SUNFISH[11]

Project Component Termination Report for the Period
June 1, 1990 to August 31, 1995

NCRAC FUNDING LEVEL: $280,577 (June 1, 1990 to August 31, 1995)

PARTICIPANTS:

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REASON FOR TERMINATION
The objectives for this work on Sunfish were completed.

PROJECT OBJECTIVES
(1) Determine the mechanisms of sex control in sunfish and to produce and evaluate polyploid sunfish and hybrids.

(2) Determine optimal stocking densities and relationships between temperature and growth for *Lepomis*, *Lepomis* hybrids, and triploid *Lepomis*.

PRINCIPAL ACCOMPLISHMENTS
An evaluation of both cold and pressure shocks of varying magnitudes, initiation times (time after mixing egg and sperm), and durations to determine the optimum treatments to produce tetraploid (organisms with twice the number of normal chromosomes) bluegill (*Lepomis macrochirus*) has been completed at Michigan State University (MSU). Tetraploidy was induced in five of the 16 cold shock treatments tested. Maximum induction rates of 40% are comparable to those achieved in other species. Of the 10 pressure treatments examined, none were successful in producing tetraploids. Relative survival ranged from <1 to 34% for bluegill exposed to cold shock treatments or pressure shock treatments, respectively.

Twenty-seven combinations of pressure (41,369, 48,264, or 55,158 kPa), shock durations (2, 3, or 4 min), and post-fertilization shock initiation times (2, 3, or 4 min) were tested at Southern Illinois University-Carbondale (SIUC) to identify treatments which would most efficiently induce triploidy in green sunfish (*L. cyanellus*) male H bluegill female F_1 hybrids. Several of the shock treatments produced 100% triploids with at least 90% survival relative to controls. The two shock treatments which appeared to be most effective were: (1) 48,265 kPa for 4 min begun 2 min postfertilization; and (2) 41,369 kPa for 2 min begun 3 min postfertilization. A paper based on this work appeared in the *Journal of the World Aquaculture Society*; it is the first publication on shock-induced triploidy in *Lepomis*.

Using starch gel electrophoresis, a diagnostic genetic technique, SIUC investigators found that they could distinguish among three species of sunfish: bluegill, green sunfish, and pumpkinseed (*L. gibbosus*). Furthermore, use of this technique made it possible to identify hybrids of these species; however, it did not allow for the identification of triploids.

Bluegill had a lower mean weight and poorer food conversion after 121 d of growth in a trial comparing bluegill, green sunfish, and male bluegill × female green hybrids. No significant differences were found between green sunfish and hybrids for final weight, specific growth rate, percent weight gain, or food conversion. Growth occurred over the entire range of temperatures tested, 8 to 28°C (46-82°F) at 5°C intervals; 23°C (73°F) was optimum.

Male bluegill × green sunfish female triploid and diploid F_1 hybrid growth performance was compared in a 230 d trial at 23°C (73°F). Diploids showed larger final weight and better specific growth rate, percent weight gain and food conversion.

In a third growth trial at SIUC, diploid male bluegill × female green sunfish F_1 hybrids and green sunfish were compared to triploid male green sunfish × female bluegill F_1 hybrids over 235 d. Diploid taxa were selected on the basis of the results of the 121-d growth trial. No significant differences in weight, specific growth rate, percent weight gain or food conversion were found. Green sunfish had lower dress out weights than either hybrid. Gonadal somatic index was higher in the green sunfish than in the diploid and triploid hybrids. The vast majority of the green sunfish became sexually mature and were producing gametes over the range of tested temperatures, 8-28°C (46-82°F). Growth occurred at all temperatures; 18°C (64°F) was optimum. Lower growth rates and reduced optimum temperature were attributed in this trial to the use of fish larger than the ones used in the all-diploid growth trial.

Given the presumption of sterility and other potential advantages, triploids are a viable alternative for intensive food fish culture; they will not reproduce in culture units and will not cause genetic contamination of wild stocks. Male green sunfish × female bluegill F_1 hybrid triploids and male bluegill × female green sunfish diploid F_1 hybrids performed similarly in growth trials at SIUC and appeared to be the best candidates for food fish production.

The pressure-induced triploidy and allozyme species identification techniques derived at SIUC were used to produce gynogens (an organism with only maternal chromosomes) in a study to investigate the genetic sex determination system in bluegill. Heterologous (green sunfish) spermatozoa were irradiated, 15-360 sec, with 1500 uW/cm^2 of 254 nm wavelength UV light to deactivate the DNA. The irradiated spermatozoa were then used to activate bluegill eggs.

Control eggs which were not shocked but activated with irradiated sperm were all (N = 37) haploid; controls which were fertilized with normal spermatozoa and not shocked were all diploid (N = 21). Sperm irradiation times of 120, 150, or 180 sec plus the hydrostatic shock produced 48 diploids (gynogens) and no individuals with other ploidy levels or green sunfish loci, indicating 100% gynogen production efficiency.

Supposed gynogen larvae (N = 150) were then produced and stocked into a pond. Seven sexually mature gynogens were recovered from the pond. All seven were pure bluegill, based on allozyme analysis, and female. The probability of obtaining seven females from a 1:1 sex ratio population is only 0.008. This is strong evidence that the female is the homogametic sex and that an XX/XY genetic sex determination system occurs in bluegill.

This is the first study reporting induced gynogenesis and gynogen sex ratios in bluegills; this provides the foundation necessary for developing a technique for all-female production in bluegill. Sex reversal of gynogens would yield phenotypic males that would produce all-female progeny when crossed with normal females. This strategy could be used to eliminate reproduction in bluegill culture units, developing techniques for eliminating reproduction is one of the more important goals of the North Central Regional Aquaculture Center (NCRAC) Sunfish research effort.

Stocking densities for sunfish in cages and ponds were also evaluated at SIUC. Hybrid sunfish (bluegill male × green sunfish female) grew better at densities of 100 (3 fish/ft^3) and 200 fish/m^3 (6 fish/ft^3) than at 400 fish/m^3 (11 fish/ft^3) in cages. Food conversion was best at the lowest density and it became worse as density was increased.

Growth of hybrid sunfish was directly related to stocking density in ponds at the tested densities of 7,410, 4,940, and 2,470 fish/ha (3,000, 2,000, and 1,000 fish/acre). Food conversion also

improved as density increased in ponds. Food conversion (weight of food fed/weight gain) ranged from 2.5 to 5.3 in the highest and lowest density ponds, respectively.

IMPACTS

Control of Sunfish Reproduction

The development of protocols for reducing reproduction in these fishes allows for the potential of increased growth of these fish in aquaculture systems as opposed to unrestrained reproduction. The two shock treatments which appeared to be most effective were: (1) 48,265 kPa for 4 min begun 2 min post-fertilization; and (2) 41,369 kPa for 2 min begun 3 min post-fertilization.

Optimal Stocking Densities

Information garnered from this work indicates the desirabilities of these fish for aquaculture in the NCR. The sunfish species and diploid and triploid hybrids which were evaluated showed optimum growth at temperatures of 18 to 23°C (64-73°F); all groups grew across a temperature range of 8 to 28°C (46-82°F). Even at 8°C (46°F), the sunfish species, diploid and triploid hybrids, that were evaluated showed 150 to 200% weight gains over 121 d, and growth rates generally increased with increasing temperature. In light of results from this research project, the male bluegill × female green sunfish diploid F_1 hybrid and the male green sunfish × bluegill sunfish triploid F_1 hybrid appeared to be the best candidates for aquacultural production.

A stocking rate of 200 fish/m^3 (6 fish/ft^3) is the recommended stocking density for sunfish in cages. In open pond culture, the recommended stocking density for sunfish is 12,355 to 17,297 fingerlings/ha (5,000 to 7,000 fingerlings/acre).

RECOMMENDED FOLLOW-UP ACTIVITIES

The male green sunfish × female bluegill triploid hybrid grew as well or better than the other four *Lepomis* taxa (green sunfish, bluegill, male bluegill male × female green sunfish hybrid, and male bluegill × female green sunfish triploid hybrid) evaluated. There is a need to evaluate growth in this triploid to a larger size, because triploids, theoretically, have their greatest growth advantage over diploids after the diploids reach sexual maturity.

One of the major impediments to open pond culture of sunfish is uncontrolled reproduction. Monosex sunfish production would eliminate this problem. An important first step has been taken in the development of monosex stock production in sunfish. Induced gynogenesis in bluegill has been accomplished at SIUC. The mating of sex-reversed XX gynogens to normal XX females would produce all-female progeny. Although females did not grow as well as males in SIUC's studies, their growth performance could be considerably enhanced in the absence of males. There is a need to explore monosex stock production in sunfish to determine if this is a viable means for controlling reproduction in culture units.

PUBLICATIONS, MANUSCRIPTS, OR PAPERS PRESENTED

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

TOTAL SUPPORT FOR THE FIRST TWO PROJECTS

YEARS	NCRAC-USDA FUNDING	OTHER SUPPORT					TOTAL SUPPORT
		UNIVER-SITY	INDUSTRY	OTHER FEDERAL	OTHER	TOTAL	
1990-92	$130,758	$96,710				$96,710	$227,468

1992-94	$149,799	$343,160	$3,200[a]		$29,830[b]	$376,190	$525,989
TOTAL	$280,557	$439,870	$3,200		$29,830	$472,900	$753,457

[a]American Fishing Tackle Manufacturing Association
[b]Illinois Natural History Survey

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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.