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Ovarian Cycling, Clutch Characteristics, and Oocyte Size of the River Shiner Notropis blennius (Girard) in the Upper Mississippi River

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ABSTRACT

Specimens of the river shiner were collected from three locations in Pool 4 of the upper Mississippi River in Wisconsin and Minnesota during June, July, and August 1995. All individuals from the first collection (21 June) displayed early maturing, late maturing or mature ovaries, indicating that spawning commenced prior to this date. Ovarian cycling continued over the next seven weeks but ended by 18 August, when 100% of the individuals were atretic. Clutch sizes varied from 436 to 2754 ova in mature females that ranged from 48.3 to 87.9 mm standard length (SL). Clutch size was significantly dependent on SL, but slopes and intercepts declined significantly over time. Mean clutch size declined significantly from 1600 on 30 June to 1056 and 1010 on 8 July and 11 August, respectively. This decline remained significant after means were adjusted for fish size. Mean mature oocyte size remained constant (0.89 mm) during the same period and was not dependent on standard length. Ovulated ripe oocytes in two fish ranged from 1.06 to 1.22 mm. The overall ovarian cycling schedule supported the hypothesis that the river shiner is a multiple clutch spawner in the upper Mississippi River.

INTRODUCTION

As concern for the environment and the integrity of its ecosystems has grown, so has the need for systematically collected life history data (Wilson 1992). In particular, Heins (see Heins et al. 1996) has repeatedly pointed out the need for accurate data regarding clutch and egg size characteristics within the two most speciose groups of the North American freshwater fishes: Cyprinidae (minnows) and Etheostomatinae (darters). The ability to test questions of historical ecology and to make informed decisions about protecting the biotic integrity of fish communities both rest on the availability and accuracy of basic life history data. Among the most important of these data are those that delimit spawning seasons, clutch production, clutch size, and size of mature eggs.

The river shiner, *Notropis blennius* (Girard), is found in large rivers in the Hudson Bay drainage from western Ontario and northern Minnesota to Alberta and in the Mississippi River drainage from Ohio and West Virginia west to Nebraska and south to Texas and Louisiana (Eddy and Underhill 1974, Lee et al. 1980). Despite its wide range and frequent high abundance levels, no one has determined whether or not it is a multiple-clutch spawner or published seasonal estimates of clutch and egg sizes. The only data regarding fecundity is that of Becker (1983), who reported mature egg counts and sizes for four specimens collected on three dates in three locations. Several additional authors have provided estimates of spawning season (Starrett 1951, Pflieger 1997, Trautman 1981, Harlan et al 1987, Cross and Collins 1995, Etnier and Starnes 1995), although none has based the estimates on examination of ovarian stages. While studying the life history of another species in Pool 4 of the upper Mississippi River, periodic capture of river shiners allowed us to elucidate some of its reproductive characteristics. Our objectives were to describe the ovarian cycle, to determine clutch size and its relationships to fish size, and to do the same for oocyte size.

MATERIALS AND METHODS

We used bag seines to collect specimens from June through August 1995 from three locations in Pool 4 of the upper Mississippi River (river km 1213.4 near Alma, WI; river km 1220.4 near Hershey Island; and river km 1223.6 near Wabasha, MN). We euthanized the specimens in a 100 mg/l solution of MS222, fixed them in 10% formalin, and rinsed them in flowing water for 24 hrs prior to making measurements.

We used a dial caliper to measure standard (SL) and total lengths (TL) to the nearest 0.1 mm. After removing surface moisture by repeated blotting, we measured total body mass (TBM), eviscerated body mass (EBM), and gonadal mass (GM--both ovaries) to the nearest 0.001 g. For the EBM measure, we removed all viscera except gills and kidneys. We calculated a gonadosomatic index (GSI) by dividing GM by EBM and multiplying by 100.

Using the ovarian development criteria of Heins and Rabito (1986) and Heins and Baker (1993), we scored the reproductive condition of each specimen as latent (LA), early maturing (EM), late maturing (LM), mature (MA), ripening (MR) or ripe (RE). We added the category of "atretic" (AT) because specimens from late August were resorbing ova. We determined clutch size by counting the number of mature and ripening oocytes in the right ovary of MA and MR individuals and multiplying by two. We measured the largest and smallest diameters of 10 mature or ripening oocytes from each fish with an ocular micrometer (nearest 0.076 mm) and used the average of these two diameters to calculate mean ovum diameter for each fish (Heins and Baker 1988).

We determined all body size relationships by least-squares regression. In cases where length and mass were the variables, we log transformed the measurements. We did not use geometric mean regressions (Ricker 1973) because previous analyses of short-lived, diminutive fishes have shown it to be unnecessary (Johnson and Hatch 1991). Since length to length and length to EBM relationships are affected proportionately during the growing season, we pooled the date-specific data. Since gondal mass does change seasonally, body size relationships involving TBM could vary through time. To test the effects of the TBM variable, we performed date-specific log-transformed TBM on SL regressions and tested for coincidence using the dummy variable-single equation method (Kleinbaum et al. 1988). Because these lines were coincident, we pooled all body size variables. We performed regressions of clutch size and ovum size on body size separately for the four dates under the assumption that these reproductive characteristics changed with time. Subsequently, we compared slopes of these lines using the dummy variable method above.

To compare clutch sizes across dates, we adjusted clutch size for SL (because they were length dependent--see Table 4) and compared size-adjusted means using a factorial design ANOVA and Fisher's PLSD. We performed a similar analysis for ovum diameters but did not adjust for SL because ovum diameter was not dependent on SL. We used Statview® 4.01 (Abacus Concepts, Inc., Berkley, CA, 1992-93) to compute all F statistics and P values.

RESULTS

Body size relationships–We developed the six body size relationships in Table 1 because fishery data for nongame species rarely include measurements beyond TL and occasionally TBM. The multiple-partial $F_{6,80}$ statistic for coincidence of the date-specific log TBM on log SL regressions was 0.025 (critical $F_{.05} = 2.21$). Thus, we pooled all body-size data and determined one predictive regression for each relationship. All equations were highly significant ($p < 0.0001$) and provided a high level ($r^2 \geq 0.968$) of predictability.

Ovarian cycle–Seventeen river shiners were collected on 2 June but all were males, some of which exuded milt. On 21 June both sexes were collected and all females were EM, LM or MA, suggesting that the spawning season began sometime earlier (Table 2.) Females clearly were cycling through yolk-loading stages until 18 August when all females captured were in atresis. We chose to

Table 1. Body size regressions for female *Notropis blennius* captured in Pool 4 of the upper Mississippi River, June through August 1995. Probability values for the F-statistic were < 0.0001 for each regression.

Regression	intercept	slope	r^2	N
SL on TL	-0.495	0.786	0.992	87
EBM on TBM	0.192	0.673	0.988	86
log EBM on log SL	-4.663	2.918	0.974	87
log TBM on log SL	-4.761	3.055	0.968	86
log EBM on log TL	-5.059	2.961	0.981	87
log TBM on log TL	-5.175	3.099	0.975	86

report these individuals as atretic instead of LA because their GSIs were much higher than those of the LA fish, mostly because they contained large numbers of mature and ripe ova. This ovarian condition is very distinct from that of an LA young-of-the-year fish (gonad mass of one 28.6 mm SL fish was less then 0.001 g) or an LA adult from later in the fall. The seasonal pattern of GSI suggests that peak reproductive readiness occurred from mid-June through early July and that reproduction continued through mid-August (Figure 1). Mean GSI was statistically similar on the last two dates ($t = 0.812$, $p = 0.424$, $df = 26$), but we know from ovarian condition that females were 100% atretic on 18 August as compared to 11% on 11 August. This result points to the importance of determining ovarian condition to delimit the reproductive season.

Clutch and ovum size–Clutch sizes varied from 436 to 2754 in females that ranged from 48.3 to 87.9 mm SL (Table 3). Clutch size was highly dependent on SL even in the small 30 June sample (Table 4). Statistical differences existed among the four equations so we did not combine them. Clutch size declined after June as evidenced by date-specific means and regression slopes. Regression slopes were not equal ($F_{3,44} = 20.440$, $P < 0.0001$) and SL-adjusted mean clutch sizes were significantly different ($F_{4,59} = 14.574$, $P < 0.0001$). Fisher's PLSD performed on SL-adjusted clutch sizes showed that the two June means were similar and both were larger than those in July and August. Adjusted mean clutch size on 8 July was similar to that of 11 August, and both were significantly larger than that of 18 August. Because 100% of the 18 August individuals showed signs of atresis, they were not used to determine clutch size characteristics but are given here to emphasize the importance of using only MA and MR individuals for determining clutch size.

Mature/ripening ovum size in non-atretic females ranged from 0.81 to 1.06 mm, with a mean of 0.89 mm on every date (Table 3). Contrary to clutch size, ovum size was not dependent on SL. A statistically significant regression was produced only for the 30 June sample, where N = 5 (Table 4.) ANOVA of the date-

Table 2. Reproductive condition of female *Notropis blennius* collected June to August 1995 in three areas of Pool 4 in the upper Mississippi River.

			Ovarian stage						
Date	Site	n	LA	EM	LM	MA	MR	RE	AT
21 Jun	Alma, WI	20	0	10	15	35	40	0	0
30 Jun	Alma, WI	5	0	0	0	60	40	0	0
08 Jul	Wabasha, MN	19	0	11	5	26	47	0	11
21 Jul	Hershey Island	4	0	0	0	25	0	0	75
11 Aug	Alma, WI	19	5[a]	0	0	21	52	11	11
18 Aug	Alma, WI	19	0	0	0	0	0	0	100

[a] 1995 year class

specific groupings in Table 3 showed homogeneity of variance and no differences among means ($F_{4,59}$ = 0.030, P = 0.9983). Ripe ova measured in two non-atretic fish ranged from 1.06 to 1.22 mm. Other ripe ova were found in atretic individuals but were not measured because of potential absorption effects. Since only two ripe, non-atretic fish were encountered, we could not determine if mature/ripening ovum size increases upon becoming fully ripe.

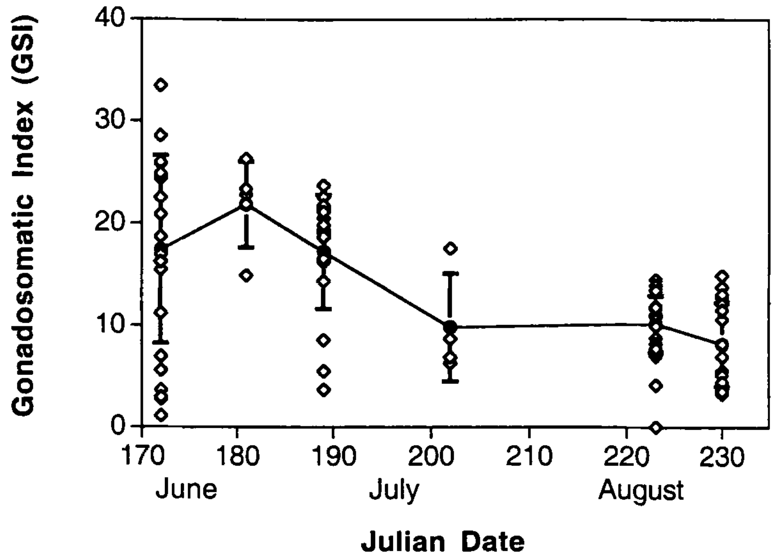

Figure 1. Seasonal variation in gonadosomatic indices (lines connect the means of the ranges) of female *Notropis blennius* collected June to August 1995 in three areas of Pool 4 in the upper Mississippi River.

DISCUSSION

In the southern portion of its range, the river shiner is reported to spawn from early June to late August (Pflieger 1997) or mid-June to mid-July (Cross and Collins 1995). Becker (1983) reported June to the latter half of August as the spawning season in Wisconsin. In our study, the onset of spawning likely occurred prior to 21 June since no LA females were encountered on that date. Even though GSI ranged from 3.4 to 14.8 on 18 August and its mean was not statistically different from that of 11 August, the ovarian condition of all females (atretic) indicates that spawning had stopped prior to this date. Workers using GSI as an indication of spawning activity likely would conclude that spawning was still in progress on 18 August, especially if a subsequent date showed a mean closer to 3 or 4, which is typical of fall LA darters in Minnesota (Hatch 1982 and 1985, Johnson and Hatch 1991). Field workers using the occurrence of distended abdomens or egg expression might have drawn a similar conclusion since several 18 August females had somewhat distended abdomens and five contained loose, (perhaps ovulated) ripe ova. Although precise spawning onset and cessation dates will vary from year to year with seasonal temperature changes (Hubbs 1985), the length of the season may or may not vary. To determine an accurate season length, frequent sampling and examination of ovarian stage are necessary.

Becker (1983) counted mature ova in two river shiners from the Mississippi River and two from the Wisconsin River that were captured in June and July (year not given). His counts ranged from 1,895 in an 82 mm TL fish to 3,005 in an 89 mm TL fish. Converting to SL and applying our June and July regressions, the same sized fish in our study produced considerably smaller clutches of 953-1450 and 1162-2002. Becker described the eggs that he did not count as small and

Table 3. Means and ranges of standard length (SL), clutch size (CS) and mature/ripening oocyte diameter (OD) from MA and MR female *Notropis blennius* captured in Pool 4 of the upper Mississippi River, June through August 1995.

Date	Site	n	SL mean	SL s.d.	CS mean	CS s.d.	OD mean	OD s.d.
21 Jun	Alma, WI	15	67.3	6.2	1485	543	0.89	0.05
30 Jun	Alma, WI	5	63.7	10.2	1600	883	0.89	0.06
08 Jul	Wabasha, MN	14	65.1	11.8	1098	561	0.90	0.04
21 Jul	Hershey Island	1	53.6	-	614	-	0.82	-
11 Aug	Alma, WI	14	68.6	7.8	1165	397	0.92	0.07
18 Aug	Alma, WI	14	68.9	7.1	506 [a]	191	0.89	0.13

[a] Counts of ova that were mature or ripening but now in atresis; cannot be used to estimate clutch size.

white. Thus, it is possible that he counted some maturing ova (i.e., those in the process of loading yolk but not yet mature), mistaking them for mature because of their yellow color. The ovum sizes he reported are comparable to ours, but we do not know if they represent means or the largest ovum encountered. Compared to other *Notropis* species that have been studied by Heins and his associates, *N. blennius* tends toward the high end of clutch size. This is not due only to the much larger average body size of river shiners. If we use our regression equations to predict clutch size at 49 mm SL, the low end of our size range, clutch sizes in June and July ranged from 458 to 509. For similar sized *Hybopsis longirostris* (formerly *Notropis*) (Heins 1991, Heins and Baker 1992), the range was 138-203. The range for similar sized *N. texanus* was 401-630 (Heins and Rabito 1988) and for *Lythrurus umbratilis* (formerly *Notropis*) was about 575-880 (Matthews and Heins 1984). Slightly smaller *N. leedsi* (47 mm SL) produced clutches of 148-238 (Heins and Rabito 1986). The mean clutch size for one population of *N. venustus* having a mean SL of 48.9 mm was 214 (Heins and Baker 1987). Other species examined to date were generally smaller than the smallest river shiners examined in this study; so size-specific comparisons are not possible.

Our results provide two lines of evidence showing that river shiners produced multiple clutches of eggs during the breeding season. First, the ovaries of all MA females contained two very distinct groups of oocytes: either yellow (mature) or orange-yellow (ripening) oocytes of fairly homogenous size (range of < 0.152 mm in a single fish) and white to yellowish maturing oocytes of heterogeneous size (range ≥ 0.608 mm in a single fish). This is the condition found in multiple clutch producing shiners (Matthews and Heins 1984, Heins and Dorsett 1986, Heins and Rabito 1986, Heins and Rabito 1988, Heins 1991, Heins and Baker 1992, Hatch 2001). Second, the seasonal distribution of ovarian stages closely matches the multiple clutch field model of Heins (1990), where the percentage of reproductive females remains near 100% and then precipitously falls to zero over a prolonged reproductive season. Multiple clutch production is especially adaptive for diminutive fishes since it offers a way to gain back annual propagule number lost by reduced body size. Further, it provides a means of maintaining propagule size in the face of an exponentially decreasing body cavity volume. Thus, it makes sense that, in studies where ovarian development was included, most shiners and darters produced multiple clutches (Hatch 1982, Heins and Baker 1989, Parrish et al 1990, Johnson and Hatch 1991, Weddle and Burr 1991, Heins and Machado 1992, Heins and Baker 1993, Heins et al 1996).

We noted above the constraint placed on egg size in diminutive fishes. According to optimizing theory, egg size should be an important fitness component (Smith and Fretwell 1974). As clutch size increases, mature ovum size must decrease if body cavity size is held constant. While our data do not refute this statement, they show that, although clutch size varies through the spawning season, egg size does not. Further, river shiners are at the high end of reported shiner clutch size and still above the middle of mature ovum size. Having a relatively large body size probably helps accommodate this combination.

What remains to be determined in order to measure fecundity and ultimately fitness in this fish is the number of clutches spawned per season. At present there is no procedure for determining this measurement in the field, and laboratory determinations suffer from the lack of environmental cues and factors that could cause spawning periodicity to vary. Heins and Rabito (1986) showed that captive *N. leedsi* on average spawned a full clutch of eggs every 4.6 days (range 3-10 days) at 22-26°C. One individual spawned 21 clutches and 15 of 20 fish spawned six or more clutches despite some individuals being sacrificed during the experiment. This is the only *Notropis* for which such extensive data are available; and since *N. leedsi* produces much smaller size-adjusted clutch sizes than *N. blennius*, an extrapolation to the latter species is unwarranted.

Table 4. Regression of fish length (SL) on clutch size (CS) and mature/ripening oocyte diameter (OD) from MA and MR female *Notropis blennius* captured in Pool 4 of the upper Mississippi River, June through August 1995.

Regression	Date	intercept	slope	r^2	N	p > F
Log CS on Log SL	21 Jun	-3.349	3.556	0.731	15	<0.0001
	30 Jun	-3.908	3.914	0.943	5	0.0058
	08 Jul	-1.361	2.403	0.834	16	<0.0001
	11 Aug	-1.031	2.202	0.520	14	0.0036
Log OD on Log SL	21 Jun	-0.344	0.161	0.080	15	0.3079
	30 Jun	-0.804	0.418	0.962	5	0.0032
	08 Jul	-0.151	0.056	0.050	16	0.4049
	11 Aug	0.050	-0.057	0.016	14	0.6694

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