

AQUATIC VEGETATION DEMONSTRATION PROJECTS IN THE RIO GRANDE VALLEY

Michael P. Masser

Nuisance aquatic vegetation clogs irrigation canals and reservoirs of the Irrigation Districts throughout the Rio Grande Valley. Aquatic vegetation not only slows the flow of water but increases evapo-transpiration, canal seepage, lowers property values, and increases breeding areas for unwanted insects (e.g. mosquito). The water from these Districts is used primarily for irrigation of crops but also for other human and industrial purposes. Demonstration projects have been developed with several of these districts to control nuisance aquatic vegetation.

Grass carp control of hydrilla demonstrations are underway with Brownsville Irrigation District, Rancho Viejo, and Hidalgo County Irrigation District Number One. Over a 14 month period in 2002-03 the Hidalgo County Irrigation District Number One spent \$137,162.36 on aquatic vegetation control (in the previous 12 months it was \$97,000.00). Their canals (139 surface acres but over 170 miles long) were completely choked with hydrilla to the point that they had to pump extra water to push the vegetation down and force the water through. The Districts total cost on the grass carp was \$50,055.00 (and this should cover at least the next 5 years). That is a savings this year of \$87,000+ and over \$100,000 for each of the next 4 years (and possibly longer) for a total savings of over a half million dollars.

Herbicide control of water hyacinth and water lettuce was done at Cameron County Irrigation District No. 6. Reward herbicide was used as the herbicide. All plants contacted by the herbicide were killed within 3 days. This represents water savings by reducing plant transpiration by 30%. In this case, that would be a conservation of approximately 16,000 gallons per day.

Funding for this project is from the US Department of Agriculture – CSREES, and Texas Cooperative Extension.

AQUACULTURE PRODUCTION IN SOUTH-CENTRAL TEXAS

Peter Woods and Michael Masser

Although aquaculture is one of the most rapidly growing segments of the domestic agriculture industry, Texans have been slow to respond to the challenge. That is until about two years ago when, in collaboration with Texas Cooperative Extension and other institutions, a few catfish farmers the Coastal Bend organized and formed the Texas Aquaculture Cooperative. Since that time, the catfish industry in the area has seen substantial growth. Two years ago there were approximately 1,000 acres of ponds – now, there are nearly 1,400 acres of water devoted to catfish. And many more are planned or currently under construction. The Cooperative now owns its own processing plant.

Other segments of the industry are growing as well. Hybrid striped bass acreage is expanding, and interest in other species such as tilapia and crawfish is high. A new 200 acre Red Drum farm has opened within the past year effectively doubling existing acreage. As traditional farm commodities falter, farmers are finding that Texas holds several competitive advantages over other southern states in the production of these temperate to tropical aquatic species. Texas has a longer growing season, good clay soils, plenty of water, and beneficial breezes that help farmers produce more for less. And since Texas consumes more catfish than any other state, no one can be closer to the markets!

SOUTHERN REGIONAL AQUACULTURE CENTER

Michael P. Masser, Verna Bartlett, and Judy Winn

Aquaculture is one of the most rapidly growing sectors of US agriculture. Producers and consumers of aquaculture products continue to desire current information on how to produce the highest quality products, reduce the cost of production, and improve environmental quality. Texas Cooperative Extension has the responsibility to solicit, review, edit, publish, and distribute practical aquaculture publications for the Southern Regional Aquaculture Center (SRAC), since 1988. These publications are distributed throughout the southern region through Land-Grant Universities and to other regional centers across the US and via the World Wide Web to producers, consumers, and educators across the globe. Publications encompass a wide range of topics such as: production (by species and methods), water quality, processing, marketing, and legal constraints. To date this project has produced more than 165 Fact Sheets, 23 Videos, 3 Project Summaries, 4 Project Results, and 3 CD's. All publications are available in hard copy, on CD, or on the Wildlife and Fisheries Sciences Extension web site at <http://wildlife.tamu.edu>, which is linked to the TAMU Extension publications web site and to many other educational institutions through the World Wide Web. These publications are in continual demand by producers, educators, and the interested public in Texas, the South, the US, and internationally.

SRAC publications are annually revised as needed and new publications are developed. A Publications Steering Committee, chaired by the TAMU project Principle Investigator decides which publications will be revised and new publications that will be developed. The Steering Committee is made up of scientists (both research and extension) and industry leaders from throughout the Southern Region. Generally, 10-12 new publications are developed and distributed each year. In 2003, seven SRAC publications were developed, published, and distributed throughout the U.S.

Funding for this project is from the 13 States in the Southern Region, the US Department of Agriculture– CSREES, and Texas Cooperative Extension.

BASS 101 SYMPOSIUM

Michael Masser and Billy Higginbotham

With over one million ponds comprising some one-half million surface acres. Texas landowners have numerous aquatic resources to manage for recreational fishing. Interest for managing these smaller impoundments for largemouth bass is of particular interest among landowners.

In order to provide intensive management information on largemouth bass, a two day fee-based program entitled "Bass 101" was conducted in conjunction with the Hays County Extension office, Texas Parks and Wildlife, Natural Resources Conservation Service and the City of San Marcos. A similar symposium was conducted in 2001 in Athens, TX.

The workshop was an unequivocal success. Feedback from participants demonstrated knowledge gains. Surveys indicated that 80% of the 190 participants were pondowners. Forty-four percent indicated that aquatic weed management was their biggest problem, followed by poor fishing (17%) and water quality (7%). Interestingly, 20% said they would spend \$50/acre to improve fishing, another 24% would spend \$50-\$100/acre, another 17% would spend \$100-\$250/acre, another 19% would spend \$250-\$500/acre, another 7% would spend \$500-\$1,000/acre and 13% would spend over \$1,000/acre.

Pre- and post-tests revealed that participants increased their knowledge by 65%. A future Bass 101 symposium is tentatively planned in the Houston area in spring 2005.

DEVELOPMENT OF A CLOSED RECIRCULATING VIRAL-PATHOGEN FREE POSTLARVAE PRODUCTION CENTER FOR NATIVE SPECIES

Ryan L. Gandy, Tzachi M. Samocha, Michael P. Masser, Delbert M. Gatlin III, Joe M. Fox, Abdul-Mehdi S. Ali, and Michael F. Speed

Induced Maturation of *Farfantepenaeus duorarum* Under Closed Recirculating Conditions

A founder (F) population of *F. duorarum* was isolated from wild populations and placed in a closed recirculating induced maturation system to study the effect of eyestalk ablation in a closed recirculating system on the reproductive performance of the shrimp. Twelve percent of the ablated females spawned each night with an average spawn size of 81,000 eggs and fertilization rate of 84%. Survival rates to Nauplius 1 and Zoea 1 sub-stages were 78 % and 70%, respectively. Un-ablated females failed to mature thus, illustrating the need for ablation to induce maturation and spawning.

Production of *F. aztecus* Postlarvae in a Closed Recirculating Larval Rearing System

Larvae of the *F. aztecus*, produced on-site, were used to test a closed recirculating larval rearing system. The data from these studies suggest that raising larvae from Zoea 1 sub-stage through five-day-old postlarval stage (PL₅) is feasible using these systems. Over six production cycles the recirculating systems performed similar to the control tanks with no discharge of the culture water at the end of each cycle. The data suggest that effluent from hatcheries can be reduced by 97% when using closed recirculating systems to control water quality during the larval rearing stage. With the increasing regulation on effluent discharged in the coastal zone and the high cost of coastal land, the use of closed recirculating systems for hatcheries may allow operators to increase production while complying with the increasingly strict effluent water regulations.

Closing the Life Cycle of *F. aztecus* in a Closed Recirculating System

The studies showed that closed-recirculating system can be used to induce maturation and spawning of this species in captivity followed by the production of F1 and F2 generations. When using a closed recirculating system 7% of the ablated females spawned each night. The average spawn size was 50,000 eggs with 69% fertilization rate and survival of 66% from Nauplii 1 to Zoea 1.

Texas Live Bait - Market Survey

A survey of live bait shrimp availability conducted early 2004 showed that the winter period from November through March has no supply from the wild. During this period a farm raised product could enter the market un-contested by wild live bait. A supply of 174,370 lbs of live bait shrimp could easily enter the market during this five-month period and be sold. During this winter period most retailers indicated they would purchase a farm raised live bait shrimp for up-to \$6.00/lbs. In addition, all bait dealers indicated that they have never had enough wild caught shrimp during the winter months to fill the winter demand and most believe they could sell double the amount they were reporting if more were available. As a whole, the Texas live bait shrimp retailers indicated their desire for a 65-70 count shrimp. This survey revealed a summer demand from April through October during which the entire Texas market could absorb 1.36 million pounds of live bait shrimp.

Funding Sources: USDA SBIR Phase II, Tom Slick Fellowship, Sea Grant Industrial Fellowship and TAES.

ORGANIC STRATEGIES FOR FISHMEAL AND FISH OIL REPLACEMENT
Robert A. Bullis, D. Allen Davis, Tzachi Samocha and Susmita Patnaik

If aquaculture is to continue to be an increasing contributor to the human food supply, it is critical that aquaculture feeds become less reliant on marine-derived fishmeal and fish oil as the preferred source of essential proteins and lipids. Not only is the wild fishery from which these products are extracted at or near sustainable levels of harvest, there is also increasing concern the fishmeal and fish oil may contain contaminants and pollutants that have bioaccumulated in marine fish. Current impetus for the replacement of fishmeal and oil in marine fish and shrimp feeds are due to nutritional, economic and social concerns.

Our goal has been to develop a strategy for the production of diets that replace fishmeal and fish oil ingredients with organically certified proteins and lipids. Prototypical diets have been produced and growth trials are currently underway in outdoor tanks and research ponds. Production results that confirm the ability of these diets to perform economically in a standard shrimp production environment were obtained. The data suggest that the ingredients of diets currently being tested can be substituted with ingredients that will qualify these diets for certification under the USDA National Organic Program. Commercial organic diets that are based on our strategies would contain no marine-sourced ingredients and could be used for both herbivorous and carnivorous species.

Funding Sources: Advanced BioNutritio and TAES

INTENSIVE NURSERY OF *LITOPENAEUS VANNAMEI* WITH LIMITED DISCHARGE USING THREE METHODS FOR PARTICULATE MATTER REMOVAL

Mike Handy, Tzachi M. Samocha, Susmita Patnaik, Ryan L. Gandy, Chris Robinson and David A. McKee

Concerns regarding disease epizootics and environmental impacts of aquaculture effluents have led the shrimp farming industry to research methods of increasing biosecurity and limiting water exchange. A nursery study was conducted at the Texas Agricultural Experiment Station, Shrimp Mariculture Research Facility, Corpus Christi, Texas to evaluate water quality, growth, and survival of the Pacific white shrimp, *Litopenaeus vannamei*, in a limited discharge, biosecure system. Three 45 m³ HDPE-lined greenhouse-enclosed raceways (RW's) were stocked with five to six-day-old postlarvae (PL) and cultured for 74 days. Each RW employed a separate method of particulate matter removal: Bead filtration (BF), Pressurized sand filtration (PSF), and Foam fractionation (FF). The results of the water quality analysis suggest that PL's could tolerate ammonium levels as high as 22.64 mg/L and nitrite levels of 8.79 ppm. Other data is summarized in the table below.

Summary of the 2003 intensive nursery study with *Litopenaeus vannamei* operated with limited-discharge at the TAES Shrimp Mariculture Research Facility, Corpus Christi.

Treat	PL/m ³	Wt ₀ [*] (mg)	Days	Wt _f ^{**} (g)	Yield (kg/m ³)	Survival (%)	New Water (% of total vol./d)	FCR
BF	3,780	0.6	74	0.65	2.42	96.3	1.35	1.70
RSF	6,540	0.6	74	0.85	5.26	100.1	0.47	1.09
FF	5,010	0.6	74	0.69	3.18	97.8	2.06	1.50

* Wt₀ – Average weight at stocking

** Wt_f – Average weight at harvest

Throughout the study, shrimp showed no signs of bacterial or viral pathogen infections. This study demonstrated that the raceway operated with the rapid sand filter outperformed the other two raceways in terms of shrimp growth, FCR, survival, yield and daily water exchange rate.

Funding Sources: USDA Marine Shrimp Farming Program, TAES, Harlingen Shrimp Farms and TAMU-CC

THE EFFECT OF DIFFERENT SYNTHETIC POLYMER LINERS ON GROWTH, SURVIVAL AND SELECTED WATER QUALITY INDICATORS IN TANK-SYSTEM STOCKED WITH *LITOPENAEUS VANNAMEI* UNDER LIMITED DISCHARGE AND IN THE PRESENCE OF NATURAL PRODUCTIVITY

Tzachi M. Samocha, Susmita Patnaik, Ami Horowitz and Sarah Horowitz

The trend of intensification in shrimp farming activities in different parts of the world has been associated with wide use of ponds and tanks lined with synthetic polymers made of different compounds and thickness. As some of these polymers can release toxic substances into the culture media, it is important to select liners that have no adverse effect on growth or survival of the targeted cultured organism. A 70-d study was designed to evaluate the effects of liners made of different Ethylene Propylene Diene Monomer (EPDM) synthetic polymers (Liner #1, #2 and #3) on growth, survival and selected water quality indicators in a static tank-system stocked with juveniles (0.66 ± 0.06 g) of the Pacific white shrimp *Litopenaeus vannamei*. A non-toxic liner made of High Density PolyEthylene (HDPE), commonly used in aquaculture, served as a liner-control. Tanks with no liner served as no-liner control. There were six tank replicates for each treatment. Tanks (610 L, 0.85 m²) were stocked at 30 shrimp/m². Every tank with test liner was provided with two 0.91 m² pieces of one of the EPDM test liners or the HDPE liner that were suspended vertically in the water column. All tanks were provided with two airstones for mixing and aeration. Dissolved oxygen, temperature, pH and salinity were monitored twice daily, while ammonium and nitrite were monitored once a week in all the tanks.

No statistically significant differences were found in the daily dissolved oxygen, salinity, pH and temperature between all treatments. Survival rates at the end of the study ranged between 88.46 and 98.46% with no statistically significant differences between treatments. Shrimp average final weight in the three EPDM test liners and the HDPE control liner ranged between 12.29 g and 13.03 g, with no statistically significant difference. The average weight of the shrimp in the no-liner control group (14.7 g) was significantly higher than in the other treatments.

These results suggest that under the conditions of this study, the growth performance of the shrimp raised in the presence of the EPDM liner was as good as the growth observed for the shrimp raised in the presence of a commonly used HDPE liner. As the tests were done at a ratio of 2.164 m²/m³ exposed liner surface area to water volume, which is 1.25 fold higher than what is commonly used in aquaculture raceways, these results should be applicable to aquaculture usage.

Funding Sources: Firestone Building Products, TAES and UPAH Tech

DIETARY ZINC REQUIREMENT OF HYBRID STRIPED BASS (*MORONE CHRYSOPS*? X *M. SAXATILIS*)

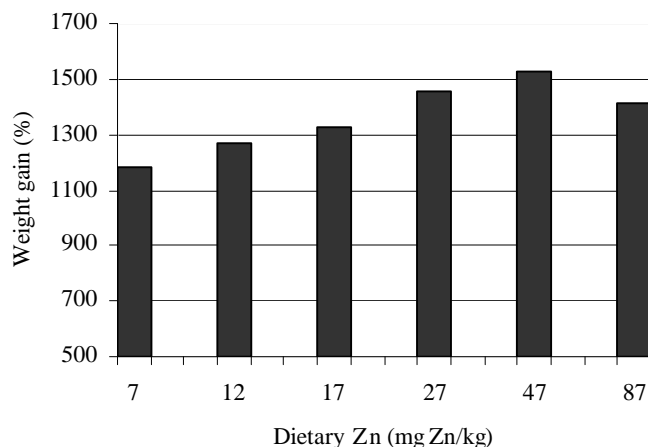
Jonathan B. Goff and Delbert M. Gatlin III

Zinc is a trace mineral element that plays an essential role in numerous biochemical processes, and has been shown to affect growth and health of several fish species. Reduced weight gain, feed efficiency and survival of fish along with dwarfism, cataracts, and erosion of fins have been noted as clinical signs of dietary zinc deficiency. Zinc also may form complexes with different dietary elements and compounds, which in turn may affect its bioavailability to fish. Dietary zinc requirements have been evaluated with many different species such as rainbow trout, *Onchorhynchus mykiss*, tilapia *Oreochromis aureus*, common carp *Cyprinus carpio*, red drum *Sciaenops ocellatus*, and channel catfish *Ictalurus punctatus*; however, the dietary zinc requirement of hybrid striped bass (*Morone chrysops* ? x *M. saxatilis*?) has not been determined. Therefore, this study was conducted to define the dietary zinc requirement for the hybrid striped bass.

Experimental diets were formulated to contain 35% crude protein, 6% lipid, and approximately 3.4 kcal digestible energy/g from purified ingredients to minimize the level of intrinsic zinc to 7 mg Zn/kg. The basal diet was supplemented with 5, 10, 20, 40 and 80 mg Zn/kg from zinc sulfate heptahydrate. All six diets were fed to triplicate groups of 15 fingerling hybrid striped bass initially weighing 0.86 ± 0.05 g/fish in 38-L glass aquaria connected as a recirculating system. The fish were fed twice daily to apparent satiation and weighed weekly in order to adjust feeding and record growth. At the conclusion of the 10-week feeding trial, blood and bone samples were obtained and analyzed for zinc concentrations. Growth performance, hematology and zinc concentrations of various tissues were subjected to analysis of variance and regression using SPSS 11.0 software with significance set at P<0.05.

Fish thrived during the trial with no overt deficiency signs besides reduced growth observed even in fish fed the basal diet. Weight gain of fish steadily increased with graded levels of dietary zinc (Fig. 1). Based on weight gain and bone zinc data, the minimum dietary zinc requirement of hybrid striped bass was approximately 25-30 mg Zn/kg diet. This requirement estimate is similar to the range of dietary zinc requirements reported for other fish species.

Funding: Texas Agricultural Experiment Station



A COMPARISON OF TWO COMMERCIAL DIETS WITH 30% AND 36% CRUDE PROTEIN FOR THE CULTURE OF *LITOPENAEUS VANNAMEI* UNDER LIMITED WATER EXCHANGE IN MEMBRANE-LINED PONDS

Tzachi M. Samocha, Susmita Patnaik, Ryan L. Gandy, David L. Brock and Addison L. Lawrence

For the last decade, more and more shrimp producers started to grow shrimp in ponds with limited discharge. In most cases, this reduced water exchange did not result in negative impact on yields. Furthermore, the decrease in water usage also helped minimize disease outbreaks and negative environmental impact. This reduction in water usage has encouraged feed manufacturers to formulate feeds that can perform better under limited discharge conditions. The use of improved diet formulations along with improved feed management can minimize waste of dietary protein, the largest expenditures in feed production. This optimization of protein utilization could reduce costs and improve water quality.

Two commercial diets containing 30% and 36% crude protein were evaluated for their perceived effect on water quality, growth and survival of the Pacific white shrimp *Litopenaeus vannamei* in membrane-lined ponds under limited water discharge. Juveniles (0.80 g) were stocked into two (2,450 m³) ponds at a density of 75 shrimp/m³ (106/m²). Paddlewheel and aspirator-type aerators (10-30 hp/ha) were used for water circulation and oxygenation in each pond. Dissolved oxygen, salinity, pH and temperature were monitored twice daily; ammonium-N, nitrite-N, nitrate-N, reactive P, five-day carbonaceous biochemical oxygen demand (cBOD₅), total suspended solids (TSS), volatile suspended solids (VSS) and settleable solids were monitored weekly.

To provide the shrimp with similar dietary nitrogen, the 36% rations were reduced to a level of 15.8% below that of the 30% rations. Commercial diets (donated by Rangen Inc., Buhl, Idaho) were formulated to contain similar nutrient levels when fed at iso-nitrogenous basis. No statistically significant differences were observed between the different diets for ammonium, nitrite, nitrate, reactive phosphorus, TSS and VSS. Only cBOD₅ was statistically significant higher in the low-protein diet treatment. There was no statistically significant difference in the average body weight of shrimps fed 30% and 36% protein diets. The harvest data is summarized in the table below. The shrimp fed the 36% protein diet had a lower FCR, greater survival and higher yield than the pond fed at 30% protein diet. This study demonstrated that under limited discharge conditions, better feed utilization can be expected when shrimp are offered a high-protein diet at a reduced ration size compared to a low-protein diet fed at a higher ration size.

Funding Sources: USDA Marine Shrimp Farming Program, Rangen Inc., TAES and Harlingen Shrimp Farms

The effect of two commercial diets with 30% and 36% CP on growth, survival and FCR of *Litopenaeus vannamei* in outdoor ponds under limited water discharge.

Diet	N	Av. Wt. ^f (g)	Time (d)	Yield (kg/m ³)	Sur.(%)	Growth (g/wk)	FCR	N. Water (%/day)
P-30% CP	1	16.4	137	0.67	55.1	0.84	2.58	0.80
P-36% CP	1	15.8	137	0.80	69.0	0.80	1.88	0.65

EVALUATION OF CRYSTALLINE AMINO ACID TEST DIETS INCLUDING PH ADJUSTMENT WITH RED DRUM (*SCIAENOPS OCELLATUS*) AND HYBRID STRIPED BASS (*MORONE CHRYSOPS X M. SAXATILIS*)

Kasey W. Whiteman and Delbert M. Gatlin III

The red drum (*Sciaenops ocellatus*) and hybrid striped bass (*Morone chrysops x M. saxatilis*; HSB) are cultured in the United States for both stock enhancement and food production. Based on previous observations in this laboratory, the HSB appeared to use pH-adjusted crystalline amino acid test diets more readily than the red drum. Therefore, the present study was conducted to directly compare responses of red drum and HSB fed crystalline amino acids with and without neutralization (pH 7) relative to an intact protein diet composed of red drum muscle. Fifteen red drum (initially 1.5-2.5 g each) and 15 HSB (initially 4.5-5.5 g each) were placed separately in each of 9, 38-L aquaria (18 aquaria total), and each diet was randomly assigned to three replicate tanks of red drum and HSB. After an 8-week feeding period, both red drum and HSB fed the intact protein diet had significantly (P<0.05) greater weight gain and feed efficiency compared to those fed the crystalline amino acid diets, although HSB tended to use the crystalline diets more efficiently. In addition, no differences were detected in red drum or HSB fed the crystalline amino acid diets regardless of pH adjustment.

Funding: The Aquaculture Protein Centre, Sunndalsora, Norway

A COMPARISON OF TWO COMMERCIAL DIETS (30% AND 36% CRUDE PROTEIN) FOR THE CULTURE OF *LITOPENAEUS VANNAMEI* UNDER LIMITED WATER EXCHANGE IN BARE BOTTOM TANKS

Tzachi M. Samocha, Susmita Patnaik, Ryan L. Gandy, David L. Brock, and Addison L. Lawrence

Viral disease outbreaks and increased environmental regulations have pressured commercial shrimp farmers to raise shrimp under limited water exchange. Excess unassimilated nitrogen, associated with surplus dietary protein, can result in poor water quality. As protein is one of the largest expenditures in feed production, unutilized dietary protein can result in increased production cost to farmers. Thus, optimization of protein utilization could reduce costs and improve water quality.

Two commercial diets containing 30% and 36% crude protein were evaluated for their effect on water quality, growth and survival of the Pacific white shrimp *Litopenaeus vannamei* in bare bottom tanks under limited water discharge. The 36% CP diet was formulated to provide similar nutrient levels when fed at iso-nitrogenous basis to the 30% CP diet. Table 1 shows the ration sizes and the number of replicates in each treatment. All diets were manufactured and donated by Rangen, Inc. (Buhl, Idaho). Juveniles (0.66 g) were stocked into twenty-two 10.5-m² (7.35-m³) tanks at a density of 75 juveniles/m³ (52/m²). Dissolved oxygen, salinity, pH and temperature were monitored twice daily; ammonium, nitrite, nitrate, reactive P, cBOD₅, TSS, and VSS were monitored weekly.

Table 1. Experimental design with 30% and 36% protein diets in bare bottom tank study with the Pacific white shrimp *Litopenaeus vannamei* under limited water exchange conditions.

Treatment ID	Replicate	% CP	Nitrogen	Ration
30% CP-100%	6	30	-	100%
36% CP-84.2%	5	36	Iso N	84.2%
30% CP-84.2%	5	30	-	84.2%
36% CP-70.9%	6	36	Iso N	70.9%

There were no statistically significant differences in daily water quality indicators between treatments. Also no statistically significant differences were found in ammonium and nitrite concentrations between treatments. Reactive phosphorus, nitrate and VSS were significantly higher in the low-protein treatments. The high phosphorus levels in the low-protein diet is a matter of concern since its level in effluents is controlled by the National Pollution Discharge Elimination System (NPDES).

There were no statistically significant differences in survival and average body weight of shrimp fed at 30% and 36% CP diets on iso-nitrogenous basis. The FCR for the two 36% CP diets were significantly lower than the 30% CP diet. The yield in the 36% - 84.2% CP diet treatment was significantly higher than the 30% - 84.2% CP diet (Table 2). These findings suggest optimization of FCR is feasible using a higher protein feed with a low ration size than a lower protein feed at a higher ration size. It suggests a more effective way of cutting the feed cost without negative impact on critical water quality indicators such as ammonia and nitrite levels between different protein tanks.

Table 2. The effect of two commercial diets (30% and 36% CP) on average weight, growth, survival, yields and FCR of *Litopenaeus vannamei* in bare bottom outdoor tank-system under limited water discharge (same superscripts within a column are not statistically different).

Treatment CP-Ration	N	Av. Wt ^f (g)	Time (d)	Yield (kg/m ³)	Sur. (%)	Growth (g/wk)	FCR	N. Water (%/day)
30% CP-100%	6	16.69 ^a	118	1.03 ^{ab}	87.7 ^a	0.95 ^a	1.82 ^a	0.88
30% CP-84.2%	5	14.46 ^b	118	0.98 ^c	94.9 ^a	0.82 ^b	1.66 ^a	0.85
36% CP-84.2%	5	16.01 ^a	118	1.14 ^a	89.2 ^a	0.91 ^a	1.38 ^b	0.91
36% CP-70.9%	6	13.92 ^b	118	1.02 ^{ab}	94.0 ^a	0.79 ^b	1.33 ^b	0.86

Funding Sources: USDA Marine Shrimp Farming Program, Rangen Inc., TAES and Harlingen Shrimp Farms

EVALUATION OF DIFFERENT SULFUR COMPOUNDS IN THE DIET OF RED DRUM *SCIAENOPS OCELLATUS*

Jonathan B. Goff and Delbert M. Gatlin III

Aquaculture production of red drum *Sciaenops ocellatus* for stock enhancement and human consumption continues to be practiced by public and private entities in Texas. Thus, refinement of diet formulations to enhance the efficiency of red drum production continues to be pursued. Based on the amino acid requirements of red drum that have been established to date, the sulfur amino acid (SAA) requirement for methionine plus cystine appears to be most limiting. Relatively large substitution of plant proteins, such as soybean meal, for animal feedstuffs will likely require supplemental methionine to meet the SAA requirement of red drum. Therefore, this study was conducted to determine the value of different sulfur compounds in diets for red drum.

A feeding trial was conducted to compare growth characteristics of red drum fed diets supplemented with L-methionine, DL-methionine, or methionine hydroxy analog (MHA) on an equal-sulfur basis. The basal diet was formulated to contain 35% crude protein and 0.54% methionine plus cystine from red drum muscle and a crystalline amino acid premix. The premix was used to ensure that all amino acids other than methionine and cystine were present at or above required levels. Three diets were supplemented with L-methionine, DL-methionine, or MHA to provide methionine plus cystine at 1.06% of diet to meet the established requirement of red drum. Another diet was supplemented with additional L-methionine to provide 1.71% of diet with a SAA:lysine ratio of 0.48, the estimated ideal ratio for red drum based on muscle composition. All five diets were then fed to triplicate groups of 12 fingerling red drum initially weighing 8.95 ± 0.33 g/fish (mean \pm s.d.) in 110-L aquaria connected as a brackish (7 ppt) water recirculating system. Feedings were administered twice daily at a rate approaching satiation for 8 weeks. Production data were evaluated using one-way analysis of variance and Duncan's multiple range test with significance set at $P < 0.05$.

Fish fed the basal diet had a significantly reduced weight gain (200% of initial weight) which was expected due to the inadequate level of methionine. The basal diet also supported lower overall survival of 52%; whereas, survival averaged over 95% for fish fed the other diets. There was no statistical difference in weight gain of fish fed diets containing L-methionine, DL-methionine, MHA, or the ideal methionine to lysine ratio. All methionine-supplemented diets supported similar weight gain (414 - 443%) over the 8-week trial. Results from this study indicate red drum are able to use DL-methionine and MHA as effectively as L-methionine to meet their SAA requirement. Also, no additional growth enhancement was observed when methionine was supplemented beyond the established requirement.

Funding: Sea Grant College Program

EVALUATION OF ALIMET[®] AND CRYSTALLINE DL-METHIONINE HYDROXYL ANALOG (MHA) AS COMPARED TO L-METHIONINE IN THE DIET OF HYBRID STRIPED BASS (*MORONE CHRYSOPS* \times *M. SAXATILIS*)

Mark C. Kelly and Delbert M. Gatlin III

Hybrid striped bass (HSB) represents a prominent aquacultural enterprise in the United States. Improvements to existing culture aspects including diet formulations are needed to increase the efficiency and profitability of culturing HSB. The protein/amino acid component of diet represents one of the most expensive constituents; therefore, reducing the cost of satisfying amino acid requirements of fish may result in more cost-effective production. This study was conducted to evaluate Alimet[®], a liquid form of MHA, as a substitute for L-methionine in the diet of HSB. This was achieved by comparing production characteristics of fish fed casein-gelatin-based diets containing 35% crude protein and supplemented with either Alimet[®] crystalline DL-MHA, or L-Methionine to meet the established requirement of HSB. A basal diet containing no supplemental methionine also was included. Groups of 15 fish initially averaging 11.8 g were graded and stocked into 12, 38-l aquaria operated as a recirculating system. Each experimental diet was randomly assigned to three replicate aquaria and fed at a rate approaching apparent satiation twice daily for 10 weeks. Based on weight gain and feed efficiency, Alimet[®] and crystalline MHA provided comparable performance of HSB, but both were statistically ($P < 0.05$) inferior to L-methionine when supplemented on an equal-sulfur basis.

Funding: The Aquaculture Protein Centre, Sunndalsora, Norway

DIETARY PROTEIN REQUIREMENT OF BLACK SEA BASS AND RELATIVE USE OF CARBOHYDRATE AND LIPID

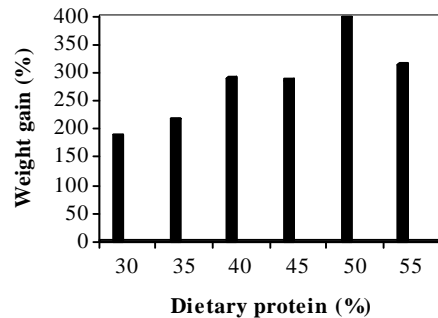
Jonathan B. Goff, Steven C. Ging, and Delbert M. Gatlin III

The black sea bass (*Centropristis striata*) is a highly desired commercial and recreational species on the Atlantic coast of the United States. Its high market value and demand along with a seasonal supply make it a good candidate for commercial aquaculture. However, more information concerning nutritional requirements of this species is needed to develop diet formulations and facilitate its aquacultural production. Quantifying dietary protein requirements of cultured species is important because protein is the most expensive nutrient and thus can dramatically influence diet costs. Excessive dietary protein that is catabolized for energy also may increase nitrogenous waste production. Different fish species have shown considerable variation in their ability to use protein, carbohydrate and lipid for energy with carnivorous fish tending to use protein and lipid preferentially to carbohydrate. Therefore, a study was conducted to determine the minimum dietary crude protein requirement of black sea bass and relative use of carbohydrate and lipid for energy.

Experimental diets were formulated with menhaden fish meal to contain different amounts of protein, soluble carbohydrate (dextrin) and menhaden oil. The control diet contained 40% crude protein, 8% lipid and 22% dextrin while five other diets contained graded levels of crude protein from 30 to 55% of diet. Dextrin and lipid were varied to provide approximately 2.9 to 3.6 kcal estimated digestible energy/g. To evaluate the relative use of carbohydrate and lipid for energy, four diets were formulated to contain 40% crude protein with different amounts of lipid and dextrin. Lipid ranged from 6 to 19% of diet and dextrin decreased from 30 to 1.3% as lipid increased. The feeding trial was conducted in 38-L aquaria connected as a recirculating system to a biofilter and sand filter with water salinity maintained at 25 ± 4 ppt using synthetic seawater and NaCl. Temperature was maintained at $24 \pm 2^\circ\text{C}$ throughout the trial. Juvenile black sea bass initially averaging approximately 1.3 g/fish were sorted into groups of 15 and stocked into each aquarium. Each diet was randomly assigned to three aquaria and fed at a rate approaching apparent satiation two times per day. Fish were group weighed once a week to assess weight gain and adjust feed quantities. The feeding trial was conducted for 7 weeks.

The minimum dietary protein requirement was determined to be approximately 50% of diet based on weight gain (Fig. 1) and feed efficiency responses. In comparing the relative value of carbohydrate and lipid, weight gain and feed efficiency responses were similar among the various treatments. Based on these data, the black sea bass requires relatively high dietary protein for optimal growth and appears to use soluble carbohydrate effectively for energy.

Funding: Sea Grant National Marine Aquaculture Initiative



DIETARY BREWERS YEAST AND THE PREBIOTIC GROBIOTIC™AE INFLUENCE GROWTH PERFORMANCE, IMMUNE RESPONSES AND RESISTANCE OF HYBRID STRIPED BASS *MORONE CHRYSOPS X M. SAXATILIS* TO *STREPTOCOCCUS INIAE* INFECTION

Peng Li and Delbert M. Gatlin III

Use of prebiotics, nondigestible dietary ingredients which beneficially affect the host by selectively stimulating the growth of and/or activating the metabolism of health-promoting bacteria in the intestinal tract, is a novel concept in aquaculture. Two separate feeding trials were conducted to evaluate graded levels of a commercial prebiotic Grobiotic™AE (GA), a mixture of partially autolyzed brewers yeast, dairy ingredient components and dried fermentation products, in the diet of hybrid striped bass, as compared to partially autolyzed brewers yeast (Brewtech®, BY). The basal diet in both trials was formulated to contain 40% protein, 10% lipid and an estimated digestible energy level of 3.5 kcal/g. Two levels (1 and 2 % of diet) of GA and BY were added to the basal diet with menhaden fish meal and menhaden oil adjusted to provide isonitrogenous and isolipidic diets. Each diet was fed to five (trial 1) or three (trial 2) replicate groups of juvenile hybrid striped bass in 110-L aquaria twice daily at rates approximating apparent satiation for 7 weeks (trial 1) or 4 weeks (trial 2).

Enhanced growth performance was generally observed in fish fed the diets supplemented with GA or BY, compared to the basal diet after 7 weeks of feeding in trial 1 (Table 1). Significantly higher ($p < 0.05$) feed efficiency was observed in fish fed diets supplemented with 1 and 2% GA. After 4 weeks of feeding in trial 2, growth and feed efficiency were not significantly affected by the various dietary treatments. Extracellular superoxide anion production of head kidney macrophages from fish fed diets with 1 and 2% BY and 1% GA was significantly ($p < 0.01$) higher than that of fish fed the basal diet. All groups of fish fed BY and GA showed a significantly ($p < 0.01$) enhanced survival (73.3-90%) after bath exposure to *S. iniae* compared to fish fed the basal diet (53.3%). Based on these data, it is concluded that GA and partially autolyzed BY can serve as functional feedstuffs in the diet of hybrid striped bass by enhancing growth performance and immunological responses. Further research is planned to investigate the mechanism(s) of action associated with these compounds and their application in aquaculture.

Funding: International Ingredient Corporation, Inc

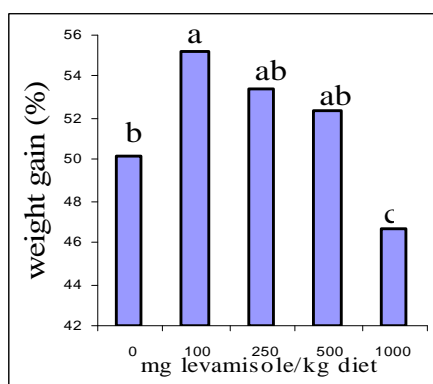
TABLE 1. Responses of hybrid striped bass fed diet containing different levels of BY and GA.

Feeding trial 1 (7 weeks)			Feeding trial 2 (4 weeks)				
Diet	Weight gain (%)	Feed efficiency	Neutrophil oxidative	Serum lysozyme	Extracellular O ₂ ⁻ (nmol)	Intracellular O ₂ ⁻ (O.D.)	Survival (%)
Basal	388	0.93b	1.20	473	7.21c	1.28	53.3b
1% BY	388	0.95ab	1.40	515	11.22a	1.55	90.0a
2% BY	404	0.95ab	1.58	472	10.55a	1.66	76.7a
1% GA	420	1.0a	1.28	448	9.71ab	1.79	73.3a
2% GA	405	1.0a	1.31	640	8.34bc	1.59	86.7a

IN VIVO AND IN VITRO EVALUATION OF LEVAMISOLE AS AN IMMUNOSTIMULANT FOR HYBRID STRIPED BASS MORONE CHRYSOPS X M. SAXATILIS

Peng Li, Xiaoxue Wang, and Delbert M. Gatlin III

Levamisole is a broad spectrum anthelmintic drug widely used to control internal parasites in livestock. It also has been used in studies on fish with the aim of enhancing innate immune response or serving as an adjuvant with a vaccine. Due to its low cost and low residue in fish, it holds promise as an immunostimulant for hybrid striped bass, one of the most important fish cultured in the USA. Thus, experiments were conducted to evaluate levamisole with hybrid striped bass. A basal diet was formulated to contain 40% protein, 10% lipid and an estimated digestible energy level of 3.5 kcal/g. Four levels (100, 250, 500 and 1000 mg/kg) of levamisole were added to the basal diet in place of cellulose. Each diet was fed to three replicate groups of hybrid striped bass (initial weight of 41.6 g/fish) at rates approximating apparent satiation for 3 weeks after which two fish per aquarium (6 per dietary treatment) were randomly chosen for immune response assays. In addition, a *Streptococcus iniae* bath challenge was executed on the remaining fish. Levamisole significantly influenced weight gain ($P < 0.05$) and feed efficiency ($P < 0.01$) after 3 weeks of feeding as shown in Fig. 1. Intracellular superoxide anion production of head kidney macrophages of fish fed 250 mg levamisole/kg diet was significantly higher than that of the other four groups. Hematocrit and serum lysozyme were not affected by dietary treatments. Fish fed 100, 250 and 500 mg levamisole/kg diet tended ($p < 0.2$) to have better survival after disease challenge than fish fed the basal diet and 1000 mg levamisole/kg diet.



In addition, a series of *in vitro* manipulations were conducted with phagocytic cells isolated from the head kidney (HK) of hybrid striped bass. The HK phagocytes were cultured in media containing 1, 10, 100, 1000 mg levamisole/ml. Extracellular and intracellular reactive oxygen intermediates were determined after 24, 48 and 72 h of incubation. No differences in reactive oxygen intermediates were observed among the treatments. However, 1000 mg levamisole/ml suppressed the reactive oxygen of HK cells ($p < 0.0001$). Also, 100 mg levamisole/ml suppressed HK cell functions after 48-h and 72-h incubations.

It is concluded that dietary levamisole is capable of enhancing growth performance of hybrid striped bass after feeding 100 mg/kg diet. The effects of levamisole on hybrid striped bass are closely dose- and time-dependent as shown in other fish species. An overdose of levamisole (approximately 56 mg/kg fish weight per day) caused chronic toxicity by retarding growth. *In vitro* results suggest that HK phagocytes of hybrid striped bass are not the target cells of levamisole, but levamisole may influence the respiratory burst by modulating the production of lymphokines from lymphocytes.

Funding: The Texas Agricultural Experiment Station

NUTRITIONAL VALUE OF FISHERIES BY-CATCH AND BY-PRODUCT MEALS IN THE DIET OF RED DRUM (*SCIAENOPS OCELLATUS*)

Peng Li, Xiaoxue Wang, Ronald W. Hardy, Delbert M. Gatlin III

Discarding of by-catch and generation of by-products from capture fisheries has long been recognized as wasteful, but greater effort is needed to make use of these resources in aquaculture as its growth throughout the world requires increased production of feeds to support the cultured organisms. Protein resources, primarily fish meal, will probably be a constraint to further development of the aquaculture industry. Fisheries by-catch or by-product meals theoretically have good potential to reduce dependence on fish meal, although research in this subject is limited. A digestibility study with sub-adult red drum and a feeding trial were conducted with juveniles to evaluate the use of various by-product ingredients in aquafeeds. A shrimp by-catch meal (SBM) from shrimp trawling in the Gulf of Mexico, a shrimp processing waste meal (SWM) from aquacultured *Litopenaeus vannamei*, two underutilized fish meals [Pacific whiting meal without soluble (PW) and Pacific whiting meal with soluble (PWS)], and a fish-processing waste [red salmon head meal (RSHM)] from Alaska were included in diets. The digestibility study employed chromic oxide as a marker and stripping for fecal collection. No differences ($P>0.05$) in organic matter, crude protein, energy and total phosphorus digestibility were observed among fish fed Special Select™ menhaden fish meal, SBM, PW or PWS, while digestibility of these nutrients and energy from RSHM was significantly lower. During the 6-week feeding trial, no significant differences were observed among fish fed diets in which 25% of the protein from menhaden fish meal was replaced with RSHM or 50% was replaced by SBM in 40% crude protein diets. Fish fed diets in which 50% or 100% of protein from menhaden fish meal was replaced with PWS, PW and SWM showed significantly ($P<0.05$) reduced growth and feed efficiency, although there was no significant difference in survival of fish fed the different diets. More research is needed to optimize the use of these ingredients and to eliminate limiting factors by improving processing techniques, amino acid availability and palatability.

Funding: Sea Grant National Marine Aquaculture Initiative

EVALUATION OF DIETARY NUCLEOTIDES AS AN IMMUNOMODULATOR FOR HYBRID STRIPED BASS (*MORONE CHRYSOPS X M. SAXATILIS*)

Peng Li, Donald. H. Lewis and Delbert. M. Gatlin III

Three feeding trials were conducted to evaluate potential immunomodulatory effects of nucleotides in the diet of hybrid striped bass. A basal diet was formulated to contain 40% protein, 10% lipid and an estimated digestible energy level of 3.5 kcal/g. In the experimental diet, nucleotides from Ascogen P® were added to the basal formulation at the manufacturer's recommended rate of 0.5% and menhaden fish meal and cellulose were adjusted to maintain the diets isonitrogenous. Each diet was fed to four replicate groups (trials 1 and 2) of juvenile hybrid striped bass twice daily at rates approximating apparent satiation for either 8 (trial 1) and 7 (trial 2) weeks, respectively. The third feeding trial was conducted to explore the efficacy of long-term (16 weeks) administration of nucleotides in the diet. After trials 1 and 2, a *Streptococcus iniae* bath challenge was executed to test the effects of diet on disease resistance. Additional *S. iniae* exposure was conducted in trial 1 because of limited mortality after the first challenge. For trial 2, eight surviving fish from each treatment were given a booster intraperitoneal injection of 6×10^6 formalin-killed *S. iniae* cells/ml and serum were taken for antibody titer quantification 10 days later.

No significant differences ($P>0.05$) in weight gain and feed efficiency were generally observed in fish fed the diets supplemented with nucleotides compared to the basal diet in both trials. In Trial 1, body composition of whole fish, hemocrit and serum lysozyme levels were observed to be within normal ranges and not influenced by dietary nucleotides. However, significantly ($p=0.017-0.091$) enhanced survival after exposure to *S. iniae* was generally observed in each controlled challenge. In trial 2, fish fed the nucleotide-supplemented diet tended to have a higher antibody response based on microtitration agglutination and slide agglutination; however, the difference was not statistically significant because of high variation between individual fish. Neutrophil oxidative radical production of fish fed the nucleotide-supplemented diet was significantly higher than that of fish fed the basal diet.

In the third trial (16 weeks), intracellular and extracellular superoxide anion production of head kidney macrophages, neutrophil oxidative radical production and serum lysozyme were not significantly affected by long-term administration of dietary nucleotides when compared to fish fed the basal diet.

Based on the result of this study, it is concluded that dietary nucleotides positively influenced immune responses of hybrid striped bass as well as resistance to *S. iniae* infection when administered for a relatively short period (7-8 weeks). Extended feeding (16 weeks) of nucleotides did not apparent adversely affect non-specific immune responses of hybrid striped bass.

Funding: Texas Agricultural Experiment Station

COMPARISON OF PURIFIED AND PRACTICAL DIETS SUPPLEMENTED WITH OR WITHOUT B-GLUCAN AND SELENIUM ON RESISTANCE OF HYBRID STRIPED BASS (*MORONE CHRYSOPS X M. SAXATILIS*) TO *STREPTOCOCCUS INIAE* INFECTION
Francisco Jaramillo, Jr. and Delbert M. Gatlin, III

This study was conducted to evaluate the efficacy of b-glucan and selenium supplements to the diet on increasing survival of hybrid striped bass (*Morone chrysops x M. saxatilis*) exposed to *Streptococcus iniae*. A 2 x 2 x 2 factorial design was employed by including purified casein/gelatin-based diets and practical menhaden fish meal-based diets with supplements of either b-glucan from barley at 0 and 0.1% of diet or sodium selenite at 0 or 0.2 mg/kg diet or a combination of these supplements. All diets were formulated to meet the nutritional needs of hybrid striped bass with the exception of selenium. The purified and practical basal diets contained 0.03 and 1.03 mg Se/kg, respectively, and the diets supplemented with sodium selenite had an additional 0.07 mg Se/kg on average. Juvenile hybrid striped bass initially averaging 2.44 ± 0.17 g/fish were fed the eight experimental diets in triplicate 110-L recirculating aquaria for 6 wk, after which they were immersed in a bath of *S. iniae* at 6.2×10^6 CFU/ml for 2 h and monitored for 21 d. Weight gain and feed efficiency were significantly ($P < 0.05$) affected by diet type and selenium supplementation with fish fed practical diets and those supplemented with selenium having the greatest values. Supplementation of b-glucan to the purified or practical type diets did not significantly affect survival of fish after experimental infection. The most notable difference ($P < 0.0001$) was in the comparison of diet type; the survival rate of fish fed the practical diets was 75% compared to 35% for those fed the purified diets. Dietary supplementation of b-glucan did not enhance disease resistance in the present study. Fish fed menhaden fish meal-based diets were significantly more resistant to *S. iniae* in comparison to the those fed the purified diets. Thus, nutritional influences on disease resistance of hybrid striped bass were evident in this study and warrant further investigation.

Funding: Texas Advanced Technology Program

HEREDITY OF TRAITS IMPORTANT FOR AQUACULTURE OF HYBRID STRIPED BASS
Kirstin E. Ross, Xiaoxue Wang, Delbert M. Gatlin III and John R. Gold

Hybrid striped bass (HSB) - male striped bass crossed with female white bass - have been shown to possess superior traits for aquaculture in comparison with parental stocks. Commercially farmed HSB is a rapidly growing industry. However, it is possible that a constraint limiting expanded production of hybrid striped bass is suboptimal production efficiency stemming from uncontrolled variation in performance (survival, growth, nutritional assimilation) of fish derived from undomesticated broodstock. Given the success of selective breeding programs in other aquacultural and agricultural areas, a selective breeding program to increase yield of HSB is warranted.

The first step in a selective breeding program requires an understanding of the heritability of traits important to production. In this study, we crossed 10 female white bass with 10 male striped bass in a factorial arrangement to estimate heritability for specific production traits. Offspring were tagged for identification and genotyped to determine parentage. Growth (total mass and length, and liver, fat, fillet, and gonad ratios), tolerance to a bacterial challenge (*Streptococcus iniae*) and protein accretion were monitored to determine parental influence on these traits. Body composition results indicate relationships between fat and fillet ratios and body mass. Estimates of heritability of traits suggest potential for a broodstock selection program.

Funding: Texas Advanced Technology Program

STOCK STRUCTURE AND EFFECTIVE SIZE OF RED DRUM (*SCIAENOPS OCELLATUS*) IN THE NORTHERN GULF OF MEXICO: IMPLICATIONS RELATIVE TO STOCK ENHANCEMENT AND RECRUITMENT
John R. Gold

Genetic analysis of stock structure of a target species typically is considered a high priority for stock-enhancement programs. The rationale is that different stocks may possess unique genetic attributes that relate to important adaptive differences, and that mixing different stocks for stock enhancement may have negative impacts. Results of genetic studies of stock structure of red drum (*Sciaenops ocellatus*), an estuarine-dependent sciaenid fish in the northern Gulf of Mexico, are presented and discussed. A stimulus for the research is the red drum stock-enhancement program carried out by the State of Texas. Significant (genetic) heterogeneity occurs among geographic samples of red drum, indicating genetically defined stocks. However, the distribution of genetic variation in red drum follows a pattern of isolation by distance where occurrence of overlapping stocks that are centered in (natal) bays and estuaries is indicated. Gene exchange among red drum stocks is hypothesized to be a function of geographic distance, with an effective geographic neighborhood size of 700-800 kilometers. Estimates of the genetic effective size of red drum in the northern Gulf of Mexico are three orders of magnitude smaller than estimates of adult census size. Implications of these findings for red drum stock enhancement and recruitment are considered.

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