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# Effects of stocking density and dietary protein on green sunfish (*Lepomis cyanellus*)×bluegill (*L. macrochirus*) hybrids overwintered in ponds

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#### ABSTRACT

Growth, survival, and feed conversion were evaluated in juvenile green sunfish (*L. cyanel-lus*) × bluegill (*L. macrochirus*) hybrids (mean wt. 40.6 g) stocked in twelve 0.04-ha ponds at densities of 12 350 and 24 700 fish  $ha^{-1}$  and fed diets containing 25 or 35% protein for 183 days. Final mean weights were higher for fish stocked at 12 350 fish  $ha^{-1}$  (53 g) than those stocked at 24 700 fish  $ha^{-1}$  (48 g). There was no difference in weights of fish fed either dietary protein level. Final biomass densities increased with increasing stocking density (range = 589–1245 kg  $ha^{-1}$ ) but were not significantly affected (P > 0.05) by dietary protein level. Percentage increase in biomass density was significantly higher (P < 0.05) for fish stocked at 12 350 fish  $ha^{-1}$  (26%) than for fish stocked at 24 700 (12%). Stocking rate and dietary protein had no significant effect on survival or feed conversion ratio which averaged 95% and 3.6, respectively. Results of this study indicate that stocking rate has a significant effect on winter growth of hybrid bluegill, possibly due to availability of natural foods. The refinement of feeding schedules and use of floating diets should be investigated.

### INTRODUCTION

Hybrid sunfish have become increasingly popular for stocking into recreational ponds. The hybrid produced by crossing the female green sunfish (*Lepomis cyanellus*) with the male bluegill (*L. macrochirus*) (GS×BG) is probably the most commonly stocked (Brunson and Robinette, 1982). Several attributes also make this hybrid a potential candidate for commercial production, including: ready acceptance of prepared diets (Lewis and Heidinger, 1971), rapid growth (Brunson, 1983), and efficient feed conversion during warm months (Brunson and Robinette, 1982). Also, their aggressive nature and susceptibility to angling could allow producers to market to, or through, fee-fishing operations (Brunson and Robinette, 1986). This could be espe-

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cially important in areas where processing infrastructure has not yet developed. Heidinger (1975) suggested that at latitudes having a short period when air temperatures are above 21°C, hybrid sunfishes may be more economical to raise for either food fish or fee-fishing markets than channel catfish.

To reach sizes desired by fee-fishing operations ( $\geq 110$  g) (Ellison and Heidinger, 1976), young-of-the-year (YOY) GS×BG hybrids must be overwintered before second year growout. Heidinger (1975) reported a weight gain of 32% for GS×BG hybrids overwintered in cages and fed a prepared diet and 30% for those overwintered in ponds and not fed. Brunson and Robinette (1982) reported an overwintering gain of 183% in GS×BG hybrids relying on natural food and 261% weight gain with a feed conversion ratio (FCR) of 1.6 in hybrids provided with supplemental feed.

In other fishes, cultural conditions and nutritional factors have been shown to affect winter growth. Robinette et al. (1982) reported that fingerling channel catfish (*Ictalurus punctatus*) fed a 25% protein diet during the winter grew as well as catfish fed a 35% crude protein practical diet. Tidwell and Mims (1991) found that, in catfish, reducing density increased winter weight gain more than low-temperature feeding.

The objective of this study was to evaluate the effects of fish density, dietary protein level, and their interactions on the growth and body composition of  $GS \times BG$  hybrids overwintered in ponds.

# MATERIALS AND METHODS

A  $2 \times 2$  factorial experiment was designed to determine the effects of stocking rate on fish growth under two different dietary protein regimes. Juvenile female green sunfish and male bluegill F<sub>1</sub> hybrids (mean individual weight  $40.6 \pm 2.3$  g) were stocked into twelve 0.04-ha ponds at rates of 12 350 and 24 700 fish  $\cdot$  ha<sup>-1</sup> and fed one of two diets containing either 25 or 35% protein (Table 1). Each of the four treatment combinations was replicated in three ponds. Diets were manufactured into sinking pellets by a commercial feed mill (Farmers Feed, Lexington, KY). Diets were analyzed for crude protein, fat, and moisture (Table 1). Crude protein was determined by a LECO FP-228 nitrogen determinator (Sweeney and Rexroad, 1987), crude fat by ether extraction, and moisture by drying a 2-g sample to constant weight (AOAC, 1990). Feeding schedules and amounts were based on a chart for winter feeding of channel catfish (Dupree and Huner, 1984). Fish weights were updated every 2 weeks based on an assumed feed conversion of 1.5 (Brunson and Robinette, 1982).

Ponds used in this study were approximately 1.5-m deep and were supplied with water from a reservoir filled by rain runoff. Water levels were maintained at a constant depth by periodic additions. Water temperature and dissolved oxygen (DO) were monitored in each pond twice daily (09.00 and

## TABLE 1

	Dietary protein (%)		
	25	35	-
Ingredients (%)		i	······································
Fish meal (67% protein)	6.50	10.00	
Soybean meal (44% protein)	34.00	58.00	
Ground corn	52.00	24.50	
Cod liver oil	1.50	1.50	
Premix <sup>a</sup>	1.00	1.00	
Monocalcium phosphate	1.00	1.00	
Binder	4.00	4.00	
Chemical analysis			
Dry matter (%)	91.5	92.3	
Protein (%) <sup>b</sup>	25.3	35.7	
Lipid (%) <sup>b</sup>	3.2	3.3	

Ingredients and chemical composition of diets, containing two levels of crude protein, fed to sunfish hybrids during the winter

<sup>a</sup>Premix supplied the following vitamins and minerals per kg of diet: retinol palimate (A), 4532 IU; cholecalciferol (D<sub>3</sub>), 2266 IU;  $\alpha$ -tocopherol (E), 75 IU; menadione (K), 11 mg; cyanocobalamin (B<sub>12</sub>), 20 mg; ascorbic acid (C), 778 mg; folic acid, 2.2 mg; riboflavin (B<sub>2</sub>), 13.2 mg; pantothenic acid, 35.2 mg; niacin, 88.0 mg; choline chloride, 516 mg; thiamine (B<sub>1</sub>), 11 mg; pyridoxine (B<sub>6</sub>), 11 mg; Zn (as ZnSO<sub>4</sub>), 173 mg; Fe (as FeSO<sub>4</sub>), 60 mg; Cu (as CuSO<sub>4</sub>), 7.5 mg; I (as CaIO<sub>3</sub>), 3.75 mg; Co (as CoSO<sub>4</sub>), 1.6 mg; Mn (as MnSO<sub>4</sub>), 180 mg; Al (as AlK(SO<sub>4</sub>)<sub>2</sub>), 1.0 mg; Se (as Na<sub>2</sub>SeO<sub>3</sub>), 0.3 mg; K (as KCl), 3474 mg; and Na (as Na<sub>2</sub>PO<sub>4</sub>), 1932 mg. <sup>b</sup>Moisture-free basis.

16.00 h) at a depth of 0.5 m. Mechanical aeration was supplied if DO was predicted (by graph) to fall below 5 mg $\cdot$ l<sup>-1</sup> during the night. Ammonia, nitrite and pH were determined weekly (16.00 h).

Total number and weight of fish in each pond were determined at harvest. Fifty fish were randomly sampled from each pond and individually weighed (g) and measured for total length (cm). Whole bodies of three fish sampled from each pond were homogenized in a blender and analyzed for protein and moisture. Protein was determined by the Kjeldahl method and moisture was measured by drying in an oven (95°C until constant weight) (AOAC, 1990). Due to problems during analysis, fat could not be determined.

Specific growth rate (SGR, % body wt/d) was calculated from:

# $SGR = 100 \times (\ln W_f - \ln W_i)/t$ ,

where  $W_{\rm f}$ =mean weight at the end of the period,  $W_{\rm i}$ =mean weight at the beginning of the period, and t=time in days of the period (Ricker, 1975). Condition factor (K) was calculated from  $K=100 \times W \cdot L^{-3}$ , where W=weight (g) and L=total length (cm) (Weatherly and Gill, 1987). Coefficients of variation (CV) of body lengths and weights for a sample of 50 fish from each replicate pond were calculated from  $CV=100\times \text{s.d.}/\bar{X}$ , where s.d.=standard deviation, and  $\bar{X}$ =mean. Feed conversion ratio (FCR) was calculated from FCR=weight of feed fed (g)/live weight gain (g).

Data were analyzed by two-way analysis of variance (ANOVA) using the Statistical Analysis System (Statistical Analysis Systems, 1988) to determine the effects of stocking rate, dietary protein, and their interactions on growth, coefficients of variation of weights and lengths, condition factor, feed conversion, body composition, and survival. All percentage and ratio data were transformed to arc-sin values prior to analysis (Zar, 1984).

# RESULTS

There were no significant differences (P > 0.05) in mean morning and afternoon dissolved oxygen concentrations, morning and afternoon temperatures, total ammonia concentrations, nitrite concentrations, and pH among treatments. Overall, these variables averaged 12.7 and 13.9 mg·l<sup>-1</sup>, 6.1 and 6.9 °C, 0.189 mg·l<sup>-1</sup>, 0.003 mg·l<sup>-1</sup>, and 8.3, respectively.

Mean individual fish weights at harvest were significantly higher (P < 0.05) for fish stocked at 12 350·ha<sup>-1</sup> (53.5 g) than for those stocked at 24 700·ha<sup>-1</sup> (49.1 g) (Table 2). No significant (P > 0.05) effect of dietary protein on final weights was observed. There was no significant (P > 0.05) effect of stocking rate or dietary protein on fish length at harvest. Survival was not significantly (P > 0.05) affected by stocking rate or protein level, and averaged 95.1% overall (Table 2). Final biomasses were significantly higher (P < 0.01) for fish stocked at 24 750·ha<sup>-1</sup> (1168 kg·ha<sup>-1</sup>) than for fish stocked at

TABLE	2
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Main effect means* of final weight and length, final biomass density, and survival of sunfish hybrids
(Lepomis cyanellus $\times L$ . macrochirus) overwintered in ponds for 183 days at two stocking densities and under two dietary protein regimes

Final weight (g)	Final length (cm)	Biomass (kg·ha <sup>-1</sup> )	Survival (%)
$52.0 \pm 1.28$	127+02	6450+1750	074+07
			$97.4 \pm 0.7$
48.0±1.5°	$13.3 \pm 0.1$	$1,167.5\pm47.5^{\circ}$	93.0±2.0
$49.6 \pm 2.0$	$13.5 \pm 0.1$	$875.0 \pm 110.0$	$96.3 \pm 1.3$
$51.4 \pm 1.5$	$13.5 \pm 0.2$	$937.5 \pm 132.5$	$93.0 \pm 2.0$
	weight (g) 53.0±1.2 <sup>a</sup> 48.0±1.5 <sup>b</sup> 49.6±2.0	weight (g)  length (cm)    53.0±1.2 <sup>a</sup> 13.7±0.2    48.0±1.5 <sup>b</sup> 13.3±0.1    49.6±2.0  13.5±0.1	weight (g)length (cm)(kg·ha <sup>-1</sup> ) $53.0 \pm 1.2^{a}$ $48.0 \pm 1.5^{b}$ $13.7 \pm 0.2$ $13.3 \pm 0.1$ $645.0 \pm 17.5^{a}$ $1,167.5 \pm 47.5^{b}$ $49.6 \pm 2.0$ $13.5 \pm 0.1$ $875.0 \pm 110.0$

\*Means ( $\pm$ s.e.) of six replicate ponds, means within a column followed by different letters are significantly different (P < 0.05).

#### TABLE 3

Main effect	Average individual gain (%)	SGR (% day <sup>-1</sup> )	Biomass increase (%)	Feed conversion ratio	Condition factor (K)
Density					
$(fish \cdot ha^{-1})$	20 2 1 2 2	0.141.0.01	25 9 1 2 08	1 2 1 0 1	22401
12 350	$28.3 \pm 2.2$	$0.14 \pm 0.01$	$25.8 \pm 2.0^{a}$	$1.3 \pm 0.1$	$2.3 \pm 0.1$
24 700	$20.2 \pm 3.4$	$0.10 \pm 0.02$	11.8±3.7 <sup>ь</sup>	$5.8 \pm 2.2$	$2.5 \pm 0.1$
Dietary protein (%)					
25	$23.2 \pm 3.4$	$0.11\pm0.02$	$19.6 \pm 4.5$	$3.8 \pm 2.1$	$2.4 \pm 0.1$
35	$25.4 \pm 3.1$	$0.12 \pm 0.01$	$18.0 \pm 4.1$	$3.4 \pm 1.6$	$2.4 \pm 0.1$

Main effect means\* of individual gain, specific growth rate (SGR), biomass increase, feed conversion ratio, and condition factor of sunfish hybrids (*Lepomis cyanellus* $\times$ *L. macrochirus*) overwintered in ponds for 183 days at two stocking densities and under two dietary protein regimes

\*Means ( $\pm$ s.e.) of six replicate ponds, means within a column followed by different letters are significantly different (P < 0.05).

12 350  $\cdot$  ha<sup>-1</sup> (645 kg  $\cdot$  ha<sup>-1</sup>) (Table 2). There was no significant (P>0.05) effect of dietary protein on final biomass (Table 2).

Differences in growth rates, expressed as average individual gain (g) and specific growth rate (%·day<sup>-1</sup>) were not statistically significant (P > 0.05) for effect of stocking rate, dietary protein, or their interaction (Table 3). Increase in biomass density (%) was significantly higher (P < 0.05) for fish stocked at 12 350 (26%) than for fish stocked at 24 700 (12%). There was no significant (P > 0.05) effect of dietary protein on biomass increase (Table 3).

Feed conversion ratios (Table 3) were lower for fish stocked at 12  $350 \cdot ha^{-1}$  (1.3) than for fish stocked at 24  $700 \cdot ha^{-1}$  (5.8). However, differences in FCR were not statistically significant (P > 0.05). Within treatment variation was high (s.e.=5.5) in ponds stocked at 24  $700 \cdot ha^{-1}$ . The effect of dietary protein on feed conversion was not significant (P > 0.05). Final mean condition factors ranged from 2.02 to 2.63 and did not differ significantly (P > 0.05) among treatments (Table 3). There were no significant (P > 0.05) effects or interaction of stocking density and dietary regime on mean coefficients of variation of final body weights (34.3%) and lengths (11.0%) or whole body protein (61.5%), or dry matter (26.7%).

# DISCUSSION

Average harvest size and pond production (percentage increase in biomass) were reduced by increased stocking rate. This suggests that hybrid bluegill should not be overwintered at stocking rates greater than  $24\ 700 \cdot ha^{-1}$  if winter weight gain is the objective. Higher stocking rates may be feasible if inventory maintenance is the primary objective.

Reduced harvest size and unit production at the higher stocking rate may also indicate that feeds are not being well utilized or that utilization varied. Feed conversion ratios ranged from 1.1 to 1.7 in low-density ponds but from 1.4 to 14.1 in high-density ponds. This indicates that in ponds stocked at 12 350 fish  $ha^{-1}$  feed was well utilized, or less important due to availability of natural foods. In ponds stocked with 24 700 fish  $ha^{-1}$  it appears that a prepared diet was more important, but feed acceptance or feed utilization was more variable. This is corroborated by the observation that in some high stocking rate ponds, fish were observed to accept feed while in others, they were not.

Overall, mean FCR was 3.6, which is higher than values reported in Mississippi (1.6) (Brunson and Robinette, 1982). Water temperature differences probably account for some differences in results. Brunson and Robinette (1982) reported an average afternoon surface water temperature of  $10.7^{\circ}$ C vs. 6.9°C in this study. Feed conversions were similar to those reported by Mims and Tidwell (1989) for channel catfish, (*Ictalurus punctatus*), overwintered under similar conditions (3.4).

Weight gains in fish stocked at 12  $350 \cdot ha^{-1}$  (28%) were similar to those reported by Heidinger (1975) in Illinois (30%), when fish were stocked at only  $143 \cdot ha^{-1}$  but not fed. Gains in the current study were much lower than reported by Brunson and Robinette (1982) (262%), possibly due to larger stocking sizes (40.6 vs. 12.3 g, respectively), higher stocking rates (24 350 vs.  $2471 \cdot ha^{-1}$ , respectively), lower temperature (6.9 vs.  $10.7^{\circ}$ C, respectively) and probably reduced availability of natural food (which they reported to be high). Condition factor (K) for fish in this study (2.41) was similar to the mean K reported by Brunson and Robinette (1982) (2.35). Overall survival was good, averaging 95%. Brunson and Robinette (1983) reported 81% survival for hybrids overwintering in monoculture.

Based on experience with overwintering channel catfish, sinking feed pellets were used in this study. Observations indicate that the use of floating pellets may have been advantageous for this hybrid, even at low temperatures. Fish in certain ponds were consistently observed to feed actively at the surface, at temperatures as low as  $8.0^{\circ}$ C, even though sinking pellets were used. In contrast, catfish do not feed consistently in ponds when the temperature drops below  $21^{\circ}$ C (Lovell, 1989). If accepted at low temperatures, floating pellets could allow better control over feed amounts, and improve feed conversion efficiency, and possibly growth.

In summary, dietary protein level had no effect on winter growth of  $GS \times BG$  hybrids. Decreased stocking rate increased winter growth, possibly due to greater availability of natural foods. The use of floating feeds and/or different

feeding regimes to improve winter growth of  $GS \times BG$  hybrids should be investigated.

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