode infects two common snails in catfish ponds, the marsh ramshorn snail (*Planorbella trivolvis*) and ghost ramshorn snail (*Biomphalaria havanensis*), which subsequently shed immature parasite stages that are infective to catfish. If the parasitized catfish is eaten by an American white pelican, the adult develops in the bird and the trematode life cycle is completed. The process begins again when parasite eggs are deposited into the pond through the bird's feces. There are currently no treatments available for trematode infestations in catfish and the American white pelican is a protected species, this leaves much of the trematode management centered on the control of the snail hosts.

Copper sulfate pond treatment is the most common mode of snail management in catfish production. However, treatment regimens are largely performed on arbitrary schedules, or based on the farmer's perceived snail density in specific ponds. Unfortunately, assessing snail densities by casual observation of pond margins can be complicated by the presence of dense vegetation along the pond margins, as well as light reflectance and pond turbidity which can obscure snails. Many times, snails are not observed on the surface of the water, even in ponds with heavy snail population. As such, assessing snail populations in ponds can be labor intensive, as it requires careful evaluation of the pond margins using dip nets to dislodge submerged snails that may be attached to vegetation. Also be aware there are multiple species of snails residing in catfish ponds, but only the marsh ramshorn and ghost ramshorn are of industry concern. Therefore, the presence of snails is not as important as which snails are present. Regardless, more targeted methods to improve timing and efficacy of snail management processes are critical for effective

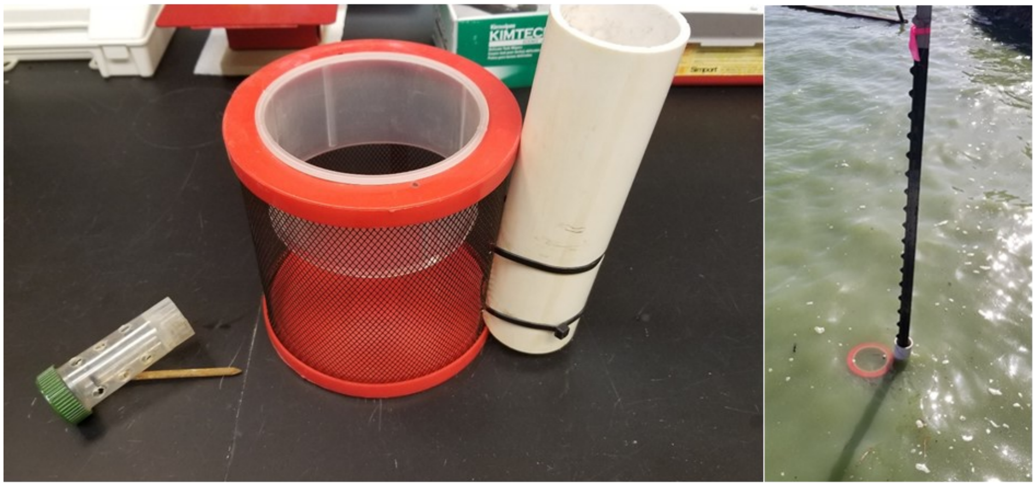


Figure 1. Basic snail trap construction (left) showing bait holder (far left). If trap was baited, a small carrot was placed inside the bait holder, which sat inside the trap itself. Traps were deployed along the pond margin, just below the water surface and secured in place using a T-post (right).

control of trematode infestations.

Studies conducted in 2021 and 2022 have investigated the use of simple, low-cost traps (Fig. 1) to monitor snail density more reliably in catfish ponds. Traps were placed in fingerling and foodfish production ponds on a commercial catfish facility and checked weekly throughout the production season (May – September). Initially traps were baited with a small, store-bought carrot placed inside a perforated 50 mL tube (Fig. 1). At each check, all snails were removed from the trap and transported back to NWAC where they were identified and counted. Later work demonstrated no differences in snail numbers between baited and unbaited traps, simplifying the monitoring protocol.

The studies have shown the timing of peak snail density tends to differ between fingerling and foodfish ponds. Fingerling ponds typically peak in mid-/ late-June and persist until mid-July before starting to decline (Fig. 2). On the other hand, foodfish ponds showed a slight delay in maximum observed snails, shifting towards mid-July and maintaining fairly consistent densities until mid-August (Fig. 3).

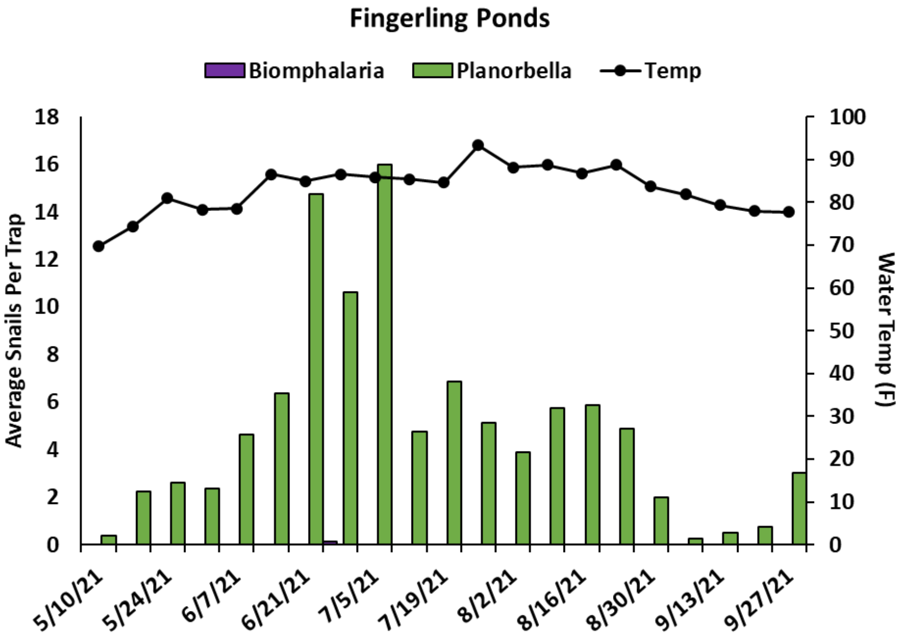


Figure 2. Histogram of average snails captured per week from four fingerling ponds during the 2021 production season. Marsh ramshorn captures are in green, ghost ramshorn are in purple. The line plot shows the average water temperature (˚F) during each sampling point.

These studies also found that pond treatments intended to control snails are often administered 3-4 weeks after snail populations peak in mid- to late-August. This suggests snail treatments may be occurring too late to have an appreciable effect on trematodes in the current production season, since infested snails have most likely already shed the parasite and negatively impacted production. Active infestations cause lost production due to poor feed consumption and increased occurrence of secondary bacterial infections. However, the detrimental effects of the parasite occur during the initial stages of infection and fish rapidly recover if the source of infection (i.e. snails) is removed or fish are transferred to another pond. As such, timely identification of ponds requiring treatment is critical to minimizing production losses. The use of snail traps to monitor snail populations is being evaluated as an early warning system to identify ponds that require treatment before detrimental trematode infestations occur. Work is being conducted to evaluate effectiveness of weekly low-dose copper treatments along with a mechanized precision delivery system to distribute granular copper sulfate evenly and accurately along pond margins in a single pass. In conjunction with this work, NWAC scientists are evaluating the effects of copper on the pond microbial community (beneficial algae, microbial pathogens, blue-green algae, etc), timing of application, sublethal impacts on snails, and impacts on fish production. Treating snails early in the season, soon after snails emerge from overwintering in the pond mud, may increase treatment efficacy as snails may be in a weakened state as they recover from winter estivation and return to normal metabolic function. Additionally, an earlier treatment may negatively impact snail reproduction or even kill immature stages of the parasite before they can infect the snail host. Any of these prospective benefits would greatly reduce the number of potential snail hosts in the pond which, in turn, would reduce the potential for *B. damnificus* outbreaks. A strategic treatment plan targeting these snail pests could have impacts that span multiple production cycles, which over time may greatly reduce the influence of *B. damnificus* on production.

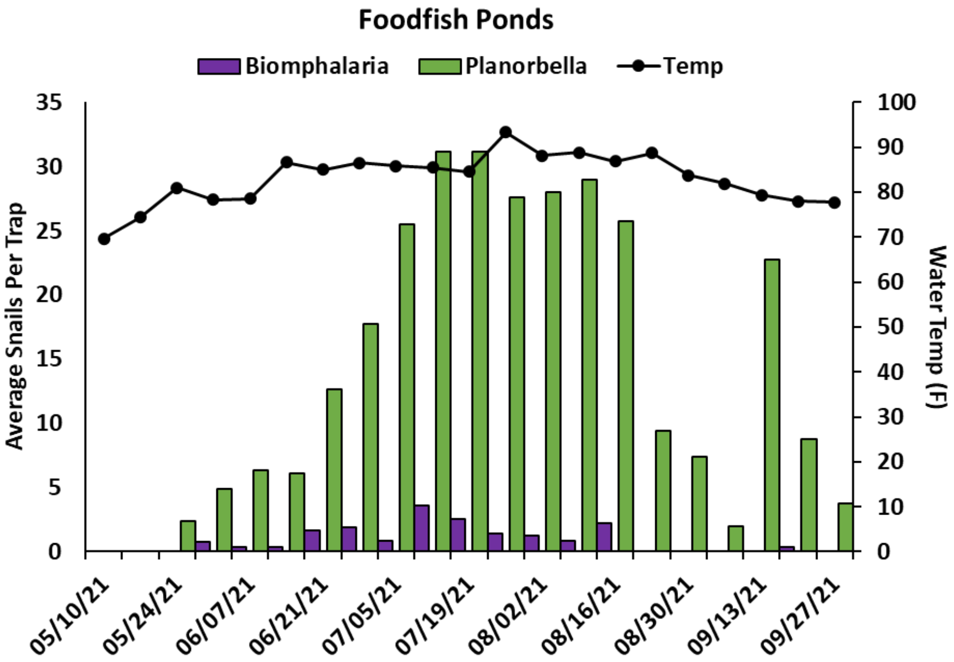


Figure 3. Histogram of average snails captured per week from four foodfish ponds during the 2021 production season. Marsh ramshorn captures are in green, ghost ramshorn are in purple. The line plot shows the average water temperature (˚F) during each sampling point.

Spring 2023 Catfish Disease Update

Anita Kelly, AFFC

Results of the annual Alabama Catfish Disease Survey are in! The survey was responded to by 65 of the 66 producers in west Alabama representing a total of 14,836 acres of production of which 3,429 acres were used to raise hybrid catfish. The survey showed that there were 1,369 ponds under commercial production with an average stocking rate of 7,523 fish per acre. West Alabama production of hybrid catfish has remained around 20% since 2020.

The reported total poundage lost to the five primary disease agents (*Aeromonas*, *Edwardsiella*, columnaris, hamburger gill, and toxic releases) was about the same as 2021 with 5.36 million pounds of fish (2022) compared to 5.09 million lost in 2021 (Figure 1). The estimated monetary loss to the Alabama catfish industry was $14,355,541 in 2022, a 19% increase from 2021. This value includes lost pounds of fish, medicated feed costs, chemical treatments, and lost feeding days. The primary cause of disease losses in Alabama continues to be from bacterial diseases; *Aeromonas hydrophila* (2.1 million lbs) followed by columnaris (1.7 million lbs) and *Edwardsiella* or ESC (0.5 million lbs). Losses due to unidentified toxins were 0.29 million lbs up significantly from 2021, which tallied 0.09 million pounds. Losses due to hamburger gill (PGD) were significantly lower in 2022 at 0.34 million pounds compared to 0.47 million lbs in 2021. In 2022, the recorded losses of fish to columnaris were the second lowest amount since 2015, while losses due to virulent *Aeromonas* was like 2021. Losses due to ESC, which had been steadily declining since 2015, showed a slight increase in 2022 (Fig. 2). Losses of catfish to toxic releases were significantly higher in 2022 (Fig. 3) and hamburger gill was lower in 2022 compared to 2021 (Fig. 4).

So far in 2023, a few cases of columnaris have been reported as well as hamburger gill and ich. Remember, if your fish have a coinfection of hamburger gill and columnaris, do not treat for columnaris with copper sulfate as you will kill the fish. Feeding medicated feed will help alleviate the columnaris if your fish are eating. Increased aeration will help alleviate the symptoms of hamburger gill until the spores break open. As always, as the water warms continue to be on the look-out for Columnaris (or cigar mouth) and *Aeromonas*. Remember early detection will prevent disease losses!

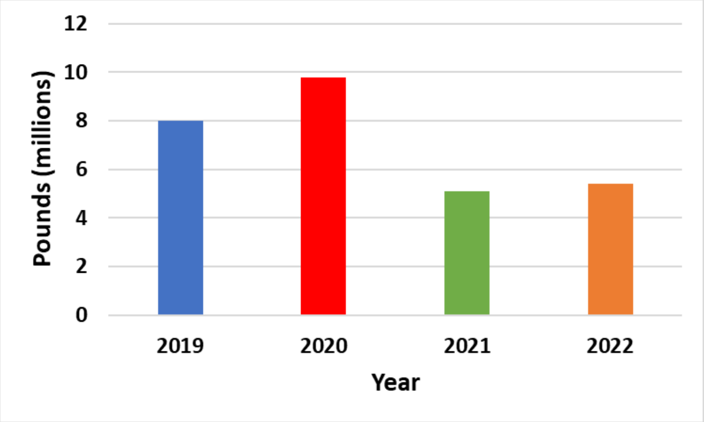


Figure 1. Total pounds (in millions) of catfish lost by year from 2019 to 2022 in west Alabama.

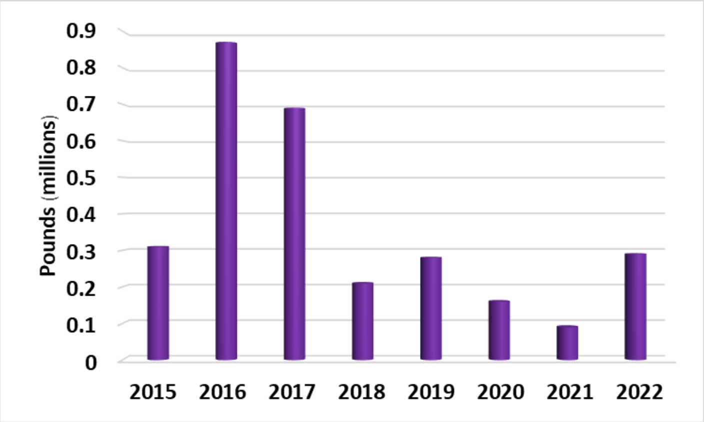


Figure 3. Total pounds (in millions) of catfish losses by year to toxic releases in west Alabama.

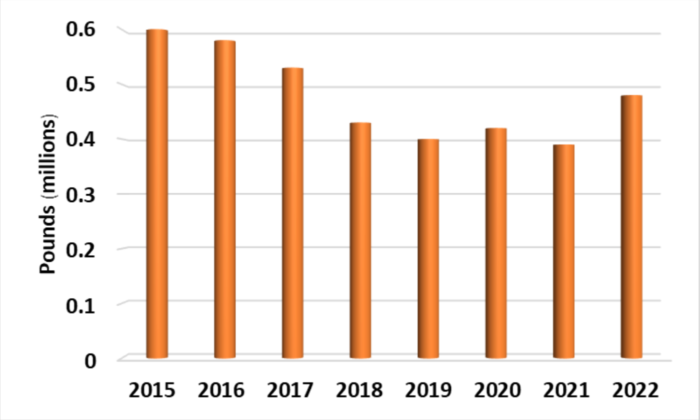


Figure 2. Total pounds (in millions) of fish lost by year to ESC,in west Alabama.

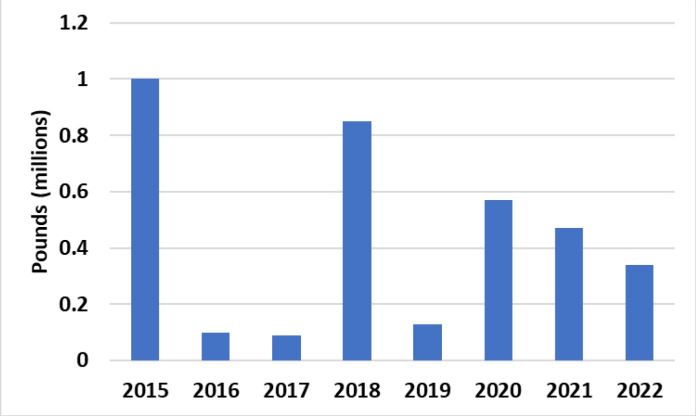


Figure 4. Total pounds (in millions) of catfish losses by year to hamburger gill, in west Alabama.

Terramycin 200® Approved for Columnaris

Anita Kelly, AFFC

On March 10, 2023, the Food and Drug Administration approved using Terramycin 200® (oxytetracycline) to control columnaris disease caused by *Flavobacterium columnare* in catfish. Like other approved antibiotics, this approval requires a VFD (veterinary feed directive). To obtain the feed, a veterinarian must fill out the VFD and send it to the feed mill of your choice. The veterinarian must also have a relationship with the producer. Terramycin 200® is currently the only antibiotic approved against the three major bacterial diseases seen in catfish.

Southern Regional Aquaculture Center Funding Has Been Secured to Evaluate the Impact of Waterbird Depredation on Catfish Farms in West Alabama and East Mississippi

Luke Roy[1], Mark Smith[2], Brian Dorr[3], Anita Kelly[1], Paul Burr[3], Carole Engle[4], and Jonathan van Senten[4]
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Figure 1. A double-crested cormorant feeding on a catfish fingerling. Photo Credit: Dr. Jimmy Avery, Mississippi State University.

With over 30,000 water acres of aquaculture production in channel catfish (*Ictalurus punctatus*) and hybrid catfish (*Ictalurus punctatus* female x *Ictalurus furcatus* male), west Alabama and east Mississippi are one of the largest catfish aquaculture-producing regions in the U.S. This area also lies within the Mississippi Flyway, a major migratory route for several species of fish-eating birds. These fish-eating birds, such as double-crested cormorants (*Nannopterum auritum*), commonly feed on commercially produced catfish, causing considerable financial losses to producers. While research has been conducted to document and quantify losses due to cormorants and other fish-eating birds in the Mississippi Alluvial Valley (West Mississippi and Arkansas), little research has been done within the Black Belt region. Research is needed to better understand and quantify the known losses to whole-farm profitability due to predation by fish-eating birds in this region. The USDA NIFA Southern Regional Aquaculture Center recently funded a two-year project to investigate this issue. The objectives of this project are to quantify the abundance and foraging patterns of fish-eating birds, the rate of fish consumption, and ultimately the economic impact of fish-eating birds frequenting catfish ponds in west Alabama/east Mississippi. This research will generate estimates of economic losses of fish to predatory birds that can be used to inform the development of management strategies to mitigate losses.

Currently, the research team is recruiting a graduate student for the project, which will be initiated in the Fall. The graduate student will be housed in the laboratory of Dr. Mark Smith at Auburn University, who serves as the project's principal investigator. The Alabama Fish Farming Center will be contacting potential farmers interested in participating in the project in west Alabama. If you have questions regarding this project or wish to be involved, contact Luke Roy at the Alabama Fish Farming Center (334-624-4016).

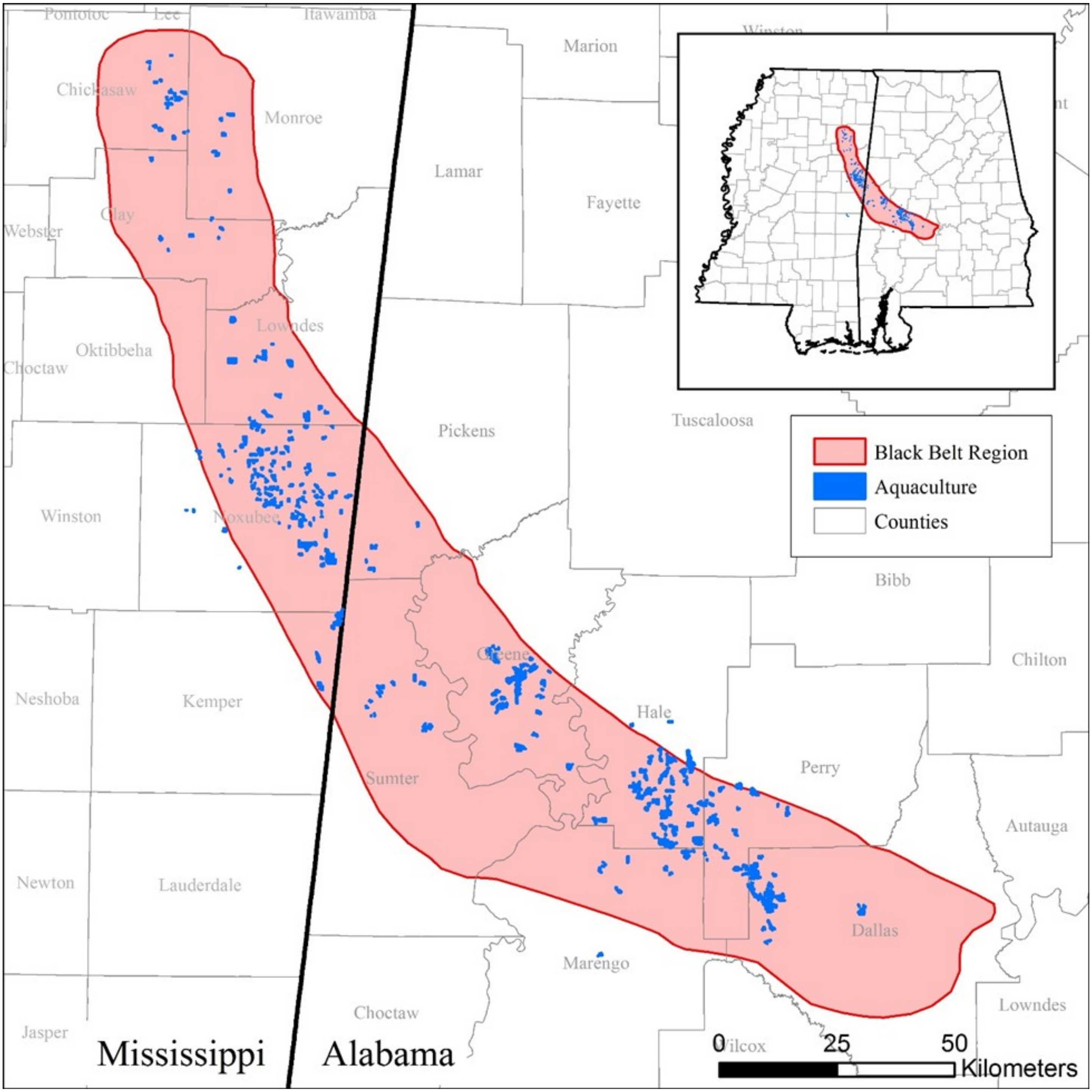

Figure 2. Study area for USDA SRAC waterbird depredation study.

RESEARCH ROUNDUP

Strategies for Channel Catfish Winter Feeding

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Several studies describe the nutritional requirements for channel catfish, leading to the formulation of high-performance feeds. However, it is difficult to predict the most economical dietary requirements for growth maintenance during winter because there are no standardized winter-feeding practices across the catfish industry. In west Alabama, catfish farmers use 28% protein, 32% protein, or a combination of both to feed their fish during winter (some use 32% in summer and then switch to 28% in winter). Feed containing 28% and 32% crude protein used for food size catfish production adds substantially to production costs, as feed costs are more than 50% of all variable costs. This study aimed to investigate different winter feed management strategies using the two most common feeds (28 and 32% protein feed) used by the west Alabama catfish industry.

To detect differences in growth performance, physiological parameters, proximate composition, and fatty acid composition of channel catfish fed with different winter-feeding strategies, two trials were carried out at two locations. Fish were fed using one of four feeding strategies: a 28% or 32% crude protein feed fed 4 or 8 times a month throughout the winter. Trial A was conducted at E.W. Shell Fisheries Center at Auburn University, testing four treatments with three replicates, totaling 12 pond-based tanks (650 gallons) at a density of 1 fish per 21 gallons (Fig. 1). Trial B was conducted at a farm in Hale County close to the Alabama Fish Farming Center in Greensboro, Alabama, testing four treatments with four replicates,

Figure 1. Trial A was conducted at E.W. Shell Fisheries Center using four treatments and three replicates, totaling 12 pond-based tanks of 650 gallons at a density of 1 fish per 21 gallons.

Figure 2. Trial B was conducted at a farm located four miles from the Alabama Fish Farming Center using four treatments and four replicates, totaling 16 pond-based tanks of 210 gallons at a density of 1 fish per 21 gallons.

totaling 16 pond-based tanks of 210 gallons at a density of 1 fish per 21 gallons (Fig. 2).

Channel catfish from both trials were raised at E.W. Shell Fisheries and stocked in mid-January 2022 with water temperatures ranging from 44 to 70 °F (Fig.3) with an average weight of 1.15 ± 0.02 lbs (Trial A; mean ± SE) and 1.12 ± 0.005 lbs (Trial B). The feeding protocol consisted of checking the weather forecast, and fish were then offered feed on the warmest day(s) of a given week. A feed response was observed to feed the set rate of 1% of body weight/day. After 86 days of culture, fish were harvested, counted, and individually sampled for length and weight. Ten fish from each tank were frozen for proximate analysis, and another 10 fish from each tank were sampled for blood collection.

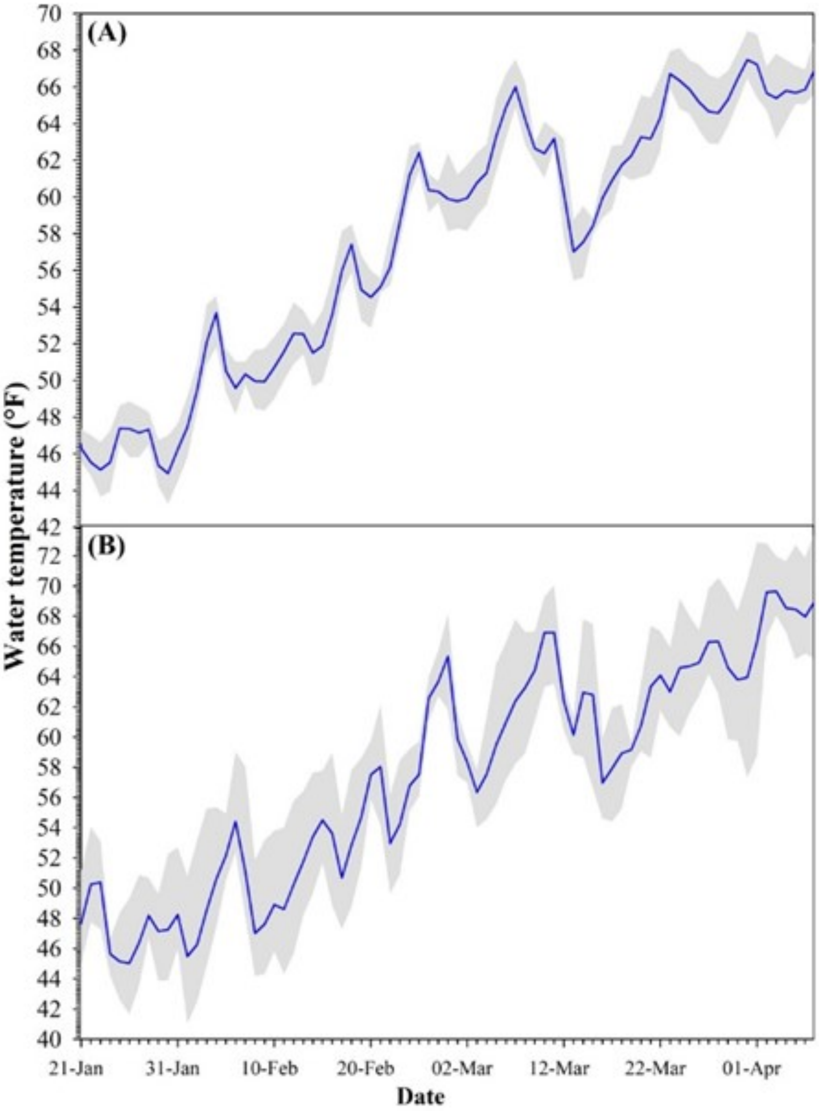

Figure 3. Water temperature of Trial A (Auburn) and Trial B (west Alabama) producing channel catfish during winter months.

The feed protein content (28 or 32%) and feeding regimes (4 and 8 times/month) affected growth in Trial A and B, however it didn’t impact final body weight, final body length, feed conversion ratio (FCR), or survival (Fig. 4 and Fig.5). Fulton’s condition factor (k), a measure of fish robustness, was better for catfish fed 8 times/month using either 28 or 32% protein feed in Trial A. Trial B had the lowest growth in the 28% protein 4 times/month treatment. The specific growth rate, weight gain (lbs and %) and thermal growth coefficient were different in Trial A, where catfish fed the 32% protein diet 4 times/month had inferior results compared to catfish fed 28% protein feed 4 and 8 times/month and 32% protein feed 8 times/month. However, no differences were observed in these metrics during Trial B. Ash (%) and moisture (%) were impacted by feed protein concentration and feeding frequencies in Trial A.

The different percentages of protein content and feeding frequencies tested in this study showed changes in physiological parameters. In Trial A, phosphorus and globulin were higher in fish fed 8 times/month, regardless of protein content. Total serum protein was not found to be different for diet type, but feeding frequency increased total blood protein, although the combination of feeding and time was not significant. Total serum protein is essential as it is responsible for aspects of the fish's immune response, maintenance of proper osmolality, regulating pH, and transferring metabolites throughout the fish. In the current trial, the increased feeding would have allowed more consumed proteins to be allocated to the blood for these physiological functions. No differences were detected in the physiological parameters for Trial B except for amylase, where higher values were detected when fish were fed 8 times/month instead of 4 times/month, regardless of protein content of the feed. Amylase is an important enzyme that breaks down larger sugars, such as starch and glycogen, into more readily utilizable energy for the fish. The increase in amylase, in this case, corresponds with the feeding frequency, with more feeding necessitating the need for more of the enzyme to make more utilizable energy. In summary, both feeding strategies and the 28% and 32% feeds yielded acceptable performance in maintaining overall fish health and robustness throughout the winter months. We want to thank the Alabama Catfish Feed Mill for donating the feeds used in this study.

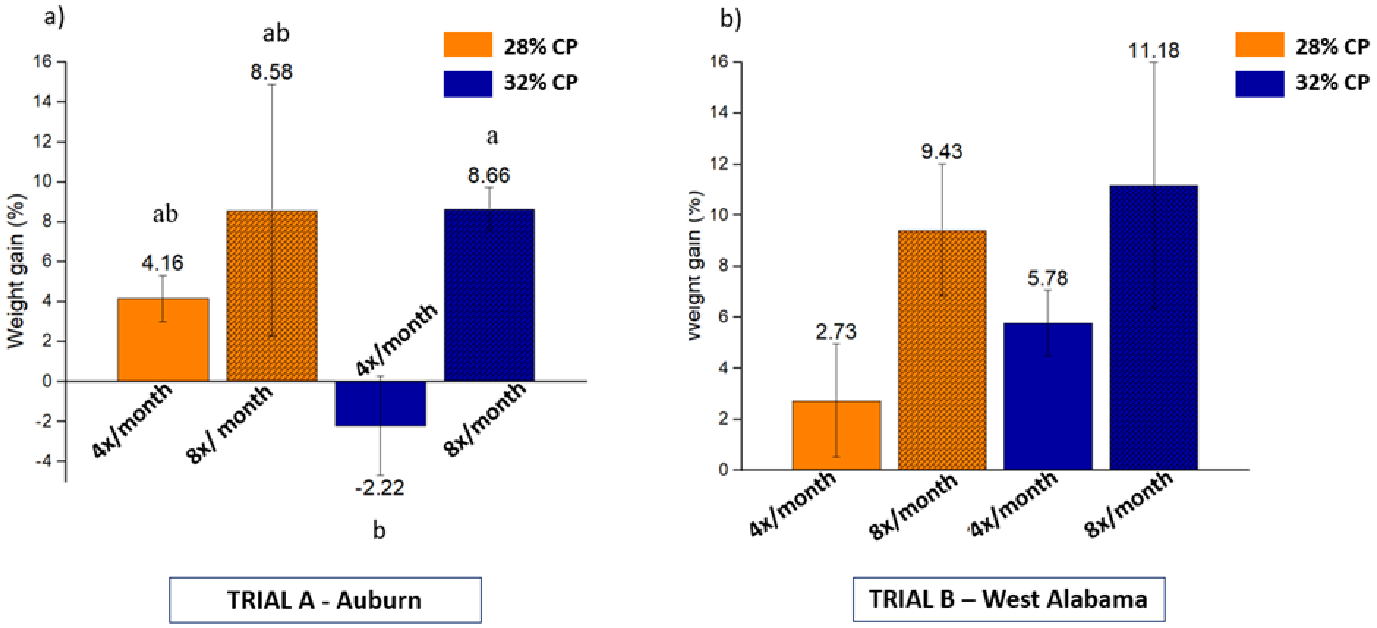

Figure 4. Weight gain (%) of channel catfish produced during winter months fed commercial feed containing 28 and 32% crude protein at Auburn (Figure a) and West Alabama (Figure b).

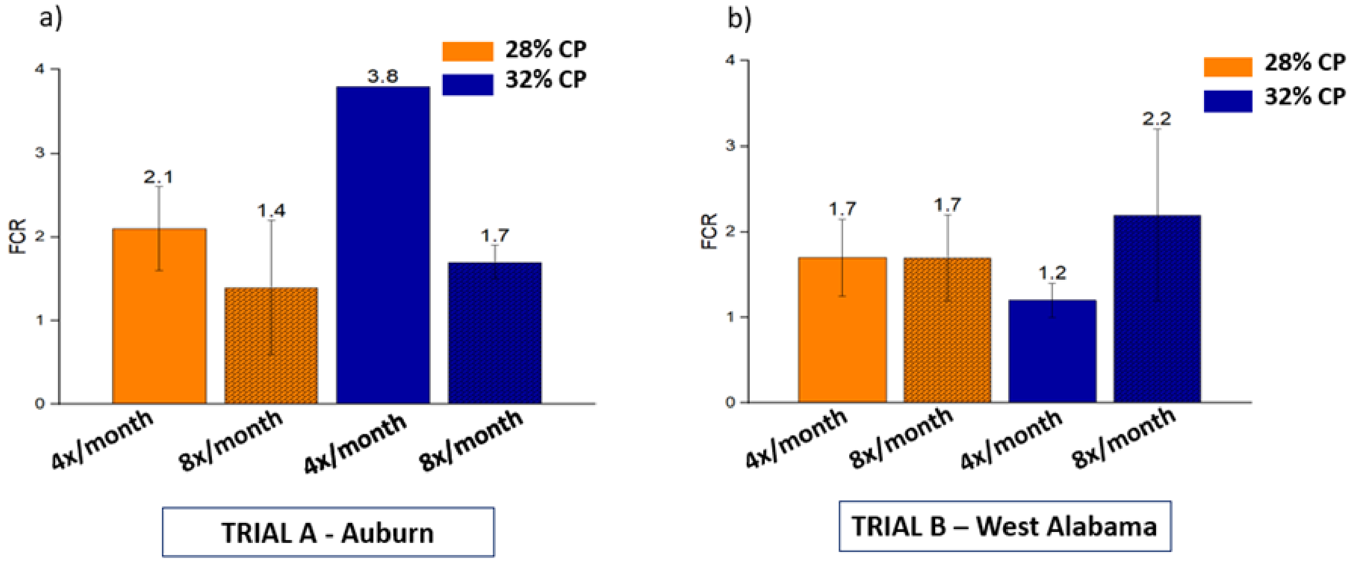

Figure 5. Feed Conversion ratio (FCR) of channel catfish produced during winter months fed commercial feed containing 28 and 32% crude protein at Auburn (Figure a) and West Alabama (Figure b).

Exploring the Relationship Between Biofloc Systems, Probiotics, and Health of Nile Tilapia

Uthpala Padeniya, Allen Davis, Timothy J. Bruce
SFAAS

Biofloc is a popular technology used to control water quality with the added advantage of some species, such as tilapia, utilizing the floc as a secondary food source, enhancing nutrient extraction. Biofloc technologies are those techniques that favor the production of complex suspended microbial communities for the processing of nutrients within the culture tank. This is considered an environmentally friendly technology as water use and waste discharge are minimized, and nutrients can be recycled by species such as tilapia consuming the floc. The proliferation of microbes is achieved by intermittent additions of carbon which shift carbon:nitrogen ratios which result in the growth of a diverse microbial community.

In addition to processing waste products, this community serves as a natural food source and may minimize the development of harmful bacteria through competition and/or the release of biological compounds. Given that the bacterial community is not controlled, and not all harmful bacteria are excluded, disease outbreaks can still occur, such as Streptococcosis. *Streptococcus iniae* is one of the bacterial pathogens that is responsible for causing this disease. Streptococcosis brings about substantial economic losses to global tilapia aquaculture.

In biofloc-type systems, the use of antibiotics could be problematic as they could negatively influence the bacterial community and/or promote the development of antibiotic resistant strains of bacteria. Hence, the use of probiotics to further protect animals from disease occurrences is a viable option. Probiotics are beneficial bacteria that can enhance the growth and health of organisms which can either be added to the water or directly to the feed. Given that biofloc systems are complex bacterial communities, it makes sense to look at the addition of probiotics to the feed allowing direct intake by the fish to potentially further enhance the health of tilapia.

Figure 1. Biofloc system used to rear Nile tilapia.

In this example, a 16-week feeding trial was conducted at the E.W. Shell Fisheries Center, Auburn, Alabama, to examine the effects of culturing fish in a biofloc system (Fig. 1) and dietary probiotics on disease resistance and immune responses against *Streptococcus iniae*. Nile tilapia of an average weight of 71.4 ± 4.4 g were stocked equally in each tank. Three treatments were tested: 1) A commercial control diet, 2) a control diet top-coated with BiOWiSH FeedBuilder Syn3, and 3) a control diet top-coated with AP193. Both are *Bacillus* spp. bacteria which have the potential to help improve disease resistance in tilapia. During the feeding trial, fish were sampled

Figure 2. Intra-peritoneal injection-based challenge trial with *S. iniae*.

every four weeks, and the water temperature was maintained around 82ºF. Water quality parameters, including DO, pH, TAN, and nitrite were kept at acceptable ranges. Following the feeding trial, the fish were sampled. A subset of fish from all three treatments were subjected to an intra-peritoneal injection-based *in vivo* challenge trial with *S. iniae* (Fig. 2), a pathogen of interest for tilapia producers.

The average weights of the fish at the end of the growth trial were 255.7 ±8.1 g (Control) 236.7 ± 5.1 (AP193), and 239.9 ±12.9 g (BiOWiSH FeedBuilder Syn3). Survivals of fish after the feeding trial were 88.1±3.9% (control), 93.1±9.2% (AP193) and 96.6±6.6% (BiOWiSH FeedBuilder Syn3). In summary, there were no clear dietary differences in growth, survival or water quality parameters which could be typical in such a setting.

However, the importance of this study was expressed when fish were subjected to disease challenges. The survival of fish fed with probiotics was significantly higher than that of fish fed the control diet (Fig. 3). This could be well explained as the cumulative percent mortality at the end of the disease challenge trial was lower in probiotic treated groups than the control group. For further examinations of the immune responses of Nile tilapia, serum lysozyme activity, and gene expression were evaluated before and after the challenge. No significant differences were found between the different dietary treatments, although some gene expressions were higher in probiotic-treated groups than in the control group.

These study results show that when Nile tilapia were provided dietary probiotics in biofloc systems, there may be increased survival when exposed to *S. iniae* pathogen. This finding could be important on a production scale as this pathogen is responsible for most disease mortalities in tilapia production. Furthermore, to understand the relationship between dietary treatments in biofloc systems, a second feeding trial, and challenge will investigate the influence of protease complexes and humic substances for Nile tilapia reared in biofloc systems. These combined studies will provide a clearer picture of the influence of probiotic applications and biofloc tilapia culture to enhance fish health in larger production systems.

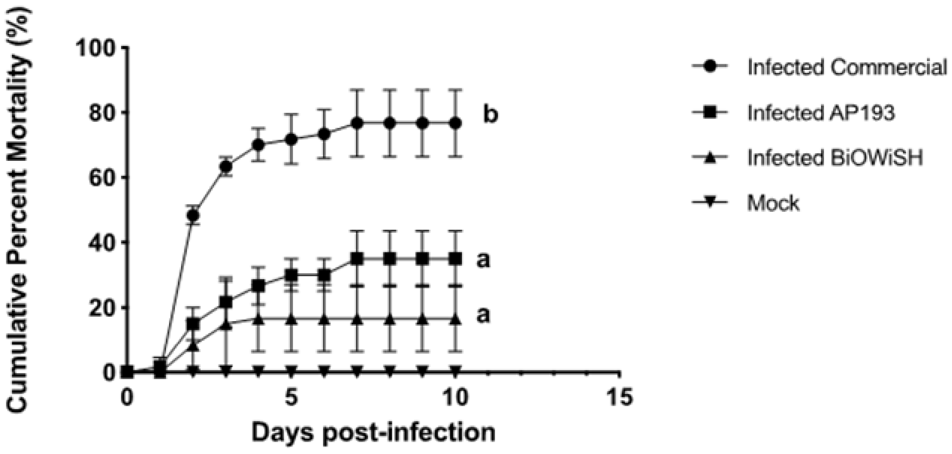

Figure 3. Mortality trends from the challenge trial with *S. iniae*.

Influence of Probiotics on the Growth and Immune Response of Fingerling Channel Catfish

Khanh Nguyen[1], Timothy Bruce[1], Oluwafunmilola Afe[1], Mark Liles[2], Benjamin Beck[3], and D. Allen Davis [1]
[1]SFAAS, [2]Biological Sciences, AU, [3]USDA ARS Aquatic Animal Health Laboratory

Figure 1. Channel catfish infected by Flavobacterium columnare.

The U.S. channel catfish industry faces significant economic losses due to columnaris disease (Fig. 1), ESC, and virulent *Aeromonas hydrophila*. These diseases can cause reduced growth rates, poor feed utilization, and increased mortality, leading to losses for farmers and higher consumer prices. In addition, with the increasing demand for fish protein, farmers struggle to control and prevent diseases, particularly during the fingerling and juvenile stages. While antibiotics have been used to reduce the impact of these diseases, their overuse can lead to antibiotic-resistant bacteria, making them less effective over time. In addition, the limitations on the types of antibiotics and strict withdrawal times remain a problem for producers.

Probiotics have recently gained attention as a promising alternative to antibiotic therapy. Due to their ease of application, they have been shown to increase growth rates, improve feed utilization efficiency, and enhance the immune system of cultured animals. However, many factors can affect the efficacy of probiotics, including strain of bacteria used, dosage, and method of application. Therefore, the results of these trials do not always translate to a production-scale environment where a more natural mode of infection occurs.

To evaluate the incorporation of probiotics in fingerling channel catfish, a study was conducted that compared two different probiotics: 1) a commercial product, BiOWiSH FeedBuilder Syn3 by BiOWish Technologies Inc., that utilizes *Bacillus subtillis* as the primary bacteria, and 2) an experimental strain, AP193, which is *Bacillus velezensis, supplied* by Dr. Mark Liles of the Department of Biological Sciences at Auburn University, under natural infection conditions. The study aimed to determine if probiotics could reduce the impact of disease outbreaks on channel catfish and improve their growth and survival. Water efflux from channel catfish production ponds with a history of disease outbreaks was introduced into an indoor laboratory flow-through system to simulate natural infection conditions. Two trials were conducted in the spring (6 weeks) and summer (8 weeks) of 2021.

In the first trial, the impact of the commercial and experimental probiotics was evaluated with an initial fish size of 8 lbs/ 1000 fish. Three diets were tested: 1) a basal diet without probiotics (Basal), 2) a basal diet that was top-coated with AP193 (B-AP), and 3) a basal diet that was top-coated with BiOWiSH FeedBuilder Syn3 (B-BW). The probiotic inclusion rates were based on the manufacturer's recommendation. The second trial, with an initial animal size of 1 lb/ 1000 fish, aimed to examine the impact of higher and lower inclusion rates of the commercial probiotics compared to the rate recommended by the manufacturer for the basal diet. Four diets were tested, including 1) a basal diet without probiotics (Basal), 2) a basal diet that was top-coated with half of the recommended inclusion rate (B-BW-L), 3) a basal diet that was top-coated following the recommendation of the manufacturer (B-BW), and 4) a basal diet that was top-coated with twice the recommended inclusion rate (B-BW-H).

The study showed that the commercial and experimental probiotics did not negatively affect the animals. The experimental probiotic AP193 had a positive effect on lowering inflammation in channel catfish under natural culture conditions. However, the commercial probiotic did not reduce inflammation and no differences in the growth and survival rates occurred for either probiotic used or for the higher and lower inclusion rates used for the commercial probiotic. Moreover, it is worth noting that the actual count of viable probiotic cells indicated a 1,000 to 10,000 times disparity between what was listed on the label and the exact number of beneficial bacteria in the diet. This suggests that not only can the strain of bacteria used affect efficacy, but the actual dosage on the feed may not be the same as indicated.

In conclusion, the study showed that the AP193-diet positively reduced inflammation in fingerling channel catfish under natural culture conditions. Further research that exposes catfish to other environmental stressors, such as changes in water quality or temperature fluctuations, could have a more significant impact on the efficacy of probiotics. As research continues, it is important to consider all of these factors when evaluating the role of probiotics in channel catfish farming,

More details regarding the study can be found at *Veterinary Sciences* (2023), https://doi.org/10.3390/vetsci10030236

Thermal tolerance study for the Pacific white shrimp

Aya S. Hussain, Kaelyn J. Fogelman, Hisham A. Abdelrahman, Luke A. Roy, and James A. Stoeckel
SFAAS

Shrimp represent a potential, high-value alternative aquaculture species in west Alabama, but few shrimp farms are currently in production due to unpredictable yields. Recent research has shown that high variation in shrimp production among ponds may be attributed in large part to differences in temperature regimes of pond water during the warm seasons. Understanding how shrimp adjust to increasing seasonal temperatures and temperatures at which shrimp begin to experience trouble are important in the broader appreciation of how shrimp cope with seasonal temperature cycles. Shrimp farmers in Alabama typically stock production ponds in May and June each year after water temperatures rise above 70 °F.

Water temperatures as high as 96.8 °F have been documented in July and August by commercial shrimp farmers in production ponds, which are approximately 3 feet in depth and can heat up considerably by late afternoon during the summer months. There has been increased mortality reported by commercial shrimp farmers in Alabama when water temperatures are highest, and shrimp are approaching harvest size.

In recent years, some west Alabama inland shrimp production ponds have had survival as low as 30–40%. A large percentage of mortality tends to occur near the end of the production cycle and has been labeled “late-term mortality”. These reductions in survival have an impact on the production and profitability of commercial shrimp farms in west Alabama and the southeastern U.S. Similar trends in late-term mortality have been reported by both Florida and Texas shrimp producers. Commercial shrimp producers believe that a combination of prolonged high summer water temperatures, suboptimal ionic profiles, and harmful algae blooms likely contribute to the observed late-term mortality.

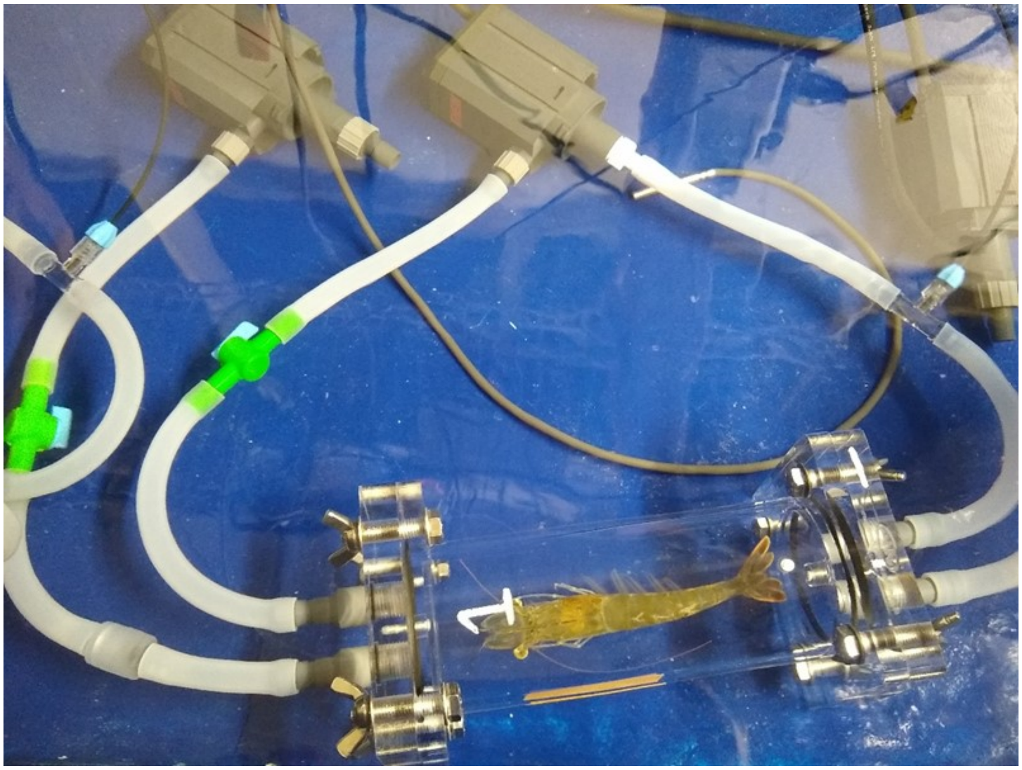

Figure 1: Shrimp placed within an acrylic respirometry chamber.

Short term (acute, less than 7 days) thermal tolerance of shrimp can be measured as the upper thermal limits of the animal. Upper thermal limits are often measured using critical thermal maxima (CT_{max}), where the animal is exposed to temperature increases at a constant rate until a critical endpoint is reached. Common endpoint responses include loss of equilibrium, sudden onset of muscular spasms, and finally "heat rigor", "coma", or "death". The upper thermal tolerance of animals such as shrimp is thought to be due to reduced oxygen supply capacity at high temperatures – meaning that while there may be sufficient oxygen available in the water for the animal to use, they may be unable to use the oxygen for important cardiac and respiratory functions. One approach to understanding what an animal is experiencing internally, or physiologically, at elevated temperatures is the concept of aerobic (or metabolic) scope. Aerobic scope is defined as the difference between maximum aerobic metabolic rate (MMR) and standard aerobic metabolic rate (SMR). SMR is the minimum rate of oxygen consumption required for basic maintenance and survival needs of the animal at a given temperature. MMR is the maximum metabolic rate that the animal is capable of under stressful conditions. So, we can think of aerobic scope as the difference between what an animal is capable of doing for MMR, and what an animal fundamentally needs for SMR. The energy that is left over for aerobic scope is the energy the animal now has for activities beyond basic survival, such as growth, movement, sexual maturity, and reproduction.

In theory, the greater the aerobic scope, the more energy the animal has for activities beyond basic survival and maintenance. Aerobic scope is greatest at optimal temperatures for the animal, but beyond those optimal temperatures, aerobic scope, and extra energy for the animal, decreases with increasing temperature. The temperature at which aerobic scope equals zero (MMR = SMR; maximum metabolic rate that the animal is capable of now is the same as the basic metabolic rate needed for maintenance and survival) represents the critical temperature. At temperatures above critical temperature the animal is no longer physiologically capable of meeting its basic metabolic costs and no longer has energy for extra activities such as growth or reproduction. The upper thermal limit of the animal (or CT_{max}), indicated by the animal's loss of equilibrium, occurs at the temperature beyond which the organism reaches critical temperature and no longer has sufficient energy for basic metabolic costs.

To understand the mechanisms behind upper lethal limits in shrimp, we evaluated linkages between directly measured thermal limits and aerobic scope, or ability to provide energy above that needed for basic maintenance. We tested whether thermal tolerance decreases with increasing shrimp age/size and whether aerobic scope is a useful concept for understanding the physiological basis of thermal tolerance in shrimp. We exposed two size classes (small: 0.07 ± 0.03 and large: 0.87 ± 0.09 oz) of shrimp to increasing temperature at a rate of 33.8 °F/ hour from 82.4–107.6 °F. At each temperature, we used intermittent respirometry (Fig. 1) to estimate resting metabolic rate and we directly measured lethal thermal tolerance by evaluating critical thermal maximum (CT_{max}; Fig. 2).

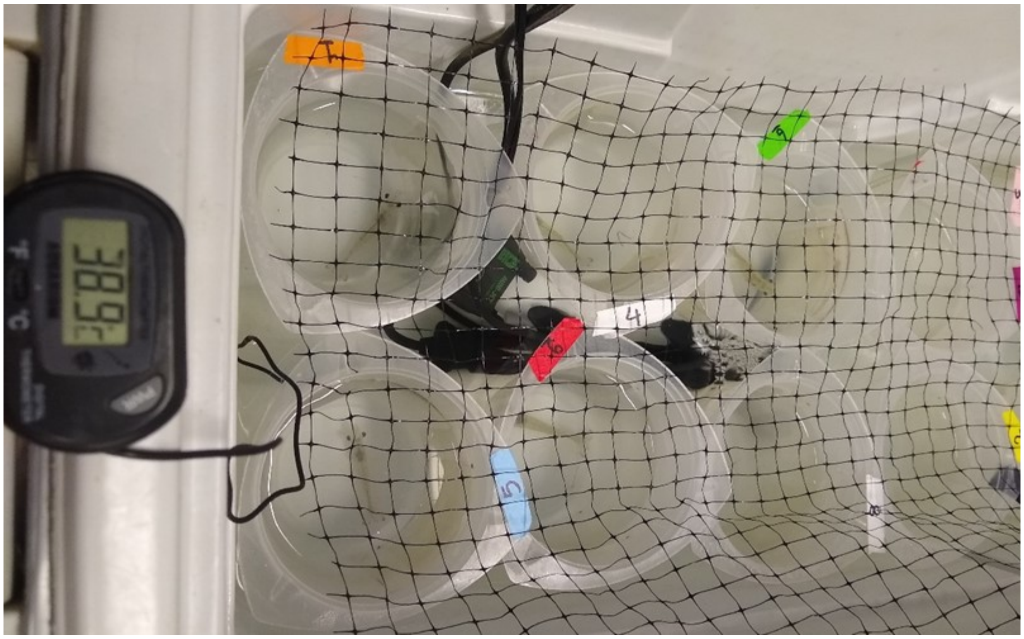

Figure 2: A shrimp placed in each of the 10 beakers during the critical thermal maximum exposure (at 102 °F).

Additionally, we used the electron transport system assay to estimate maximum metabolic rate at temperatures from 48.2–113 °F. Small shrimp had a higher CT_{max} than large shrimp, with upper lethal limits of 105.08 and 102.2 °F, respectively. Large shrimp exhibited a lower temperature at minimum aerobic scope than that of the smaller shrimp (Fig. 3). We found that the upper thermal limits of *L. vannamei* in low-salinity aquaculture ponds are likely driven in part by reduced oxygen supply capacity at high temperatures – meaning that they may be unable to use the oxygen available in the water for important cardiac and respiratory functions. Upper thermal limits were preceded by temperature-dependent declines in aerobic scope and occurred within a couple of degrees of peak resting metabolic rate. Reductions in aerobic scope appear to be one of the underlying physiological drivers of thermal tolerance in *L. vannamei* and an indicator of increasing thermal stress. Changes in the temperature at which aerobic scope reaches its minimum may be a useful predictor of shifts in thermal tolerance among shrimp size-classes.

Understanding the physiological underpinnings of thermal tolerance may ultimately be of use in evaluating thermal tolerance of genetic lines of *L. vannamei* for low-salinity aquaculture, with temperatures at minimum aerobic scope and peak resting metabolic rate being particularly relevant endpoints. Additional studies are needed to determine whether this framework is useful for understanding the thermal tolerance of other high-valued aquaculture species experiencing unexplained mortalities, or even reductions in performance, particularly in situations where culture water temperature is high. These relationships may also be useful in understanding the physiological basis of performance (lethal limits, growth, and reproduction) in the presence of additional stressors such as suboptimal ionic balance or suboptimal water quality parameters that may also affect metabolic rates.

For further reading, please consult Aquaculture (2023), https://doi.org/10.1016/j.aquaculture.2023.739402.

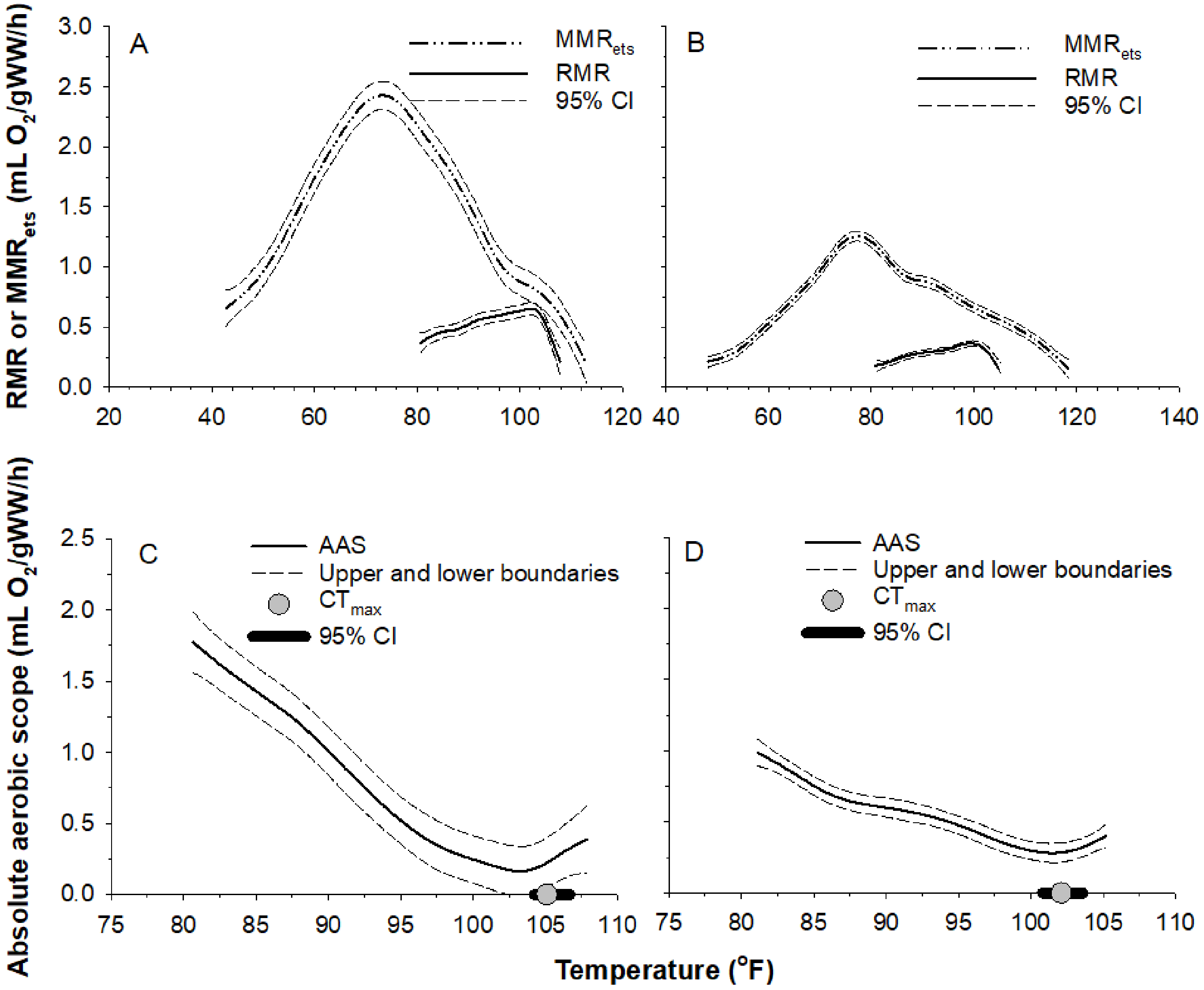

Figure 3: Relationships between activity of the electron transport system representing maximum metabolic rate (MMR$_{ets}$), resting metabolic rate (RMR), and temperature for (A) small and (B) large-size classes of shrimp. Relationships between absolute aerobic scope (AAS), CT$_{max}$, and temperature for (C) small- and (D) large-size classes of shrimp. Gray circles and solid lines on the X-axes indicate the median and 95% confidence intervals (CI) for CT$_{max}$.

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