FINAL REPORT: ASPECTS OF REPRODUCTION IN A CAPTIVE POPULATION OF THE STATE THREATENED IRONCOLOR SHINER (NOTROPIS CHALYBAEUS)

SUBMITTED TO

DIVISION OF NATURAL HERITAGE
ILLINOIS DEPARTMENT OF CONSERVATION
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EXECUTIVE SUMMARY

1. Ironcolor shiner populations in Illinois are near the northern edge of the range of the species. With respect to conservation, the protection of these populations is paramount because most other northern populations have been extirpated.
2. The ideal habitat of the ironcolor shiner in Illinois is generally small, clear, low-gradient streams with a sand/organic matter substrate and an abundance of aquatic macrophytes.
3. The breeding season length in a pond-held population extended from May through June; the peak breeding period is mid-June. Apparently most spawning occurs at water temperatures ranging from approximately 24 to 33 C.
4. The sex ratio is 1 male:1 female and both sexes are mature at about 32 mm SL.
5. Mean clutch size is 109 eggs per mature female; clutch weight accounts for about 7% of the body weight.
6. The ironcolor shiner is amenable to pond culture producing high numbers of offspring, exhibiting rapid growth, and overwintering successfully for two years and three reproductive seasons.
7. The efficacy of pond culture for the management of the ironcolor shiner and perhaps other endangered/threatened fish species should be a primary consideration in development of a recovery plan. To be effective as a management tool, pond culture of endangered/threatened

species should be initiated and evaluated before natural populations reach critically low levels. We recommend that a recovery plan be developed for the ironcolor shiner, including establishment of populations in former habitats, before this species meets the fate of other Illinois minnows (e.g., bluehead shiner, cypress minnow).

INTRODUCTION

The ironcolor shiner, Notropis chalybaeus (Cypriniformes, Cyprinidae), an Illinois threatened species (IESPB 1990), has a very restricted distribution in Illinois. It is limited to the sand areas of the Kankakee River drainage, Kankakee and Iroquois counties, and the sand areas of the middle Illinois River drainage, Mason and Tazewell counties (Smith 1979). It formerly (1901) occurred in the Des Plaines River, Cook County, but this badly polluted stream no longer provides suitable habitat for this fish. Recent collections from the Mason County (SIUC 1988, 1989) and Kankakee River areas (Seegert 1989) indicate that the species is present in adequate numbers, but it has been found nowhere else in Illinois. Because of its limited range and the susceptibility of its habitat to siltation and drainage, the Illinois Endangered Species Protection Board initiated a study of its reproductive potential and habitat as well as a review of its status and range (Burr et al. 1989). That study documented various reproductive characteristics of the species from Crane Creek, Mason County, Illinois, and the reproductive success of introduced stock from this locality in a culture pond at SIUC. In a continuing effort to document reproductive characteristics of the species, the study was extended for an additional year through funding from the Illinois Nongame Wildlife Conservation Fund.

This final report is submitted in fulfillment of a contract entered by and made between the State of Illinois, Department of Conservation, Division of Natural Heritage and Southern Illinois

University at Carbondale. This report details results of investigations to quantify reproductive characteristics of a captive population of the ironcolor shiner.

METHODS

Individuals of the ironcolor shiner were collected from Crane Creek, 1.5 mi. W of Easton, Mason County, Illinois, T21N, R7W, Sec. 27 & 34, on 25 May 1989. Approximately 200 individuals were transported alive to SIUC in plastic bags supplemented with oxygen and 109 specimens were preserved for use in determining aspects of reproduction under natural conditions (see Burr et al. 1989 for analysis of preserved individuals). Living individuals were stocked into fish culture pond No. 12 (surface area = 0.1 acre) at SIUC on 25 May after having acclimated from a stream temperature of 63 F to a pond temperature of 70 F. Methods and results of the first years findings are presented in Burr et al. (1989).

We conducted the present study from 29 June 1990 through 17 July 1991. We visited the culture pond at approximate two week intervals from 29 June 1990 through 11 September 1990, monthly intervals through 10 April 1991, and two week intervals thereafter. We did not sample in January 1991 due to icing over of the culture pond. On each visit surface and bottom temperature of the pond was recorded, and a sample of ironcolor shiners was collected with standard small-mesh minnow and/or bag seines and preserved and stored in 5% formalin.

We sexed preserved specimens from each sample by visual examination of the gonads and measured each individual to the

nearest 0.1 mm standard length (SL). We performed gross examination of ovaries to determine reproductive condition of females following criteria proposed by Heins and Rabito (1986). We classified ovaries into one of five developmental stages: latent (LA), early maturing (EM), late maturing (LM), mature (MA), and ripe (RE). We classified testes into two stages: LA (small, translucent testes) or MA (enlarged, opaque, white testes).

We removed ovaries and body organs from each female, dried ovaries and specimens at 55 C for 24 hours, separately weighed these to the nearest 0.0001 gm, and calculated a gonadosomatic index (GSI) as ovarian weight divided by adjusted body weight (i.e., dried bodies without organs) times 1000. We determined characteristics of a clutch (i.e., a group of eggs that develops synchronously and is spawned within a relatively short period of time [Heins and Rabito 1986]) from up to five MA females from each sample during the spawning season. For each of these females, we counted all mature oocytes, measured 15 randomly selected mature oocytes (average of largest and smallest dimensions) to the nearest 0.086 mm with an ocular micrometer, and dried and weighed all mature oocytes as above. We determined mean oocyte dry weight by dividing dry weight of the total mature oocytes by the number of mature oocytes from a particular female. We calculated relative clutch mass (RCM) by dividing total oocyte dry weight by dry body weight and converting the data to percentages.

We evaluated length-weight relationships for females. We determined length-weight relationships using the formula: $\log_{10}W = \log_{10}(a) + b \log_{10}(SL)$, where W = adjusted body weight, SL is standard length, and a and b are constants. All significance tests were performed at the 0.05 level of probability.

RESULTS

Sex Ratio.--We obtained 208 females and 187 males during our sampling period. This ratio was not significantly different from 1:1 (chi-square = 1.116; d.f. = 1; $0.10 < p < 0.25$).

Length-Weight Relationship.--Regression of log-transformed length and adjusted body weight for females (27.5-42.9 mm SL; n = 175) was: log (W) = -4.762 + 2.459 log (SL), $r^2 = 0.670$.

Reproductive Condition.--The MA females we examined contained at least two different groups of developing eggs (small maturing oocytes and larger mature oocytes). Classification of females by gross examination of the ovary, revealed two to three principal stages of reproductive condition during May, June, and early July (i.e., LM, MA, and RE stages)(Table 1). Among females (31.9-42.9 mm SL) classified in these three categories, there was no significant correlation between reproductive condition and SL ($r = 0.014$; n = 133; $p < 0.876$). Shifts in frequencies of reproductive condition occurred from LA/EM to LM/MA categories from late March to mid-April. Females classified as MA were in the highest percentages of any category from mid-May through mid-July in 1991, but in 1990 frequencies of MA females dropped considerably by the later date.

Table 1. Reproductive condition of ironcolor shiner females in samples taken from an SIUC culture pond in 1990-1991. The total number of females examined from each sample (n) and the percentage of occurrence for each stage of reproductive condition are shown. LA, latent; EM, early maturing; LM, late maturing; MA, mature; RE, ripe.

		Reproductive Condition				
Sample Date	n	LA	EM	LM	MA	RE
29 June 1990	13	0	0	23	77	0
16 July 1990	19	0	21	68	11	0
30 July 1990	16	56	13	25	6	0
13 Aug 1990	7	86	14	0	0	0
1 Sept 1990	9	78	22	0	0	0
16 Oct 1990	11	55	45	0	0	0
13 Nov 1990	4	50	50	0	0	0
20 Dec 1990	3	100	0	0	0	0
20 Feb 1991	11	0	100	0	0	0
23 Mar 1991	12	0	75	25	0	0
10 Apr 1991	11	0	55	44	0	0
24 Apr 1991	17	0	0	94	6	0
12 May 1991	19	0	0	11	84	5
1 June 1991	19	0	0	5	84	11
15 June 1991	21	0	0	29	71	0
3 July 1991	8	0	0	37	63	0
17 July 1991	8	0	0	0	100	0

Mean gonadosomatic index (GSI) values for 26 females (32.4-42.9 mm SL) are highest from May through June and lowest from July to April (Figure 1). Variation of the GSI, as reflected in minimum and maximum values, is greatest from May to June and relatively small from July to April. Notable increases and decreases in the mean GSI occur from March to May and June to July, respectively. These increases are concomitant with the increases in water temperature from 24 C to 33 C (Figure 1).

Males classified as MA first appeared in samples in March and constituted the majority of males (> 64%) in samples through July (only 1 MA male was present in the August sample). The transition from LA male to MA male occurred in March (Figure 2). There was a significant positive correlation between SL and reproductive condition of males ($r = 0.329$; $n = 187$; $p < 0.0001$).

Clutch Characteristics.--Mature eggs were cream to light yellow, the vitelline membrane was often raised slightly above the yolk, and the eggs were randomly distributed throughout the ovary. A summary of clutch characteristics and egg size is given in Table 2. Mean clutch size (i.e., the number of mature ova) was 109 and ranged from 70 to 147. Mean mature ova peaked in June and had decreased rapidly by 3 July; the 15 June sample contained the largest range in clutch size (Figure 3). The mean relative clutch mass was 7.1% and ranged from 2.3 to 17.5%. As with clutch size, relative clutch mass peaked on 15 June and steadily decreased thereafter (Figure 4). Mean diameter of mature eggs was 0.91 mm and ranged from 0.71 to 1.0 mm; mean diameters were

Table 2. Correlations of SL and adjusted body weight with clutch and oocyte characteristics and means ± 1 SD (standard deviation) and ranges of clutch and oocyte characteristics of mature ironcolor shiner females (n = 26; range of SL = 32.4 - 42.9) sampled in May, June, July, and August. Females examined for the 29 July 1990 sample were combined with females collected in May through August 1991 for these analyses.

	Correlation with	
Clutch Characteristics	SL	Adjusted Body Wt.
Clutch Size (No. MA ova)	r = 0.597* (p < 0.001)	0.185 ns
$\bar{x}$ = 109 + 20.2		
range = 70 - 147		
RCM (%)	r = 0.216 ns	----
$\bar{x}$ = 7.1 + 3.29		
range = 2.3 - 17.5		
Ovary weight (mg)	r = 0.589* (p < 0.001)	0.468* (p < 0.001)
$\bar{x}$ = 17.0 + 11.06		
range = 5.5 - 60.6		
Oocyte weight (gm x 10^{-6})	r = 0.241 ns	0.256 ns
$\bar{x}$ = 79.9 + 28.49		
range = 32.8 - 175		
Oocyte diam. (mm)	r = - 0.119 ns	-0.089 ns
$\bar{x}$ = 0.91 + 0.712		
range = 0.71 - 1.0		

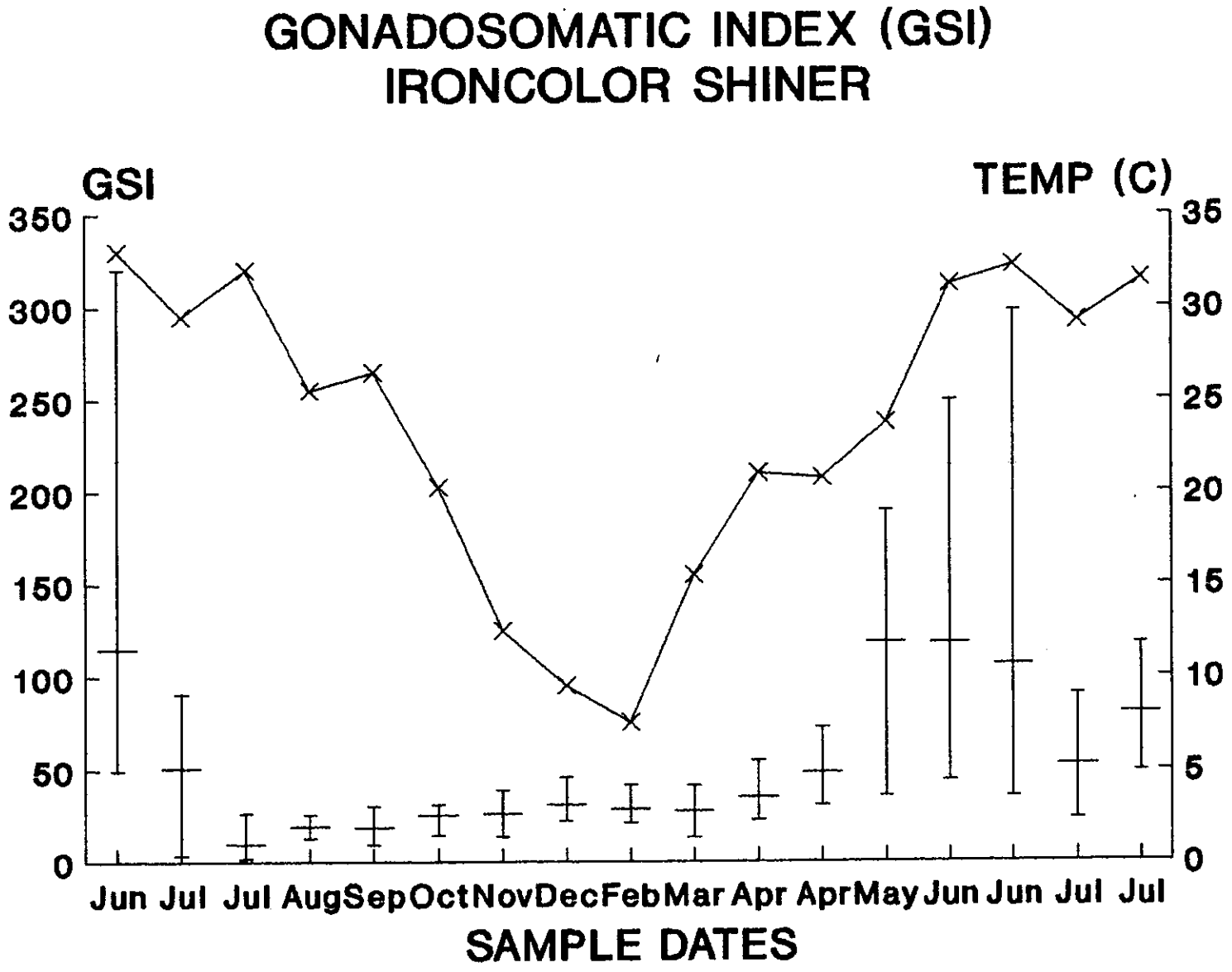

Figure 1. Monthly variation in the gonadosomatic index in female pond-held ironcolor shiners. The top line indicates the temperature profile, under which are the minimum, mean, and maximum values for a given sampling date.

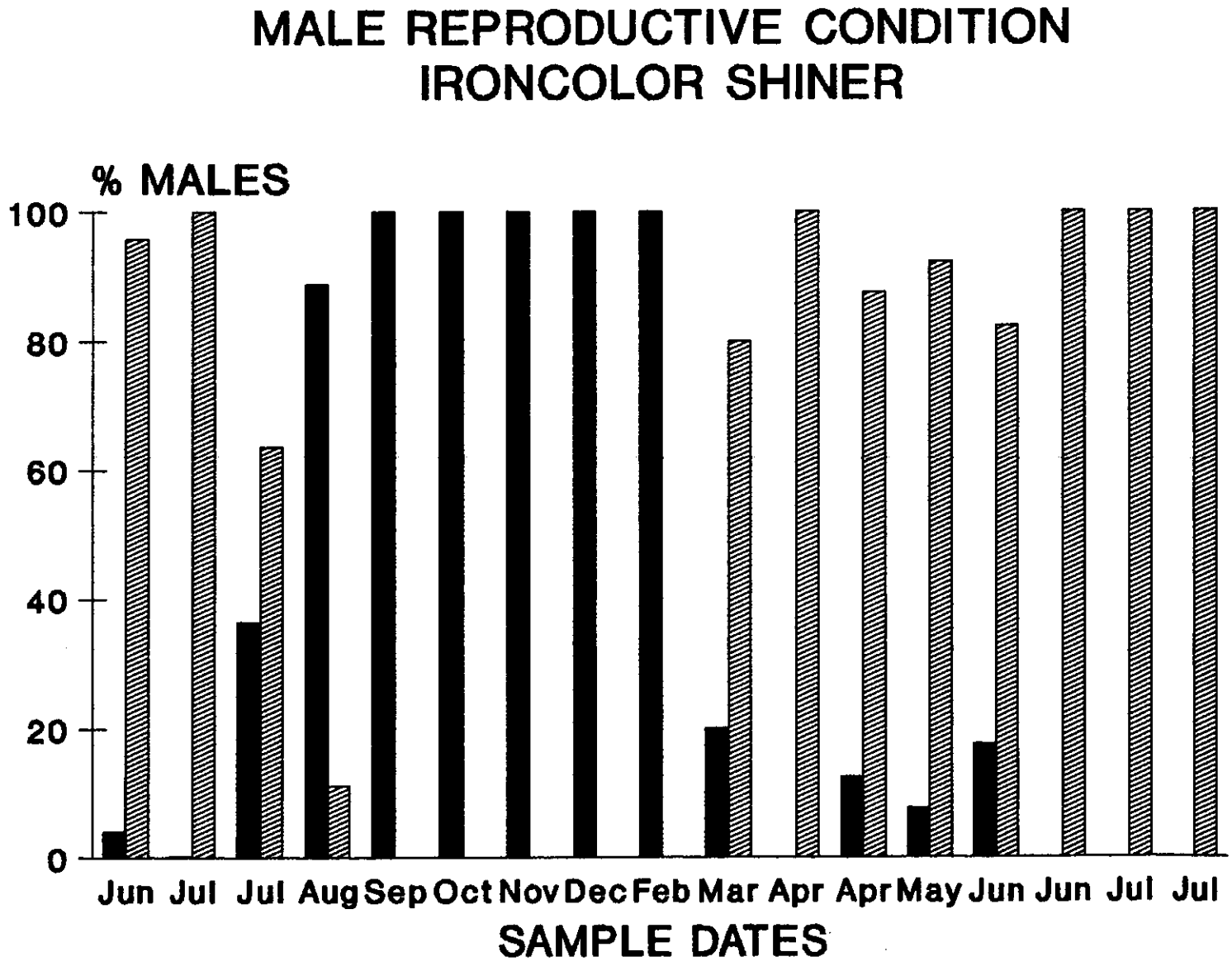

Figure 2. Monthly variation in reproductive condition in male pond-held ironcolor shiners. Black bars indicate latent (LA) males, striped bars indicate mature (MA) males.

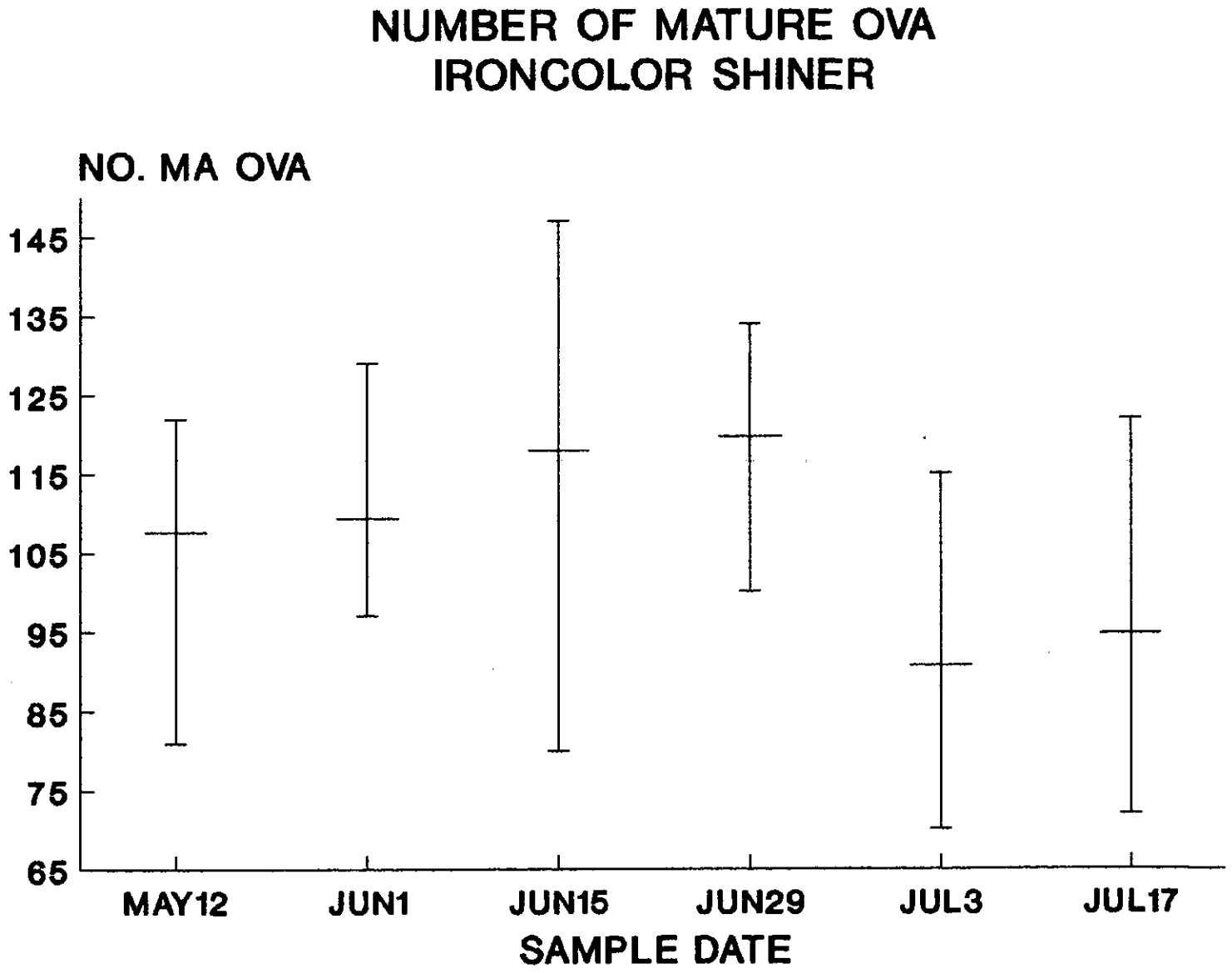

Figure 3. Variation in number of mature ova in female pond-held ironcolor shiners. Minimum, mean, and maximum values are indicated on the vertical lines.

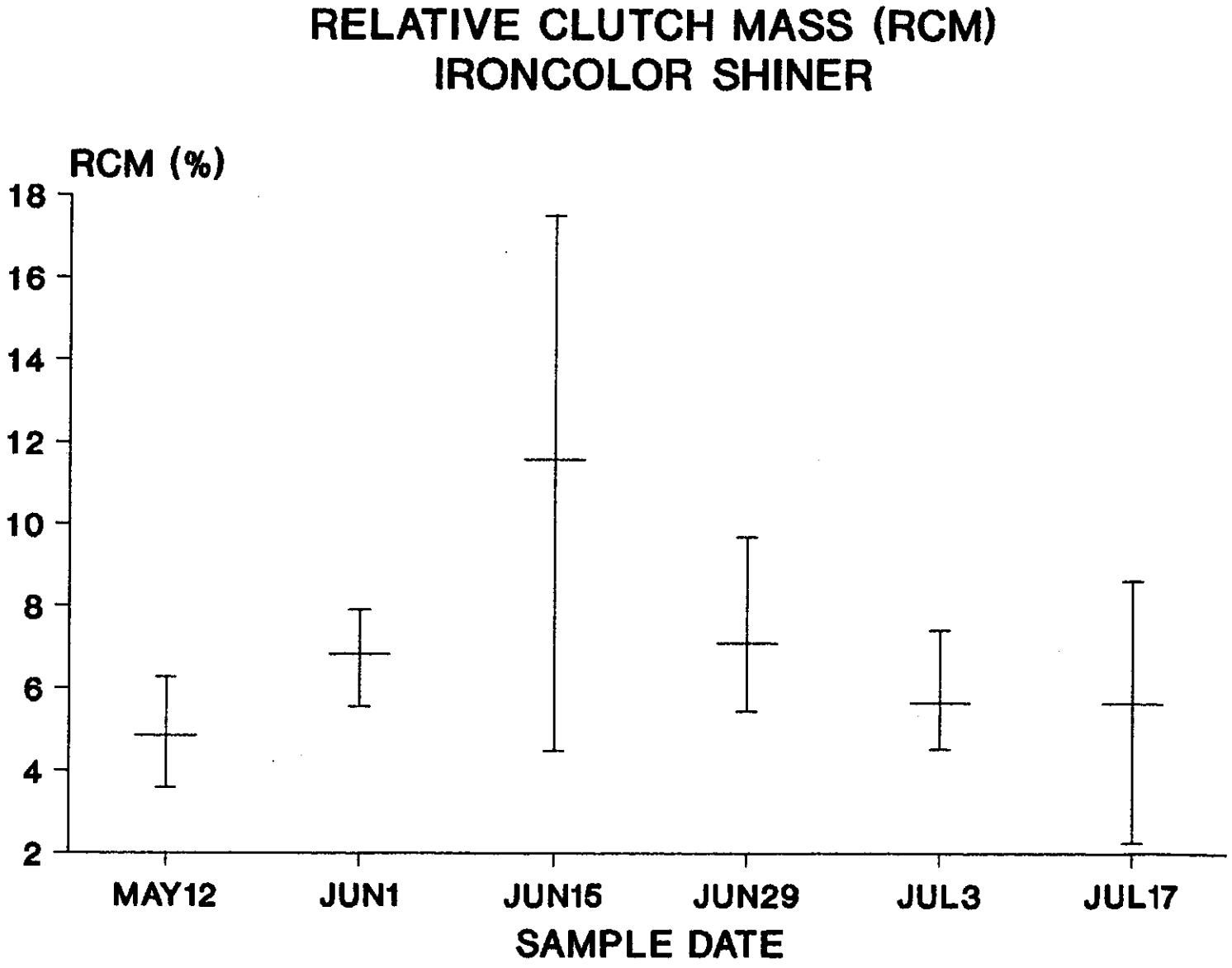

Figure 4. Variation in relative clutch mass in female pond-held ironcolor shiners. Minimum, mean, and maximum values are indicated on the vertical lines.

largest from 12 May to 15 June and decreased slightly in late June and July (Figure 5). Mean egg weight was 79.9 x 10^{-6} gm, ranged from 32.8 to 175 x 10^{-6} gm, and was highest on 15 June, successively increasing prior to this date and decreasing afterward (Figure 6). Mean ovary weight was 17 mg and ranged from 5.5 to 60.6 mg. There was no correlation between adjusted body weight and clutch size, egg weight, or egg diameter; the relationship between adjusted body weight and ovary weight was significantly and positively correlated (Table 2). There was no significant correlation between SL and RCM, egg weight, and egg diameter; the relationships between SL and clutch size and SL and ovary weight were significantly and positively correlated.

DISCUSSION

The largest documented populations of the ironcolor shiner in the entire upper Mississippi Basin occur in the sand areas along the Illinois River, Illinois, and the Kankakee River, Illinois and Indiana. Illinois localities represent the northern limit of the range of the species and harbor the few remaining reproducing populations in the region. Inclusion of the ironcolor shiner on the Illinois threatened list should be maintained, especially because in Wisconsin (Becker 1983) and Iowa (Harlan and Speaker 1987) the species has been extirpated within historic times.

We plan additional comprehensive analyses on reproductive biology and early life history. Nevertheless, it is of interest here to compare known reproductive characteristics of the pond culture stock of the ironcolor shiner with individuals from a

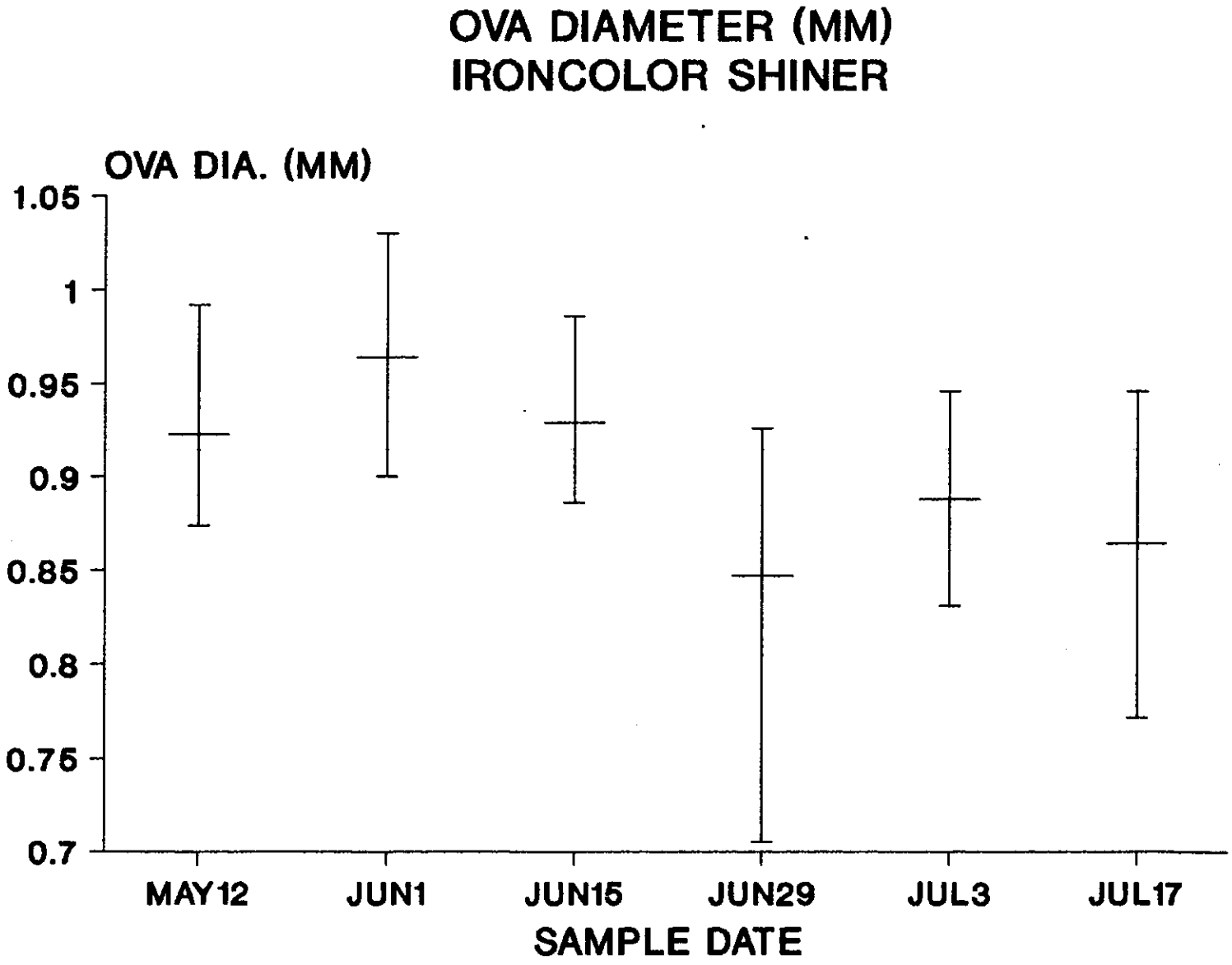

Figure 5. Variation in mature ova diameters in female pond-held ironcolor shiners. Minimum, mean, and maximum values are indicated on the vertical lines.

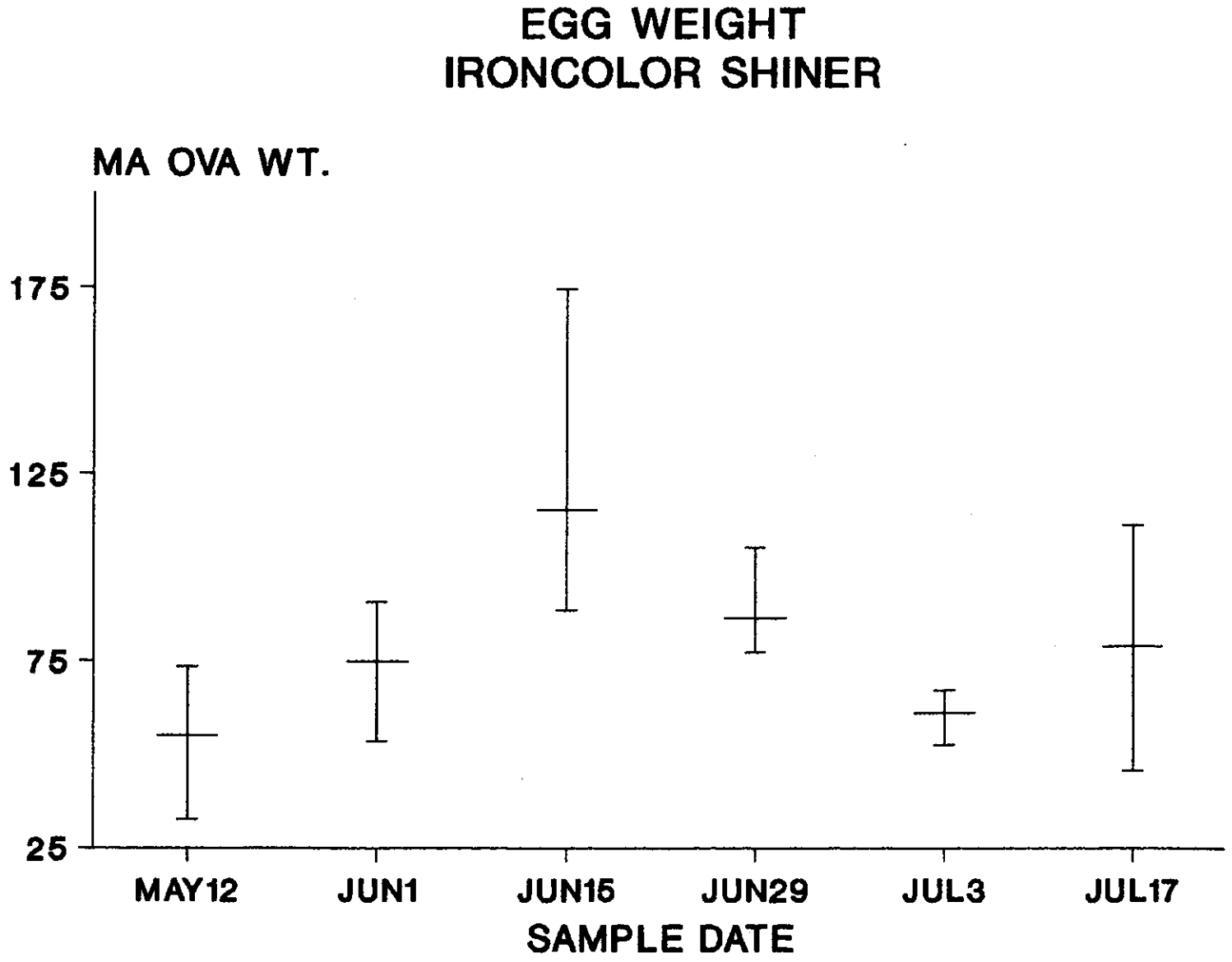

Figure 6. Variation in mature egg weight in female pond-held ironcolor shiners. Minimum, mean, and maximum values are indicated on the vertical lines.

wild population sampled from Crane Creek, Mason County, Illinois (Burr et al. 1989).

Burr et al. (1989) concluded that breeding season length extended from late May through July in Illinois streams. As judged from reproductive condition (Table 1, Figure 2), GSI (Figure 1), and clutch characteristics (Figures 3-6), breeding season length in pond stock is essentially identical to wild populations; both have peak breeding periods in June. Our observations indicate that pond culture has not abbreviated or extended breeding season length in this species.

Pond individuals reached maturity at about 32 mm SL, those from the wild population were mature at about 30 (males) and 28 (females) mm SL. It appears that nearly all ironcolor shiners probably reach sexual maturity during the reproductive season following hatching regardless of their environment.

A comparison of sex ratios from a wild population (Burr et al. 1989) vs pond stock indicates a ratio not significantly different from 1:1 in either situation. In the pond population, adult males average larger than females (36.5 vs 32.4 mm SL, respectively). In the natural population females also weighed less than males of comparable lengths.

Mature females from the pond contained at least two different groups of developing eggs (small maturing oocytes and larger mature oocytes) as did the wild population (Burr et al. 1989). According to Heins and Rabito (1986) this ovarian profile is typical of cyprinids that produce multiple clutches. In wild females there was a significant correlation between reproductive

condition (i.e., LM, MA, and RE females) and SL, but this correlation was nonsignificant for pond-held females. The disparity in sample sizes (30 females from the wild, 133 from the pond) may account for this discrepancy and the fact that the sample examined from the wild included smaller females (range = 28.3–37.2; $\bar{x}$ = 33.3 mm SL) than did the sample from the pond population (range = 31.9–42.9; $\bar{x}$ = 36.2 mm SL).

A comparison of clutch characteristics between wild and pond-held females revealed that mean egg diameters were approximately equal. However, the wild sample contained a greater number of heavier eggs, but the clutch mass represented proportionately less of the adjusted body weight than in the pond samples (Table 2). Although significance tests of the means of various clutch characteristics and egg sizes have not been performed, differences between wild and pond-held females may result from: 1) combining pond-held samples from across the reproductive season, and 2) pond conditions may have resulted in physiological adjustments in egg weight and clutch size.

With this report and the one completed earlier (Burr et al. 1989) we now have a plethora of data on the reproductive cycle of the ironcolor shiner in both natural stream and artificial pond environments. Our work has shown that this species is amenable to pond culture; it has overwintered, successfully reproduced, and recruited for over two years and three reproductive seasons in a 0.1 acre pond.

The efficacy of pond culture for the management of the ironcolor shiner and perhaps other endangered/threatened fish

species should be a primary consideration in development of a recovery plan. To be effective as a management tool, pond culture of endangered/threatened species should be initiated and evaluated before natural populations reach critically low levels. We recommend that a recovery plan be developed for the ironcolor shiner, including establishment of populations in former habitats, before this species meets the fate of other Illinois minnows (e.g., bluehead shiner, cypress minnow).

WORK ELEMENTS REMAINING

1. Examine length-weight relationships of males, compare the regression equations for males and females, and estimate potential clutch numbers via manipulation of the regressions. Examine length-weight relationships of natural vs captive populations by sex, compare the regression equations for the respective population, and estimate potential clutch numbers via manipulation of the regressions.
2. Identify internal parasites infecting pond-held ironcolor shiners.
3. Examine ova size-class frequencies for selected MA females during the spawning months to quantify batch development.
4. Quantify extent and timing of tubercle development in males.
5. Describe and illustrate larval ironcolor shiners at various sizes.

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