FEED FORMULATION AND FEEDING STRATEGIES FOR BAIT AND ORNAMENTAL FISH

Reporting Period
June 1, 2005 - August 31, 2009

Funding Level

Year 1	$102,913
Year 2	$107,198
Year 3	$124,952
Total	$335,063

Participants

University of Arkansas at Pine Bluff (Lead Institution)	Rebecca Lochmann
University of Arkansas at Pine Bluff (Extension)	Nathan Stone
Texas A & M University	Delbert Gatlin
University of Florida	Craig Watson
University of Georgia	Gary Burtle

Administrative Advisor
Dr. Ron Lacewell
Texas A & M University
College Staion, Texas

PROJECT OBJECTIVES

1. Manipulate diet composition and/or feeding strategy for economical production of "jumbo" golden shiners.

2. Manipulate diet composition and feeding strategy to increase immunocompetence and resistance to stress in bait and ornamental fish during:

   a. Production
   b. Transport and Live Display

3. Determine the relative contribution of natural foods and prepared diets to growth, response to low dissolved oxygen, and other health indices for bait and ornamental fish in different production systems.

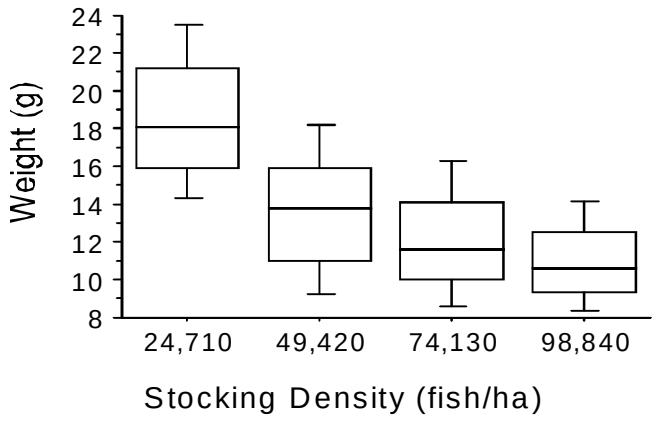
PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

Objective 1. *Manipulate diet composition and/or feeding strategy for economical production of "jumbo" golden shiners*

University of Arkansas at Pine Bluff. The first objective was to determine an appropriate stocking density for juvenile golden shiners to maximize the production of jumbos (12 g and larger) within a single growing season. This density would then be used for a subsequent study evaluating feeding frequency and diet composition. Golden shiner juveniles (0.5 g) were stocked on July 25 into 12, 0.04-ha fenced and netted earthen ponds at four densities (24,710; 49,420; 74,130; and 98,840/ha) and cultured for 105 days. Fish were fed to satiation once daily with a commercial 42% protein extruded pellet. Ponds were aerated 10 hours nightly using 0.37-kW aerators. Secchi disk visibility was measured every 2 weeks, and total ammonia nitrogen, pH, chlorophyll *a*, dissolved oxygen and zooplankton were determined monthly. Recording thermographs were installed in two ponds and recorded water temperature every 6 hours. Fish were sampled monthly. Ponds were harvested November 7-8. Average fish weight and survival were estimated by weighing and counting five subsamples of at least 25 fish. Weights (g) and lengths (mm) of a sample of at least 50 fish per pond were measured to determine condition and size variation. Remaining fish were bulk-weighed.

Average fish weight declined with increasing stocking density (Figure 1). At the lowest density, 98.4% of the weight at harvest was composed of jumbo fish. Survival ranged from 53 to 87% and was not significantly different among treatments. Gross yield increased with density from 366 to 753 kg/ha and was highly variable among ponds. Net yield of jumbos did not differ among the three higher density treatments. The 74,130/ha (30,000/acre) treatment resulted in an average gross yield of 639 kg/ha, of which 54% by weight was comprised of fish that weighed more than 12 g, and this density was selected for the next trial. Advanced fry were found in six ponds by August, documenting previously undescribed sexual maturity at 3 months

Figure 1. Box plots showing the distribution of fish weights at harvest.



of age in golden shiners. Juveniles stocked into study ponds had been raised from hatchery fry that were obtained on May 11, at 1 to 2 days of age.

Stocking juvenile golden shiners in late July, as was done in this study, results in lower single-season yields of jumbos when compared to direct stocking of hatchery fry at low densities. Previous work showed that direct stocking of fry in early May resulted in about 650 kg/ha of jumbos in a single season. However, the extra production of jumbos must be balanced against other uses for the ponds; juveniles used in this study were produced by stocking fry at 3.7 million/ha for 9 weeks, resulting in yields of about 900 kg/ha.

Results at a glance...

- *Stocking juvenile golden shiners in late July resulted in lower single-season yields of jumbos when compared to direct stocking of hatchery fry at low densities. However, the extra production of jumbos produced by stocking fry must be balanced against other uses for the ponds. Growout diets with no fish meal fed once daily to golden shiners supported yields similar to those obtained with more expensive diets and more frequent feeding.*

A second trial evaluated the effects of diet composition and feeding frequency on the growth and production of golden shiners. Juvenile golden shiners (average weight of 0.46 g) were stocked into 12, 0.04-ha earthen ponds at a rate of 74,100 fish/ha. Fish were fed either once or twice daily with one of two diets (Table 1); a control diet (Diet 1) and an experimental diet (Diet 2), with the intent of matching the performance of fish fed the control diet but at a lower cost. The feed form was an slow-sinking, extruded pellet. Fish were fed at 3% body weight per feeding, adjusted weekly based on an assumed

Table 1. Composition of the diets[1] being tested for producing jumbo golden shiners in a single growing season.

	Amount (g/100g as fed)	
Ingredient	Diet 1 (control)	Diet 2 (no fish meal)
Menhaden fish meal (62%)	26.0	0.0
Poultry by-product meal (60%)	15.0	34.0
Soybean meal (48%)	30.0	40.0
Corn	7.0	5.0
Wheat midds	13.8	12.8
Vitamin C (Stay-C)	0.146	0.146
Choline	0.58	0.58
Vitamin premix	0.4	0.4
Mineral premix	0.1	0.1
Poultry fat	7.0	7.0

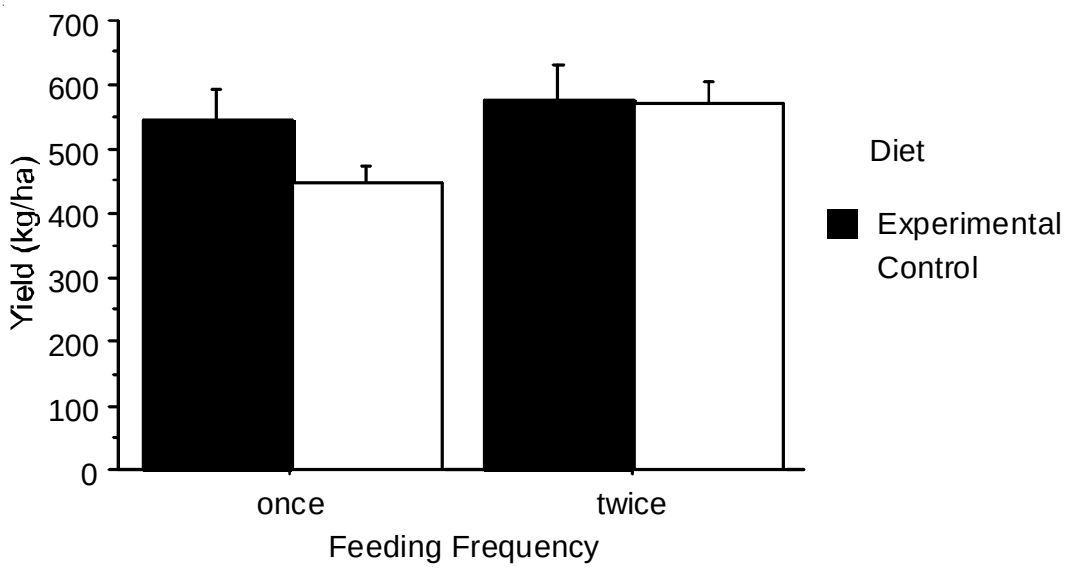
[1] Diets contain approximately 42% total protein and 9-10% lipid by calculation.

feed conversion ratio (FCR) of 1:1 and by sampling every 2 weeks. Ponds were aerated 10 h nightly (2200 – 0800 hours) with 0.37-kW aerators. Secchi disk visibility was measured every 2 weeks, and total ammonia nitrogen, pH, chlorophyll *a*, dissolved oxygen and zooplankton were determined monthly. Ponds were harvested November 19-20 after 80 days.

Average fish weight and survival were estimated by weighing and counting five sub-samples of at least 25 fish. Weights (g) and lengths (mm) of a sample of at least 50 fish per pond were measured to determine condition and size variation. Remaining fish were bulk-weighed.

At harvest, there was no difference in yield (Figure 2), average weight, or survival due to diet or feeding frequency. Yield averaged 535 kg/ha with a standard error of ± 24 kg/ha. Survival ranged from 57 to 80% and mean weight per fish was 10.2 ± 0.4 g. Results showed that feeding a diet with fish meal did not improve yields over a comparable diet formulated with poultry by-products, and that feeding twice a day instead of once a day provided no benefits.

Figure 2. Yield of golden shiners stocked at 74,100/acre for 80-d and fed either once or twice daily at 3% body weight with a 42% crude protein diet with fish meal (Control) or without fish meal (Experimental).



Objective *2.* *Manipulate diet composition and feeding strategy to increase immunocompetence and resistance to stress in bait and ornamental fish under simulated commercial conditions.*

Objective 2a. *Production*

Texas A&M University in collaboration with University of Arkansas at Pine Bluff. Three feeding trials were conducted at Texas A&M in recirculating systems with golden shiners to evaluate various potential immunostimulatory diet supplements. These trials were largely inconclusive,

possibly due to limited utilization of the practical basal diet formulation that was used to standardize methodology among institutions. Extrusion processing or a different method of particle size reduction may be needed to increase the utilization of these experimental diets by golden shiners.

We also supplemented the work originally planned by developing methodologies to quantitatively measure the immunocompetence and stress responses of baitfish under various conditions. A series of evaluations was conducted to define effective biological endpoints and/or physiological indicators of golden shiner health in response to various stimuli for rapid assessment of fish quality, and development of nutritional, pharmacological or husbandry strategies to enhance production efficiency. Some measurements of immunological and physiological responses have been developed for golden shiner including differential blood leucocyte counts, serum complement and cortisol assays. In Year 1, our group (TAMU) demonstrated that serum lysozyme activity of golden shiner and goldfish is very sensitive to the pH of the bacterial suspension (*Micrococcus lysodeikticus*), compared to hybrid striped bass and channel catfish. The optimal pH for lysozyme assay of golden shiner and goldfish was determined to be 5.9 and 6.0, respectively.

Our research team also found that neutrophil oxidative radical production could be analyzed according to a previously described procedure, but the isolation of head kidney from golden shiner is nearly impossible because that organ is almost invisible.

Our laboratory completed a 10-week feeding trial in a recirculating system with goldfish to evaluate potential immunostimulatory supplements including a commercial dairy/yeast prebiotic (GroBiotic®-A) at either 1 or 2% of diet, the amino acid arginine, and three different nucleotide preparations. After the 10-week feeding period, none of the supplements conferred increased growth, feed efficiency or immunocompetence as measured by oxidative radical production of blood neutrophils. Representative samples of fish fed each diet also were subjected to low dissolved oxygen (DO) stress as originally proposed. However, the goldfish were so tolerant of low DO conditions that we could not kill fish in any treatments even when lowering the DO concentration to below 0.5 mg/L by bubbling nitrogen into the water.

Results at a glance...

- *Prebiotics, immune stimulants, and differences in protein or lipid content of diets had only limited impacts on general performance of golden shiners. However, the prebiotic GroBiotic®-A significantly improved survival of golden shiners exposed to the bacterium that causes columnaris disease. In systems with natural foods (pools or ponds), it was necessary to impose a stressor (crowding) on golden shiners before exposure to bacteria to get a statistically significant increase in survival of fish fed diets with prebiotics. Prophylactic use of the prebiotic should be economically feasible based on a partial budget analysis of data from the golden shiner pond trial.*

University of Arkansas at Pine Bluff in collaboration with Texas A&M University. In Year 1, a 14-week feeding trial was conducted at the University of Arkansas at Pine Bluff with golden shiner in aquaria to determine whether practical diets supplemented with GroBiotic®-A, extra lipid, or both could improve growth, survival, feed conversion, body composition, or survival upon exposure to low dissolved oxygen. Six diets similar to a commercial diet (30% protein and 9.6 kg energy/gram of protein) were formulated. Two diets contained the same protein components (primarily fish and poultry meals) and differed only

in the amount of added lipid (4 and 10% poultry fat).

The diet with 4% fat was the control. Two other diets were similar to diets 1 and 2 except they contained 2% GroBiotic®-A. Two additional diets contained poultry meal in place of fish meal on an estimated digestible protein basis. Twenty-five fish (1.2 ± 0.001 g average weight) were stocked into each of four replicate 110-L tanks per treatment in a flow-through system. Fish were fed twice daily to apparent satiation and group-weighed every 2 weeks to track growth. Weight gain, survival, and feed efficiency are shown in Table 2. Fish fed diets with GroBiotic®-A or no fish meal +4% poultry fat had slightly lower feed conversion ratio (feed offered/ fish growth) than fish fed other diets. Statistical analysis of fish weight over time showed some transient differences, but final weight gain did not differ by diet. Post-trial fish were exposed to low dissolved oxygen for 24 hours with no mortality.

Whole-body lipid was analyzed and there were no differences among treatments (Table 2). Because the golden shiners were not large enough to obtain blood for health assays at the end of the feeding trial, a subset of fish was maintained on their experimental diets for 12 more weeks. Alternative complement activity in these larger fish did not differ by diet.

Due to the lack of effect from the low-DO stress test, we performed a columnaris disease challenge on a subset of golden shiners fed the control diet (basal + 4% poultry fat), the basal + 10% poultry fat diet, or the GroBiotic®-A + 10% poultry fat diet. GroBiotic®-A significantly enhanced survival of golden shiner relative to diets with 4 or 10% poultry fat and no GroBiotic®-A.

In Year 3, an 8-week feeding trial was conducted at UAPB with goldfish in aquaria to determine whether practical diets supplemented with GroBiotic®-A, extra lipid, or both could improve growth, survival, feed conversion, body composition, or survival upon exposure to the bacteria that causes columnaris disease. Four diets similar to a commercial diet (30% protein and 9.6 kg energy/gram of protein) were formulated. Two diets contained the same protein components (primarily fish and poultry meals) and differed only in the amount of added lipid (4 and 10% poultry fat). The diet with 4% fat was the control. Two other diets were similar to diets 1 and 2 except they contained 2% GroBiotic®-A.

Table 2. Performance of juvenile golden shiners fed diets containing different concentrations of poultry fat (PF), Grobiotic-A® (GROB), or menhaden fish meal (FM) for 14 weeks[1]

Diet	Mean individual weight gain (g)	Feed conversion	Survival (%)	Whole-body lipid (%)
Basal - 4% PF	1.00±0.06	5.7±0.2b	80.0±2.8	4.1±0.7
GROB - 4% PF	1.15±0.04	5.2±0.2ab	78.0±1.2	4.8±0.7
No FM - 4% PF	1.06±0.05	5.0±0.2a	83.0±3.0	5.3±1.3
Basal - 10% PF	1.06±0.05	5.8±0.2b	88.0±2.3	3.7±0.6
GROB - 10% PF	1.18±0.03	5.0±0.1a	85.0±3.4	6.9±1.2
No FM - 10% PF	1.09±0.09	5.7±0.2b	84.0±2.8	5.7±0.6

[1]Means in columns with different letters are significantly different (P<0.10, Fisher's LSD).

Twenty-five fish (0.57 ± 0.002 g average weight) were stocked into each of four replicate 110-L tanks per treatment in a recirculating system. Fish were fed twice daily to apparent satiation and group-weighed every 2 weeks to track growth. Weight gain, survival, and feed conversion are shown in Table 3. Weight gain, feed conversion and survival of goldfish did not differ among diets. Whole-body lipid was higher in fish fed the 10%-fat diets than in those fed the 4%-fat diets.

After the feeding trial we performed a columnaris disease challenge on goldfish fed each of the diets. Although the goldfish were exposed to higher densities of bacteria than were golden shiners, there were no differences in mortality among goldfish fed different diets. The growth rate of bacteria was so high that it is possible that the nutrients in the broth were depleted, and the bacteria might have been dead at the time of the challenge. To avoid this possibility in the future, fresh broth will be added when the culture is nearing peak optical density for the challenge. Signs of columnaris disease were also seen in some goldfish during the feeding trial. Although infected fish were not used in the challenge, the remaining fish may have been exposed to the bacteria and developed resistance before the challenge.

Table 3. Performance of goldfish in aquaria fed diets containing different concentrations of poultry fat (PF) or GroBiotic®-A (GROB) for 8 weeks. Means were not significantly different (P>0.05, Fisher's LSD).

Diet	Mean individual weight gain (g)	Feed conversion	Survival (%)
Basal - 4% PF	2.13 ± 0.15	1.8 ± 0.1	83.3 ± 4.1
GROB – 4% PF	2.30 ± 0.05	1.7 ± 0.0	85.8 ± 4.2
Basal – 10% PF	2.10 ± 0.09	1.8 ± 0.1	76.7 ± 1.4
GROB – 10% PF	2.31 ± 0.12	1.7 ± 0.1	79.2 ± 4.4

Objective 2b. *Transport and Live Display*

University of Georgia. Whole-cooked soybeans are being compared to soybean meal in diets of golden shiners, feeder goldfish and fathead minnows. Golden shiners were stocked into aquaria in Years 1 and 2 but were subject to excessive mortality within a few days of stocking under a variety of culture conditions. Antibiotics applied to the golden shiners had no significant positive effect on survival. However, increasing salinity of the systems to 3 parts per thousand by addition of artificial sea salts improved survival of the golden shiners.

Subsequent feeding trials with golden shiners in aquaria were improved by the addition of salt to the culture water when using golden shiners from a commercial source or by using golden shiners from a breeding population established on site. Survival of commercial golden shiners was 0% after 14 days in aquaria versus 97% survival for Tifton-reared golden shiners after 56 days in fresh water. When 3,000 mg of sodium chloride was added per liter of water, the commercial golden shiner survival was improved to 85% over 56 days. Fathead minnows

obtained from the same commercial source did not show signs of disease and survived at the rate of 95% for 56 days in aquaria in fresh water.

Weight gain for golden shiners fed a complete diet (gain = 0.52 g) or whole-cooked soybeans (gain = 0.57 g) was not statistically different over 56 days when 0.5 gram golden shiners from Tifton ponds were fed to satiation in aquaria. Similarly, weight gain for fathead minnows (1.5 g initial weight) was not significantly different when fed a complete diet (gain = 1.06 g) or whole-cooked soybeans (gain = 1.01 g). Goldfish trials have not been completed.

Pond trials with golden shiners fed roasted full-fat soybean meal show similar growth to golden shiners fed complete diets. Consumption of natural food appeared to provide essential nutrients that are not present in the simple soybean meal diet. Further analyses are in progress.

Economics of feeding for baitfish has changed over the course of this project. Roasted soybeans obtained for $500 per ton in 2006 cost $630 per ton in 2008, FOB Missouri. While 48% protein soybean meal is available for $342 per ton, complete feed costs range from $360 to $640 per ton, depending on quantity and location. At on-the-farm prices of $11 to $12 per bushel, soybean roasting would put feed value between $407 and $444 per ton for whole roasted soybeans. Roasting costs another $25 to $40 per ton. Therefore, baitfish producers, who also raised soybeans, could consider on-the-farm roasting in order to reduce dependence on the feed milling industry during periods of price uncertainty.

Results at a glance...

- *Golden shiners in ponds fed roasted full-fat soybean meal show similar growth to golden shiners fed complete diets. Consumption of natural food appeared to provide essential nutrients that are not present in the simple soybean meal diet. During periods of price uncertainty, baitfish producers who also raise soybeans could consider on-farm roasting to reduce dependence on the feed milling industry.*

Supplemental objective. *Development of methodologies to quantitatively measure the immunocompetence and stress responses of baitfish under various conditions.*

Texas A&M University. An assay of whole-body cortisol has been developed and shown to be quite responsive in measuring stress of shiners subjected to various handling procedures. Samples of golden shiners obtained from a study conducted during year 1 in which fish were subjected to normal harvesting, handling and distribution practices have been analyzed for whole-body cortisol as well as zinc and ascorbic acid as potential indicators of stressful conditions. The whole-body cortisol assay was determined to be a most sensitive measure of stress in golden shiners; whereas, whole-body zinc and ascorbic acid were not readily altered by the various harvesting, grading and transportation stressors.

An additional study examined the effects of four prebiotics, GroBiotic®-A (a mixture of partially autolyzed brewers yeast, dairy ingredient components and dried fermentation products), mannanoligosaccharide (MOS), galactooligosaccharide (GOS), and the fructooligosaccharide (FOS) inulin on digestibility of soybean-meal-based diets by goldfish. A basal diet was formulated so that 50% of the protein was provided by soybean meal and the other 50% was from menhaden fishmeal. Each prebiotic was supplemented to the basal diet at 1% by weight. A diet containing all of its protein from menhaden fish meal also was prepared as a control diet. Chromic oxide was

added to the diets at 1% as an inert marker. Each diet was fed to adult goldfish in duplicate 110-L aquaria for a total of 8 weeks. The dried fecal material from each aquarium was pooled over time and analyzed for protein, lipid, organic matter and chromium in order to compute coefficients of apparent digestibility. Results of the study revealed that none of the prebiotics affected apparent digestibility coefficients of the soybean-meal-based diet compared to the basal diet, although the diet supplemented with MOS consistently yielded the lowest values. In addition, goldfish digested the soybean-meal-based diets as well as the control diet. Denaturing gradient gel electrophoresis analysis revealed no differences in microbiota of goldfish fed the various prebiotics. These results are in contrast to those obtained with carnivorous fish species such as the red drum (*Sciaenops ocellatus*) in which the prebiotics increased digestibility coefficients of soybean-meal-based diets and altered GI tract microbiota.

Objective 3. *Determine the relative contribution of natural foods and prepared diets to growth, response to low dissolved oxygen, and other health indices of bait and ornamental fish in different production systems.*

Bait species

Texas A&M University. Based on the positive responses of golden shiners to GroBiotic®-A observed in the first year of this project year at UAPB, two separate feeding trials with goldfish have evaluated the effects of GroBiotic®-A in the presence or absence of natural productivity. Fish have been fed a commercially prepared basal diet and one supplemented with GroBiotic®-A in both a recirculating system containing well water and an outdoor system receiving a continuous supply of pond water. Significantly increased feed efficiency was noted in both feeding trials for goldfish fed GroBiotic®-A when compared to the basal diet. However, no significant differences were noted in regards to percent weight gain or survival over the course of the feeding trials, nor during a controlled disease challenge with *Aeromonas* spp. Goldfish in the presence of natural foods did exhibit significantly greater feed efficiency and survival during the feeding trial, as well as in the disease challenge. Denaturing gradient gel electrophoresis (DGGE) was performed on 16S ribosomal DNA isolated from digesta samples collected from intestinal sections of representative goldfish after each feeding trial to evaluate the relatedness of the GI tract microbiota. These analyses revealed no difference in the GI tract microbiota in the anterior or posterior intestinal sections regardless of diet, unlike previous studies in this laboratory with other species. Additionally, fish fed the diet supplemented with GroBiotic®-A showed a reduced stress response as measured by whole-body cortisol after net confinement.

A feeding trial was conducted with juvenile goldfish initially averaging 3 g per fish in which they were fed diets supplemented with either a dairy/yeast prebiotic

Results at a glance...

- *GroBiotic®-A also enhanced performance (growth, survival, condition index, or feed efficiency) of goldfish in systems with natural foods, and reduced cortisol response of goldfish to crowding stress. Interestingly, no differences in gut microflora were detected in fish fed diets with or without prebiotic, and the mechanism of action still needs to be determined.*

(GroBiotic®-A), a nucleotide product (Ascogen®), or arginine at 1% by weight using gelatin as a carrier to spray the supplements on a practical diet formulation. The fish were reared in the presence or absence of natural productivity, in two culture systems consisting of 38-L aquaria by supplying them with either a continuous flow of pond water or recirculated well water, respectively. The experimental diets were randomly appropriated to three units in each culture system. The goldfish were fed at a fixed percentage of body weight throughout the 10-week trial.

No differences were observed in percent weight gain or survival of fish fed the various treatments at the end of the trial. Feed efficiency was significantly better in goldfish fed the GroBiotic®-A supplemented diet in both culture systems. Blood neutrophil radical production levels as a measure of non-specific immunity were significantly higher in fish reared in the presence of natural productivity regardless of dietary treatment. In addition, during a separate disease trial with *Aeromonas hydrophila*, goldfish fed diets supplemented with either GroBiotic®-A or Ascogen® experienced significantly higher survival rates compared to fish fed the basal diet. Thus, these results indicate disease resistance of goldfish can be improved with dietary supplementation of commercial dairy/yeast prebiotic and nucleotide preparations.

University of Arkansas at Pine Bluff. A 10-week feeding trial with golden shiner in outdoor pools was conducted at the University of Arkansas at Pine Bluff using the same diets described in Objective 2a (see Table 2 for list of diets). Methodological differences from the tank trial included less frequent fish sampling (monthly) to avoid mortalities due to handling stress, and monitoring natural food abundance through Secchi depth and chlorophyll *a* readings. Four hundred (0.46 ±0.002 g in individual mass) fish were randomly stocked into each of four plastic-lined 4.1-m3 pools that were fertilized 1 week before stocking and maintained static during the study. Fish were fed twice daily to apparent satiation and group-weighed every 2 weeks to track growth. Chlorophyll *a* was measured to assess phytoplankton abundance and other water quality parameters were acceptable for golden shiner. Weight gain and feed conversion did not differ by diet (Table 4). There were slight differences in condition factor and survival that were not consistently associated with diet variables (Table 4). Whole-body lipid was significantly higher

Table 4. Performance of golden shiners in pools fed diets containing different concentrations of poultry fat (PF), GroBiotic®-A (GROB), or menhaden fish meal (FM) for 10 weeks. Means in columns with different letters are significantly different (P<0.10, Fisher's LSD).

Diet	Mean individual weight gain (g)	Feed conversion	Condition index	Survival (%)	Whole-body lipid (%)
Basal - 4% PF	1.32 ± 0.13	2.4 ± 0.2	0.85 ± 0.01^{c}	99.8 ± 0.2^{a}	7.9 ± 0.3^{b}
GROB – 4% PF	1.47 ± 0.10	2.6 ± 0.1	0.88 ± 0.01^{b}	96.6 ± 1.3^{b}	7.8 ± 0.4^{b}
No FM – 4% PF	1.56 ± 0.10	2.4 ± 0.1	0.88 ± 0.01^{b}	98.9 ± 0.5^{a}	8.5 ± 0.3^{b}
Basal – 10% PF	1.50 ± 0.21	2.4 ± 0.1	0.91 ± 0.01^{a}	99.1 ± 0.7^{a}	10.0 ± 0.6^{a}
GROB – 10% PF	1.50 ± 0.12	2.5 ± 0.1	0.89 ± 0.01^{b}	99.2 ± 0.6^{a}	9.6 ± 0.2^{a}
No FM – 10% PF	1.43 ± 0.03	2.4 ± 0.1	0.90 ± 0.01^{a}	99.6 ± 0.4^{a}	9.3 ± 0.3^{a}

in fish fed the 10% poultry fat diets compared to those fed the 4% poultry fat diets, regardless of other diet variables.

After harvest, shiners fed the control diet or the diet with 2% GroBiotic®-A were acclimated to indoor tanks and challenged with *Flavobacterium columnare* (trial 1). In trial 2, shiners from the same treatments were subjected to confinement stress or left unmolested, then exposed to *F. columnare*. Mortality (mean ± SE) was not significantly different for the control diet (23.4 ± 3.4%), GroBiotic®-A diet (10.0 ± 3.3%), or GroBiotic®-A diet with stress (16.7 ± 3.4%) treatments. Mortality for the control diet with stress (50.0 ± 3.3%) treatment was significantly greater than the other treatments. This suggests that prebiotic supplementation in golden shiner feeds prior to a stressful event could reduce associated mortality from *F. columnare* significantly compared to control diets.

We evaluated the performance of juvenile golden shiners in ponds fed a control diet or the same formula with 2% GroBiotic®-A. Golden shiner juveniles (0.1 g) were stocked on June 28, 2007 into 10, 0.04-ha fenced and netted earthen ponds at 21.9 kg/ha. Fish were fed to satiation twice daily (4 to 7% body weight) with custom-made 35%-protein diets extruded as 1.5-mm pellets. The diet formulation was similar to a commercial catfish diet. Temperature and dissolved oxygen concentrations were measured daily, Secchi disk visibility and chlorophyll *a* were measured weekly, and total ammonia nitrogen, nitrite, alkalinity, and pH were determined monthly. Ponds were aerated 10 hours nightly using 0.37-kW aerators. Subsamples of fish (100 per pond) were counted and weighed at 2-week intervals to track growth and adjust feed rations. Due to small initial fish size and the relatively low stocking density, growth was very rapid and the study was harvested after 7 weeks to avoid reproduction. At harvest there were no differences in average fish weight, net yield, or feed conversion ratio between treatments (Table 5).

After harvest, 100 fish per pond were moved to indoor tanks for acclimation prior to a bacterial challenge with *Flavobacterium columnare*. Fish were maintained on their respective diets during acclimation and the challenge. Each pond replicate received three experimental treatments: confinement stress for 30 minutes prior to *F. columnare* exposure (stressed); left un-molested prior to F. columnare exposure (un-stressed); or left un-molested and not exposed to *F. columnare* (control). Stress was induced by netting all 24 shiners in a tank and placing them in a small basket suspended within each aquarium. Pre-stress and post-stress fish samples were frozen for subsequent whole-body cortisol analysis to document stress. Confinement stress induced a significantly higher cortisol response compared to unstressed fish, regardless of diet. After the fish were released into the tank they were exposed to a virulent strain (PB02) of *F. columnare* for 18 hours. Mortality was

Table 5. Performance of golden shiners in ponds fed a control diet or a diet with 2% GroBiotic®-A (GROB) for 7 weeks. Means were not significantly different (P>0.10, 1-way ANOVA).

Diet	Average individual weight gain (g)	Net yield (g)	Feed conversion
Control	2.93 ± 0.29	287.0 ± 586.75	1.34 ± 0.08
2% GROB	2.89 ± 0.28	690.3 ± 623.0	1.47 ± 0.10

monitored and recorded for 14 days. Mortality ranged from 0 to 35.0% and was not significantly different for fish in the control or un-stressed treatments fed either the control or 2% GroBiotic®-A diets. The stressed shiners fed the 2% GroBiotic®-A diet also had similar mortality rates compared to control and un-stressed shiners, but the stressed fish that received the control diet had significantly greater mean (± SE) mortality (26.7 ± 4.4%). A partial budget analysis based on the results of the challenge indicate that the increased cost of feed containing 2% GroBiotic®-A would be justified based on increased survival of golden shiners exposed to stress and pathogens.

A 10-week feeding trial with goldfish in pools was conducted using the same diets described in the goldfish aquarium trial at UAPB (see Table 3). Four hundred (0.36 ±0.002 g in individual mass) fish were randomly stocked into each of four plastic-lined, 4.1-m3 pools that were fertilized 1 week before stocking and maintained static for most of the study. Fish were fed twice daily to apparent satiation and subsamples of 100 fish per pool were group-weighed every 2 weeks to track growth. Natural food abundance was monitored through Secchi depth and chlorophyll *a* readings. Other water quality parameters were acceptable for goldfish, except for one instance of high pH (> 9) where fish were showing signs of stress. All pools were flushed with fresh water for 2 hours to restore water quality. At 10 weeks all fish were counted and group-weighed by pool. Fifty individual fish per pool were also euthanized for individual measurements of length and weight to calculate condition index (Fulton's K). These fish were frozen and used for proximate analysis.Whole-body lipid was higher in fish fed the 10%-fat diets than in those fed the 4%-fat diets. Weight gain and condition index of goldfish fed diets with 10% poultry fat, 2% GroBiotic®-A + 4% poultry fat, or 2% GroBiotic®-A + 10% poultry fat were higher than those of fish fed the control diet with 4% poultry fat and no prebiotic (Table 6). Feed conversion and survival did not differ among diets. One hundred goldfish per pool were retained live in the pools where they were fed their experimental diets until they were moved to indoor tanks for acclimation prior to a columnaris challenge. The challenge was conducted in two parts: 1) Fish fed 4% fat diets with or without prebiotic; 2) Fish fed 10% fat diets with or without prebiotic. Within each part, half of the fish in each dietary treatment were stressed by 30 minutes of confinement prior to exposure to columnaris, while the other half were not stressed. Serum cortisol values were higher in confined fish than in unconfined fish. Results were different for fish fed the 4% fat diets or the 10% fat diets. Stressed fish fed the 4% fat

Table 6. Performance of goldfish in pools fed diets containing different concentrations of poultry fat (PF) or GroBiotic®-A (GROB) for 10 weeks. Means in columns with different letters are significantly different (P≤0.05, Fisher's LSD).

Diet	Mean individual weight gain (g)	Feed conversion	Condition index	Survival (%)
Basal - 4% PF	2.26 ± 0.12[b]	1.2 ± 0.03	1.47 ± 0.02[b]	84.1 ± 2.0
GROB – 4% PF	2.54 ± 0.09[a]	1.2 ± 0.04	1.53 ± 0.02[a]	82.9 ± 2.4
Basal – 10% PF	2.74 ± 0.07[a]	1.2 ± 0.03	1.58 ± 0.02[a]	85.0 ± 1.5
GROB – 10% PF	2.56 ± 0.07[a]	1.3 ± 0.02	1.54 ± 0.02[a]	86.9 ± 2.6

diet with prebiotic had lower mortality than those fed the same diet without prebiotic. However, stressed fish fed the 10% fat diets had higher mortality than unstressed fish, regardless of prebiotic inclusion. The basis for the protective effect of the prebiotic in 4%-fat diets but not in the 10%-fat diets is unknown.

University of Georgia. Pond feeding trials are in progress using fathead minnows. At that time, transport hardiness will be evaluated.

Ornamental species

University of Florida. During this project we submitted five species of fish (*Brachydanio rerio*, zebra danios; *Xiphophorus helleri*, swordtails; *Hypostomus* sp., common plecostomus; *Cichlasoma meeki*, firemouth cichlid; and *Moenkhausia sanctaefilomenae*, red-eye tetra) to the treatments outlined in the original proposal. The original objective was to determine the relative contributions to ornamental fish growth of direct consumption of manufactured feed and natural foods produced as an indirect result of feeding. At the time the proposal was submitted there was at least one large feed supplier selling farmers unprocessed meal diets, but at a relatively expensive cost, and this work was designed to determine whether use of a processed (i.e., pelleted and reground) diet would provide better growth and survival. Two fertilization regimes were also added to the tests to determine the ability of these species to utilize primary and secondary productivity. Each trial consisted of 6 replicated ponds of each species, with four treatments: 1) cottonseed meal; 2) liquid fertilizer; 3) unprocessed, 33%-protein meal-type diet; and 4) processed, 33%-protein diet. Ponds were stocked at rates consistent with industry standards, and trials were each conducted for 12 weeks. A similar tank trial was conducted with each species to compare growth and survival using the two diets. Ten replicate tanks of fish were fed each diet for 12 weeks and growth and survival measured and compared. Other measurements included water quality (ammonia, nitrite, pH, temperature, and dissolved oxygen), and weekly chlorophyll *a* samples from pond studies.

A "low dissolved oxygen" stress test was conducted on three of the five species, but was discontinued as it showed no significant measurement of the fish's ability to handle stress due to deprivation of dissolved oxygen.

There were significant differences in the growth and survival of zebra danios produced in ponds receiving treatments of liquid fertilizer, cottonseed meal, an unprocessed meal diet, and a processed diet. Growth on the processed diet was best, followed by unprocessed diet, cottonseed meal, and liquid fertilizer. Although growth was best with the addition of processed diets or organic materials, liquid fertilizer alone produced a good number of market-sized fish with minimal costs. The economics of each level of input need further analysis. Zebra danios fed a processed diet in tanks also outperformed fish fed an unprocessed diet.

Survival of swordtails in all pond studies was greater than 100% due to reproduction in the pond during the 12 weeks. Overall survival was based on number of fish at final harvest with the processed

Results at a glance...

- *Zebra danios, swordtails, plecostomus, firemouth meeki cichlids, and red-eye tetras performed similarly on processed or unprocessed diets in ponds, while results were generally less favorable for liquid fertilizer or cottonseed meal treatments. In some species, a large number of fish could be produced with fertilizer alone, but fish size was reduced. Except for zebrafish, these species also performed similarly on processed and unprocessed diets in aquaria.*

diet treatment being highest followed in order by the unprocessed diet, liquid fertilizer and cottonseed meal treatments. Overall weight of fish produced differed among treatments. There was an increase in overall weight of fish produced in each treatment, with the processed diet providing the highest overall weight, followed by the unprocessed diet, cottonseed meal, and liquid fertilizer. No significant difference in production were seen in tanks studies for swordtails.

Yields of plecostomus in pond studies also differed among treatments, with yields decreasing in the following order: unprocessed diets > processed diets > cottonseed meal > liquid fertilizer. Survival varied dramatically, with survival in the liquid fertilizer treatment being only 10%, compared to 65% survival for the unprocessed diet. No significant differences in growth or survival between the unprocessed and processed diet treatments were found in the tank study.

Highest yield of firemouth meeki cichlids in ponds was obtained on the processed diet treatment, followed in order by the unprocessed diet, liquid fertilizer, and cottonseed meal. Firemouth meeki cichlids readily spawn in ponds, and reproduction and survival of offspring was best in the liquid-fertilized ponds, but fish size was significantly smaller than in the ponds receiving either diet. There was no significant difference in growth or survival of Firemouth meeki cichlids fed either diet in the tank study.

The processed diet treatment provided highest yield of red-eye tetras in ponds, followed in order by the unprocessed diet, cottonseed meal, and liquid fertilizer treatments. However, the total weight of fish was less with the unprocessed diet than with cottonseed meal, and the total number of fish was greatest with cottonseed meal, followed by processed diets, unprocessed diets, and liquid fertilizer. Tank studies for red-eye tetras showed no significant difference in growth or survival between the two diets.

Chlorophyll *a* values were consistent throughout the study for all species. A significant difference in primary productivity (based on chlorophyll *a*) was also observed, consistent with anticipated results (i.e. fertilized ponds were high in primary productivity, with unprocessed and processed diet treatments showing a lower level of chlorophyll *a*.)

The small size of most ornamental species allows them to utilize primary and secondary productivity, but the impact of this source of nutrition alone was unknown for most species. This project demonstrated that pond fertilization alone can produce a significant number of fish, and at a relatively low cost, but the size of fish grown on fertilizer alone is significantly smaller than for fed fish. There also was a general trend toward increasing growth and survival in ponds with processing of the diet, but not for all species (e.g., plecostomus fed an unprocessed diet had a 13% increase in survival). Selection of type of "fertilization" from an organic fertilizer such as cottonseed meal to use of an inorganic fertilizer also affected yield, but again there was variation in this trend between species. Survival and total yield of firemouth meeki cichlid was dramatically increased in ponds with inorganic fertilizer relative to ponds fertilized with cottonseed meal. Chlorophyll *a* levels and water quality parameters were consistent with expectations, showing higher productivity in the pond water when fertilization was used rather that a diet.

Several issues related to the overall value and impact of this study should be addressed in future work. All fish except the firemouth meeki cichlids and the danios were procured for the study from local producers. Red-eye tetras were received at a very small size from a local hatchery, and their counting methodology was obviously flawed. We attempted to stock 7,500 fry per pond, and physically counted over 30,000 fish in some ponds at harvest. Future studies should not rely on external parties counting fish, and there is an obvious need to assist farms with how they are enumerating their inventories while stocking.

WORK PLANNED

UAPB and Texas A&M University (TAMU) are doing supplemental collaborative work on the effects of prebiotics in baitfish species.

IMPACTS

The overall goal of this project is to develop diets, feeding practices, and production strategies that enhance stress resistance and prolong survival of bait and ornamental fishes. Production diets with no fish meal fed once daily to golden shiners support yields similar to those obtained with more expensive diets and more frequent feeding. The use of a dairy/yeast prebiotic has shown consistently positive results in baitfish and the cost should be offset by improved survival under production conditions. The prebiotic was effective in golden shiners in ponds even at a low stocking density, where natural foods typically have a greater impact on performance. Similarly, the prebiotic improved survival of goldfish raised in outdoor pools with 4%-fat diets and natural foods, followed by stress and exposure to columnaris. The economics of these feed additives look promising, but need further verification due to the small scale of the baitfish industry.

In addition to the positive effects of a commercial dairy/yeast prebiotic on disease resistance of golden shiners observed by co-investigators on this project, this prebiotic improved stress resistance of goldfish based on a reduction in whole-body cortisol after net confinement. Thus, this prebiotic appears to have considerable potential as a feed additive to protect baitfish from stressors and diseases commonly encountered in production.

Whole roasted soybean, when ground into a meal, can produce good growth in golden shiners, fathead minnows, and goldfish, provided they are in ponds with natural food available. Soybean economics should be considered before using whole roasted soybean meal. However, this product could allow on-the-farm production of baitfish feeds.

Performance of golden shiner in ponds fed full-fat soybean meal or a nutritionally complete diet was comparable. Therefore, full-fat soybean meal can be used to reduce production costs when complete diets are more costly. Baitfish producers who also produce soybeans may realize additional profits by roasting the soybeans on-farm.

With ornamental species, pond fertilization alone produced a significant number of fish, and at a relatively low cost, but the size of these fish was significantly smaller than when feeds were used. Several ornamental fish producers have altered their stocking densities and feeding regimes based on the findings of the pond studies in this project. Overall, there was a general improvement in growth and survival of ornamental species using a processed diet. Perhaps most importantly, there were no advantages to using the expensive, unprocessed meal that was used previously by the ornamental fish industry, which should discourage farms from renewing a market for these diets.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

Publications

Li, P. B. Ray, D. M. Gatlin III, T. Sink, R. Chen and R. Lochmann. 2009. Effect of handling and transport on cortisol response and nutrient mobilization of golden shiner Notemigonus crysoleucas. Journal of the World Aquaculture Society 40:803-809.

Lochmann, R.T., T.D. Sink, and H. Phillips. 2009. Effects of dietary lipid concentration, a dairy/yeast prebiotic, and fish and non-fish protein sources on growth, survival, and non-specific immune response of golden shiner, Notemigonus crysoleucas, in indoor tanks and outdoor pools. North American Journal of Aquaculture 71:16-23.

Lochmann, R.T., T. Sink, N. Kinsey, and E. Marecaux. 2010. Effects of a dairy/yeast prebiotic on performance of golden shiners in ponds. North American Journal of Aquaculture, in press.

Savolainen, L. C. and D.M. Gatlin III. 2009. Evaluation of dairy/yeast prebiotic supplementation in the diet of juvenile common goldfish *(Carassius auratus)* in the presence or absence of phytoplankton/zooplankton. Journal of Aquatic Animal Health 21:156-163.

Sink, T.D., S. Kumaran, and R.T. Lochmann. 2007. Development of a whole-body cortisol extraction procedure for determination of stress in golden shiners, Notemigonus crysoleucas. Fish Physiology and Biochemistry 33:189-193.

Sink, T.D., R.T. Lochmann, A.E. Goodwin, and E. Marecaux. 2007. Mortality rates in golden shiner, Notemigonus crysoleucas, fed high-fat diets with or without a dairy/yeast prebiotic prior to challenge with Flavobacterium columnare. North American Journal of Aquaculture 69:305-308.

Sink, T.D., and R.T. Lochmann. 2008. Preliminary observations of mortality reduction in stressed, Flavobacterium columnare, challenged golden shiners after treatment with a dairy-yeast prebiotic. North American Journal of Aquaculture 70:192–194.

Theses and Dissertations

Raggi, T. (2009) Prebiotics have limited effects on nutrient digestibility of a soybean meal-based diet by goldfish *Carassius auratus*. Masters Thesis, Texas A&M University.

Manuscripts in preparation

Li, P., R.T. Lochmann, and D.M. Gatlin, III. Determination of optimal pH for measurement of serum lysozyme activities of various warm water fishes. Fish and Shellfish Immunology.

Lochmann, R., T.D. Sink, H. Phillips, and R. Chen. The effects of a dairy/yeast prebiotic on growth and health of goldfish in indoor tanks and outdoor pools. North American Journal of Aquaculture.

Savolainen, L. C., T. Raggi and D. M. Gatlin III (2009) Evaluation of arginine, nucleotides and a dairy/yeast prebiotic on resistance of juvenile common goldfish (*Carassius auratus*) to *Aeromonas hydrophila*. Journal of the World Aquaculture Society.

Presentations

Chen, R., H. Phillips, and R. Lochmann. 2009. Effects of standard and high-fat diets with or without a dairy/yeast prebiotic on performance of goldfish *Carassius auratus* in aquaria. Aquaculture America 2009, February 16-18, Seattle, Washington.

Gatlin, D.M., III. 2008. Review of prebiotic research with fish and shrimp. Texas Aquaculture Association Conference, January 16-18. El Campo, Texas.

Gatlin, D. M., III, P. Li, B. Ray, R. Chen, T. Sink, and R.T. Lochmann. 2007. Review of immune and stress responses of golden shiner, Notemigonus crysoleucas. Aquaculture America 2007, February 26-March 3, San Antonio, Texas.

Gatlin, D. M., III, P. Li, X. Wang, G. S. Burr, F. Castille A. L. Lawrence and A. Buentello. 2007. Potential application of prebiotics in fish and shrimp culture. Thirty-first Fish Feed and Nutrition Workshop, May 16-17, Auburn, Alabama.

Li, P., T. Sink, B. Ray, R. Chen, R. Lochmann and D. M. Gatlin III. 2008. Effect of handling and transport on cortisol response and nutrient mobilization of golden shiner Notemigonus crysoleucas. Aquaculture America 2008, February 9-12, Lake Buena Vista, Florida.

Lochmann, R.T., T. Sink, N. Kinsey, and E. Marecaux. 2008. Effects of a dietary prebiotic on performance of golden shiners in ponds. Aquaculture America 2008, February 9-12, Lake Buena Vista, Florida.

Lochmann, R., T. Sink, and H. Phillips. 2008. Effects of a dairy-yeast prebiotic on performance of golden shiner in tanks, pools and ponds. Arkansas Bait and Ornamental Fish Grower's Association Meeting, February 7, Lonoke, Arkansas.

Lochmann, R., T. Sink, H. Phillips, and F. Bearden. 2008. Effects of a prebiotic, lipid concentration, and protein source on performance of golden shiner (Notemigonus crysoleucas) in outdoor pools. Aquaculture America 2008, February 9-12, Lake Buena Vista, Florida.

Lochmann, R.T., N. Stone, A. Kachowski, and A. Gannam. 2008. Effects of diets with and without fish meal and feeding frequency on the growth of juvenile golden shiners. Aquaculture America 2008, February 9-12, Lake Buena Vista, Florida.

Lochmann, R. 2007. Overview of shiner prebiotic studies. Thirty-first Fish Feed and Nutrition Workshop, May 16-17, Auburn, Alabama.

Lochmann, R., T. Sink, H. Phillips, P. Li, and D.M. Gatlin, III. 2007. Effects of a feed additive, lipid concentration, and protein source on performance of golden shiner (*Notemigonus crysoleucas*). Aquaculture America 2007, February 26 - March 3, San Antonio, Texas.

Lochmann, R., N. Stone, and A. Kachowski. 2007. Stocking rates for juvenile golden shiners to maximize single-season production of jumbo (>12 g) fish. Aquaculture America 2007, February 26 - March 3, San Antonio, Texas.

Lochmann, R.T., T.D. Sink, H. Phillips, P. Li, and D.M. Gatlin, III. 2006. Effects of a feed additive, lipid concentration, and protein source on performance of golden shiner in tanks. UAPB Aquaculture/Fisheries Field Day, October 5, Pine Bluff, Arkansas.

Phillips, H., N. Kinsey, and R. Lochmann. 2009. Effects of standard and high-fat diets with or without a dairy/yeast prebiotic on performance of goldfish *Carassius auratus* in outdoor pools. Aquaculture America 2009, February 16-18, Seattle, Washington.

Raggi, T., G. Burr, B. Ray, A. Buentello and D. Gatlin III. 2009. Effects of prebiotics on nutrient digestibility of a soybean meal-based diet by goldfish *Carassius auratus* Aquaculture America 2009, February 16-18, Seattle, Washington.

Savolainen, L. C. and D. M. Gatlin III. 2009. Evaluation of the prebiotic Grobiotic®-A in the diet of juvenile common goldfish (*Carassius auratus*) in the presence or absence of natural productivity. Aquaculture America 2009, February 16-18, Seattle, Washington.

Savolainen, L.C. and D.M. Gatlin III. 2008. Evaluation of the prebiotic GroBiotic®-A in the diet of juvenile common goldfish (Carassius auratus) in the presence or absence of natural productivity. Texas A&M Student Research Week, Texas A&M University, College Station, Texas.

Sink, T.D., R.T. Lochmann, and S. Kumaran. 2008. Development of a whole-body cortisol extraction procedure for determination of stress in golden shiners Notemigonus crysoleucas. Aquaculture America 2008, February 9-12, Lake Buena Vista, Florida.

Sink, T., R. T. Lochmann, A. E. Goodwin, and E. Marecaux. 2007. Mortality rates in golden shiners, Notemigonus crysoleucas, fed diets high in fat and the prebiotic GroBiotic®-A prior to challenge with Flavobacterium columnare.Aquaculture America 2007, February 26 - March 3, San Antonio, Texas.

Sink, T.D., R. T. Lochmann, A. E. Goodwin, and E. Marecaux. 2006. Mortality rates in golden shiners, Notemigonus

crysoleucas, fed diets high in fat and the prebiotic GroBiotic®-A prior to challenge with Flavobacterium columnare. Poster presentation, UAPB Aquaculture/Fisheries Field Day, October 5, Pine Bluff, Arkansas.

Stone, N., A. Kachowski, and R. Lochmann. 2006. Evaluation of low stocking rates for juvenile golden shiners to maximize single-season production of jumbo fish. Poster presentation, UAPB Aquaculture/Fisheries Field Day, October 5, Pine Bluff, Arkansas.

Watson, C. 2008. Diet and feeding strategies for ornamental fish; feed the pond or feed the fish, year 2. Aquaculture America 2008, February 9-12, Lake Buena Vista, Florida.

Watson, C. 2007. Growth and survival of ornamental fish based on pond fertilization and feeding. Aquaculture America 2007, February 26 - March 3, San Antonio, Texas.

◆ ◆ ◆