SUNFISH[13]

Progress Report for the Period
September 1, 1994 to August 31, 1996

NCRAC FUNDING LEVEL: $174,999 (September 1, 1994 to August 31, 1996)

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Non-Funded Collaborators:		
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Fountain Bluff Fish Farms		Illinois
Illinois Department of Conservation	Little Grassy Fish Hatchery, Carbondale	Illinois
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Ron Johnson	Spruce Creek Fish Farm	Minnesota
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Missouri Department of Conservation		Missouri
Tribal Council	Red Lake Band Chippewa	Wisconsin
National Biological Service	Midwest Science Center (formerly USFWS National Fisheries Contaminant Research Laboratory)	Missouri

PROJECT OBJECTIVES

(1) Produce a production manual, accompanying videos and other information as necessary to demonstrate the technology for culturing centrarchids.

(2) Determine the major nutritional requirements for centrarchids and to compare their growth and performance using available commercial feeds in laboratory and field settings.

(3) Determine the best feeding management strategies for culturing centrarchids in laboratory and field settings.

ANTICIPATED BENEFITS

At the 1993 Program Planning Meeting held in Madison, Wisconsin, the North Central Regional Aquaculture Center (NCRAC) Industry Advisory Council specifically requested the development of extension educational materials in the form of a production manual and accompanying video tapes, as a high priority need for demonstrating the commercial feasibility of centrarchid sunfish aquaculture in the region. Such information is needed to enable this industry to enlarge.

Defining the critical nutritional requirements for targeted sunfish will enable development of diets that meet, but not exceed, their requirements. Feed costs are typically the largest annual variable cost; thus, minimizing nutrient concentrations decreases costs without impairing weight gain or health of individuals. Protein requirements of sunfishes are poorly understood, which hinders

their economic potential in food fish culture. Accurate estimates of protein requirements for hybrid sunfish that have sex ratios skewed towards males may prove useful in promoting maximal growth rates as well as minimizing feed costs.

Significant progress has been made with regard to sunfish brood stock development (bluegill and black crappie), spawning, acceptance of prepared diets, and good growth response. Most of the research and commercial production of sunfish has focused on utilizing pond systems (extensive aquaculture). However, to a lesser extent this same effort has been directed at intensive aquaculture. With a better understanding of the early life stage feeding strategies the aquaculture industry will be able to broaden the scope of sunfish aquaculture to include rearing these fish under intensive conditions.

PROGRESS AND PRINCIPAL ACCOMPLISHMENTS

During the 1994-96 period University of Nebraska-Lincoln (UNL) researchers were to produce two 10-20 minute educational video tapes on selected topics covered in the new sunfish production guide. However, due to time constraints at UNL these videotapes are postponed until 1997. Michigan State University (MSU) and ISU personnel have completed drafts of the new sunfish culture guide. The individual chapters will be reviewed during winter 1996; the guide is scheduled for completion by summer 1997.

There have been numerous sunfish hybrids produced by both researchers and private aquaculturists; these hybrids have varying percentages of male offspring and growth rates. The hybrid sunfish used by NCRAC researchers is the F_1 offspring resulting from crossing a female green sunfish (*Lepomis cyanellus*) with a male bluegill (*L. macrochirus*).

At Southern Illinois University-Carbondale (SIUC), researchers used practical diets containing crude protein levels of 32, 36, 40, and 44% and compared their ability to promote growth of hybrid sunfish in two culture systems: recirculating culture system and culture ponds.

Recirculating Culture System

Year 1 adult hybrid sunfishes (source: Fountain Bluff Fish Farms, Illinois; mean initial weight = 37.1 g; 1.3 oz) were stocked at a density of 28 fish per 300-L (79.3-gal) circular tank (three replicates per treatment). Flow rates were 30 L/min (7.9 gal/min) and water temperature was maintained at approximately 24°C (75.2°F). Feeding rates were 2%/day divided into two feedings during the 98-day growth trial. Survival ranged from 93 to 100% and did not differ significantly between treatments ($P > 0.05$). Weight increase and feed conversion efficiency were highest for the 44% crude protein diet and were significantly greater than the 36 and 32% diets (0.39 versus 0.33 and 0.27, respectively). These data indicate that optimal crude protein levels are likely to be in excess of 40% for hybrid sunfish in recirculating culture systems. The poor feed conversion efficiencies observed may be due to the experimental animals being sexually mature and directing considerable amount of their food intake towards gamete production and reproductive behavior. Proximate analysis of feeds and fish whole bodies is now under way.

Pond Culture

Juvenile hybrid sunfish (mean weight = 12 g; 0.04 oz), were stocked (May 23, 1995) at a rate of 5,504 fish/ha (2,228 fish/acre), into 16 ponds averaging 0.04 ha (0.10 acre) (four treatments/four replicates per treatment). Ponds were supplied with one of four practical diet formulations containing crude protein levels of 32, 36, 40, or 44%. Feeding rate was initially 3% of the estimated biomass once a day except on days of sampling. All ponds exhibited nest building activities by June 6 and recruitment of F_2 hybrids in some ponds was apparent by July 18. Feeding rates were reduced to 2% (August 15 through September 26, 1995) when a large amount of feed was noticed left from the previous feedings. This reduction in feeding activity coincided with high temperatures of 30°C (86°F). Resulting data was of limited use due to natural recruitment of F_2 offspring.

Year 1 adult hybrid sunfishes (source: Fountain Bluff Fish Farms, Illinois; mean initial weight = 40 g; 1.41 oz) were stocked April 16, 1996 into 16 ponds averaging 0.04 ha (0.10 acre) (four treatments/four replicates per treatment). Stocking density was 13,875 fish/ha (5,615 fish/acre). All ponds were limed and fertilized two weeks prior to stocking to promote plankton blooms. Feeding to apparent satiation was carried out two times per day except during times of rain and strong winds. Aeration to ponds with dissolved oxygen levels of less than 2.0 mg/L was applied with a tractor driven paddle wheel. Harvest is to be carried out October 29, 1996 following a complete draw down.

Researchers at MSU have empirically determined the optimal energy level for growth and protein retention in 125-mm (4.- in) hybrid sunfish utilizing a saturation kinetics model for curve fitting. Results demonstrate the semi-purified diet developed for these trials is well accepted by these fish; this results in a slightly lower but comparable growth to that obtained using a commercial control diet. There were no significant differences in growth or net protein utilization (NPU) between the experimental diets and the control diet; hence the semi-purified diet is suitable for the remaining phases of these trials.

The whole body indispensable amino acid (IAA) profile of 50- and 125-mm (2.0- and 4.9-in) hybrid sunfish, green sunfish, and bluegill has been determined. The data obtained has been used for predicting the IAA requirements for these species using the A:E ratio ([individual IAA content/total IAA content+Cys+Tyr] $\times$ 1000) of whole fish tissue. These predicted IAA requirements will be used in the preparation of diets for the remaining phases.

MSU researchers are currently beginning a trial evaluating growth, NPU, protein retention, and energy retention in 125-mm (4.9-in) hybrid sunfish fed graded levels of protein in isocaloric diets using the optimal energy level predicted in the previous trial. Diets have been formulated to meet IAA requirements for hybrid sunfish determined by researchers at Purdue University (Purdue) with the unknown requirements incorporated at levels predicted by the A/E ratio. This trial will be completed the first of the year; results will be used to predict the optimal P:E ratio for 125-mm (4.9-in) fish.

Research at Purdue was initially focused on quantifying key nutritional requirements of hybrid sunfish. Through three separate studies with the hybrid sunfish, growth was relatively low despite offering a broad variety of diets. Prior to conducting the next series of studies on critical nutritional requirements, an evaluation of pure bluegill was conducted. Growth of pure bluegill was double the growth observed with hybrid bluegill. The studies were conducted in the same experimental systems in the same conditions with the same broad variety of feeds. There was also differential use of commercial diets. Results of those studies clearly indicated that diets formulated for trout and salmon were better than diets formulated for catfish. Further, there were clear distinctions within the trout diets. That is, all trout diets are not the same nor is the response in the hybrid sunfish comparable to the pure bluegill. Both the optimum lipid:carbohydrate ratio and quantitative phosphorus requirements are underway. The optimum lipid study was expanded to include both hybrid sunfish and bluegill. Results will be known by December 1996.

Researchers at the University of Missouri have examined the potential to increase growth rates of hybrid sunfish during grow-out by using feeding schedules that bring out these fishes' compensatory growth response (increased growth following a period of fasting). Hybrid sunfish were held individually in experimental enclosures submerged in larger water-recirculation tanks. Water temperature was maintained at 24°C (75.2°F) as was a 15-h light/9-h dark photoperiod regime. Mealworms (*Tenebrio molitor*) were used as the food in these initial experiments so that daily consumption by individual fish could be accurately determined. Over the 105 day experiment, mean growth rates of hybrid sunfish in the 2 and 14 day no feeding cycle groups were 2.1 and 1.5 times faster than the controls that were fed *ad libitum* every day.

These results represent the first demonstration that fish can be grown significantly larger than daily-fed controls over identical time periods by eliciting the compensatory growth response.

Growth improvements from compensatory growth appeared to result from increases in both consumption rate and growth efficiency. While best results were observed for the shortest off/on feeding cycle, there was some suggestion from growth responses that longer off/on cycles (>14 days) may be of value.

The primary goal of the University of Wisconsin-Milwaukee (UW-Milwaukee) researchers was to utilize the early life stage feeding technology developed for yellow perch and apply this approach to centrarchids, specifically, black crappie. The researchers selected two early life stages as their starting points for the development of intensive aquaculture strategies. Young-of-the-year (YOY) Wisconsin pond-raised black crappie (*N* = 1,200) were obtained in fall 1994. Under laboratory conditions these fish accepted adult frozen brine shrimp as a transitional food within 3 days and were habituated to commercial starter feed within 14 days. Survival to present was greater than 65%. In addition, UW-Milwaukee researchers obtained several hundred YOY black crappie from a commercial producer in Iowa. Initially these fish were fed "green tank" water organisms, which included copepods, ostracods and smaller cladocerans. These organisms are all much larger than those fed to yellow perch at first feeding. Later on, brine shrimp nauplii (BSN) (*Artemia franciscana*) and a beef liver mixture was added to the feeding schedule. This group of black crappies habituated to a formulated starter diet within 26 days. This group of fish (*N* = 73) was terminated on September 25, 1995; mean length and weight was 66.8 mm (2.63 in) and 3.92 g (0.14 oz), respectively.

Since the last report, UW-Milwaukee researchers have continued to expand their efforts to habituate YOY black crappie to formulated diets. Past efforts to spawn adults in the laboratory or to collect wild adults have not been successful. They have continued to maintain the group of YOY black crappies acquired in October 1994 for use as a captive brood stock. These fish were habituated to commercial formulated diet within 14 days of arrival and have been maintained on a rearing regime that is intended to promote gonadal development. It is anticipated that these fish will be fully mature and available for spawning in the spring of 1997.

As a back-up to their efforts to produce YOY from laboratory and wild spawns, UW-Milwaukee researchers obtained 2,741 pond-spawned YOY black crappies (mean length = 26 mm; 1.0 in; mean weight = 0.1-0.5 g; 0.004-0.018 oz) from the Gavin's Point National Fish Hatchery in Yankton, South Dakota. The fish were stocked into a circular flow-through rearing tank and the photoperiod was set at 13-h light. When offered BSN on the day of arrival approximately half the fish accepted the food. Trial feedings with formulated diets on the day of arrival were unsuccessful. These fish took longer to habituate to formulated diet than either the slightly larger YOY brought to the lab in October 1994, those habituated to a formulated diet within 14 days, or the larval crappies tested in July 1995 that habituated to formulated starter diet within 26 days. These results suggest that there is a strong preference for BSN, and that habituation is not readily achieved by merely offering the formulated diet along with the transitional live food. This group of YOY crappie was very reluctant to feed in the presence of observers. Although there was limited interest in formulated foods as early as 6 days after the beginning of the trial, the general population consumed mainly the BSN. Full habituation to formulated diet appeared to closely follow the forced restriction of the live food. Survival during the trial was excellent, 99% over a rearing period of 103 days. UW-Milwaukee researchers intend to continue rearing this group of fish to demonstrate the growth that can be achieved under intensive flow-through culture with formulated diets. Growth information has been obtained at 0 days (26 mm; 1.0 in); 12 days (34 mm; 1.3 in), 57 days (55 mm; 2.2 in) and 105 days (75 mm; 3.0 in) since the start of the trial.

One objective of the ISU researchers was to spawn sunfish out-of-season through temperature and photoperiod manipulation under laboratory settings (bluegill and hybrid sunfish). ISU researchers stocked adult fish at a ratio of two males to four females (170 g; 6.0 oz) per 640-L (169-gal) tanks in a recirculation system. After an acclimation period, temperature and photoperiod were maintained at 24°C (75.2°F) and 14-h light/10-h dark. They were able to spawn bluegills during a six month period (December 1994 - May 1995); 40 spawns averaging

20,000 larvae each were obtained from 24 females. Hybrid sunfish were successfully produced the following fall.

The second objective of the ISU study was to develop a procedure for tank-rearing larval bluegill and larval hybrid sunfish. In the first set of experiments, seven commercial diets were used for feeding larval bluegill from the onset of exogenous feeding to 28 days posthatch. Although all diets were consumed by the larvae, none were digested and survival was essentially zero. In the next set of experiments, bluegill larvae were able to digest commercial diets by feeding them BSN for an initial 7 day period and then switching to commercial feed over a 3 day period. Using this protocol, three feeds (Fry Feed Kyowa® B-250, Hatchery Encapsulon® Grade II, and Larval AP-100®) were compared over a 28 day interval. There were no significant (P # 0.05) differences in growth (length and weight) among the three diets at the end of 28 days, but survival was significantly higher for fish fed Fry Feed Kyowa® B-250. In another experiment, Fry Feed Kyowa® B-250 was fed to larval bluegill after feeding them BSN for 3, 7, or 14 days with an additional 3 day weaning period with mixed feeding. Larvae fed BSN for 14 days had significantly higher growth and survival than did larvae in the 3 day and 7 day treatment groups. In a final experiment, Fry Feed Kyowa® B-250 was fed to larval hybrid sunfish after feeding them brine shrimp for 0, 3 or 7 days with an additional 3 day weaning period of mixed feeding. The larvae fed brine shrimp for only 0 or 3 days initially grew slower than did the larvae in the 7 day treatment; however, by the end of the experiment (28 days posthatch), there were no significant differences among lengths or weights in the three treatments. At 28 days posthatch, larvae fed brine shrimp for 7 days had a significantly higher survival rate than larvae in either the 0 or 3 day treatments. Results indicate that the protocol for tank-rearing larval bluegill and larval hybrid sunfish should include using brine shrimp prior to using a commercial diet. It appeared that larval hybrid sunfish could digest the commercial diet at the onset of exogenous feeding. However, without BSN much lower survival rates resulted. Survival rates of about 25 and 37% can be expected for bluegill and hybrids, respectively, by following this protocol.

WORK PLANNED

UNL will produce videos in 1997 related to the upcoming sunfish culture guide. This guide will be competed during 1997.

Critical nutritional requirements for targeted species reduces feed costs and overall cost of production of fishes will continue to be defined by Purdue and MSU researchers. SIUC researchers will compile data from their recirculation and pond studies. These data will be important pieces of information for manufacturers of feed.

UW-Milwaukee researchers will attempt the laboratory spawning of their captive black crappie brood stock by manipulating temperature and photoperiod. If necessary they will use spawning induction substances in spring 1997. If successful, the YOY black crappie produced from this brood stock will be used in the new NCRAC sunfish project. Researchers at ISU will continue to do research into sunfish culture by growing hybrid sunfish up to food-size and to evaluate a sunfish hybrid produced by crossing a female redear sunfish (*L. microlophus*) with a male bluegill.

IMPACTS

- Coupled with the NCRAC-sponsored development of improved intensive larval sunfish culture techniques at ISU under the direction of Morris, commercial fish farmers have the tools to establish stocks of polyploid sunfishes.
- NCRAC funding permitted SIUC to leverage funding from the American Fishing Tackle Manufacturing Association to evaluate benefits of triploid sunfish in recreational fishing ponds. The supply of triploids to recreational fisheries could provide a new market for regional producers.
- Developing diets specifically for targeted species results in maximum performance at the lowest possible cost. Purdue research directed at minimizing costs of feeds will help to maximize profit to the producer.

- It now appears that the intensive culture technology developed for yellow perch can be applied to black crappie. Also, YOY (30-60 day old) pond-produced black crappie can habituate to prepared diets within 26 days; YOY (100 day old) pond-produced black crappie can habituate to prepared diets within 14 days. The potential for the intensive culture of black crappie looks very promising.
- It is now possible to produce bluegills and hybrid sunfish in the laboratory out-of-season by manipulation of temperature and photoperiod without the use of hormones. This protocol allows for the production of these fish, regardless of season, for both laboratory studies and aquaculture stocking.
- The potential for the intensive culture of black crappie will provide an alternative to seasonal pond rearing and could expand the growth and production to an annual basis in conjunction with recirculating aquaculture system technology.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

See the Appendix for a cumulative output for all NCRAC-funded Sunfish activities.

SUPPORT

YEARS	NCRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
1994-96	$174,999	$177,300	$12,012[a]			$189,312	$364,311
TOTAL	$174,999	$177,300	$12,012			$189,312	$364,311

[a]Farmland Industries, Inc.

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Publications in Print

Bryan, M.D., J.E. Morris, and G.J. Atchison. 1994. Methods for culturing bluegill in the laboratory. Progressive Fish-Culturist 56:217-221.

Miller, S. 1995. Tetraploid induction protocols for bluegill sunfish, *Lepomis macrochirus*, using cold and pressure shocks. Master's thesis. Michigan State University, East Lansing.

Mischke, C.C. 1995. Larval bluegill culture in the laboratory. Master's thesis. Iowa State University, Ames.

Montes-Brunner, Y. 1992. Study of the developmental stages of bluegill (*Lepomis macrochirus*) eggs using selected histological techniques. Master's thesis. Michigan State University, East Lansing.

Read, E.R. 1994. Cage culture of black, white and F_1 hybrid crappie (*Pomoxis* species). Master's thesis. Pittsburg State University, Pittsburg, Kansas.

Tetzlaff, B., and P. Wills. 1991. Current trends in the culture of hybrid sunfish. Pages 214-218 *in* Proceedings of North Central Aquaculture Conference, Kalamazoo, Michigan, March 18-21, 1991.

Thomas, G.L. 1995. Culture of white crappie *(Pomoxis annularis)* in a Recirculating System. Master's thesis, Pittsburg State University, Pittsburg, Kansas.

Westmas, A.R. 1992. Polyploidy induction in buegill sunfish (*Lepomis macrochirus*) using cold and pressure shocks. Master's thesis. Michigan State University, East Lansing.

Westmaas, A.R., W. Young, and D. Garling. 1991. Induction of polyploids in bluegills and chinook salmon. Pages 110-112 *in* Proceedings of North Central Aquaculture Conference, Kalamazoo, Michigan, March 18-21, 1991.

Wills, P.S., J.P. Paret, and R.J. Sheehan. 1994. Induced triploidy in *Lepomis* sunfish and hybrids. Journal of the World Aquaculture Society 25(4):47-60.

Manuscripts

Mischke, C.C., and J.E. Morris. In review. Comparison of growth and survival of larval bluegill in the laboratory under different feeding regimes. Progressive Fish-Culturist.

Mischke, C.C., and J.E. Morris. In review. Out-of-season spawing of bluegill in the laboratory. Progressive Fish-Culturist.

Papers Presented

Brown, P.B., and K. Wilson. 1994. Experimental and practical diet evaluations with hybrid bluegill. 25th Annual Meeting of the World Aquaculture Society, New Orleans, Louisiana, January 12-16, 1994.

Mischke, C.C., and J.E. Morris. 1996. Growth and survival of larval bluegill (*Lepomis macrochirus*) and hybrid sunfish (green sunfish, *L. cyanellus* H bluegill) in the laboratory under different feeding regimes. Iowa-Nebraska American Fisheries Society Meeting, Council Bluffs, Iowa, January 29-31, 1996.

Mischke, C.C., and J.E. Morris. 1996. Early spawning of bluegill. Midcontinent Warmwater Fish Culture Workshop, Council Bluffs, Iowa, February 7, 1996.

Mischke, C.C., and J.E. Morris. 1996. Growth and survival of larval bluegill, *Lepomis macrochirus*, in the laboratory under different feeding regimes. American Chapter of the World Aquaculture Society, Arlington, Texas, February 14-17, 1996. (Awarded Best Student Poster)

Morris, J.E. 1995. Hybrid bluegill culture update. Combined North Central and Ninth Annual Minnesota Aquaculture Conference and Trade Show, Minneapolis, Minnesota, February 17-18, 1995.

Morris, J.E. 1995. Culture of bluegills under laboratory conditions. Nebraska Aquaculture Conference, North Platte, Nebraska, March 25, 1995.

Paret, J.M., R.J. Sheehan and S.D. Cherck. 1993. Growth performance of *Lepomis* diploid hybrids, triploid hybrids and parental species at five temperatures. Meeting of the Illinois and Iowa Chapters of the American Fisheries Society, Bettendorf, Iowa, February 16-18, 1993.

Read, E.R., and J.R. Triplett. 1994. Cage culture of crappie. 56th Midwest Fish and Wildlife Conference, Indianapolis, Indiana, December 4-7, 1994.

Read, E.R., and J.R. Triplett. 1995. Cage culture of black, white and F_1 hybrid crappie (*Pomoxis* species). Kansas Commercial Fish Growers Association, McPherson, Kansas, February 2, 1995.

Sheehan, R.J., J.P. Paret, P.S. Wills, and J.E. Seeb. 1993. Induced triploidy and growth of *Lepomis* parental species, hybrid, and triploid hybrid at five temperatures, 8 to 28EC. Prospects for Polyploid Fish in Fisheries Management Symposium, 123rd Annual Meeting of the American Fisheries Society, Portland, Oregon, August 29 - September 2, 1993. (Invited paper)

Thomas, G.L., and J.R. Triplett. 1994-1995. Close-loop white crappie (*Pomoxis annularis*) culture. 56th Midwest Fish and Wildlife Conference, Indianapolis, Indiana, December 4-7, 1994. Also presented at the Kansas Commercial Fish Growers Association Meeting, McPherson, Kansas, February 2, 1995 and Kansas Academy of Science Annual Meeting, Pittsburg State University, Pittsburg, Kansas, April 7, 1995.