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Early Detection of *Edwardsiella ictaluri* in NWAC103 Channel Catfish Fingerlings

Lanie Bilodeau, David Wise, and Bill Wolters

Enteric septicemia of catfish (ESC), caused by the bacterium *Edwardsiella ictaluri*, is the most prevalent disease affecting farm-raised channel catfish and is responsible for up to 50% of total losses per year costing farmers millions of dollars. ESC outbreaks occur primarily in fall and spring when water temperatures are between 22°C and 28°C. Current management practices include fry vaccination, reducing feed at times of likely ESC outbreak, and offering feed with Romet® and/or Terramycin®. These methods have varying degrees of success based on the severity of the ESC outbreak and timing of treatment.

Diagnostic methods currently used for ESC are time consuming and unreliable at low levels of infection, when successful treatment is most likely. A DNA-based assay has been developed for rapid and reliable detection of *E. ictaluri* in blood and other tissue samples. This assay enables detection of as few as the equivalent of 2.5 cells of *E. ictaluri* in less time and with more accuracy than traditional bacterial culture. The assay takes approximately 3 hours to run from the time of sample collection.

This assay may allow diagnostic labs to provide farmers with a means for early detection of ESC outbreaks. If *E. ictaluri* infection is detected prior to the appearance of physical signs of ESC in fish, effective management strategies can be applied that may improve survival. In order for the assay to be utilized effectively for early detection of ESC in commercial catfish ponds, a strategy of routine sampling will be necessary for accurate assessment of the health status of the population.

The feasibility of sampling a small proportion of fish in a pond and detecting changes in concentrations of *E. ictaluri* was tested. NWAC103 channel catfish were collected from six fingerling ponds stocked at 120,000 fish per acre at the USDA Catfish Genetics Research Unit to track changes in the amount of *E. ictaluri* present in blood samples over a three week period. Each week 10 fish were selected randomly from each pond. A blood sample from each fish was tested with the ESC detection assay to determine the quantity of *E. ictaluri* DNA (expressed as cell-equivalents) present.

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Early Detection of *Edwardsiella ictaluri*
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Water temperatures dropped below the ideal temperature range for *E. ictaluri* growth from September 24 through September 27 (Figure 1). At this time, *E. ictaluri* concentrations decreased in fish from pond 154 and remained undetectable in the remaining five ponds (Figure 1). There was an increase in bacterial levels during week 3 of the trial (October 3) in ponds B5, 260, and 317 (Figure 1). Bacterial levels continued to decrease in pond 154. Water temperatures remained below 18°C after October 14, 2003 at which time sampling ended.

Although no mortalities were reported throughout the entire testing period, high mortalities had been reported for pond 154 in August 2002 and fish collected on September 19 were symptomatic for ESC, based on external signs of the disease. Fish collected in all subsequent samples showed no signs of ESC infection, including those in which low levels of *E. ictaluri* DNA was measured with the genetic assay. While this data is preliminary, it shows that the presence of *E. ictaluri* and changes in concentrations of *E. ictaluri* can be detected with the ESC genetic detection assay.

This research will be expanded in Spring and Fall 2003 to further monitor NWAC103 ponds at the USDA Catfish Genetics Research Unit and determine the utility of this assay in disease management of genetically improved catfish lines released to commercial producers.

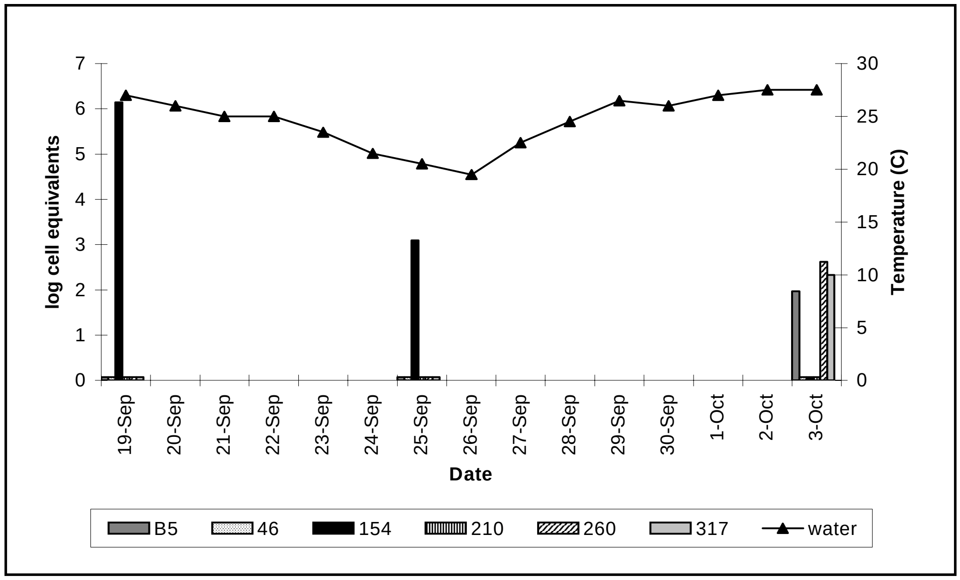

Figure 1. Log values of *Edwardsiella ictaluri* cell-equivalents detected in six ponds with ESC genetic detection assay and average water temperature (°C) throughout testing period.

Florfenicol Antibiotic Studies Conducted

Pat Gaunt

Florfenicol (FFC) is an antibiotic developed by Schering-Plough Animal Health Corporation (SPAH) (Union, New Jersey) and approved for use in beef cattle and swine in the U.S. Internationally, FFC is approved for use in aquaculture under the trade name of Aquaflor® for control of susceptible bacterial disease in Japan (yellowtail, red sea bream, coho salmon, horse mackerel, rainbow trout), South Korea (yellowtail, eel), Norway (salmon), Canada (salmon), and the United Kingdom (salmon). SPAH's desire to gain approval for this aquaculture product in the U.S. brought the company to the NWAC for research trials. SPAH asked Mississippi State University College of Veterinary Medicine (MSU-CVM) to assess the efficacy of FFC against *Edwardsiella ictaluri* (*E. ictaluri*), the causative agent of enteric septicemia of catfish (ESC). The tests began with an *in vitro* study and advanced to aquarium and field trials.

The *in vitro* study involved laboratory work in which MSU-CVM took 12 bacterial isolates from cases submitted to the Fish Diagnostic Laboratory and positively identified them as *E. ictaluri* based on biochemical tests. All isolates were found to be sensitive to FFC as determined by Kirby Bauer zones of inhibition. The Minimum Inhibitory Concentration (MIC) of FFC against *E. ictaluri* was determined to be 0.25 mg/mL. This value is important in determining the concentration of the drug that must be attained in the body to fight bacteria, and the low value obtained in the study indicated that the organism was very sensitive to FFC. The FFC values for most fish pathogens range from 0.3-1.6 mg/mL.

Since results from the laboratory study were so promising, MSU-CVM proceeded with testing the efficacy of FFC against *E. ictaluri* in aquarium studies. Three separate efficacy trials

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New Publications Produced by the Southern Regional Aquaculture Center

Craig Tucker and Sarah Harris
Southern Regional Aquaculture Center

When the Regional Aquaculture Centers were established in the late 1980s, one of the mandates from Congress was to develop and disseminate information needed to solve problems in the aquaculture industry. The Southern Regional Aquaculture Center (SRAC) is effective at meeting that goal because it provides a unique mechanism for assessing regional needs and establishing priorities for developing educational materials.

The efficiency and effectiveness of the SRAC "Publications Project" is rooted in the benefits of having access to a region-wide pool of experts to develop educational materials and then allowing the Center to cover the cost of development and initial publication. This process assures that the best available talent is brought to bear on each subject, it insures against duplication of effort among states, and it makes efficient use of personnel and funds at the State level.

To facilitate development of educational materials for the Southern Region, SRAC established the "Publications, Videos and Computer Software" project to assess and prioritize publication needs and develop appropriate publications to meet those needs. This project, which is under the leadership of Dr. Michael Masser at Texas A&M University, has become one of our most successful activities.

Since the initiation of this project, SRAC has published 154 fact sheets, 16 research summaries and 19 videos. More than 121 scientists have contributed to SRAC publications, with representation from all 13 states and 2 territories in the Southern Region. Each publication is thoroughly reviewed by producers, administrators, and scientists for accuracy and style before publication.

For information on all SRAC Projects, visit our website at:

http://www.msstate.edu/dept/srac

In addition to the wide variety of information offered, you can print copies of all SRAC publications, obtain the address of your state Aquaculture Extension Specialist, and link to many other useful aquaculture sites.

During the past two years, research and extension scientists from the following institutions and agencies have contributed to SRAC publications: Auburn University, Clemson University, Kentucky State University, Louisiana State University, LSU Sea Grant Program, Mississippi State University, North Carolina State University, Purdue University, Texas A&M University, and University of Arkansas at Pine Bluff.

You will find information on just about any topic you can imagine from the list of publications available from SRAC.

To give you some idea of the variety of material available, here are some recent titles:

- Toxicities of Agricultural Pesticides to Selected Aquatic Organisms
- Measuring Dissolved Oxygen in Catfish Ponds
- Cultivating Eastern Oysters
- Water Gardens
- Watershed Fish Production Ponds: Guide to Site Selection and Construction
- Processing Channel Catfish
- Baitfish: Feeds and Feeding Practices
- Artemia Production for Marine Larval Fish Culture
- Bighead Carp
- Mutton Snapper
- Southern Flounder
- Sturgeon

All SRAC Fact Sheets, as well as a variety of other printed materials, are readily available. If you have Internet access, the easiest way to obtain SRAC publications is to visit the SRAC website (see the box above) and browse the list of publications. When you find the publication of interest, simply click on the title and print. If you do not have access to the Internet, copies of SRAC publications can be obtained from Dr. Jimmy Avery, Aquaculture Extension Professor at the NWAC or your local Aquaculture Extension Specialist.

Diuron Residues in Catfish Exposed in Consecutive Years

Craig Tucker, Susan Kingsbury, and Reba Ingram

Most off-flavors in pond-raised catfish are caused by earthy-musty chemicals produced by blue-green algae. Experience has shown that blue-green algae are difficult to eradicate from catfish ponds without using chemical algicides.

Until 1999, copper-based products were the only algicides labeled for use in catfish ponds. Although copper algicides can be effective at controlling blue-green algae, obtaining consistent results is a hit-or-miss proposition because copper interacts strongly with certain water quality variables.

In spring of 1999, the United States Environmental Protection Agency (EPA) granted an emergency exemption under Section 18 of the Federal Insecticide, Fungicide, and Rodenticide Act authorizing the use of diuron to manage blue-green off-flavors in catfish. Diuron is a good candidate for off-flavor management because it is inexpensive, easy to apply, and works consistently across a wide range of environmental conditions. The major problem with diuron is its tendency to accumulate in fish living in treated waters. In fact, the label directions for using diuron in ponds—which calls for no more than nine weekly applications of 10 parts per billion (ppb) of diuron in a year—was developed to assure that residues in catfish fillets stayed below the tolerance level of 2.0 parts per million (ppm) established by EPA.

EPA established the tissue tolerance level from data obtained in pond studies conducted by scientists at Mississippi State University. In those studies, catfish were exposed to diuron over a 90-day period and diuron residues in fish fillets were measured during and after the treatment period. That study was based on the premise that catfish would be exposed to only one set of diuron applications before harvest. However, in the "topping" growout system used by most catfish farmers, fish may remain in ponds for more than one growing season and may be exposed to more than one series of diuron applications. We therefore conducted a study to determine whether diuron residues in fish carry over from one year to the next and, if so, whether the cumulative exposure resulted in residues exceeding the tolerance level.

We stocked three ponds with fish previously unexposed to diuron. We then treated each pond in the late fall using the full course of nine weekly treatments of 10 ppb diuron. We retreated the ponds early in the following spring with another full course of nine diuron treatments. The timing of the two diuron treatment series was intentionally chosen to be a "worst-case scenario" that minimized the time between the end of the first treatment series and the beginning of the second. This treatment schedule should maximize the opportunity for carryover of residues from one treatment series to the next. Samples of catfish were collected before and after each treatment series, and sent to the Mississippi State Chemical Laboratory for analysis of diuron levels in fillets.

No diuron was detected in catfish fillets prior to the initial diuron treatment series. Immediately after the ninth weekly diuron application in the fall, diuron levels in fillets averaged 0.35 ppm, with the highest level in an individual fillet being 0.72 ppm. Even the highest value was well below the 2.0 ppm tolerance level set by EPA.

On the day before the second series of diuron treatments the following spring, diuron concentrations had fallen to undetectable levels in fillets from all fish sampled. Immediately after the spring treatment series, tissue diuron levels averaged 0.13 ppm, and the highest individual value was 0.191 ppm—approximately a tenth of the EPA tolerance level.

In summary, diuron residue levels in fillets remained well below 1 ppm after nine consecutive weekly treatments with 10 ppb diuron. Diuron levels in fillets became undetectable within 2 to 4 months after the final application in the fall and there was no carryover of residues in fish from one year to the next. Exposure of fish to diuron at the label-specified rate in consecutive years will not cause residues in fillets to exceed the 2.0 ppm tolerance level established for the chemical in catfish fillets. Under no circumstances, however, should the annual treatment rate on the label be exceeded.

Comparison of Two-phase with Traditional Three-phase Production of Hybrid Striped Bass in Earthen Ponds

Louis R. D'Abramo

A three-phase growout process is commonly used in the commercial production of hybrid striped bass in earthen ponds. Phase I fingerlings, 2.2 to 3.3 lb/1000, are stocked into phase II ponds at a density of approximately 12,000/acre. Harvest of fish (2.2 to 4.5/lb) from phase II ponds is followed by size grading that precedes stocking into ponds (3,500/acre) for the final growout phase. Size grading is designed to reduce the degree of variation in harvest weight after the final stage of growout has been completed and may result in significant mortality. The prospect of completely bypassing phase II of growout in favor of a direct stocking of graded, phase I juveniles into ponds has been investigated for the past four years. Fish larger than those traditionally stocked into phase II growout ponds would be used in this direct stocking procedure.

This management practice would offer many advantages that can translate into a more efficient enterprise. Mortality that often arises from the inability of smaller fish to transfer from a live to a formulated diet in phase II ponds after stocking would be minimized. Larger juveniles would already be on a formulated feed before stocking into the final growout ponds. In addition, stocking larger fish would reduce the total growout time necessary to produce an acceptable market size.

Recent experiments at the Eastern Unit of the NWAC have demonstrated that this proposed management practice potentially has application and is economically practical. Juveniles harvested from phase I of growout were graded to produce two relatively uniform individual weight classes, 6.6 lb/1000 (3 g each) and 11.0 lb/1000 (5 g each). These fish were then stocked at either 3,500/acre or 4,500/acre into ponds that represented the second (final) stage of growout in the direct stock procedure. After stocking, the fish were fed commercially manufactured > 40% crude protein feeds daily to satiation for 17 months. At harvest, survival of fish in all ponds ranged from 51 to 99%, but was consistently highest in ponds stocked with 5-g juveniles (83% versus 70%). Average production for the 3,500/acre and 4,500/acre stocking densities, 5-g stocking weight, was 4,020 and 4,952 lb/acre, respectively.

A comprehensive economic analysis revealed that the treatment consisting of a 4,500/acre stocking density and 5-g stocking weight yielded the highest net return. Variable costs increase due to the higher purchase costs of larger juveniles to stock. However, this cost is of little consequence when compared to costs incurred from mortality and the labor to harvest fish from phase II ponds and to grade them for stocking into phase III ponds.

Of the total population of fish harvested from ponds stocked with 5-g juveniles, 18 to 30% weighed more than 1.25 lb, the size generally in highest demand. A 4 to 5 month extension of this final stage of production, equivalent to the total combined time commonly needed for traditional second and third phases of production, would probably have yielded 50% of all harvested fish being market size. For the 17-month experimental growout period, economic analysis indicated that the direct stock, two-phase growout management practice is more profitable than the traditional three-phase growout.

This successful experimental demonstration of the direct stocking procedure for commercial production of hybrid striped bass lays the foundation for future investigations. For example, using lower stocking densities and a stocking weight even greater than 5 g should reduce the time to market size to approximately 12 or 13 months, rather than the current combined 20 to 24 months for phases II and III. Despite the lower overall production per pond resulting from lower stocking densities, more crops can be harvested per unit of time, and significantly higher returns over time would eventually be realized. In addition, if the time to harvest is reduced, then the risk of crop loss due to disease or adverse environmental conditions is correspondingly reduced.

Scaling up the direct stocking procedure from small experimental ponds to commercial ponds poses a management problem. It may be difficult for fish stocked at low densities into ponds of 5 to 10 acres to encounter a sufficient amount of formulated diet for maximum growth. Slower growth would then be expected and a greater variation in the individual size of fish within the pond population would probably develop. A seemingly

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Treating Channel Catfish Eggs with Hydrogen Peroxide Can Improve Hatching Success

Brian Small

Fungal and bacterial egg infections remain a significant problem for hatcheries, reducing hatching success. Chemical therapeutics such as povidone iodine and formalin are often used to control potential infections. Povidone iodine is designated by the U.S. Food and Drug Administration (FDA) as a low regulatory priority (LRP) aquaculture drug when used as an egg surface disinfectant, and formalin is approved by the FDA for the control of fungi of the family *Saprolegniaceae* on the eggs of all finfish. Although formalin is an effective therapeutic, concerns of safety exist among users due to its suspected carcinogenicity and strong odor. Last year, we reported on the successful use of daily hydrogen peroxide baths to improve catfish hatching success (NWAC NEWS, July 2001). Here we present further research on the use of hydrogen peroxide for improving catfish hatching success, and suggest methods for commercial use.

Compared to a one-time bath with povidone iodine (100 ppm) for 10 minutes and daily 15 minute formalin baths (1,600 ppm), hatching success of channel catfish eggs was significantly improved with daily hydrogen peroxide treatment as a 15 minute 250 ppm bath. Using this procedure, hatching success increased between 26 and 40%, with an average 31% improvement. The highest concentration of hydrogen peroxide tested (500 ppm) resulted in a significant decrease in hatching success as a result of chorionic deterioration causing the embryos to be prematurely released into the water column. Although formalin has been shown to be effective as a bath treatment for controlling *Saprolegnia* infections on catfish eggs, treatment with formalin in this study had no impact on hatching success relative to the controls, perhaps due to pretreatment with povidone iodine.

Incubation troughs in commercial catfish hatcheries commonly contain about 100 gallons of water and average one complete water exchange in 45 to 60 minutes. In commercial hatcheries, turning off the water for a 15 minute therapeutic treatment everyday can be a substantial risk, with millions of eggs being lost in the event the water flow is not restored. As an alternative, we sought to identify a concentration of hydrogen peroxide which would provide a beneficial treatment in a continuous-flow situation. Eggs were treated with 0, 35, 70,100, 200, and 300 ppm daily until eyed (Figure 1). Hydrogen peroxide was added all at once at the water inlet side of the trough. The 70 ppm treatment yielded the highest hatching success. Premature hatching was observed in both the 200 and 300 ppm treatments. Available literature for other fishes suggests that hydrogen peroxide treatment beyond embryo eye pigmentation (eyed stage) does not have a detrimental effect on hatching success; however, this has not yet been verified with channel catfish eggs in our laboratory.

Our experiments were conducted in a 100 gallon trough with a water flow of 2 gpm. If the water flow is lower or higher, the amount of hydrogen peroxide needed to acheive the same effective concentration will be different.Recommended hydrogen peroxide flow-through treatments for 100 gallon hatching troughs with different water flows are presented in Table 1.

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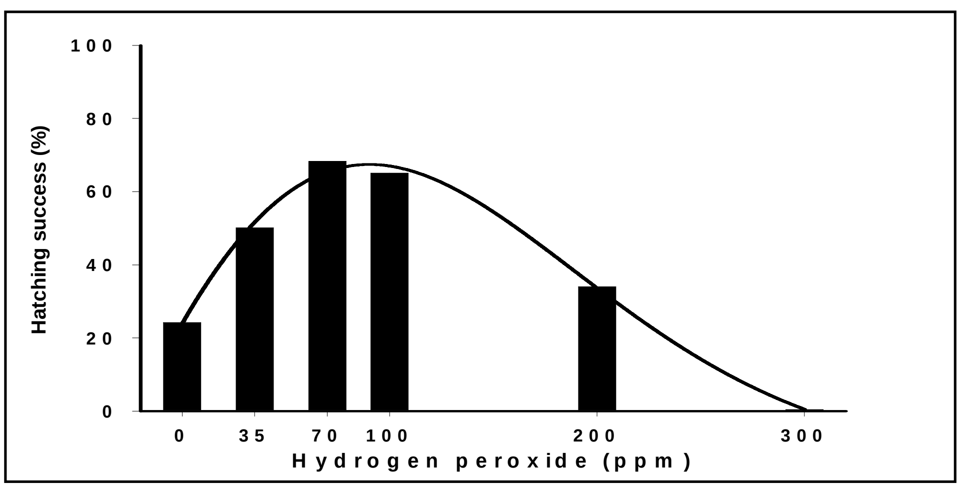

Figure 1. Average hatching success for channel catfish eggs treated with increasing concentrations of hydrogen peroxide in a 100 gallon hatching trough with continuous water turnover (2 gpm).

Alternatively, you can calculate the required hydrogen peroxide treatment for 100 gallon troughs using these formulae:

- milliliters H_20_2 = (GPM flow) x (1250) / (% H_20_2);
  ex. (2.0) x (1250) / 50 = 50 mL
- fluid ounces H_20_2 = (GPM flow) x (43.9) / (% H_20_2);
  ex. (2.0) x (43.9) / 30 = 2.9 oz.

The cost of treating 100 gallons of hatchery water with 70 ppm hydrogen peroxide is about $0.08/day (based on $300/55 gallon of 50% H_20_2).

The U.S. FDA has approved the use of hydrogen peroxide treatment to control infections on fish eggs at levels up to 500 ppm through a low-regulatory ruling. In our studies, treating eggs in a 100 gallon trough having a 2 gpm flow with 70 ppm hydrogen peroxide was found to increase hatching success by approximately 180% above untreated controls. Considering that annual catfish fry and fingerling production is approximately 1.6 billion, this could mean an increase of several hundred million fry and fingerlings annually.

Table 1. Hydrogen peroxide (H_20_2) treatment for channel catfish eggs in a hatching trough containing 100 gallons of water.

Water flow (GPM)	Milliliters (fluid ounces) 50% H_20_2	30% H_20_2
1.0	25 (0.9)	42 (1.5)
2.0	50 (1.8)	83 (2.9)
3.0	75 (2.6)	125 (4.4)
4.0	100 (3.5)	167 (5.9)
5.0	150 (4.4)	208 (7.3)
6.0	200 (5.3)	250 (8.8)

Florfenicol Antibiotic Studies Conducted

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were conducted testing dosages ranging from 10 to 40 mg FFC/kg body weight for 5 days in the range-finding study to 5 to 15 mg FFC/kg body weight for 10 days in the dose-titration study. From the results of these two preliminary studies, a dose of 10 mg FFC/kg body weight for 10 days was chosen for the dose-confirmation study. Fish from the FFC-treated tanks showed statistically significant differences from fish in the untreated tanks.

In addition, a tolerance trial to assess the palatability and toxicosis of the FFC-medicated feed was conducted with dose rates ranging from 0 to 100 mg FFC/kg body weight. There were no statistical differences in palatability between untreated feed and FFC-medicated feed. In addition, histopathology performed on all fish in this experiment showed that there were no treatment-related effects.

The dose rate of 10 mg FFC/kg body weight for 10 days was established from the dose-confirmation study as the effective dose for the field study.

The field trial was conducted by MSU-CVM at Delta Western Research Center (Indianola, MS) in fourteen, 0.1-acre ponds. One of the ponds experienced a natural outbreak with *E. ictaluri*. The fish in the remaining ponds were infected with an inoculum from this pond according to protocol. Treatment of the fish with medicated feed began after mortalities from ESC reached 0.3% per pond. Fish in each pond were fed either control or medicated feed for 10 days. The treatment period was followed by 14 days of unmedicated feed then termination of the trial. Samples of fish from each pond were counted and weighed to get an estimate of the number of fish per pound. Then the fish from each pond were seined, weighed, and an estimate of the number of survivors was made. Bacteria from dead fish were cultured and the carcasses were evaluated for clinical lesions of ESC. Each pond was evaluated for differences in the numbers of surviving fish. FFC-treated fish showed a statistically significant increase in survival.

In a separate residue-depletion study, a 0.1-acre pond at Delta Western Research Center was stocked with food size fish and medicated with 10 mg FFC/kg body weight for 10 days. Twenty fish were sampled from the pond at 1, 2, 4, 7, 14, and 21 days post-treatment, and fillets were submitted for FFC analysis. FFC metabolites were below the maximum residue limits by day 7.

SPAH took the reports from these studies together with other documents and submitted them to the U.S. Food and Drug Administration in August 2002 in an application for approval of FFC for use in aquaculture in the US. These studies are currently under the review process.

In summary, these studies conducted by the team of scientists at MSU-CVM demonstrated that FFC could be incorporated into a commercially acceptable catfish feed that is safe, palatable, and efficacious in preventing mortality from ESC. If approved, this antibiotic will be an important addition to the limited number of pharmaceuticals and therapeutics currently available to the aquaculture industry.

Zooplankton Nutritional Value: Nursery Pond Fertilization Effects

Charles C. Mischke, Menghe H. Li, David J. Wise, and Paul V. Zimba

Catfish fry readily consume zooplankton and selectively forage on larger zooplankton such as copepods, cladocerans, and ostracods but do not consume rotifers or copepod nauplii. Zooplankton are assumed to play a major role in catfish nutrition for several after fry are stocked into ponds, whereas prepared feeds serve primarily as fertilizer for natural pond productivity.

If nutritional requirements of channel catfish fry are met by zooplankton, early management practices for nursery ponds may be impacted. Changes in zooplankton nutritional value related to fertilization practices would also impact management decisions. This study was conducted to determine the nutritional value of large zooplankton from channel catfish nursery ponds and if changes in nutritional value occur with fertilization.

Methods

Eight one-acre nursery ponds at the NWAC were used for the study. Management of these ponds was similar to industry practices. Ponds were dry during the winter and filled in May. At filling, four ponds received an initial application of 75 pounds cottonseed meal, 0.5 gallon liquid phosphorus (0-50-0), 40 pounds urea (49-0-0), and 1 pound silica followed by twice per week applications of 25 pounds cottonseed meal, 0.25 gallon phosphorus, 20 pounds urea, and 0.30 pound silica. Four ponds (controls) did not receive fertilizer applications. Ponds were fertilized for one month and then large zooplankton were captured from each pond. Zooplankton from the samples were identified and analyzed for pigment composition (chlorophyll and carotenoids), crude protein and fat, amino acids, fatty acids, vitamins and minerals.

Results and Discussion

Although other studies have determined nutritional value of specific zooplankton—typically cultured zooplankton—we wanted to determine pond fertilization effects on the population of wild zooplankton that are consumed by channel catfish fry and small fingerlings. Zooplankton captured in this study included copepods, cladocerans and ostracods; there were no significant differences in zooplankton composition between fertilized and non-fertilized ponds. However, fertilization did increase zooplankton abundance by three-fold (based on capture efficiency).

Catfish fry raised from swim-up to about one week of age require 58% protein for maximum growth. The minimum protein requirement appears to decline with fish growth and size to about 55% at 0.2 g and to 46 to 50% from 3 to 5 g. The zooplankton in the present study did not differ between fertilized and non-fertilized ponds in their protein and fat contents. Zooplankton contained 65% crude protein on a dry matter basis (Table 1), which was in excess of the protein requirement determined for channel catfish fry. Zooplankton in the present study contained about 9% fat, which is slightly lower than that in typical catfish starter diets and higher than that in typical fingerling feeds.

Mineral analyses from the fertilized and non-fertilized pond zooplankton samples are presented, without statistical analysis (Table 1). All analyzed minerals except cobalt (the requirement for cobalt by channel catfish has not been determined) were in excess of the requirements determined for catfish fingerlings.

Analysis of composite vitamin samples is presented (Table 1). The zooplankton captured in the present study were excellent sources of niacin and vitamin E with concentrations being several times higher than the requirements determined for fingerlings. Only pantothenic acid and vitamin B_6 in the zooplankton sample collected from the non-fertilized ponds were below requirements. Other vitamins were either at or slightly above the requirement levels.

Amino acid composition was similar between zooplankton from fertilized ponds and those from non-fertilized ponds (Table 1). All indispensable amino acids were in excess of the requirement determined for fingerling catfish. The table represents total amino acid composition and not available amino acids; digestibility of these zooplankters is not known. However, protein digestibility of rotifers is reported to be high—89 to 94%. Assuming digestibility of these zooplankton is 80% or greater, all amino acid requirements for channel catfish fingerlings are met.

Only two fatty acids were significantly different in zooplankton from fertilized

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and non-fertilized ponds—16:0 and 20:4n-6. In both treatments the 16:0, 18:1, and 20:5n-3 were the dominant fatty acids. Channel catfish do not appear to be as sensitive to fatty acid deficiency as some other species, but require n-3 highly unsaturated fatty acids (HUFA) for optimum growth.

It appears that 1 to 2% dietary linolenic acid (18:3 n-3) or 0.75% n-3 HUFA will satisfy the n-3 fatty acid requirement of fingerling catfish. The n-3 HUFA from zooplankton in the present study averaged 18% of total fat or 1.6% of dry matter, which is in excess of the requirement. The large size fraction of zooplankton captured from catfish nursery ponds compares favorably to other n-3 HUFA sources.

Phytopigments (chlorophylls and carotenoids) from zooplankton were also analyzed. Carotenoids indicate the taxa of algae consumed by

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Table 1. Comparison of mean proximate nutrients, mineral composition (dry matter), vitamin composition (dry matter), and indispensible amino acid composition (% of protein) of zooplankton captured from control ponds and fertilized ponds.

Variable	Control	Fertilized	Requirement
Proximate nutrients			
Crude Fat (%)	9	9	
Crude Protein (%)	65	65	
Minerals			
Calcium (%)	1.6	3.3	None
Phosphorus (%)	0.81	1.01	0.03-0.04
Cobalt (ppm)	<1.5	<1.5	ND
Copper (ppm)	21.2	42.4	4.8
Iron (ppm)	600	1000	20
Manganese (ppm)	116	135	2.4
Selenium (ppm)	2.05	0.80	0.25
Zinc (ppm)	100	100	20
Vitamins (ppm)			
Folic Acid	1.8	2.2	1.5
Niacin	108.0	107.5	7.4-14.0
Pantothenic Acid	4	14	10-15
Vitamin B6	1	6	3
Ascorbic Acid	39	18	11-60
Vitamin E	178	109	25-50 IU
Thiamin	1	2	1
Riboflavin	11	17	6-9
Amino Acids (%)			
Arginine	6.5	5.9	4.3
Histidine	2.3	2.2	1.5
Isoleucine	4.2	3.9	2.6
Leucine	7.3	6.7	3.5
Lysine	7.0	6.5	5.1
Methionine + Cystine	3.6	3.3	2.3
Phenylalanine + Tyrosine	0.8	10.1	5.0
Threonine	4.4	4.2	2.0
Tryptophan	1.4	1.3	0.5
Valine	5.7	5.4	3.0

Reducing Weigh-Backs with Liquid Oxygen

Eugene L. Torrans, Charles D. Hogue, Jr. and Sam Pilkinton[a]
[a]Pilkinton Brothers Farms

Food-size channel catfish are harvested by pulling long seines with tractors and hydraulic seine reels, a method that has not changed much since the 1960s. As the seine is pulled toward shore and the fish are crowded, they move through a connecting throat into an 8 to 10 foot wide by 50 to 100 foot long sock. The sock is then disconnected from the seine, the end tied off, and the fish held in the sock until they are transported to the processor. Over 50,000 pounds of catfish may be held in a sock at densities exceeding 20 pounds per cubic foot.

Fish may be loaded on transport trucks immediately after seining, but in most cases they are left in the sock overnight, allowing sub-marketable fish to grade out through the mesh of the sock. In some cases they may be held in the sock for as long as three days, with the plant hauling a portion of the fish each day.

This system works well most of the year, but in the heat of the summer, when water temperatures are high and dissolved oxygen may decrease overnight to less than 1 ppm, the possibility of major fish loss from low oxygen is great. Every catfish farmer has a horror story about losing thousands of pounds of food fish being held in a sock during warm weather.

Water is normally slowly circulated through the sock, but additional aeration is required during periods of low dissolved oxygen. However, while existing commercial aerators increase dissolved oxygen, they also increase the water velocity through the sock. This increased current may cause greater stress on the fish than the benefits derived from the slight increase in dissolved oxygen. In the worst case, an entire sock of fish may be lost. Less extreme but more typical summer conditions may result in several hundred pounds of "weigh-backs".

Often fish are loaded in the early morning, after they have already been stressed by swimming in low oxygen water for several hours. The additional stress caused by the physical crowding during loading may further increase fish losses. We believed that liquid oxygen (LOX) could be used in this situation to increase dissolved oxygen in the sock without increasing current on the fish. If so, this could greatly reduce fish losses associated with harvest and transport.

Most private live haulers and fingerling producers now use LOX stored in small insulated tanks or dewars on their transport trucks, and many farmers use it on their farm trucks when moving fish from pond to pond. LOX availability has increased throughout the industry with increased demand. Some farms (including the one where this project was conducted) have their own bulk tanks for LOX storage, from which they can refill the smaller dewars used on their trucks.

We designed a "sock-saver", a single-axle trailer that could be moved around the farm with either a pickup truck or small tractor. The trailer was built with brackets to hold three 50-gallon LOX dewars. The three dewars were connected to a steel manifold with 1/4-inch grade "R" oxygen hoses. One oxygen hose connected this manifold through a single oxygen regulator to a second steel manifold. Pressure in this second manifold was held at 30 PSI with the regulator. Eight acrylic-block air flow meters with individually rated capacities of 200 standard cubic feet per hour (SCFH) were connected in parallel to the second manifold through 12-inch lengths of oxygen hose.

Each flow meter was connected to a 6-foot, self-weighted Bio-Weave diffuser tube through a 100 foot length of oxygen hose with a quick-disconnect coupling. In use, the diffusers were placed inside the sock where they sink to the bottom (Figure 1). After use the diffusers are removed and stored in a box on the trailer. The eight lengths of oxygen hose are stored coiled on hooks welded on the trailer.

Bio-Weave diffusers were chosen because they are self-weighted, flexible, and very durable. In tank trials with water 3.5 feet deep at a temperature of 81ºF, this diffuser had an oxygen transfer efficiency of over 15%. The transfer efficiency would be greater in the 4 to 6 foot water depths where socks are normally positioned. The system was designed to deliver oxygen at a rate of up to 1600 SCFH (13.9 liquid gallons or 132 pounds per hour). With a transfer efficiency of 15%, in excess of 20 pounds of oxygen per hour would actually be transferred to the water. Based on literature

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values for metabolism, this would satisfy 80% of the oxygen demand of 50,000 pounds of catfish.

A harvest crew routinely used this unit on several commercial fish farms in east Mississippi during the 2002 growing season. They felt that this system made a major difference in their ability to hold fish overnight without losses. They also reported the fish had darker color (blanching of the skin is a sign of oxygen stress in channel catfish) and more energy while being loaded after a night (or two or three nights) in a sock.

Statistical data is difficult to collect on commercial fish farms since water temperature, water depth, current velocity, pond dissolved oxygen and fish density in the sock varies considerably with every harvest. There is also considerable variation in dissolved oxygen within a sock due to fish movements and uneven water flow through the sock. However, measurements typically showed an increase of up to 0.9 ppm in dissolved oxygen inside the sock when the system was being used. This should have a major positive impact on the ability to hold fish when pond dissolved oxygen is low.

The need for supplemental oxygen varied a great deal over the summer. Not all diffusers were used every night, and the oxygen flow rates were often reduced to as little as 15 to 25% of maximum flow. In most cases, less than 50 gallons of liquid oxygen was normally used overnight. At a delivered cost (to our test farm) of $0.62/gallon, less than $30 of oxygen was used most nights. Most farmers would consider this a minor cost compared to the potential fish losses.

The total cost of the complete sock-saver with trailer, dewars, pressure/flow regulators and diffusers was approximately $8,400. The sock-saver has proven to be simple to operate, durable and virtually maintenance-free. Since it does not require an electrical or mechanical power source, it can be set up anywhere. It has a small "footprint" and does not interfere with other equipment normally used when loading fish. Individual flow regulators allow use of one, several or all eight diffusers as needed. The diffusers and hoses are self-weighted, tangle-free, and do not interfere with the normal process of crowding the fish in the sock and dipping them up in the loading basket. While we tested this equipment only with food fish, it should be equally valuable to a fingerling producer harvesting fingerlings in warm water.

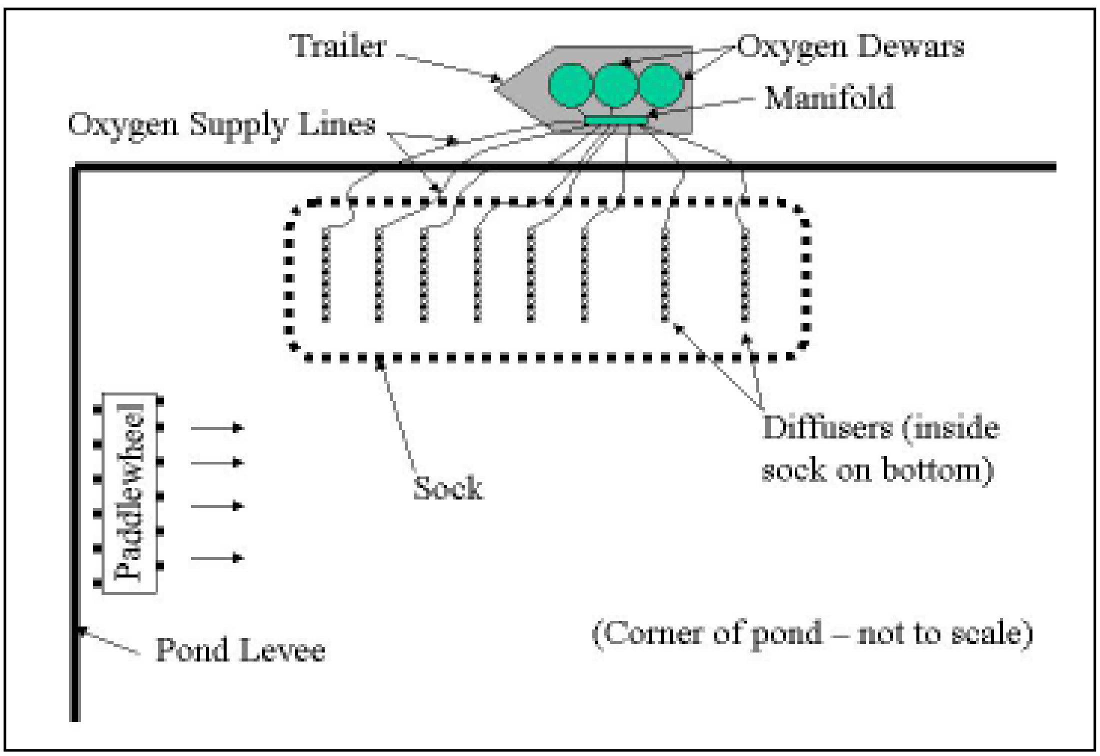

Figure 1. Placement of "sock-saver" unit in relation to pond levee and sock.

Comparison of Hybrid Striped Bass Production

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practical solution to this problem is to confine the originally stocked fish temporarily (2 to 4 weeks) to a small section of the pond. The reduction in feeding area achieved by this "penning" procedure should serve to enhance access to feed and the higher density within the confined area should also help to stimulate feeding activity.

The continued success and development of the aquaculture industry in the United States require the incorporation of management practices that improve economic return to the producer through an increase in the efficiency of the use of resources. The direct stock management practice currently under investigation for hybrid striped bass production is a response to this need.

Zooplankton Nutritional Value: Nursery Pond Fertilization Effects

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zooplankton, but may also be biologically important for fry. Carotenoids may influence fry survival, immunity, and have an antioxidant role. If pond fertilization altered zooplankton composition, the phytopigments would give an indication of grazing by zooplankton on various algae and possibly indicate preferred algal populations. However, fertilization had little effect on zooplankton nutritional value and no differences in phytopigments were detected from zooplankton between treatments. Chlorophyll *a* was the most abundant chlorophyll detected. Three carotenoids, echinenone, canthaxanthin, and β-β-carotene were also abundant in the zooplankton; echinenone and canthaxanthin indicate grazing on blue-green algae.

Summary

Zooplankton density was increased through fertilization, but there were few effects of fertilization on zooplankton nutritional composition. Effects may be more pronounced in different pond systems. The ponds used in this study have levels of nitrogen and phosphorus, before fertilization, which are relatively high. In newly constructed ponds, or in different soil types, fertilization may have a greater impact on zooplankton nutritional value, composition, and density.

Because of the high nutritional value of zooplankton present in channel catfish nursery ponds, the standard practice of feeding fry prepared diets as soon as they are stocked may not be necessary. If fertilization practices maintain large numbers of zooplankton, all fry nutritional requirements should be met through the natural biota. Additionally, it may be beneficial to offer zooplankton to fry while still in the hatchery. These large zooplankton are high in protein, contain essential amino and fatty acids and are excellent sources of vitamins and minerals.

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