CONTROL OF BLUE-GREEN ALGAE IN AQUACULTURE PONDS

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PROJECT OBJECTIVES

1. Develop chemical control methodologies to prevent the establishment of noxious blue-green algal communities.

    a. Evaluate novel selective blue-green algicides identified through laboratory screening.

    b. Isolate, identify, and test allelopathic chemicals produced by competing blue-green algae and other micro-organisms found in local aquatic communities.

2. Evaluate nutrient manipulation to promote desirable phytoplankton community structure.

    a. Increase nitrogen-to-phosphorus ratios in the water.

    b. Reduce the availability of phosphorus from pond bottom muds.

    c. Enhance the availability of inorganic carbon.

    d. Manipulate trace metal availability.

    e. Increase potassium levels in the water.

    f. Increase salinity levels in the water.

3. Evaluate water circulation as a means of altering the environment to promote desirable phytoplankton community structure.

4. Evaluate the use of plankton-feeding fish to alter the environment to promote desirable phytoplankton community structure.

5. Evaluate the development of phytoplankton communities in the Partitioned Aquaculture System.

ANTICIPATED BENEFITS

The overall goal of this project is to identify methods of controlling or eliminating blue-green algae from aquaculture ponds. The ability to control algal communities in ponds could benefit farmers in several ways. Excessive abundance of blue-green algae, especially when combined with their habit of growing in surface scums, can cause low dissolved oxygen concentrations and other water quality aberrations that affect fish growth and health. Therefore, the ability to control the composition of blooms could result in better fish growth and lower costs for aeration and other water quality management procedures.

The largest and fastest growing segment of aquaculture in the United States is farm-raised channel catfish. Catfish that are off-flavor are unmarketable, and farmers are forced to hold those fish in inventory until composition of the pond microbial community changes and flavor improves. Holding market-sized fish in inventory imposes an economic burden on farmers, and off-flavor is estimated to cost the industry well over $20 million a year.

Baitfish mortalities associated with blue-green algae are common in the early summer. Historical use of high rates of granular fertilizers may be a factor in these excessive algae blooms, especially in baitfish ponds that have been in production for years and have accumulated sediments. Phosphorus in the groundwater in Lonoke County, Arkansas, also contributes to nutrient loading. In addition, golden shiners may stimulate phytoplankton production by foraging on larger species of zooplankton that would otherwise feed on the phytoplankton. Goldfish may indirectly contribute to phytoplankton production through phosphorus release due to their bottom feeding activities. Evaluation of methods with the potential to reduce excessive blooms (pond renovation, sodium nitrate application) will provide farmers with information to make informed decisions when weighing possible benefits against the costs.

Most of the treatments and management practices considered in this project have been promoted for controlling blue-green algae, but their effectiveness has not been documented. It is anticipated that this research will reveal which, if any, of these treatments are beneficial. Any practice demonstrated to be effective in controlling blue-green algae has considerable potential for improving aquaculture management and enhancing profits.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. *Develop chemical control methodologies to prevent the establishment of noxious blue-green algal communities.*

University of Mississippi. More than 4,000 plant and algae extracts have been prepared from several thousand collections of plants, cyanobacteria (blue-green algae) and algae. These collections now include more than 300 collections of tropical cyanobacteria and marine algae, and 450 collections of aquatic and wetland plants, cyanobacteria (collections and cultures), algae, and thousands of collections of higher plants. These extracts were evaluated for selective blue-green algicidal activity in a rapid bioasay using 96-well microtiter plates.

Initial extract evaluations were conducted using *Oscillatoria agardii* as the cyanobacterial test organism, and *Selenastrum capricornutum* as a chlorophyte control for nonspecific algicidal activity. These organisms were grown in continuous flow culture and provide uniform algal material for biological evaluation. Lipid extracts of 33 tropical marine cyanobacteria and algae were evaluated in these assays at an initial concentration 100 ppm. Two related species of marine green algae and one species of marine brown algae were found to contain substances that were selectively algicidal against *O. agardii.*

While these results are promising, the cyanobacterium *O. agardii* is not known to produce odorous compounds that cause off-flavor problems in aquaculture. We have since established cultures of the *O. perornata*, a filamentous blue-green alga that produces 2-methylisoborneol (MIB), the major tainting substance in catfish grown in northwest Mississippi. These cultures are suitable for high-throughput extract evaluation.

In the first phase of the project, 356 extracts from collections of aquatic and marine cyanobacteria, plants and algae have subsequently been evaluated in replicate bioassays using *O. perornata*. Forty-three extracts (12%) were found to be strongly active and cyanobacterial-selective at the concentration of 100 ppm, used for initial screening. Dose response data was obtained for these extracts at half-log concentrations. Twelve extracts were found to be effective at 30 ppm or lower. The two most potent extracts were confirmed to be two of the related marine green algae species first identified in the initial bioassays that used *O. agardii* rather than *O. perornata*.

Bioassay-guided fractionation of several extracts has resulted in the purification of unusual cyanobacterial-selective natural products that are effective against both *Oscillatoria* strains at concentrations in the parts-per-billion range. The chemical structures of several of these metabolites have been solved and are undergoing additional toxicological and antimicrobial evaluation.

The second phase of the program focused on the evaluation of plants found in tropical rainforests and temperate regions throughout the world for cyanobacterial-selective algicidal activity. Initial evaluations of plant extracts at 100 ppm picked up some activity associated with less selective antimicrobial substances such as tannins. In order to reduce the incidence of "false-positive" hits and to select for only those plants that contain potent and potentially more selective compounds, the primary phase of the high-throughput screening program evaluated plant extracts at 20 ppm. Extract plates were evaluated in duplicate. Plant extracts that showed cyanobacterial-selective activity at 20 ppm were reconfirmed by secondary evaluations at 20, 10 and 2.0 ppm. A bio-genetically diverse repository of plant extracts from over 170 plant families collected was examined. These plants were obtained from Peru, New Guinea, and the United States. This repository contained chemically distinct crude lipophilic extracts of separate plant parts (roots, leaves and stems, flowers, etc.) of each species collected. Over 2,300 crude extracts of more than 1,050 species of higher plant extracts were evaluated in this high-throughput screening system.

Over 70 plant species showed some level of selective activity against *O. perornata*. Since the major goal of this project was to identify cyanobacteria-specific agents, a process of "deselection" was undertaken to exclude compounds with broad activity against other microorganisms (blue-green algae are gram-negative bacteria). Thus, the active extracts that showed broad antimicrobial activity against a panel of biomedically important microorganisms (bacteria and fungi) were used to deselect extracts from further study. The microorganisms used to dereplicate antibiotic-containing extracts were *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Trichophyton mentarophytes*. In addition, all active extracts that showed mammalian toxicity in the monkey kidney Vero cell cytotoxicity assay were also deselected for further examination.

The most potent cyanobacteria-selective extracts (that were not significantly antibacterial and were non-toxic) were those obtained from the roots and stems of a Peruvian collection of *Dulacia candida* (family = Olacaceae). *Dulacia candida* is a widely distributed tropical Amazonian shrub. The crude *D. candida* extracts showed extremely potent anti-cyanobacterial activity, approaching the parts per billion range. The components of the extract were separated and the bioactivity was confined to several moderately non-polar chromatographic fractions. However, the substance (or substances) responsible for the potent anti-cyanobacterial have not yet been identified.

In addition, more than one hundred pure biologically active compounds from marine invertebrates and algae have been examined for anti-cyanobacterial activity. Some of these substances inhibited algae growth, but were either cytotoxic or not selectively toxic to nuisance blue-green algae.

USDA-ARS. Proprietary analogs of a promising natural product found in certain plants were tested in ponds for selective activity against blue-green algae. Efficacy testing yielded promising results, and one of the best proprietary compounds (as determined by laboratory screening studies) significantly reduced the MIB-producing cyanobacterium *Oscillatoria perornata* and the cyanobacterium *Raphidiopsis* *brookii* at application levels of 125 ppb. The abundance of green algae and diatoms increased dramatically two days after application of 125 ppb of this proprietary compound. Based upon the results, this proprietary compound is promising as a selective algicide to help prevent the growth of *O. perornata* and *R. brookii* in catfish aquaculture ponds, but it is not a broad-spectrum blue-green algicide. The half-life of the proprietary compound in pond water has been determined to be 19 hours, much less than the synthetic herbicide diuron. Efficacy testing of the proprietary compound using 0.25-acre catfish ponds stocked with channel catfish is currently being performed 1) to confirm effective application rates for obtaining acceptable flavored catfish; 2) to determine if the proprietary compound accumulates in catfish flesh; and 3) to observe for any negative effects on the catfish crop from application of the proprietary compound.

Results at a glance...

- *A derivative of a natural compound found in certain plants is undergoing patent application for use as a selective algicide to help prevent musty off-flavor in farm-raised catfish.*

Additional testing of several of the most promising proprietary compounds has determined that they are neither carcinogenic, antifungal, antibiotic, nor anti-protozoal. Analogs were also evaluated in the laboratory for acute toxicity and histopathology using channel catfish fingerlings. Acute toxicity was determined through measurement of mortality following 96 hours of aqueous exposure. The 96-hour LC50 for the analogs tested ranged from approximately 2-6 ppm. Histological analysis revealed abnormalities in the gills of treated fish. It was determined that the lethality of the analogs is related to histological changes in the gills of the catfish. Additional screening of pure natural compounds and crude plant extracts has identified several other leads for blue-green algicides.

Objective 2. *Evaluate nutrient manipulation to promote desirable phytoplankton community structure.*

Mississippi State University. In experiments repeated over two years, eight 1,450-gallon enclosures (limnocorrals) were placed in a small research pond or a commercial earthen fish pond in which a dense phytoplankton community dominated by *Oscillatoria agardhii* was present. The N:P ratio of four enclosures was adjusted by addition of KNO_3 to provide a N:P ratio of about 30 and chelated iron was added to provide 1 ppm iron. Four enclosures did not receive nutrient additions. All enclosures were supplied with diffused aeration to produce gentle turbulence. Water samples collected every 2 to 3 days were analyzed for nutrients, solids, and indices related to phytoplankton biomass and community composition. After 2 weeks, the combination nutrient addition did not result in a shift of the phytoplankton community from dominance

Results at a glance...

- *Studies in Mississippi and Alabama indicate that various manipulations of waterborne plant nutrients have little promise for controlling phytoplankton community composition in catfish ponds with high feeding rates.*

by *O. agardhii*. Nitrite concentrations increased and soluble phosphorus concentrations declined in nitrate-treated enclosures. Algal biomass in untreated enclosures declined, suggesting that continued nutrient supply is necessary to sustain high algal biomass. Nutrient manipulation does not appear to hold promise as a technique to effect phytoplankton community structure in hypereutrophic aquaculture ponds.

Auburn University. An initial laboratory study considered the effectiveness of the chelating agents ethylenediamine tetra-acetic acid (EDTA), lignin sulfonate, and citric acid for maintaining iron in solution. EDTA was the most promising of the chelating agents, because iron remained at concentrations above 0.5 ppm for 30 days in soil-water systems treated with 1 ppm iron from iron-EDTA. Thus, it was decided to use EDTA chelated metals for pond research.

Pond studies in 1999 had three treatments: (1) chelated iron (0.5 ppm) plus chopped legume hay (40 kg/ha per week); (2) chelated iron, chopped legume hay, plus a trace element mix (1 kg/ha per week); (3) control. The hay applications resulted in low dissolved oxygen concentrations, and no benefits related to blue-green algae control were observed.

Results at a glance...

- ✰ *Phosphorus-only fertilization was as effective as nitrogen plus phosphorus fertilization in bait minnow ponds. Phosphorus-only fertilization is less expensive, it conserves nitrogen, and it lessens the possibility for nitrogen pollution of natural waters by pond effluents. Removal of soft sediment from old (25 + years) bait minnow ponds should improve bottom soil quality.*

In 1999, potassium chloride treatments of 0, 30, 90, and 120 ppm did not result in significant differences in blue-green algal abundance. A sodium nitrate based fertilizer containing 8% N, 24% P_2O_5, and 15% K_2O was as effective as a standard, 10-34-0 liquid fertilizer in promoting sunfish production.

In 2000, two treatments (legume hay at 20 kg/ha per week and legume hay plus 0.5 ppm chelated iron and 1 kg/ha per week of trace element mix) were compared to the control. No benefits of treatments on water quality or blue-green algae control were observed.

Studies conducted in bait minnow ponds failed to show a need for nitrogen fertilizer. Phosphate-only fertilization was as effective as nitrogen plus phosphorus fertilization. However, sodium nitrate was shown to be a more “environmentally-friendly” source of nitrogen than ammonium sulfate.

In 2001, additional experiments were conducted on nitrogen fertilization in sunfish ponds that were also fertilized with phosphorus. These findings suggest that fertilization with nitrogen may be more important in older ponds than in younger ones, because earlier studies in the same ponds in 1971, 1978, and 1987 had not revealed a benefit to fish production of nitrogen applications in ponds fertilized with phosphorus. No effects of nitrogen and phosphorus fertilization ratios on blue-green algae abundance were observed.

Work on the pond soil cores from bait minnow ponds in Arkansas showed that the major effect of pond aging on sediment quality was the accumulation of soft sediment of high phosphorus concentration over time. There was a drastic decline in sediment quality in 30- to 35-year-old ponds as compared to 7- to 25-year-old ponds. Laboratory experiments indicated that sodium nitrate treatment would not improve sediment quality in older ponds. The problem of impaired sediment quality in old ponds can probably be best resolved by removing soft sediment from ponds to provide firmer bottoms.

University of Arkansas at Pine Bluff. A laboratory study was conducted to evaluate the effects of incorporating sodium nitrate into pond bottom soils. Two common soils (Perry-Portland, known locally as gumbo, and Calloway-Calhoun-Loring, or crawfish) were collected from the bottoms of 20- to 45-year-old commercial baitfish ponds. A layer (4.5 L) of gumbo or crawfish soil was added to each of 24 microcosms (13-L buckets) and sodium nitrate was soil-incorporated at rates of 0, 25, 50 or 75 g N/m^2. Buckets were filled with pond water, and sodium acetate was added throughout the study as a source of organic matter, to create anoxic conditions and simulate pond bottom waters.

Results at a glance...

- *No benefit was found from using sodium nitrate to suppress phosphorus release from pond sediments.*

Results showed that incorporating sodium nitrate suppressed phosphorus release in both soils for 11-19 days. However, elevated nitrite concentrations were also found during this period. Nitrite levels as high as 180-250 ppm were measured in the high rate treatment. The higher the rate of sodium nitrate, the longer was the duration of phosphorus suppression, but the additional time was not proportional to the dose.

A second laboratory study was conducted to evaluate the effects of adding different forms of sodium nitrate through the water column on pond bottom soil. A layer of gumbo soil collected from a 45-year-old commercial baitfish pond was added to each of 25 microcosms (13-L buckets), buckets were filled with pond water, and sodium acetate was added as a source of organic matter to create anoxic conditions and simulate pond bottom waters. Sodium nitrate was added to the buckets at a rate of 50 g N/m^2 in the form of a powder, prill, or two types of coated prills (short-term and long-term). Coated prills were suggested as a mechanism to permit application of sodium nitrate through the water column in established ponds. The short-term coating was designed to release 20% the first day and to finish release by day 10 to 15, while the long-term coating was intended to slowly release sodium nitrate over a 2- to 3-month period. Results showed that compared to controls, adding sodium nitrate suppressed phosphorus release regardless of product form, with the long-term coated prill treatment lasting 10 days. Elevated nitrite levels were found in all buckets treated with sodium nitrate, however, nitrite concentrations were lowest in the long-term release prill treatment (highest concentration for this product form was 13 ppm NO_2). Nitrite levels as high as 180-250 ppm were measured in containers with the other three product forms.

A field study was conducted in eight 4.7-m^2 fiberglass limnocorrals within a 0.04-ha goldfish pond located at the University of Arkansas at Pine Bluff Aquaculture Research Station. Coated sodium nitrate prills (time-release coating was 6% total weight) were sprinkled uniformly over the water surface in four corrals at a rate of 50 g N/m^2. A low-pressure blower was used to direct an air current over the surface of each corral to provide mixing without aeration. Soluble reactive phosphorus, nitrite, and Secchi disk visibility were measured at intervals before and after application of sodium nitrate. Oxidation-reduction potential (silver/silver chloride) was measured weekly in the upper 0.5 cm of soil cores extracted from three locations within each corral. Although oxidation-reduction potential results indicated less reduced soil conditions in treated corrals for a 3-week period, there were no significant water quality benefits. Nitrite levels in treated corrals did not exceed 3 ppm throughout the experiment. Sodium nitrate additions do not appear to be a practical treatment for baitfish pond soils.

Water quality and plankton community data were collected from 12 commercial golden shiner ponds at monthly intervals. Study ponds ranged in size from 7 to 25 acres (3 to 10 ha) and represented two soil types and two ages (20-25 years and 40-45 years). Using a column sampler, water samples were collected from a single location in each pond. Results showed that for parameters linked to phytoplankton abundance (i.e., chlorophyll *a*, COD, TP, TN), seasonal water quality changes in golden shiner ponds were similar to those reported for channel catfish culture. Dissimilar results were found for dissolved inorganic nitrogen, which was highest in the fall rather than in the winter, as has been reported for catfish. This may reflect the relatively low feeding rates used for baitfish. In addition, relatively higher SRP concentrations were found in the summer months, perhaps a result of powdered feed used for young fish.

Pond renovation was evaluated as a technique to reduce problematic blue-green algae blooms and associated water quality problems. Monthly water quality and plankton community data were collected from 12 commercial goldfish ponds for a year. Six of the 12 ponds were renovated during the winter (1999-2000) and returned to production in late spring. Results indicated little difference in water quality in renovated ponds as compared to controls. Soluble reactive phosphorus was significantly higher in renovated ponds for the first two months after ponds were returned to production, reflecting pond management practices for rearing new crops of fish. Pre- and post-renovation soil testing showed no overall significant difference in sediment phosphorus concentrations. This may reflect the fact that sediment from inside ponds is used to re-build ponds levees. However, pond renovation significantly reduced the phosphorus level in the upper 2 cm of sediment. Sulfate-sulfur concentrations in pond bottom sediments averaged 98 ppm, and ranged from 3 to 417 ppm. Compared to typical levels in terrestrial soils in the study area, these concentrations are very high. Sulfate sulfur results from the decomposition of organic matter and typically is higher in clayey soils.

Results at a glance...

☆ *Pond renovation had little effect on subsequent levels of soluble reactive phosphorus in baitfish pond waters or on average concentrations of phosphorus in bottom sediments. A possible explanation for these results is that pond bottom soils are used to rebuild levees during renovation and not removed from ponds. Bottom soils are mixed in the pond reconstruction process, and renovation decreased the average soil phosphorus concentration in the upper 2-cm of sediment.*

Louisiana State University. Concentrations of 2-methylisoborneol and geosmin were determined from March 1999 through February 2000 in fifteen 0.2-acre channel catfish experimental catfish ponds located at the Aquaculture Research Station, Louisiana State University Agricultural Center, in Baton Rouge. Ponds were assigned randomly to three mineralization (salinity) levels): 0.0 parts per thousand (ppt) - control, 1.5 ppt, and 3.0 ppt.

Nominal mineral concentrations were established and maintained with periodic additions of salt (NaCl). Water samples were collected twice a week and analyzed by solid phase micro-extraction gas chromatography mass spectrometry for MIB and geosmin concentrations. All ponds contained little or no MIB until late May. All ponds experienced off-flavor episodes at one time or another. Only trace levels of geosmin were observed throughout the year. Three major increases in the concentration levels of MIB were observed; one in early June, a second in early August, and a third in late September. Increases were not observed in all ponds. In several cases, concentrations of MIB increased rapidly from barely quantifiable levels (<0.1 ppb) to very high levels (>30 ppb) over a 3-4 day period. A slight reduction in the concentrations of MIB was observed in the ponds with 3.0 ppt NaCl. Ponds with 1.5 ppt salinity routinely contained higher levels of MIB than the control (0 ppt NaCl); but there was insufficient data to determine the impact on the mitigation of off-flavor episodes by using low levels of NaCl.

A simplified method for analyzing for the muddy, musty off-flavor in catfish was developed that uses the liver of the fish rather than the muscle tissue. Slurried liver tissue permits partitioning of the analytes directly from the sample, thus eliminating the need for the microwave distillation step. Sample preparation takes less than five minutes per sample and GC/MS analysis takes 15 minutes from start to finish, for a total analysis time of 20 minutes. A comparison of the concentrations of MIB and geosmin from fish fillet tissue and the liver was made by dosing fish (previously depurated for 96 hours) with MIB and geosmin at a final concentration of 3 ppb. Fish were sampled at 0, 2, 24, 48, and 72 hours after dosing to assess uptake of off-flavor compounds. The amount of 2-MIB and geosmin recovered directly from the liver was near 5%, while the recovery from muscle tissue employing microwave distillation approaches 60%. Fish were off-flavor after only one hour of exposure. The concentrations determined from the analysis of the livers are consistent with those determined from the fillets.

An instrumental method for determining the presence of the earthy, muddy or blue-green off-flavor in catfish was compared with four professional flavor checkers. The odor threshold for a theoretical average flavor checker was between 0.1 and 0.2 ppb for 2-methylisoborneol. The lower end of the range (0.1 ppb) was selected as the instrumental cut-off for determining off-flavor fish. Results from the instrumental method were highly correlated to flavor checker results.

North Carolina State University. In 1999, a study was conducted at the Tidewater Research Station to determine the effect of applications of trace metals and organic matter on water quality and phytoplankton population dynamics in hybrid striped bass ponds. Twelve 0.25-acre hybrid striped bass (*Morone chrysops* × *M. saxatilis*) ponds were stocked (4,000/acre) in the spring and managed according to standard commercial hybrid striped bass culture procedures. Chelated iron, a mineral mix, and alfalfa pellets were periodically applied to six of the ponds during an entire growing season. Ponds were harvested in late fall. Water samples were taken weekly and analyzed for nutrients and phytoplankton composition, and soil samples taken at the beginning and end of the study were analyzed for total nitrogen, total carbon, organic matter, pH and metals. Blue-green algae began to appear in samples during the second week of September 1999 and dominated phytoplankton composition in all treatments during the month of October 1999. Application of iron, minerals, and organic matter did not result in any differences in phytoplankton species composition, fish production, soil quality or water quality when compared to control ponds.

Objective 3. *Evaluate water circulation as a means of altering the environment to promote desirable phytoplankton community structure.*

Louisiana State University. Twelve 0.1-acre ponds were stocked with multiple cohorts of channel catfish at a nominal stocking density of 10,000/acre. In eight ponds, fish were restricted to approximately one-quarter of the pond area by a barrier placed across the pond width. In these ponds, a continuously-operating, horizontally-mounted pump mixed water between the area containing fish with the open area of the pond. In four of the mixed ponds, threadfin shad were stocked at 200/acre in the open area of the pond. Although the experiment is on-going, there are no differences in water quality, phytoplankton community composition, or feeding rate among the three treatments.

Three water management practices were evaluated, each at two levels (presence or absence), alone, and in combination, to determine their effects on blue-green algal community composition and water quality in experimental mesocosms managed to simulate commercial catfish production practices. In one treatment, aluminum sulfate (alum) was applied weekly at 3 ppm to reduce phosphorus (chemical control). In a second treatment, the water column was destratified by continuous vertical mixing in contrast to conventional surface aeration (physical control). In the third treatment, planktivorous gizzard and threadfin shad (*Dorosoma* sp.) were stocked at 25,000 juveniles/acre with channel catfish to evaluate their ability to control blue-green algae (biological control).

The eight treatment combinations, arranged in a 2^3 factorial design, were randomly assigned to twenty-four 3,000-gallon fiberglass tanks with soil bottoms (mesocosms) with three replicates per treatment combination. Catfish juveniles (mean = 52 g) were stocked in May at 10,000/acre, fed a 32% crude protein commercial feed daily at rates ranging from 40 to 150 pounds/acre, and harvested in November. Water samples were collected biweekly for nutrient and phytoplankton analysis.

Mean catfish survival was 88.5% and yield averaged 7,133 pounds/acre, with no observed differences related to water management practices. Shad biomass averaged 625 pounds/acre at harvest. The alum reduced soluble reactive phosphorus in October, but had no effect on phytoplankton density or community composition. Suspension of sediments in the water column from vertical mixing increased total nitrogen, total phosphorus, nitrate, and pH but had no discernible effect on the phytoplankton community. The presence of shad significantly reduced total algal biomass as evidenced by reductions in total nitrogen, total phosphorus, chemical oxygen demand, and chlorophyll *a*. Although the percentage of blue-green algae in the phytoplankton community was not significantly reduced compared to mesocosms without shad, odorous species of blue-green algae (*Oscillatoria perornata* and *Anabaena* spp.), known to cause off-flavor in catfish, were nearly eliminated by the presence of shad. Mesocosms with shad never had odorous species of blue-green algae that accounted for more than about 3% of the blue-green algal community, while odorous blue-green algae in mesocosms without shad accounted for as much as 20% of the community. Shad had no impact on catfish production.

North Carolina State University. Twelve ponds at the Tidewater Research Station were stocked in spring of 2000 with hybrid striped bass at 4,000/acre to evaluate the effectiveness of water circulation for controlling blue-green algae abundance in hybrid striped bass ponds. Ponds were managed according to standard procedures and harvested late fall. In six of the ponds, circulators were placed to produce whole-pond horizontal water circulation. Effectiveness of the water circulators in creating currents that circulated throughout the pond was evaluated with gypsum blocks. However, the water circulators used were very unreliable, and repeated mechanical problems throughout the duration of the study resulted in both decreased water circulation and the loss of several replicates in the treatment receiving circulation. Although water quality and phytoplankton population analysis revealed no differences among treatments, frequent breakdowns of circulators resulted in inconsistent water circulation and prevented a meaningful evaluation of the circulation treatment. Consequently, a second evaluation of water circulation in hybrid striped bass ponds was planned for the 2001 production season.

Results at a glance...

- ✰ *Artificial daytime water circulation of hybrid striped bass ponds had no effect on the incidence of blue-green algae in the phytoplankton community and did not measurably improve water quality.*

In May 2001, hybrid striped bass were stocked at 4,000/acre into twelve 0.25-acre ponds at the Tidewater Research Station. In six of the ponds, water was circulated during daylight hours (from 0900 to 1600) with a 0.5-hp pump (70 gallons/minute) placed on the pond bank. The pumps drew water from near the pond bottom and discharged at the surface approximately a third of the way down the length of the pond. Water samples were analyzed weekly or biweekly for concentrations of total and soluble reactive phosphorus, ammonia, nitrite, nitrate, total suspended solids, chemical oxygen demand, biochemical oxygen demand, pH, and chlorophyll *a*. In addition, phytoplankton abundance and composition were measured biweekly. Ponds were harvested in November 2001. There were no differences among treatments in fish production or in any of the measured water quality parameters. Uncirculated ponds had higher numbers of diatoms, but there were no differences among treatments in incidence of blue-green algae or overall phytoplankton abundance. This study showed no benefits resulting from water circulation in hybrid striped bass ponds.

Mississippi State University. In three experiments, twelve 0.1-acre ponds were stocked with multiple cohorts of channel catfish at a nominal stocking density of 10,000/acre. In the first experiment, fish in eight ponds were restricted to approximately one-quarter of the pond area by a barrier placed across the pond width. In these ponds, a continuously-operating, horizontally-mounted ½-hp pump mixed water between the area containing fish with the open area of the pond. In four of the mixed ponds, threadfin shad were stocked at 200/acre in the open area of the pond. In the second and third experiments, a baffle oriented along the long axis of the pond was placed in eight ponds. One ½-hp pump mixer was placed in each of four baffled ponds and two mixers were placed in each of the other four baffled ponds. Four ponds did not have a baffle and were not mixed. Results were similar in the three experiments: there were no differences in water quality, phytoplankton community composition, or feeding rate among the three treatments. Ponds with two mixers were more turbid than ponds in the other treatments. Turbidity in ponds with two mixers was dominated by suspended mineral matter.

Objective 4. *Evaluate the use of plankton-feeding fish to alter the environment to promote desirable phytoplankton community structure.*

Auburn University. Ten 0.1-acre earthen ponds were stocked with 9-g channel catfish at a density equivalent to 6,000/acre with 0.3-pound grass carp at 20/acre. Five randomly selected ponds were stocked with 8-g threadfin shad at 800/acre. Each pond was fed once daily to apparent satiation with a commercial floating feed (32% crude protein). All ponds were harvested 8 November 1999 and fish were identified, sorted and weighed. One channel catfish was randomly selected from each pond for flavor analysis.

Total threadfin shad mortality occurred in one of the shad ponds (9 September 1999). One of the no-shad treatment ponds experienced a catfish kill during the final two weeks of September when they had attained an average weight of 0.7 pounds. Observed mortality during this period was 31% of the original stock and only 48% of the original stock was recovered at harvest. Following the shad mortality, that pond was eliminated for further consideration of water quality and phytoplankton analysis for the shad treatment.

The presence of shad had no effect on temperature, dissolved oxygen concentrations, pH, or total alkalinity of pond waters. Total organic carbon concentrations ranged from 6.3 ppm in April to 30.5 ppm in October in the shad treatment and from 4.7 ppm in April to 34.1 ppm in September in the no-shad treatment. Total organic carbon levels increased in both treatments during the growing season and were higher in the shad treatment on 27 April and 25 May and higher in the no-shad treatment on 19 August.

Total ammonia-nitrogen (TAN) concentrations increased in both treatments throughout the growing season, but reached higher levels in the no-shad treatment. In the shad treatment, TAN concentrations ranged from 0.03 ppm in April to 1.92 ppm in October. In the no-shad treatment, TAN ranged from 0.03 ppm in June to 3.88 ppm in September. TAN concentrations in the no-shad treatment were significantly higher than concentrations measured in the shad treatment for the period 1 September through the end of the study. Nitrite-nitrogen concentrations in both treatments remained below 2.0 ppb until July and then began to increase, reaching a maximum of 37.2 ppb in the shad treatment in September and 94.5 ppb in the no-shad treatment in October. For the period 1 September through 25 October, nitrite-nitrogen levels were significantly higher in the no-shad ponds.

Phytoplankton abundance (as indicated by chlorophyll *a* levels) were low initially and increased progressively throughout the study to highs of 263 ppb chlorophyll *a* in the shad treatment in September and 285 ppb in the no-shad treatment in August. Chlorophyll *a* levels were significantly higher in the no-shad treatment on only one sampling date. There were no differences overall in phytoplankton abundance when data for chlorophyll *a* levels for all

Results at a glance...

- *In both Alabama and Georgia, stocking threadfin shad with channel catfish resulted in lower ammonia and nitrite levels late in the growing season. The improved environmental conditions apparently resulted in significantly better survival of catfish in the presence of shad in the Alabama study.*

sampling dates in September and October were combined.

Ponds with shad had significantly higher abundance (36.6 organisms/mL) of phytoplankton than ponds without shad (22.2 organisms/mL), as well as significantly higher number of taxa present (ponds with shad = 17.2 taxa; ponds without shad = 15.6 taxa). However, there was no significant difference in diversity indices between treatments (ponds with shad = 2.02; ponds without shad = 1.97).

When at least 60% of the phytoplankton present in a sample were measured for the period August through October, phytoplankton in ponds without shad were larger (average greatest axial linear diameter, GALD = 63 μm) than in ponds with shad (average GALD = 39 μm). The percentage of green algae and blue-green algae in the phytoplankton community did not differ between treatments. The percentage of other algae, which included diatoms, euglenophytes, and dinoflagellates, was significantly lower in phytoplankton communities of ponds with shad compared to ponds without shad.

Survival of channel catfish in ponds with threadfin shad was 92%, which was higher than the 77% survival in ponds without shad. Catfish production was also higher in ponds with shad (4,651 pounds/acre) than in ponds without shad (3,980 pounds/acre). However, average weight at harvest was similar in both treatments (overall mean = 0.80 pounds). Feed conversion was marginally better in ponds with shad (1.30) than in ponds without shad (1.40).

Off-flavor analysis was performed on one catfish from each pond. The catfish were filleted with the skin-on, microwaved, and served to a panel of three taste testers. Slight off-flavor was detected by two of the three panelists in both treatments, but no significant differences were found.

In 2000, eight commercial channel catfish ponds were selected for analysis on three catfish farms in West Alabama. Four ponds with catfish and established threadfin shad populations were selected along with four similar ponds stocked only with channel catfish. All ponds were managed with common commercial practices. Nightly aeration was used in all ponds to prevent fish loss caused by low dissolved oxygen concentrations. Copper sulfate was added periodically to six of the eight ponds for algal management. Partial harvests of catfish were carried out in ponds throughout the study. Water samples were collected twice a month from May through October 2000.

Results at a glance...

- ✰ *Polyculture of threadfin shad with channel catfish in relatively small experimental ponds resulted in improved water quality conditions and enhanced catfish survival. The stocking of threadfin shad, in four commercial channel catfish ponds in West Alabama, did not result in improved water quality when compared to four similar ponds with catfish, but no threadfin shad.*

Mean total organic carbon (TOC) increased gradually through the entire growing season in both treatments. The TOC concentrations in the shad treatment (mean = 34.26 ppm; range = 22.1 to 56.5 ppm) were significantly higher than TOC concentrations in the no-shad treatment (mean = 27.57 ppm; range = 20.8 to 38.4 ppm).

Mean total ammonia-nitrogen concentrations also were higher in the shad treatment (mean = 1.6 ppm; range = 2.3 ppm in June to 0.3 ppm in September) than in the no-shad treatment (mean = 0.7 ppm; range = 0.3 ppm in October to 1.2 ppm in September). Nitrite nitrogen (NO_2-N) concentrations did not differ significantly between treatments, remaining below 0.1 ppm throughout most of the growing season.

Mean chlorophyll *a* concentrations (corrected for phaeophytin) varied throughout the growing season in both treatments, but the mean concentration for the entire growing season was significantly higher in the shad treatment (mean = 299 ppb; range =153 ppb to 407 ppb) than in the no-shad treatment (mean = 155 ppb; range = 66 ppb and 308 ppb). Phytoplankton community structure and size distribution are currently being examined.

This study did not reveal an improvement in water quality of commercial channel catfish ponds containing threadfin shad. In fact, concentrations of TOC, total ammonia, and chlorophyll *a* were significantly higher in the shad treatment than in the no-shad treatment. These ponds were stocked, harvested, fed, aerated, and chemically treated (with copper sulfate) independently by three different farm managers. As such, water quality differences measured between treatments were likely not solely caused by the presence or absence of threadfin shad.

University of Georgia. Threadfin shad or fathead minnows were stocked with catfish in 0.25-acre earthen ponds at Tifton, Georgia, and compared to ponds with only channel catfish. At Cohutta, Georgia, two treatments were started comparing threadfin shad and channel catfish to channel catfish alone in 0.1-acre earthen ponds. Three replicate ponds were used for each treatment for a total of nine ponds at Tifton and six at Cohutta. Channel catfish were stocked as fingerlings in multiple sizes at 44,500/acre. Threadfin shad were stocked at about 2,500/acre and were 1.7 to 4 inches long. Fathead minnows were stocked at about 10 pounds/acre (8,900 to 10,000/acre) and were 1.4 to 2 inches long.

Threadfin shad stocking was difficult due to the fragility of this species during handling, hauling, and transfer into receiving waters. Five attempts were made to stock threadfin shad at both locations. The most successful method of threadfin shad stocking was to obtain 1.5 to 2 inch shad from local ponds in the months of January to April. Even under the best conditions, it was difficult to determine the survival of the threadfin shad after stocking. Stocking threadfin shad into holding ponds and seining after one or two weeks indicated that 30-90% of the threadfin shad could die a short time after stocking due to loss of scales during handling, temperature shock, alkalinity shock, salinity shock, or other stress due to handling or transfer. Sorting threadfin shad from gizzard shad, which often is found together with the threadfin in lakes, rivers, and aquaculture ponds, causes an increase in threadfin shad losses. Cast nets or seines can be utilized for capturing threadfin shad. However, each method of capture has disadvantages. Casting nets near paddlewheel aerators appear to be successful for monospecific harvests of threadfin shad. Hauling aids should be utilized during transport and may include an anesthetic, sodium chloride, calcium chloride, anti-foaming agents, or a buffer of pH 8.0 to 7.0. Tempering should be extended to two hours of gradual exchange of hauling water with receiving water, even when similar water temperatures are found in the two water sources.

Over 50 algal species have been identified from Georgia ponds during the growing season. Blue-green algal blooms are denser at the Tifton location than at the Cohutta location. Water temperatures are cooler at Cohutta and the water source is a spring from limestone caverns. The water source at Tifton is the Floridan aquifer. No differences among treatments were observed in 1999. All ponds had blue-green algae in abundant populations. Establishment of threadfin shad populations was variable and appeared to affect the observed phytoplankton population densities. Off-flavors were not detected in channel catfish harvested from the study in 1999 or 2000. Off-flavors were detected in fish from control ponds at Tifton in 2001.

In 1999, total ammonia concentrations were lower in the shad and minnow treatments than in the ponds with only channel catfish. Also, nitrite concentrations were lower in the minnow treatment than in the other two fish combinations. Soluble reactive phosphorus was similar in all ponds.

In 2000 at Tifton, blue-green algae became abundant in ponds with channel catfish only as early as April, in May with fathead minnows and catfish, and in June with threadfin shad and catfish. Blue-green populations reached 100 million cells/mL in ponds with channel catfish only, 80 million cells/mL with fathead minnows and catfish, and 35 million cells/mL with threadfin shad and catfish. At the Cohutta location, blue-green algae did not become abundant until August. It was apparent that blue-green algae were less abundant in the ponds with threadfin shad; however, all ponds had blue-green algae blooms by late summer.

In 2001 at Tifton, the presence of threadfin shad reduced the number of blue-green algae colonies versus the control or fathead minnow treatment. Blue-green algal numbers were higher in fathead minnow ponds from March through August than in control ponds. At Cohutta, the blue-green algae, *Microcystis aeruginosa*, became abundant later than at the Tifton location. Few blue-green algal species were observed in ponds containing threadfin shad by August. The number of hours of aeration was reduced in ponds containing threadfin shad.

Although threadfin shad were successfully maintained at Tifton in catfish ponds, the final harvest at Cohutta showed that green sunfish had displaced the threadfin shad over the course of the three year project. It is apparent that channel catfish eat many of the threadfin shad and may completely eliminate the population. It is also apparent that threadfin shad do not necessarily spawn in channel catfish ponds, and may need added spawning substrate.

Results at a glance...

- ✰ *Blue-green algae numbers were reduced by threadfin shad over a three-year period in intensively-managed catfish ponds.*

Louisiana State University. Eighteen 0.1-acre earthen ponds at the ARS-LAES were stocked with channel catfish fingerlings in May 2000 at 10,000 fish/acre to study the efficacy of threadfin shad for control of blue-green algae. Juvenile and adult threadfin shad were captured from a local lake for stocking in the experimental ponds, but physiological stress associated with high water temperatures resulted in >90% mortality in the shad. Surviving shad were held in ponds and were to be stocked into catfish ponds in November-early December 2000. However, the study was irreversibly compromised when a flock of 300 white pelicans entered the research facility in December and dramatically reduced catfish and shad populations, thereby requiring repetition of the experiment.

The 18 ponds were re-stocked with catfish fingerlings in April 2001 at 10,000/acre, and six 1-acre ponds were stocked with mixed size classes of channel catfish and channel x blue catfish hybrids at densities of 7,000 fish/acre in summer. Threadfin shad were stocked in half the replicate ponds (0.1 and 1-acre) in November/December 2001 when temperatures were sufficiently cool to safety transport and stock the fish. Heavy monofilament with reflectors was placed over all experimental ponds to minimize depredation by avian predators.

A ¾-acre demonstration-size partitioned aquaculture system (PAS) was stocked with channel catfish, blue catfish, and channel x blue catfish hybrid fingerlings into individual raceways (~5,000 fish/raceway) in June and July 2001, at a collective stocking density of 19,600 fish/acre. Nearly 1,400 Nile tilapia (*Oreochromis nilotica*) weighing 860 pounds (average weight = 0.6 pounds) were stocked for algal control in late May. The presence of tilapia as biological filter-feeders in the open area of the PAS stabilized oxygen concentrations, and odorous species of blue-green algae were rarely observed. Tilapia were not re-stocked into the PAS in 2002, and incidences of low dissolved oxygen episodes and odorous communities of blue-green algal populations occurred with regularity. The absence of the filter-feeding fish appeared to be a major contributing factor to algal community and dissolved oxygen instability.

Three 1-acre ponds at the Aquaculture Research Station were stocked with 7,000 catfish fingerlings in March 2002. One of the 1-acre ponds will be managed with catfish only, one with a combination of catfish and filter-and bottom-feeding fishes (golden shiners, goldfish and freshwater drum and buffalofish), and the third with catfish, filter- and bottom-feeding fishes, and one or more floating aquatic macrophytes for nutrient removal. A combination of golden shiners and goldfish, and freshwater drum and buffalofishes will be stocked in winter when the catfish standing crop exceeds 5,000 pounds/acre and feeding rates exceed 100 pound/acre per day. A process control water quality monitoring system was built to measure oxygen, temperature, pH, nitrite, and ammonia on a continuous basis in the 1-acre ponds. Water samples are being collected from each pond monthly and analyzed for algal species composition, algal density, and off-flavor in catfish. Nutrients, organic matter, and bottom organisms (benthos) will be determined from standardized sampling protocols. Geosmin and MIB in water and fish will be analyzed by the USDA Southern Regional Research Center in New Orleans.

Objective 5. *Evaluate the development of phytoplankton communities in the Partitioned Aquaculture System.*

Clemson University

Results from the1999 Season

The 2-acre commercial scale PAS unit was brought into production in the spring of 1999 (Figure 1).

The unit was stocked with 33,000 catfish fingerlings in May. Stocked fingerlings averaged 15, 31, 48, 61 and 80 g. The catfish were stocked in two raceways consisting of four sections each. In addition, 1,320 pounds of tilapia (15,000 fish at 40 g each) were stocked into the 2-acre system. An additional 216 breeding tilapia (250-300 g) were stocked into the algal basin. The 2-acre unit successfully produced 14,500 pounds/acre of catfish at a carrying capacity of 17,000 pounds/acre. Feed applications reached 260 pounds/acre-day (Table 1). The 2-acre unit was dominated by green algal populations throughout the season and off-flavor in 1999 ranged from 1.0 to 1.5 (out of 5) at the time of harvest. This mild off-flavor was described as being “grassy”.

Figure 1. Overview of 2-acre (above) and six-1/3 acre PAS units at Clemson University.

Table 1. Summary of 1/3 acre PAS performance for growing seasons 1995-2000.

Year	Avg. max. catfish carrying capacity lb/acre	Avg. seasonal catfish yield lbs/acre	Avg. feed application lbs/acre	Avg. max. feed application lbs/acre	Tilapia co-production lbs/acre	Catfish feed conversion ratio
1995	3,258	3,078	20	60	0	1.34
1996	8,811	8,151	60	150	0/805[a]	1.53
1997	14,054	12,965	94	200	1,826	1.41
1998	16,694	14,952	113	210	2,266	1.40
1999	10,479	11,474	100	260	3,843	1.60
2000	14,108	17,232	115	260	5,407	1.50

[a/] In 1996, three of the units contained tilapia (0 lbs/acre production) and three of the units contained tilapia (805 lbs/acre production).

Six 0.33-acre PAS units were stocked with both adult tilapia alone (breeding pairs) and with tilapia fingerlings and adults to see if successful algal species control could be sustained with reducing stocking requirement through the use of breeding pairs. By mid-season, four of the six 0.33-acre PAS units shifted from early blue-green dominance back to populations of more desirable green algae as the tilapia breeding pairs expanded in numbers and weight. In two of the 0.33-acre PAS units, late season algal populations shifted to predominantly blue-green populations suggesting that use of tilapia breeding pairs alone at these feed application rates is close to, or slightly beyond, the limit of blue-green population control.

Stocking density experiments were conducted showing that raceway catfish stocking could be increased from 4-5 pounds/cubic foot to 8-10 pounds/cubic foot with no adverse effect on growth. These results demonstrated that overall system costs can be reduced by using a single high-density raceway and fewer raceway paddlewheels. A preliminary economic analysis projects that 40 acres of PAS units would produce catfish at a 5 to15¢/pound lower cost than conventional pond culture (Table 2). However, this analysis is based on the assumption the net production would exceed 22,000 pounds/acre. Because of loss of winter fish carryover as a result of spring proliferative gill disease (PGD), this potential yield has yet to be realized.

Flow experiments were conducted in 1999 to determine the uniformity of the water velocity field that can be sustained with different combinations of paddles and paddle speeds. The results suggest that sufficient mixing and flow velocity in the algal channel can be maintained with 50% of algal channel width coverage by paddlewheels (Figure 2).

Table 2. Estimated PAS Annual Ownership Costs, Operating Costs, and Unit Costs Comparison Summary (1999).

	160-Acre Conventional Ponds	20, 2-Acre PAS Unit Farm
Annual ownership cost	$ 95,301	$114,181
Annual operating cost	418,325	418,617
Total Annual Cost	513,626	532,798
Total Annual Cost/Acre	3,669	13,319
Annual pounds harvested	700,000	909,840
Ownership cost/pound	0.136	0.125
Operating cost/pound	0.598	0.460
Total Cost/Pound	0.734	0.585
TOTAL REVENUE	$525,000	$681,630

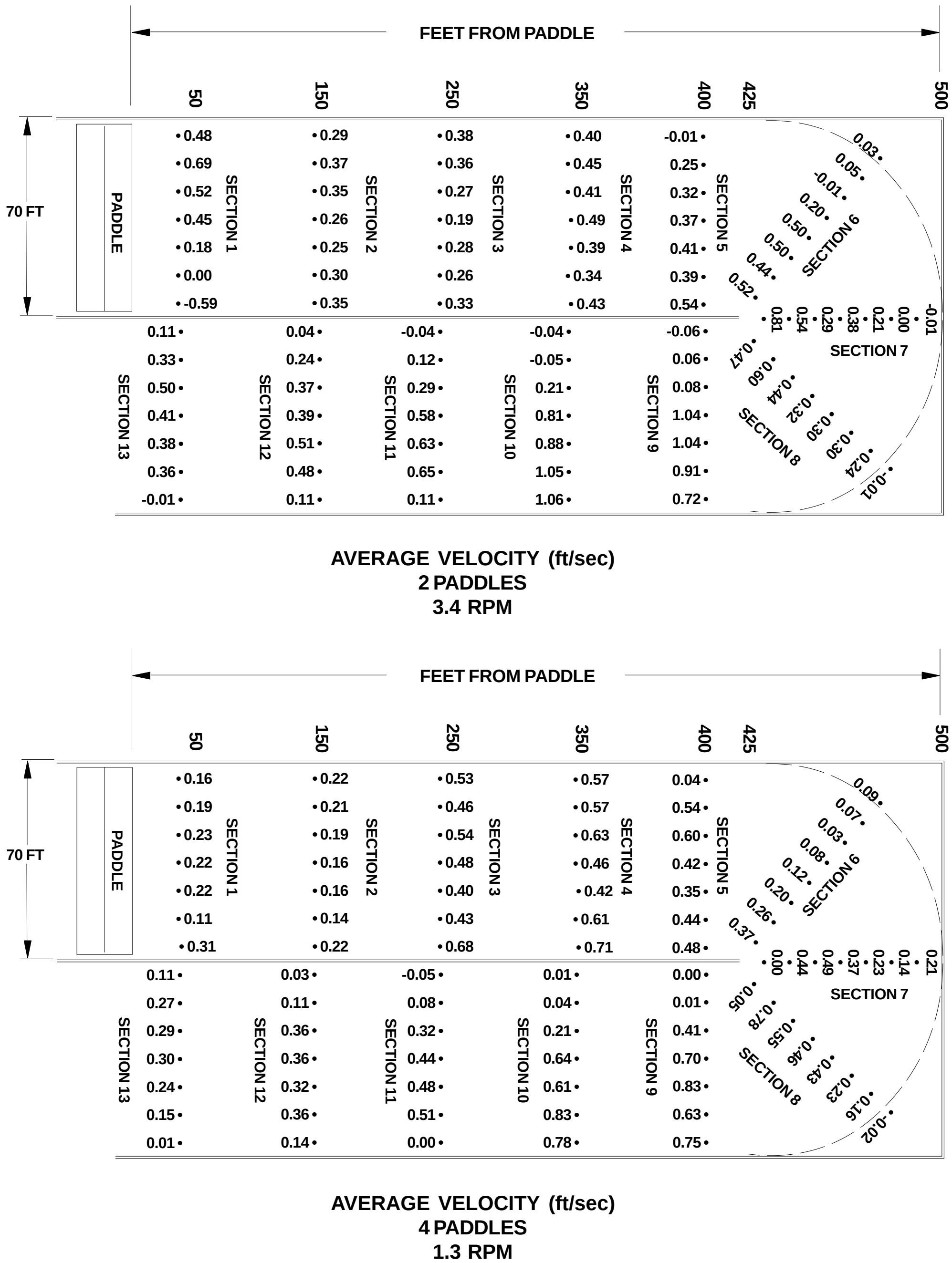

Figure 2. Velocity profiles in 2-acre PAS with 2 paddles and with 4 paddles.

<u>Results from the 2000 Season</u>
In May, the 2-acre PAS unit was stocked with 33,419 catfish fingerlings (2,050 pounds/acre) in size classes of 20, 65, and 88 g. In addition, 110 pounds/acre of tilapia breeding pairs were stocked at large in the algal basin (150 - 250 g males and 200 - 250 g females). The 1999 carryover fish (250 g each) were lost in spring 2000 in the 2-acre unit due to proliferative gill disease (PGD). Treatment of pond sediment with hydrated lime was observed to be effective in reducing the occurrence of PGD, although treatment was initiated too late to be effective.

The use of "breeding pairs" of tilapia alone in the 2-acre PAS produced marginal water quality results. By the third week of August, the phytoplankton community was completely dominated by blue-green algae. However, at the end of August, communities were undergoing a shift to green algal populations, an apparent result of increased filter-feeding activity of the expanding tilapia population.

In contrast, four 1/36-acre PAS units operating in August of 2,000 at 12,000 pounds/acre catfish carrying capacity, with 1,600 pounds/acre of native mussel in two units and 2,300 pounds/acre of silver carp in two units, exhibited a dominance of green algal populations. However, the mussels were not as effective as silver carp or tilapia at controlling blue-green algal populations and, as a result, the visual color difference between the mussel and silver carp unit was obvious (Figure 3). In August 2000 the 0.33-acre units shifted to green algal populations using tilapia breeding pairs.

Figure 3. Visual algal differences between mussel PAS unit (right) and silver carp PAS unit (left).

In Spring 2000, the net production for this season reached a 6-year average high in the six 0.33-acre units of 17,232 pounds/acre of catfish with 5,407 pounds/acre of tilapia. Average feeding rates reached 115 pounds/acre per day, with a maximum feed application of 260 pound/acre per day (Table 1). Because of excessive over-winter losses, the 2-acre prototype only produced 12,480 pounds/acre of catfish at the end of 2000.

Results from 2001 Season

In Spring 2001, the six 0.33-acre PAS units were stocked with an average of 2,931 carryover catfish (mean weight = 394 g), 3,484 large catfish fingerlings (mean weight = 71 g), and 2,092 small catfish fingerlings (mean weight = 26 g). The six 0.33-acre units were also stocked with 25 male tilapia (160 g) and 50 female tilapia (76 g). The 2-acre prototype PAS unit was stocked with 10,500 carryover catfish (318 g) along with 8,734 (132 g), 9,872 (114 g), and 11,159 (21 g) catfish fingerlings. The 2.0-acre system was also stocked with 450 breeding pairs of tilapia. Prophylactic treatments for control of early spring PGD in the winter carryover fish were conducted in winter/spring of 2000. The incidence of PGD was significantly reduced in the carryover fish in 2001. By the end of July of 2001, the 2-acre prototype system shifted to predominantly green algal population (Figure 4). Seasonal average net catfish production for 2001 averaged 16,735 pounds/acre with 2,375 pounds/acre of tilapia co-production at maximum feed application rates of 240 pounds/acre.

Summary of 3 Years of PAS Studies

Three years of catfish/tilapia co-culture in a single commercial scale 2-acre PAS unit and six 1/3-acre PAS units have demonstrated the capacity of the PAS technique for reduction of blue-green algal dominance within the culture systems.

From 1999 to 2001 annual catfish production ranged from 11,474 pounds/acre to 17,232 pounds/acre with a tilapia co-production ranging from 2,375 to 5,407 pounds/acre (Figure 5). Maximum feed application rates exceeded 250 pounds/acre per day with average application rates of 100-130 pounds/acre per day.

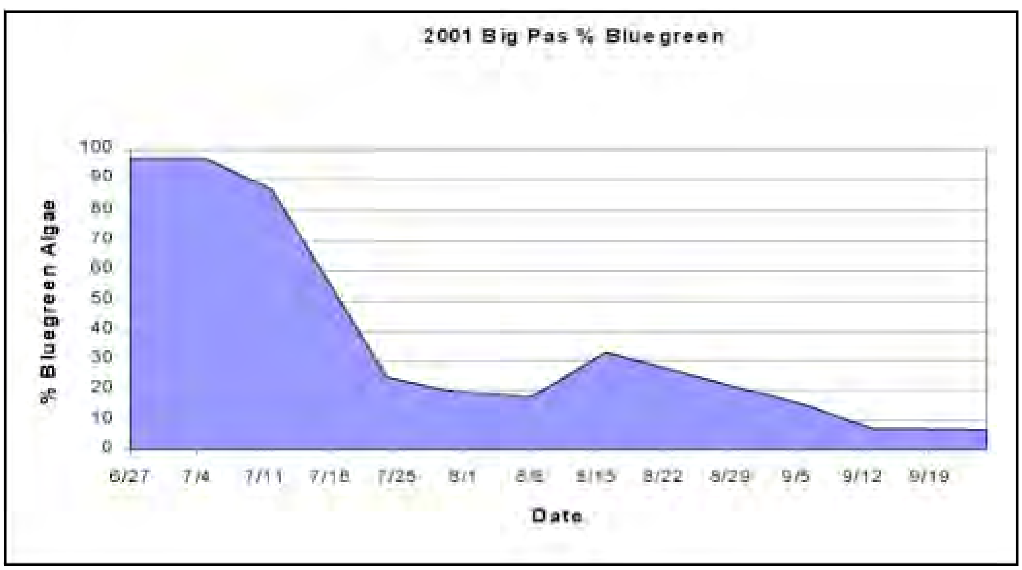

Figure 4. Blue-green algae occurrence in 2-acre PAS prototype in 2001.

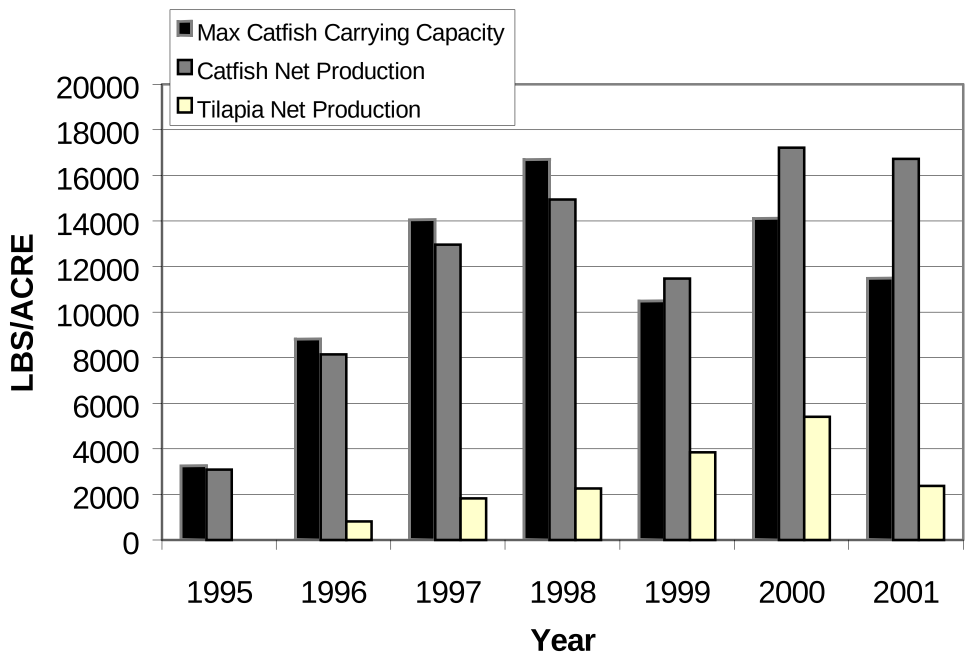

Figure 5. Six 1/3-acre PAS catfish production and maximum carrying capacities and tilapia production from 1995 to 2001.

Previous experimental trials (in 1997) had shown blue-green algal dominance could be eliminated from the PAS water column throughout the growing season by stocking the unit with large numbers of tilapia early in the season. However it was felt that this requirement may not be practical for most fish farmers. For this reason, all experimental PAS trials since 1998 have employed the technique of stocking with tilapia "breeding pairs" early in the season (typically, 150 adult females and 75 adult males/acre).The major advantage of this technique lies in the small number of tilapia needed in early spring. The disadvantage of this technique is in the overall reduction in system water quality control, particularly during mid-season. In 1997, full tilapia stocking resulted in system total ammonia-nitrogen (TAN) levels of 1-4 ppm at maximum feed application rates of 200 pounds/acre per day. As feed application rates were progressively increased (using the tilapia breeding pair stocking technique), system TAN ranged from 0.3-8 ppm (1998) to 1-12 ppm by 2001 season. Blue-green dominance is typically reduced to less than 30% of total algal population by the end of the season (with a reduction in associated off flavor). Fortunately, a harvest window of reduced blue-green occurrence is typically observed in July (at the time of the carryover fish harvest, Figure 6). This is the result of lower water temperatures and lower feed application rates at this time. As the season progresses, increased feed applications and increased water temperatures lead to a dominance by blue-green algae by the end of July to early August. As tilapia numbers and biomass expand at the end of August, blue-green numbers are again seen to decline (Figure 6).

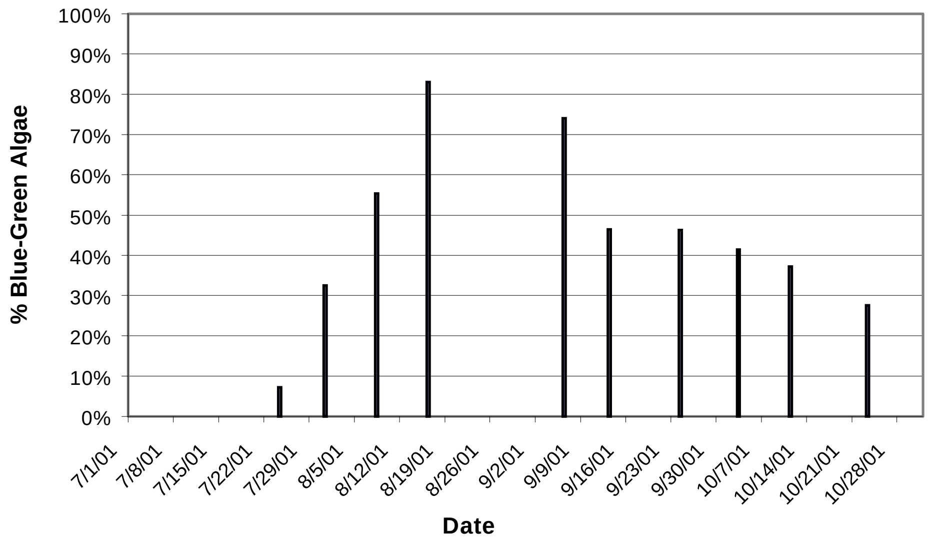

Figure 6. Typical blue-green algae abundance (as % of total) in PAS population in 2001 using tilapia "breeding pairs".

As of September 2002, the performance of the PAS units is typical of that observed in the previous three years; catfish carrying capacity has passed 10,000 pounds/acre and feed application rates exceed 250 pounds/acre per day. System pH has declined from a high of 10 early in May to typical values of 7.0-7.8 by September 10 as increased organic carbon addition and respiration rates increase CO_2 supply to the PAS water columns. The increase in tilapia numbers impacts the water quality of the system dramatically. From late July to early September 2002, system TAN levels drop from 5-8 ppm to 4-6 ppm (with very low free NH_3 at pH of 7.0-7.8). The driving force for the reduction in TAN is the much improved reduction in algal standing crop reflected by the increase in Secchi disk transparency from 6-8 cm to 12-16 cm being driven by the expanding tilapia numbers and biomass. In June 2002, seasonal blue-green algal dominance in the six 1/3-acre units peaked at 82% of total algal population; however, by September blue-green population were less than 1% of total. As of mid-September, the 2-acre unit still consisted of 41% blue-green (of total algal population).The 2-acre unit was typically observed to "lag" behind the 1/3-acre units in Secchi disk transparency and algal species control, suggesting that the larger PAS units may need more tilapia breeding pairs per acre than the small units to ensure adequate tilapia numbers to control

Results at a glance...

- *Early spring stocking of only 150-200 tilapia "breeding pairs" per acre into PAS units allows for a minimum cost control of late season blue-green algal dominance allowing for catfish harvest during a "window" of reduced fish off-flavor. This technique promotes acceptable PAS water quality limits supporting observed net catfish productions in 1999-2001 of 11,500 to 17,200 pounds /acre with a tilapia co-production of 2,400 to 5,400 pounds/acre.*

WORK PLANNED

Much of the proposed research is completed; the remaining work as noted below is proceeding on schedule and no major changes in the work plan have occurred.

Auburn University. All pond and laboratory studies have been completed, but we still are analyzing data collected during the project and will prepare two additional manuscripts.

University of Georgia. Planktivorous fish have not yet been examined for digestive tract contents. Further publications will be developed when those data are collected. A follow-up study was begun to compare threadfin shad stocked together with fathead minnows in catfish ponds with copper sulfate application for control of blue-green algae.

Mississippi State University/USDA-ARS. A request for biopesticide classification/ registration of the proprietary compound currently undergoing patent application for use as a selective algicide in catfish aquaculture will be made to the U.S. Environmental Protection Agency.

North Carolina State University. Additional studies of pond water circulation using gypsum blocks are planned as described in the proposed project objectives. A comprehensive analysis of the phytoplankton composition and nutrient concentrations of the ponds in the current study will be finished by the end of this year.

IMPACTS

Selected plant and algae extracts have shown strong anti-cyanobacterial activity. The findings indicate that natural products (small biologically active organic compounds) produced by organisms that live and compete in cyanobacteria-rich environments are a valuable source of new cyanobacterium-selective algicides and may be of use in the control of blue-green algae in aquaculture ponds. The occurrence of both noxious and toxic blue-green algal blooms in fish aquaculture is a worldwide problem. The idea that chemicals in trees (and marine algae) may have the ability to control blue-green algal blooms is intriguing, especially in extremely poor regions (such as developing countries) where one could envision harvesting these natural materials for use in aquaculture ponds. A rather unexpected outcome of this research is the discovery of several new substances with herbicidal activity. As a result of natural products screening efforts, extracts from several plants and marine organisms were found to be selectively toxic only to the green alga control (*S. capricornutum*) and to specific types of higher plants. Some of the chemical constituents of these anti-algal/herbicidal extracts may hold promise as new natural product-based means of agricultural weed control.

A proprietary natural-based compound derived from certain plants is undergoing patent application for use as a selective algicide in catfish aquaculture. This proprietary compound may eventually be used as an alternative to the synthetic algicidal compounds (e.g., diuron and copper sulfate) that catfish producers are currently using to help prevent musty off-flavor in farm-raised catfish.

Results of studies of baitfish ponds have increased our understanding of water quality and pond bottom sediments in this important sector of aquaculture in the southeast. Despite a history of long-term use of granular fertilizer in study ponds, sediment-bound phosphorus did not appear to exert a discernible effect on water quality under conditions of commercial production. Discovery of high soil sulfate sulfur levels in pond bottom samples contributed to affected baitfish farmers changing pond management practices to reduce chances of hydrogen sulfide problems.

Research at Auburn University showed that EDTA is an excellent chelating agent for iron (and presumably other metals) for use in pond aquaculture. Application of potassium chloride, legume hay, and iron and other trace elements to ponds do not appear to be useful for controlling blue-green algae in ponds. Sodium nitrate is a good nitrogen fertilizer for ponds; it has been shown to be environmentally superior to ammonium-based nitrogen fertilizers for use in aquaculture and sportfish ponds. Nitrate is not acid-forming, does not exert an oxygen demand, and its presence in effluents is less objectionable than ammonia. Pond fertilization work also revealed that nitrogen fertilization was not needed in bait minnow ponds at Auburn University, but older (25 years or more) sunfish ponds may benefit from nitrogen fertilization. All ponds needed phosphorus fertilization. Sediment removal seems to be the only feasible way of improving soil quality in old bait minnow ponds.

Research at Mississippi State University showed that nutrient manipulation techniques consisting of simultaneous adjustment of N:P ratios and addition of chelated iron do not affect phytoplankton community structure. Results from mixing studies suggest that some threshold level of turbulent mixing is necessary to overcome light limitation of phytoplankton production and to cause a shift of phytoplankton community composition from dominance by cyanobacteria. Application of turbulent mixing should attempt to develop a uniform flow field to avoid areas of concentrated turbulence that can suspend pond soils.

Research conducted at NCSU demonstrated no benefit from enhanced water circulation in hybrid striped bass ponds. Water circulation provided no improvement in water quality or fish production and did not affect the phytoplankton composition of the pond. Manipulation of micro-nutrient and carbon availability (through additions to the pond water of chelated iron, mineral premix, and organic matter) did not reduce blue-green algae abundance or change the phytoplankton community composition in hybrid striped bass ponds.

Shad stocking is being considered by catfish farmers in Georgia who had off-flavor catfish and who could not utilize herbicides for control of blue-green algae. Shad stocking started in the winter of 2000 and spring of 2001 in Georgia. Shad stocking is limited by the availability of threadfin shad. Information from this study has helped with the fathead minnow/channel catfish stocking program for proliferative gill disease control. Behavior of fathead minnows in channel catfish ponds indicated a need to encourage spawning by adding spawning substrate or to restock the fathead minnows at regular intervals in order to maintain at least 1,500 minnows/acre.

The Partitioned Aquaculture System (PAS) design and associated fish culture and management techniques provide a method to quadruple current fish production from a system which can eliminate blue-green algal dominance sufficiently to provide a "window of time" for fish harvest during a period of reduced fish off-flavor occurrence. The PAS also permits the elimination of nitrogen and phosphorus discharges from aquaculture production systems, which currently pose a potential eutrophication threat to surface and groundwater supplies. Economic projections suggest that PAS catfish production costs are 15 to 17 cents/pounds lower than conventional pond production costs providing strong motivation for widespread adaptation of PAS fish culture as a better management practice for the aquaculture industry.

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