AN ABSTRACT OF THE THESIS OF

Angela G. Bulger for the Master of Science
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Title: Population structure, habitat use, and breeding behavior of the Neosho madtom, *Noturus placidus*
Abstract approved:

Population structure, habitat use, behavior, and breeding biology of the threatened Neosho madtom, *Noturus placidus*, were studied from 1996 to 1998. Two populations of Neosho madtoms were monitored on the Neosho and Cottonwood rivers in Lyon and Chase counties, Kansas. Age and sex structure were investigated by using length-frequency histograms to determine age-classes and external characteristics to determine the sex of fish during breeding season. Two age-classes were observed, which suggests that Neosho madtoms breed at Age 1. Operational sex ratios suggested a female bias in the Neosho River. Young-of-year (YOY) inhabited areas with slower flow, shallower depth, and lower substrate compaction than adults. Breeding adults were more often found in shallower areas with loosely compacted substrate than non-breeding adults. Use of shallow areas with loosely compacted gravel demonstrates the importance of such habitat to these critical life stages, and illustrates the need to protect this habitat for maintenance of populations.

In the lab, effects of photoperiod on behavior were investigated. Individuals held in a long daylight photoperiod spent a higher proportion of time performing cavity enhancement, and courtship behaviors were seen more often in the long daylight photoperiod. The relationship between a long photoperiod and increased cavity enhancement and courtship behaviors demonstrates the influence of photoperiod on the Neosho madtom reproductive cycle. Courtship behaviors recorded included the "carousel" and "tail curl" in which the fish spun in circles head to tail then quivered, with the male's tail wrapped around the female's head. These behaviors were recorded on time-lapse video prior to a spawning event. A second spawning event occurred after two days of injection with synthetic hormone. Both clutches were laid in nest cavities and consisted of approximately 30 eggs. Dissection of the breeding females revealed previtellogenic eggs in their ovaries. Sexual dimorphism during the spawning season was also investigated and differences were described.

POPULATION STRUCTURE, HABITAT USE, AND BREEDING BEHAVIOR OF THE NEOSHO MADTOM, *NOTURUS PLACIDUS*

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Master of Science

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by
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PREFACE

The federal recovery plan for the threatened Neosho madtom, *Noturus placidus*, listed understanding its reproductive biology, behavior, and habitat use as critical for its recovery. My thesis is comprised of three investigations of *N. placidus* breeding characteristics. Each chapter was prepared for submission to appropriate scientific journals and is thus written in the style dictated by the journal to which it will be submitted. Chapter One is to be submitted to The Southwestern Naturalist, Chapter Two to Environmental Biology of Fishes, and Chapter Three to The American Midland Naturalist. This required that some background information be repeated and that format vary among chapters. The three manuscripts discuss population structure and habitat use, effects of photoperiod on activity, behavior, and courtship, and life history characteristics such as clutch size, egg diameter, and embryonic and larval development of this threatened fish. This information will contribute to understanding the breeding biology of the Neosho madtom.

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Chapter 1

NEOSHO MADTOM (NOTURUS PLACIDUS) POPULATION STRUCTURE AND HABITAT USE

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ABSTRACT--The Neosho madtom, Noturus placidus, is listed as threatened by the federal government and the state of Kansas, and as endangered by the states of Missouri and Oklahoma. The federal Neosho madtom recovery plan characterized understanding the species' biology as critical for its recovery. To investigate age and sex structure and to compare adult and young-of-year (YOY) habitat use I monitored two populations in the Neosho and Cottonwood rivers, Kansas, from August 1996 to September 1998. I sampled riffles, runs, pools, and backwaters, and measured current speed, water depth, and substrate compaction/composition at the location of capture for 443 fish. Operational sex ratios in the Neosho River differed from 1:1, with a female-bias, but this was not seen in the Cottonwood River. Length-frequency distributions showed only two age-classes, which suggests most Neosho madtoms breed at Age 1 then die. To investigate location of adults during the breeding season I divided study sites into upper, middle, and lower sections. No significant difference in spatial distribution of adults among sections was observed and Neosho madtoms were not found in pools. Breeding adults were more often found in shallower areas with loosely compacted substrate than non-breeding adults. In the Neosho River, YOY inhabited areas with significantly slower flow, shallower depth, and lower substrate compaction than adults. Breeding adult and YOY N. placidus use of shallow areas with loosely compacted gravel demonstrates the importance of such habitat to these critical life history stages, and illustrates the need for managers to protect this habitat for maintenance of populations.

INTRODUCTION--The Neosho madtom, Noturus placidus, is a small catfish with a distribution limited to mainstems of the Neosho River in eastern Kansas and northeastern Oklahoma, the Cottonwood River in eastern Kansas, and the Spring River in southwestern Missouri and southeastern Kansas (U.S. Fish and Wildlife Service, 1991; Luttrell et al., 1992; Wilkinson et al., 1996). The fish inhabits loosely compacted gravel bars in areas of high to moderate flow usually associated with riffles (Wenke et al., 1992; Fuselier and Edds, 1994). In 1990, the U.S. Fish and Wildlife Service (USFWS) listed N. placidus as threatened (55 FR 21148), primarily due to loss of habitat caused by mainstem impoundments, and characterized the need to study the species' breeding biology and habitat use as critical for its recovery (USFWS, 1991). Due to high river turbidity and flow, however, field observations are nearly impossible during the presumed spawning season, May through July; thus, little is known of the mating system of Neosho madtoms, or of the habitat use of breeding adults or young-of-year (YOY). No Neosho madtom spawning or nests have been observed in the wild. Fuselier and Edds (1994) compared the species' habitat use in spring, summer, winter, and fall, but did not examine differences in breeding season or age-class. Understanding habitat requirements for all life stages, including YOY and breeding adults, is crucial for management and recovery of the species.

Cochran (1996) suggested that cavity enhancement, especially during spawning season, may be a behavioral trait of many madtom species, and other researchers have documented the use of cavities as nests for spawning by various Noturus species (e.g., N. albater, Mayden et al., 1980; N. elegans, Burr and Dimmick, 1981; N. nocturnus, Burr and Mayden, 1982; N. flavater, Burr and Mayden, 1984; N. hildebrandi, Mayden and Walsh, 1984; N. eleutherus, Starnes and Starnes, 1985; N. phaeus, Chan, 1995; Cochran, 1996; N. baileyi and N. flavipinnis, Dinkins and Shute, 1996). In N. placidus, this behavior has been observed in the laboratory (C. Wilkinson, Emporia State Univ., pers. comm.; Bulger et al., 1998), with eggs laid in depressions the fish had made in gravel under larger substrate. Fuselier and Edds (1994) suggested that Neosho madtoms may spawn at the head or crest of gravel bars where there is an abundance of large cobble.

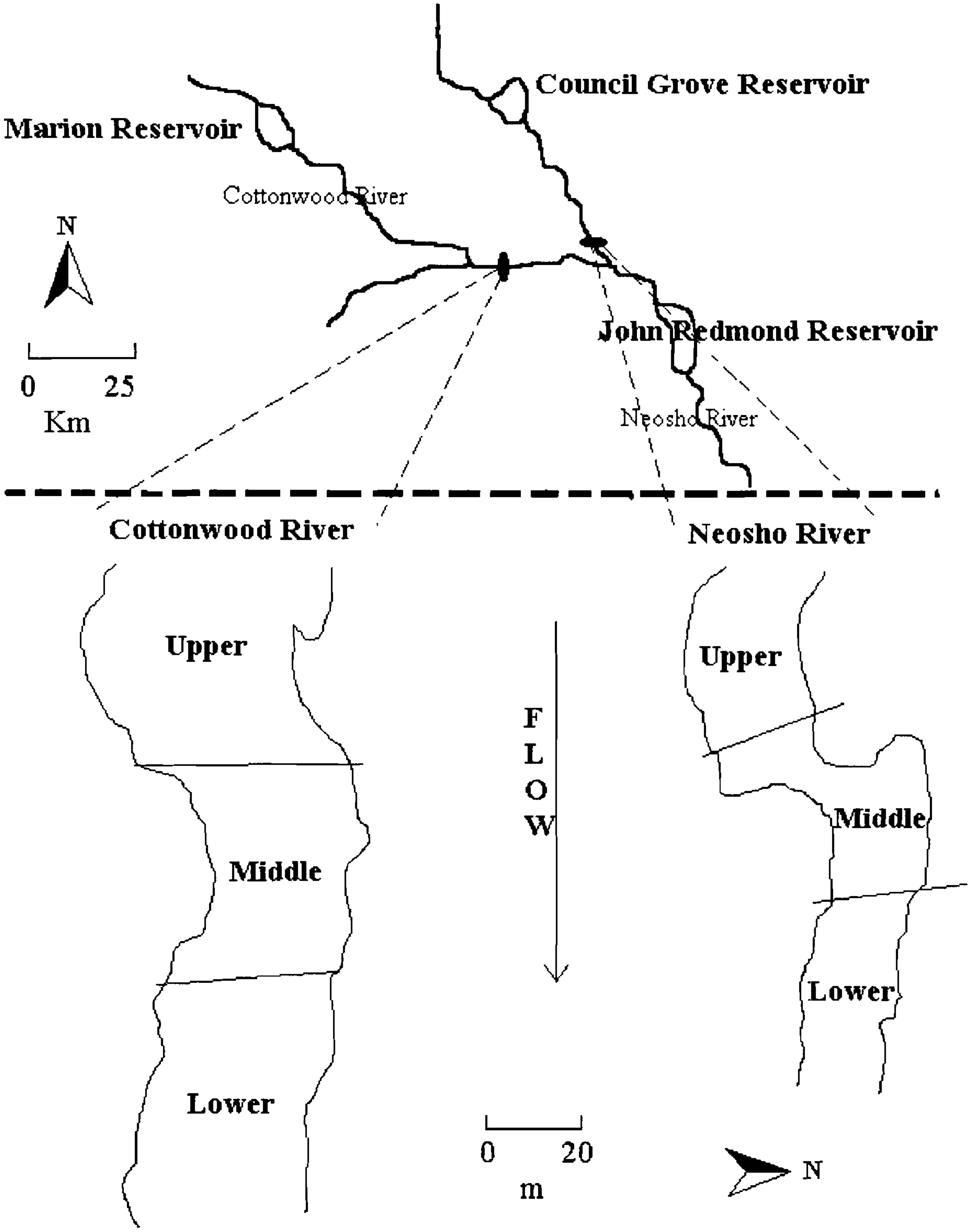
Understanding of Neosho madtom breeding biology must include consideration of population age and sex structure. Age at maturity and maximum lifespan could affect the number of times an individual can breed during a lifetime (Wootton, 1990; Matthews, 1998) and male:female ratios could influence reproductive strategies by limiting the number of males or females available for breeding (Borgia, 1979, Krebs and Davies, 1993). Neosho madtom populations could be highly susceptible to environmental perturbation if individuals breed only once, as suggested by Fuselier and Edds (1994). I studied N. placidus in the Neosho and Cottonwood rivers, Kansas, to characterize YOY and adult habitat use, compare habitat of breeding and non-breeding adults, examine male:female ratios, and determine age-class structure in two populations.

METHODS AND MATERIALS--Study areas were located on the Neosho River, Lyon County, Kansas (SE 1/4, Sec. 8, T19S, R12E; N 38^{o} 24.4 min., W 96^{o} 06.2 min.) and the Cottonwood River, Chase County, Kansas (NW 1/4, Sec. 28, T19S, R8E; N 38^{o} 22.6 min., W 96^{o} 31.8 min.). The Neosho River site measured 120 m x 20 m, and included pools, backwaters, and ca. 2100 m^2 of riffles and runs. The Cottonwood River site, a riffle artificially restored in 1992 (Fuselier and Edds, 1995), measured 150 m x 30 m, and included pools, backwaters, and ca. 3000 m^2 of riffles and runs. Both sites were bordered by agricultural fields and a riparian corridor of woody vegetation. Each site included one large gravel bar, which was divided into an upper, middle, and lower section to assess Neosho madtom spatial distribution during breeding versus non-breeding season (Fig. 1).

I sampled each site once a month, during daylight hours, from August 1996 to October 1997 and April to September 1998, water levels permitting, and twice each month from May to August to assess habitat use during breeding season. Standardized

Fig. 1

Study sites on the Neosho and Cottonwood rivers, Lyon and Chase counties, Kansas.


Council Grove Reservoir
Marion Reservoir
Cottonwood River
N
0 25
Km
John Redmond Reservoir
Neosho River
Cottonwood River
Neosho River
Upper
Upper
F L O W
Middle
Middle
Lower
Lower
0 20
m
N

sampling involved performing 30 kick hauls with a 4.6-m x 1.8-m, 4.7 mm mesh seine in riffles and runs (10 each beginning in the lower, then middle, and upper sections of each gravel bar) and three sweep hauls in each of three pools or backwaters at each site. Kick-seining began 2 m upstream from the seine, and yielded 9.2 m^2 hauls. I calculated Neosho madtom density of occurrence (species-specific density, or number of Neosho madtoms per 100 m^2 in seine hauls containing Neosho madtoms) and overall density (number per 100 m^2 in total area sampled in riffles and runs) for each visit (Wenke et al., 1992). I measured all Neosho madtoms captured and examined each for development of secondary sex characteristics, such as reddening of the premaxillary tooth patch and mouth region in males and females, swollen epaxial muscles on the head of males, swollen genital papilla on males, swollen genital pore on females, and distended abdomen on females (Pfingsten and Edds, 1994; Bulger et al., 1998), before releasing them at the site of capture.

For each seine haul in which Neosho madtoms were captured, I recorded location on the gravel bar (upper, mid, or lower section), and measured the following habitat characteristics: mesohabitat, water depth, current speed at the substrate and at mid-column, substrate compaction and percent composition, and predominant substrate. Mesohabitat was coded 1 backwater, 2 pool, 3 run, or 4 riffle. Water depth was measured with a meter stick, and current velocity at the substrate and at mid-column was measured with either a Teledyne Gurley pygmy current meter no. 625 or a Global Flow Probe model FP 101 (Global Water, Fair Oaks, California). I sampled substrate with a shovel (Grost et al., 1991) and made visual estimates of percent composition based on a modified Wentworth scale (Cummins, 1962). Predominant substrate type was coded 1 mud, 2 sand, 3 gravel, 4 small cobble, 5 large cobble, and 6 boulder, and substrate compaction was coded 1 loose, 2 moderate, and 3 compacted.

To compare habitat use by YOY and adults in breeding and non-breeding seasons I calculated weighted averages for each habitat variable, based on the number of individuals captured in each kick-haul (Sokal and Rohlf, 1995; Monzyk et al., 1997). I characterized individuals as adult or YOY by length-frequency distribution, which involved grouping members of common length-classes into cohorts, which were charted through time (Busacker et al., 1990). I defined breeding season by presence or absence of secondary sex characteristics, and included individuals showing sexual development in analysis of breeding season habitat use; sexual development in April was ambiguous so these fish were omitted from analyses. I compared habitat use of adult versus YOY, and breeding versus non-breeding adults with Wilcoxon-Mann-Whitney two-tailed tests (Sokal and Rohlf, 1995), using Mann-Whitney U: Z values, with a sequential Bonferroni correction (Rice, 1989). To compare the number of individuals captured in each section (upper, middle, and lower) during breeding and non-breeding season I used two-way ANOVA (Sokal and Rohlf, 1995), with section on each site as the experimental unit and the two rivers as replications. The number of adult Neosho madtoms captured in the upper and lower sections was normally distributed and, although those in the middle sections were not, I accepted the premise that ANOVA is a robust test, allowing for some deviation from normality (Cody and Smith, 1991). Because sample sizes were similar, ANOVA was judged to be effective whether or not variances were homogeneous (Milliken and Johnson, 1984). I used chi-square goodness of fit to test the hypothesis of 1:1 operational sex ratios (Emlen and Oring, 1977) at each site during breeding season.

RESULTS--Twenty-five samples from the Neosho River yielded 293 Neosho madtoms for habitat analysis: 119 YOY and 174 adults. Mean density of occurrence ($N = 309$) in the Neosho River was 19.8/100 m^2. Sample means ranged from zero on 22 January 1997 to 32.6/100 m^2 on 7 December 1996. Mean overall density was 4.5/100 m^2 and ranged from zero on 22 January 1997 to 9.8/100 m^2 on 2 July 1998. Neosho madtoms were found in temperatures ranging from 3 to 31°C in riffles and runs with loose to moderately compacted substrate consisting of mainly gravel (median = 84%,

Table 1 -- Habitat use by YOY (N = 119) versus adult (N = 174) Neosho madtoms captured in the Neosho River, Lyon County, Kansas, 1996-1998. Mann-Whitney U: Z values and P-values, with significance after sequential Bonferroni correction indicated by an asterisk (*).

Habitat Variable	YOY Median Range	Adult Median Range	Mann-Whitney U: Z	P-Value
Mesohabitat	3.0 2.0 - 4.0	3.0 3.0 - 4.0	-0.72	0.47
Water depth (cm)	29.0 5.0 - 81.0	38.0 7.0 - 104.0	-4.93	0.0001*
Current speed at substrate (cm/sec)	19.0 0.0 - 64.0	27.5 0.0 - 71.0	-3.75	0.0002*
Current speed at mid-column (cm/sec)	46.0 0.0 - 116.0	57.0 0.0 - 155.0	-4.80	0.0001*
Substrate Compaction	1.5 1.0 - 2.5	2.0 1.0 - 2.5	-2.96	0.0031*
Predominant Substrate	3.0 1.0 - 5.0	3.0 1.0 - 4.0	-0.30	0.76

range = 40 - 98%). Median water depth at capture locations was 34.0 cm and ranged from 5 to 104 cm, median current speed at the substrate was 23.0 cm/sec and ranged from 0 to 71 cm/sec, and median current speed at mid-column was 52.0 cm/sec and ranged from 0 to 155 cm/sec. Four of six habitat variables differed between locations where YOY and adults were captured (Table 1). Water depth was lower at YOY sites compared to adult sites (Mann-Whitney U: $Z = -4.93$, $P = 0.0001$), as were current speeds at the substrate and at mid-column ($Z = -3.75$, $P = 0.0002$ and $Z = -4.80$, $P = 0.0001$, respectively), and substrate compaction ($Z = -2.96$, $P = 0.0031$) (Table 1). Adults and YOY were found in riffles and runs with abundant gravel. Breeding individuals were found in areas with shallower depth and loosely compacted substrate ($Z = -2.54$, $P = 0.01$ and $Z = -3.33$, $P = 0.0009$, respectively) (Table 2). All adults were found in riffles and runs with abundant gravel. Twenty-six samples in the Cottonwood River yielded 150 Neosho madtoms for habitat analysis: 54 YOY and 96 adults. Mean density of occurrence ($N = 156$) was 11.9/100 m^2. Sample means ranged from 0 on three dates (13 and 26 June, and 8 July, 1997) to 24.8/100 m^2 on 30 October 1996. Mean overall density was 1.9/100 m^2 and ranged from 0 to 5.8/100 m^2 on the same dates. Neosho madtoms were found in temperatures ranging from 2 to 29°C in riffles and runs with loose to moderately compacted substrate consisting of mostly gravel (median = 79.5%, range = 50 - 95%). Median water depth was 59.5 cm and ranged from 9 to 100 cm, median current speed at the substrate was 24.0 cm/sec and ranged from zero to 62 cm/sec, and median current speed at mid-column was 62.5 cm/sec and ranged from 5 to 113 cm/sec. Current speed at mid-column was lower at YOY sites than at adult sites ($Z = -2.89$, $P = 0.004$) (Table 3). Habitat use by breeding and non-breeding adults did not differ in the Cottonwood River (Table 4). Mesohabitat (riffles and runs) and predominant substrate type (gravel) was the same for all life stages in both rivers.

Table 2 -- Habitat use by breeding adult (N = 47) versus non-breeding adult (N = 48) Neosho madtoms captured in the Neosho River, Lyon County, Kansas, 1996-1998. Mann-Whitney U: Z values and P-values, with significance after sequential Bonferroni correction indicated by an asterisk (*).

Habitat Variable	Breeding Median Range	Non-breeding Median Range	Mann-Whitney U: Z	P-value
Mesohabitat	4.0 3.0 - 4.0	4.0 3.0 - 4.0	-0.70	0.48
Water depth (cm)	30.0 15.0 - 104.0	46.0 7.0 - 91.0	-2.54	0.01*
Current speed at substrate (cm/sec)	28.0 2.0 - 66.0	31.0 0.0 - 71.0	-2.02	0.04
Current speed at mid-column (cm/sec)	63.0 12.0 - 122.0	60.0 10.0 - 155.0	-0.21	0.83
Substrate Compaction	1.5 1.0 - 2.5	2.0 1.5 - 2.5	-3.33	0.0009*
Predominant Substrate	3.0 3.0 - 4.0	3.0 1.0 - 3.0	1.72	0.09

Table 3 -- Habitat use by YOY (N = 54) versus adult (N = 96) Neosho madtoms captured in the Cottonwood River, Chase County, Kansas, 1996-1998. Mann-Whitney U: Z values and P-values, with significance after sequential Bonferroni correction indicated by an asterisk (*).

Habitat Variable	YOY Median Range	Adult Median Range	Mann-Whitney U: Z	P-value
Mesohabitat	4.0 3.0 - 4.0	4.0 3.0 - 4.0	-0.86	0.39
Water depth (cm)	55.5 9.0 - 89.0	48.0 10.0 - 100.0	1.04	0.30
Current speed at substrate (cm/sec)	20.0 3.0 - 62.0	26.0 0.0 - 57.0	-1.37	0.17
Current speed at mid-column (cm/sec)	44.5 9.0 - 85.0	57.0 16.0 - 113.0	-2.89	0.004*
Substrate Compaction	2.0 1.0 - 2.5	2.0 1.0 - 3.0	0.72	0.47
Predominant Substrate	3.0 3.0 - 3.0	3.0 3.0 - 3.0	0.00	1.00

Table 4 -- Habitat use by breeding adult (N = 19) versus non-breeding adult (N = 83) Neosho madtoms captured in the Cottonwood River, Chase County, Kansas, 1996-1998. Mann-Whitney U: Z values and P-values, with significance after sequential Bonferroni correction indicated by an asterisk (*).

Habitat Variable	Breeding Median Range	Non-breeding Median Range	Mann-Whitney U: Z	P-value
Mesohabitat	4.0 3.0 - 4.0	4.0 3.0 - 4.0	0.05	0.96
Water depth (cm)	46.0 11.0 - 94.0	55.0 10.0 - 100.0	-2.21	0.03
Current speed at substrate (cm/sec)	23.0 7.0 - 57.0	25.0 0.0 - 53.0	1.13	0.26
Current speed at mid-column (cm/sec)	65.0 20.0 - 88.0	63.0 16.0 - 113.0	-0.08	0.94
Substrate Compaction	1.5 1.0 - 2.5	2.0 1.0 - 3.0	0.56	0.58
Predominant Substrate	3.0 3.0 - 3.0	3.0 3.0 - 3.0	0.00	1.00

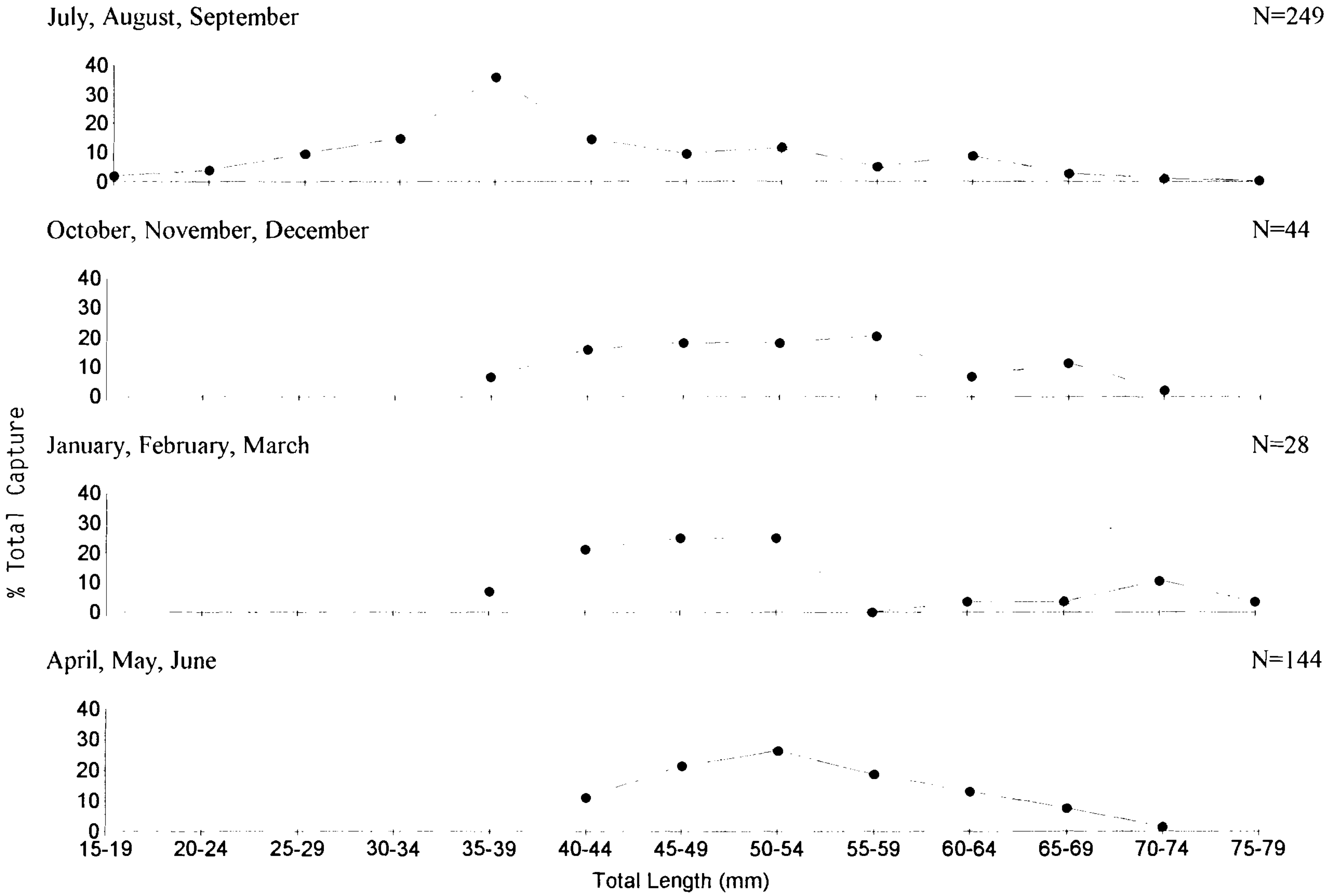
No significant difference was observed in spatial distribution on the gravel bar in breeding versus non-breeding season. ANOVA showed no significant interaction between section and season ($F_{5,11} = 0.53$, $P = 0.62$), nor was season or section effect significant ($F_{5,11} = 0.35$, $P = 0.58$ and $F_{5,11} = 1.61$, $P = 0.27$, respectively), consistent with the null hypotheses that the number of fish captured was equal among seasons and that individuals were evenly distributed among sections of the gravel bars.

Secondary sex characteristics began to develop in April (two individuals with slightly pink tooth patches) and were present until August, which suggested the breeding season had ended. In the Neosho River, 51 individuals showed development of secondary sex characteristics: 39 females, nine males, and three of undetermined sex with only slightly pink tooth patches. This operational sex ratio differed significantly from 1:1 ($\chi^2 = 10.39$, *d.f.* = 1, $P = 0.001$). Sexually developed females ranged 47 - 70 mm TL and sexually developed males ranged 57 - 70 mm TL. In the Cottonwood River, 17 individuals showed development of secondary sex characteristics: 11 female and six male, not significantly different from 1:1 ($\chi^2 = 0.75$, *d.f.* = 1, $P = 0.38$). Females showing sexual development ranged 60 - 69 mm TL and sexually developed males ranged 56 - 70 mm TL.

Pooled length-frequency data of 465 Neosho madtoms captured from both rivers showed only two age-classes (Fig. 2) and suggested that most N. placidus bred as Age 1 individuals and few, if any, survived to breed at Age 2. Because Figure 2 reports percentages it illustrates size classes but is not a good indicator of mortality. In each year the first YOY were captured in July at 19 - 41 mm TL; YOY captured in August and September ranged 27 - 45 mm and 36 - 49 mm TL, respectively. Adult Neosho madtoms ranged 38 - 79 mm TL.

Fig. 2

Length-frequency distribution for 465 Neosho madtoms collected in the Neosho and Cottonwood rivers, Lyon and Chase counties, Kansas, 1996-1998.



July, August, September N=249

October, November, December N=44

January, February, March N=28

April, May, June N=144

DISCUSSION--Moss (1983) captured 435 Neosho madtoms exclusively in riffles in the Neosho and Cottonwood rivers, and Fuselier and Edds (1994) captured 99 % of 257 N. placidus in riffles and runs in the Cottonwood River. I captured Neosho madtoms exclusively in riffles and runs in the Neosho and Cottonwood rivers, where current speed was moderate (~25 cm/sec at the substrate), water depth was less than 50 cm, gravel comprised more than 75% of the substrate, and substrate compaction was low.

Habitat use by adult and YOY Neosho madtoms differed in four of six variables measured, though both life stages used areas with abundant loose gravel. In the Neosho River, YOY, presumably seeking food, refuge from swift current, and/or avoiding negative inter- and intraspecific interactions, used shallower areas with slower flow and looser substrate than adults. Although Vives (1987) reported no significant difference in current speed, depth, or substrate use by small and large slender madtoms (N. exilis), Mayden and Burr (1981) found YOY slender madtoms in shallower areas of riffles and pools than adults. Clark (1978) reported YOY speckled madtoms, N. leptacanthus, inhabited shallow areas with slow current; Starnes and Starnes (1985) reported the same for mountain madtoms, N. eleutherus. Such intraspecific habitat segregation is common among fishes in general, though little is known about such behavior in small-bodied stream fishes (Matthews, 1998). Degradation of juvenile habitat has been suggested as one possible cause of the decline of freshwater mussels (Fuller, 1974); similar loss of habitat for critical life history stages could impact the Neosho madtom. To maintain Neosho madtom populations, managers must understand and protect specific habitat used during critical life stages rather than simply managing for "mean" habitat, which may not suffice for breeding or YOY survival.

In the Neosho River, breeding adults were found over more loosely compacted substrate than non-breeding adults. This is consistent with the hypothesis that N. placidus is a cavity spawner, as loose gravel could be more easily manipulated than compacted gravel. Fuselier and Edds (1994) found the best predictors of Neosho madtom presence in the Cottonwood River to be moderate current speed at the substrate, low variance of that current, shallow water depth, and low substrate compaction. Loosely compacted substrate was also reported as an indicator of "good" Neosho madtom habitat by Wenke et al. (1992). My data demonstrate the importance of loose gravel to the life history of the Neosho madtom, including YOY and breeding adults. Loose gravel may, in fact, be a limiting factor for this species. During drought and other periods of low water, exposed gravel may become compacted due to drying of organic material in interstitial spaces; such "cementation" causes compaction to remain high following return to normal water levels, which may force substrate-dwelling fishes such as the Neosho madtom into less suitable habitat where survival rates could be lower (Deacon, 1961; Gagen et al., 1998). Breeding adults, possibly minimizing predation risks while caring for developing eggs and larvae, also were found in shallower areas than non-breeding adults.

No difference in habitat use by breeding and non-breeding adults was observed in the Cottonwood River. Variation between the two rivers could be a function of smaller sample size from the Cottonwood River. In addition, the Cottonwood River site had been manipulated in 1992 to create an artificial riffle as habitat mitigation for gravel mining (Fuselier and Edds, 1995); according to these authors, within a year after construction the gravel bar had physical features similar to those of two nearby natural riffles. In the current study, dissimilarities in habitat use by YOY and breeding adults between the two sites could suggest a change in the artificial riffle since that time.

Authors of other madtom studies have reported a breeding season habitat shift from pools to riffles, especially to crests of riffles (Mayden and Burr, 1981; Dinkins and Shute, 1996) or from riffles to pools (Burr and Mayden, 1982; Starnes and Starnes, 1985). No such shift was observed in N. placidus. ANOVA indicated no significant difference in the spatial distribution of adults on the gravel bars during breeding versus non-breeding season, and no individuals were captured in pools. This was consistent with the null hypothesis and suggested that breeding individuals do not move from one area to another during spawning season, nor do they concentrate in a specific area of gravel bars for spawning. Specific microhabitat may be more important than distribution on the bar. However, the way breeding season was defined in my study may have masked differences in distribution of breeding versus non-breeding adults. Some individuals developed secondary sex characteristics early or late in the season and may breed at different times. Baker and Heins (1994) suggested that larger female least madtoms (N. hildebrandi) spawn before smaller females. Thus, because different individuals may spawn at different times, from early to late in the season, and because development and loss of secondary sex characteristics is not an instantaneous process, an intensive study investigating habitat use during only June, when the majority of individuals are likely breeding, is desirable. Alternatively, differences in spatial distribution on gravel bars during breeding season could be related to gender; perhaps males move to different locations to prepare nests while females remain spread throughout the gravel bar. Testing of this hypothesis was not possible in my study due to small sample size, especially of breeding males. Future studies should investigate sex-specific habitat use during breeding season.

Most individuals captured were female. Based on lab observations (Bulger et al., 1998), males are less likely to vacate a spawning cavity than females; thus, males may be less likely to be captured by kick-seining. Clugston and Cooper (1960) and Clark (1978) also suggested that male madtoms are less likely to be captured during spawning season because they are guarding nests. Chi-square analysis of operational sex ratios suggested the Neosho River population was female biased, but that of the Cottonwood River was not. Populations of N. exilis and N. hildebrandi are slightly female biased (Mayden and Burr, 1981; Mayden and Walsh, 1984), and Clark (1978) reported an April sample of N. gyrinus in which the ratio of ripe female tadpole madtoms to ripe males was 17:6. Such bias could imply a polygynous mating strategy. A third possibility is that breeding males move to deep water, such as in deep pools or the main channel, where kick-seining is impossible. However, determining the sex of individuals by using external characteristics is difficult in many fishes (Moyle and Cech, 1996), including madtoms (Burr and Mayden, 1984; Simonson and Neves, 1992), even when secondary sex characteristics are well developed; thus, observed sex ratios could have resulted from errors generated by using external characters to sex fish.

Most Noturus species have a two- to three-year lifespan (Clark, 1978; Mayden et al, 1980; Simonson and Neves, 1992; Dinkins and Shute, 1996) with a few living up to five years (Mayden and Burr, 1981; Burr and Mayden, 1982; Starnes and Starnes, 1985) and one, N. flavus, living up to nine years (Mayden et al., 1980). In contrast, N. hildebrandi lives only one year (Mayden and Walsh, 1984). Length-frequency distributions were consistent with the hypothesis that most N. placidus live one year, a consideration critical for the conservation biology of this threatened species. Relying on one or two cohorts for reproduction is risky because environmental disturbances can cause extreme population fluctuations, which can result in local extirpation (Simonson and Neves, 1992). Ninety percent of madtom species under consideration for federal listing, or already listed as endangered or threatened, spawn only once or twice during their lifetime, yet madtom species not federally listed spawn up to six times (Simonson and Neves, 1992). Understanding age-class structure and reproductive life span of the Neosho madtom is crucial to its recovery; there is need to age the fish more accurately by examining otoliths. Because of the species' limited range and potential vulnerability to environmental perturbation, future research should focus on breeding biology, including mating strategy, reproductive life span, and shifts in habitat use of all life stages, both in normal conditions and during disturbances such as floods and drought.

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Chapter 2

Effects of photoperiod on behavior and courtship of the Neosho madtom (*Noturus placidus*)

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Key words: catfish, cavity enhancement, captive propagation, spawning, ethogram, life history, courtship

ABSTRACT--The Neosho madtom, *Noturus placidus*, is a small catfish listed by the U.S. Fish and Wildlife Service as threatened. Understanding the species' reproductive biology and environmental variables that influence its spawning is critical for its recovery. Captive propagation could become necessary, and laboratory studies are critical because turbid water and high flows during its spawning season impede study in the wild. Photoperiod triggers the breeding cycle of many teleost fishes and may play an important role in the life cycle of the Neosho madtom. To test effects of long and short day-length on behavior of this species, six pairs were held under 16 L, 8 D and six pairs under 12 L, 12 D photoperiod. An ethogram was created and behavior was recorded 24 h per day. Two-min intervals for each hour in two 8-day collection periods (early and late summer) were examined, and proportion of time active and performing specific behaviors in each tank was analyzed to compare differences between treatments. Individuals held under 16 L, 8 D were more active during the light cycle in late summer than those in 12 L, 12 D. Specific behavior types examined included resting, swimming, feeding, aggression, cavity enhancement, and courtship. A higher proportion of time was spent performing cavity enhancement, cavities were deeper, and gravel size in cavities was smaller for those fish in 16 L, 8 D than in 12 L, 12 D. Throughout the experiment various courtship behaviors (e.g., 'carousel,' 'tail curl,' 'jostle,' 'fan') were observed 129 times in male-female pairs held in 16 L, 8 D, but such behaviors were not observed in 12 L, 12 D. The relationship between a long photoperiod and activity, cavity enhancement, and courtship behaviors illustrates the influence of photoperiod on the Neosho madtom reproductive cycle.

INTRODUCTION--Little is known about the behavior of diminutive stream fishes (Matthews 1998), especially the madtoms, a group of small, nocturnal North American catfishes of the genus *Noturus*. Information regarding the effects of photoperiod on activity and behavior is lacking in this group, especially with regard to spawning. The Neosho madtom, *Noturus placidus*, is listed by the U.S. Fish and Wildlife Service (USFWS, 55 FR 21148) and the state of Kansas as threatened, and by the states of Missouri and Oklahoma as endangered. The USFWS (1991) Neosho madtom recovery plan regarded understanding the species' reproductive biology and behavior as critical for its recovery. However, much remains to be learned about its reproduction, especially spawning behavior and environmental cues that trigger breeding.

Due to high river turbidity and flow, behavioral observations in the field are nearly impossible during the presumed spawning season (late May through early July), thus no Neosho madtom spawning or nests have been observed in the wild (Pfingsten & Edds 1994). Attempts at captive propagation have had limited success. Of four clutches laid in captivity, one did not develop and was likely never fertilized (Pfingsten & Edds 1994), one resulted in 43 surviving fish (Wilkinson & Edds 1997), one was presumably consumed by the spawning male (Chapter 3), and one resulted in two surviving Neosho madtoms (Chapter 3).

Understanding environmental cues that trigger spawning could increase success of captive propagation, which is essential not only for studying *N. placidus* breeding biology and behavior, but could also be necessary for possible reintroduction efforts. Photoperiod is one important factor in stimulating sexual maturation and ovulation in many fishes (Wootton 1990), including madtoms (Dinkins & Shute 1996), and may play an important role in triggering captive spawning of this threatened catfish. In addition, there is need to understand how manipulating photoperiod affects other behaviors important to the fish in captivity. For example, effects of increased photoperiod on feeding and aggression need investigation, as well as influences on overall activity levels.

My research focused on the effects of a long and short photoperiod on behavior of the Neosho madtom. Objectives were to investigate the influence of photoperiod on captive propagation by examining activity and the following specific behaviors: resting, swimming, feeding, aggression, cavity enhancement, and courtship.

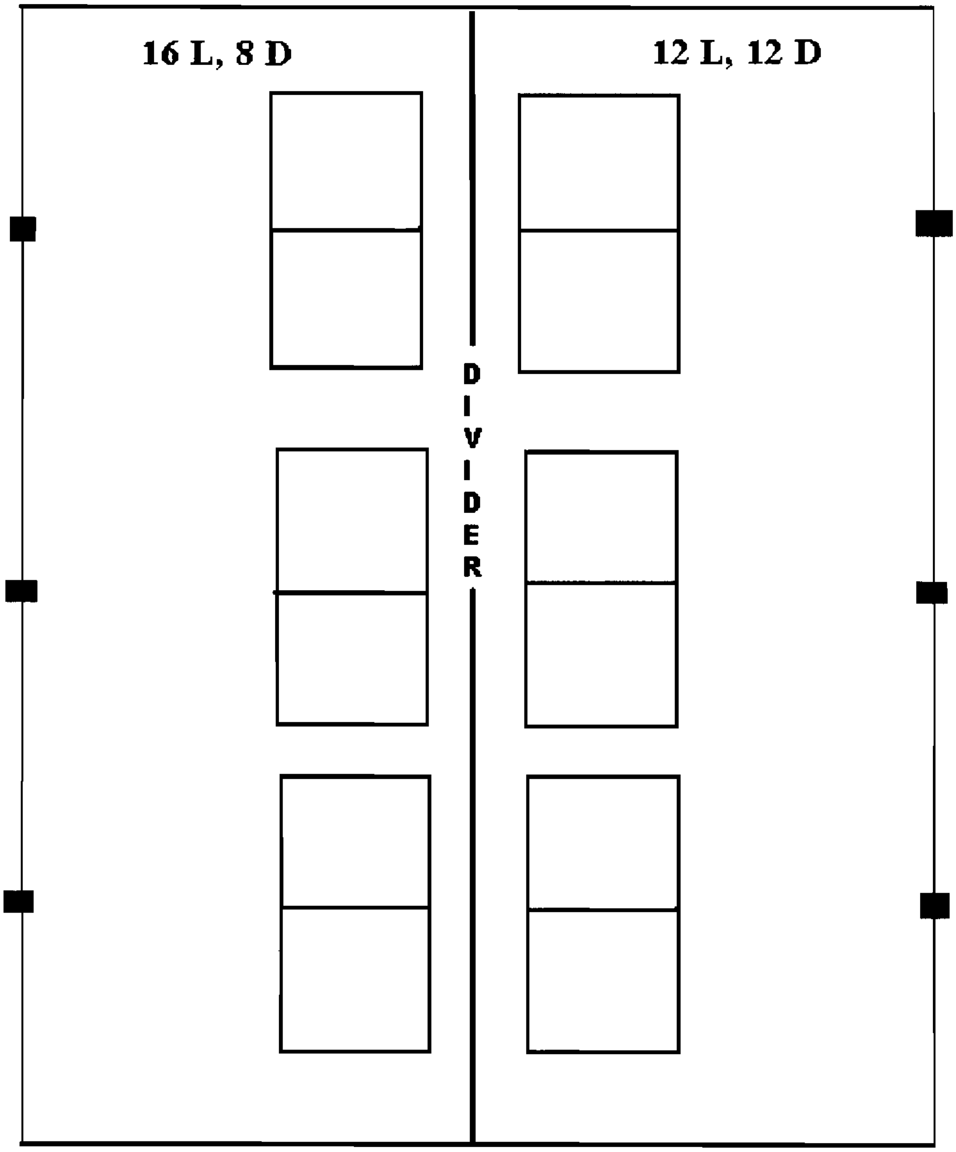
METHODS AND MATERIALS--Fish were collected from the Cottonwood River, Chase and Lyon counties, Kansas, and transported to the Columbia Environmental Research Center (CERC) in Columbia, Missouri. Eight individuals were captured on 13 August 1996 and 21 individuals were collected on 17 and 18 May 1997. Fish were kept in four 59-l holding tanks at 13.5 L, 10.5 D at water temperatures of 18 to 21°C.

I placed twelve 29.5-l aquaria in an isolation chamber in order to regulate photoperiod and dampen sound disturbances. The chamber was divided down the middle with black plastic to create two treatment groups (Figure 1). Well water (pH = 7.5) was pumped into each tank, maintained at 20 cm depth, and drained at each end, which created a slight flow with a turnover rate of approximately 8.6 l h^{-1}. I maintained water temperature at 25 to 27°C with an aquarium heater in each tank. The bottom of each tank was covered approximately 4 cm deep with 2 to 24 mm diameter gravel. Structure was provided by cutting 12.5-cm PVC pipes (10 cm diameter) in half lengthwise, which resulted in a U-shaped shelter; use of the PVC provided cover while allowing observation from the front of the tank. Photoperiod was held at 16 L, 8 D in one treatment group and at 12 L, 12 D in the other. Light was provided by fluorescent bulbs mounted in the chamber and regulated by a timer.

On 28 May 1997, I placed two fish in each tank (18 to 21°C, 13.5 L, 10.5 D) within the isolation chamber. Attempts were made to determine sex of each individual based on development of secondary sex characteristics (Pfingsten & Edds 1994) so that each tank would contain one male and one female. Individuals not placed in study tanks remained in holding tanks. I raised temperatures 1°C every three to five days beginning

Figure 1.

Arrangement of study tanks in isolation chamber (2.8 m x 1.9 m x 0.9 m) at CERC, Columbia, Missouri.



Key

■ = video camera

3 June, and by 24 June the temperature in all holding and study tanks was 25°C. To test effects of photoperiod on activity, I adjusted day-length to treatment settings (16 L, 8 D and 12 L, 12 D) on 16 June. Fish were fed a diet of live amphipods (*Hyalella azteca*) and blackworms (*Lumbriculus* sp.) every two to three days throughout the study.

Three Panasonic closed circuit black & white TV cameras, model WB-BP310, were mounted on each side of the isolation chamber so that each camera recorded activity in two tanks (Figure 1). I mounted American Dynamics 30-W LED infrared illuminators, model AD 1020/30, above the tanks to illuminate and allow recording of nighttime behaviors. Using a Toshiba virtual real-time and time-lapse VCR, model KV-7168-A, I recorded Neosho madtom behavior 24 h per day.

As a control, I collected data for three days (12 to 14 June) while both groups had a water temperature of 21°C and a photoperiod of 13.5 L, 10.5 D. Experimental data were then collected during two 8-day cycles while groups were held under treatment day-lengths. Each experimental period consisted of two 4-day data collection periods, separated by two days. By analyzing 2 min of each hour for each tank during the control and two experimental periods, the data set consisted of between 437 and 456 2-min observations, chosen randomly, for each tank; observations were not made during feeding or tank maintenance. Each time the behavior of either individual changed during the 2-min interval, I recorded the time and new behavior. Attempts were made to record the behaviors of each individual separately, but due to poor film quality and the small size of the fish this was not always possible; however, this collection method allowed me to determine the time spent performing each behavior in each tank. Behaviors recorded (Table 1) were taken from an ethogram created from a combination of observations of Neosho madtoms by me, observations made during previous attempts at Neosho madtom captive breeding (Pfingsten & Edds 1994; Wilkinson & Edds 1997), and from descriptions of spawning behavior of the brown madtom, *Noturus phaeus* (Chan 1995).

Table 1. Ethogram of Neosho madtom behaviors performed by either or both fish. Each behavior is labeled as A = active or I = inactive (resting), and categorized as a behavior type: resting, swimming, feeding, aggression, cavity enhancement (cav enhan), and courtship.

Behavior	Description	Activity	Category
Performed by either fish:			
Upside down	resting upside down under structure	I	resting
Quiet in	resting quietly under structure	I	resting
Quiet out	resting quietly out of structure	I	resting
Restless in	moving slightly about under structure	I	resting
Restless out	moving slightly about outside of structure	I	resting
Circle alone	swimming in circles against glass at front, back, or side of tank	A	swimming
Swim	swimming in no particular pattern	A	swimming
Feeding	feeding	A	feeding
Headstand	vertical in water nudging rocks with head	A	cav enhan
Rock move	moving a rock in its mouth (picks up rock and drops it in another place)	A	cav enhan
Spin	swimming in circular pattern under structure	A	cav enhan
Fanning	fanning tail while resting under structure	A	courtship
Performed by both fish:			
Quiet in both	both fish resting quietly under structure	I	resting
Quiet out both	both fish resting quietly outside structure	I	resting
Restless in both	both fish slightly moving about under structure	I	resting
Restless out both	both fish slightly moving about outside structure	I	resting
Circle chase	one fish chases other in circular pattern in front, back, or side of tank. Individuals periodically meet and have some sort of physical contact (rub, bite, or nudge)	A	swimming
Bite	one fish bites at body of other fish	A	aggression
Chase	one fish chases other in no particular pattern	A	aggression
Nudge	one adult nudges resting individual and swims away or rests next to it. Nudged individual may swim or remain resting	A	aggression
Jostle	fish switch positions back and forth under structure between short periods of rest	A	courtship
Carousel	fish swim together head to tail in small circular pattern under structure	A	courtship
Tail curl	fish lay side by side, head to tail; male has tail wrapped around head of female and both fish quiver. This behavior was only seen following carousel	A	courtship

On 23 June, I removed five individuals due to health problems or lack of development of secondary sexual characteristics, and replaced them with individuals from the holding tanks. After a 5-day acclimation period, the first experimental period (early summer) began (28 June to 1 July and 4 to 7 July). On 9 July, I again replaced nine fish with individuals from the holding tanks. After a 5-day acclimation period, the second experimental period (late summer) began (14 to 17 July and 20 to 23 July). On October 3, 1997, I euthanized all fish and sexed them internally to verify sex of each individual.

I classified each behavior as either active or inactive (resting) (Table 1). The proportion of time spent active in each tank in control groups was compared by using a Wilcoxon-Mann-Whitney two-tailed test of ranks with the null hypothesis that there was no difference in activity between the two treatments (α=0.05 for all analyses). Similarly, I compared the proportion of time spent active in each treatment during early and late summer, with the null hypothesis that activity did not differ between photoperiods. The proportion of time spent active in light versus dark hours was also tested in each treatment group by using a Wilcoxon-Mann-Whitney one-tailed test under the alternative hypothesis that activity of this nocturnal fish was higher during the dark than during the light cycle of the photoperiod.

To assess effects of photoperiod on behavior, I assigned each behavior from the ethogram one of six specific behavior types: resting, swimming, feeding, aggression, cavity enhancement, and courtship (Table 1); resting behaviors were not included in analysis, as they are the complement of active behavior. I compared the proportion of time spent performing each behavior type in light and dark hours between treatment groups by using a Wilcoxon-Mann-Whitney two-tailed test.

To examine effects of photoperiod on cavity enhancement for nesting, I placed gravel evenly in each tank at the beginning of the study and measured depth of the gravel substrate under and outside the structure at the end of the study. In addition, at the end of the study I measured the diameter of three randomly chosen pieces of gravel from under the structure, and three from the rest of the tank. These measurements allowed me to compare the depth of the cavity and gravel size within the cavity in each tank, compared to the rest of the tank bottom, and to compare cavity depth and gravel size between treatments by performing Wilcoxon-Mann-Whitney two-tailed tests. A Pearson correlation coefficient was calculated to examine strength of the relationship between cavity depth and gravel size within the cavity.

RESULTS--Throughout the control, early summer, and late summer collection periods, a total of 36 pairs of Neosho madtoms was observed within the isolation chamber. Subsequent internal examination allowed sexing of 23 pairs used in the study and revealed 12 as male/female, 10 as female/female, and one as male/male; sex of one or more individuals in other pairs was equivocal (Table 2). Each treatment group had two male/female pairs during each data collection period (Table 2).

Results from the control period (13.5 L, 10.5 D in both groups) showed no significant difference between groups in the proportion of time spent active in the dark or light (Mann-Whitney U: Z=-0.88, P=0.38 and Z=-0.61, P=0.54, respectively). I performed analyses on each experimental period separately because the Wilcoxon-Mann-Whitney test showed a significant difference in the proportion of time spent active by individuals in 12 L, 12 D; more activity was seen during late summer than during early summer in the dark cycle of the photoperiod (Z=-2.24, P=0.03). In addition, during early summer, camera failure caused uneven sample sizes; tanks 9 and 10 (12 L, 12 D) were not monitored.

Neosho madtoms spent a significantly higher proportion of time active during dark hours versus light hours (16 L, 8 D: early summer Z=2.80, P=0.005; late summer Z=2.80, P=0.005; 12 L, 12 D: early summer Z=2.17, P=0.03; late summer Z=2.80, P=0.005; Figure 2). Comparison of the proportion of time spent active during late summer showed individuals in 16 L, 8 D were more active during the light cycle than those in 12 L, 12 D

Table 2. Sex of Neosho madtoms in study tanks at the CERC during collection periods (M=male, F=female, U=undetermined), with depth of cavity (mm) under structure (depth of gravel outside structure - depth of gravel under structure) and difference between mean diameter (mm) of gravel outside structure and under structure (mean gravel diameter out - mean gravel diameter under) at end of experiment.

	Tank	Control Period	Period 1	Period 2	Cavity Depth	Gravel size Difference
16 L, 8 D	1	M/F	M/F	M/F	15	4.3
	2	F/F	F/F	F/F	37	7.3
	3	F/F	F/F	F/F	25	2.3
	4	M/F	M/F	M/F	33	4.4
	5	U/U	U/U	U/F	11	4.0
	6	U/U	U/F	F/F	22	5.0
12 L, 12 D	7	M/F	M/F	M/F	5	2.3
	8	M/U	M/U	M/U	4	-3.7
	9	U/U	U/U	M/M	1	-2.0
	10	M/F	U/F	M/F	16	1.0
	11	U/F	U/F	F/F	3	7.7
	12	F/F	M/F	F/F	11	1.5

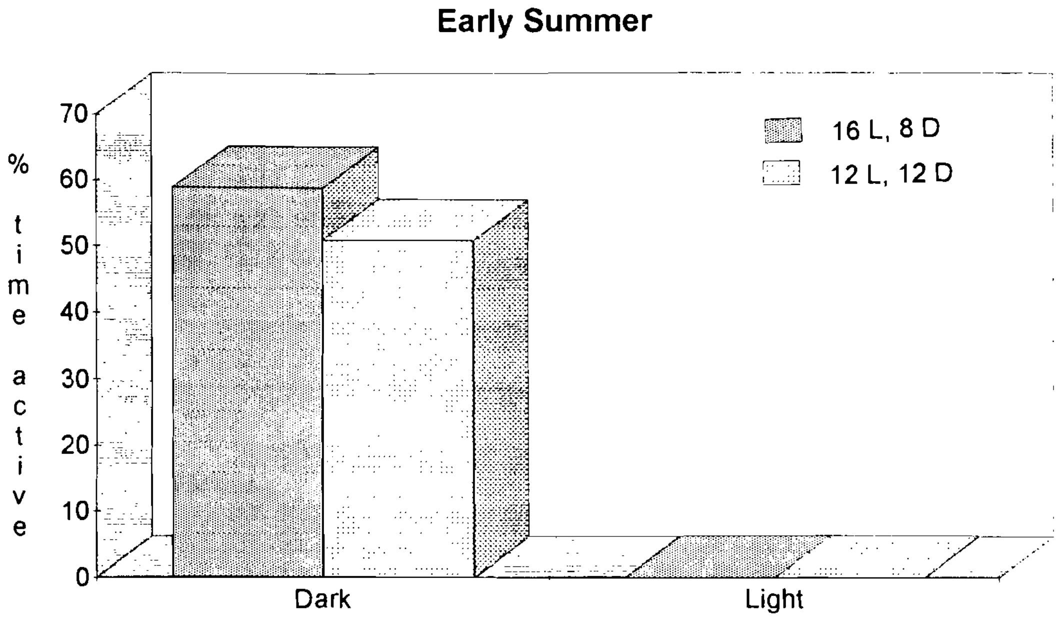
(Z=2.00, P=0.05). I observed individuals in 16 L, 8 D swimming about or foraging for food 1 to 2 h before the light cycle ended. This difference in activity was not significant during early summer (Z=-1.81, P=0.07). During dark hours, there was no significant difference in activity levels (early summer Z=-0.96, P=0.34; late summer Z=0.40, P=0.58) (Figure 2).

I compared the proportion of time spent performing the following categories of behavior between the two treatment groups in dark and light hours for each collection period: swimming, feeding, aggression, and cavity enhancement. The proportion of time spent swimming, feeding, and performing aggressive behaviors was not different between treatments (Table 3). During late summer individuals held under 16 L, 8 D spent a higher proportion of time performing cavity enhancement in dark hours than those in 12 L, 12 D (Z=2.00, P=0.05) (Table 3). Although not significant in early summer or in light, the proportion was consistently higher in 16 L, 8 D. Specific cavity enhancement behaviors included the 'spin,' in which one individual would spin in circles just above the gravel under the structure; the 'headstand,' in which one individual would hover at approximately 45° and nudge rocks from under the structure by using its head (Figure 3); and the 'rock move,' in which gravel was carried in the mouth from under the structure and dropped outside. Both males and females were observed performing the 'spin' and the 'headstand,' but only males were observed doing the 'rock move.'

No spawning was observed during my study; however, based on observations of the brown madtom (Chan 1995), slender madtom, *N. exilis* (Fitzpatrick 1981), freckled madtom, *N. nocturnus* (Fitzpatrick 1981), brindled madtom, *N. miurus* (Bowen 1980; Fitzpatrick 1981), and observations during previous Neosho madtom breeding studies (Pfingsten & Edds 1994; Wilkinson & Edds 1997), behaviors were seen in my study that indicated courtship (Chapter 3). No statistical analyses were performed on courtship behaviors because of small sample size. However, throughout the course of the study male/female pairs held in 16 L, 8 D were observed performing courtship behaviors

Figure 2.

Percent of time active in dark and light of each treatment (16 L, 8 D & 12 L, 12 D) during early and late summer.


Early Summer
% time active
70
60
50
40
30
20
10
0
16 L, 8 D
12 L, 12 D
Dark
Light

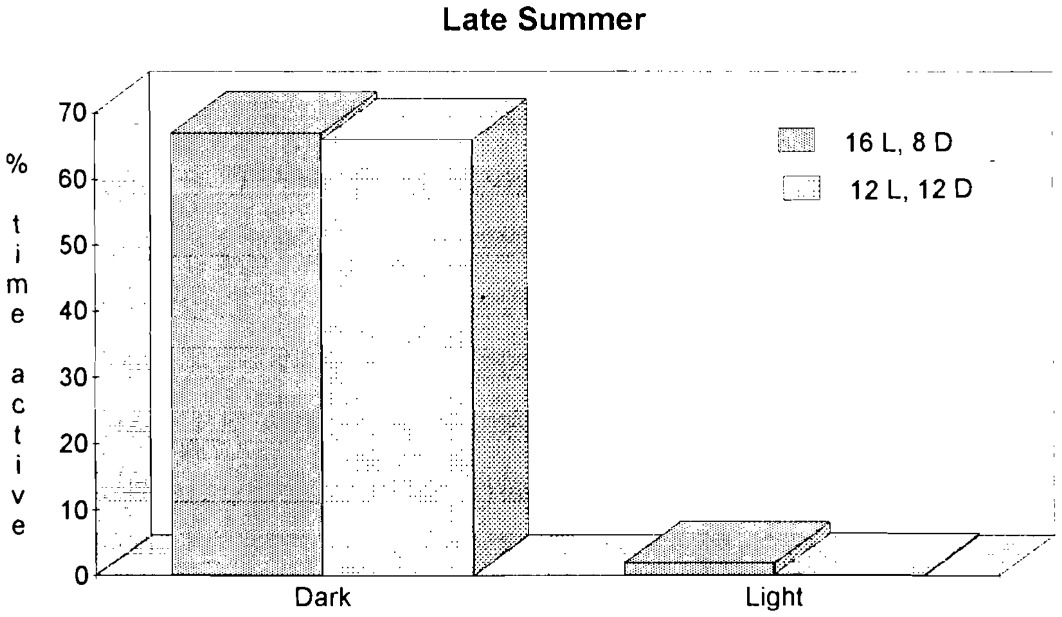

Late Summer
% time active
70
60
50
40
30
20
10
0
16 L, 8 D
12 L, 12 D
Dark
Light

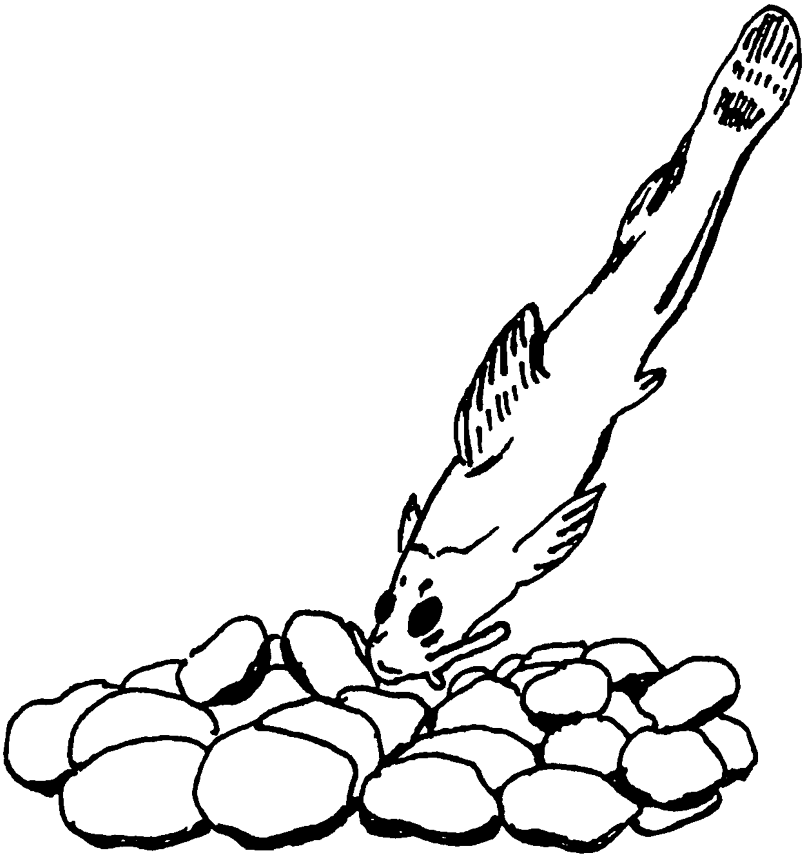
Table 3. P-values and ratio of observed to expected sum of ranks 16 L, 8 D; 12 L, 12 D) from Wilcoxon-Mann-Whitney test of proportion of time spent performing specific behaviors in tanks of each treatment in dark and light hours during early and late summer. Because expected sum of ranks differed due to differing sample size (N = 6, 4 for early summer and N = 6, 6 for late summer) ratio is provided to indicate treatment with greater proportion of time spent performing behavior. SW = swimming, FE = feeding, AG= agression, and CE = cavity enhancement.

	SW	**FE**	**AG**	**CE**
Early summer				
Dark	0.24 (1.18; 0.73)	0.46 (0.88; 1.18)	0.75 (1.06; 0.91)	0.75 (1.06; 0.91)
Light	0.17 (1.21; 0.68)	0.54 (1.06; 0.91)	0.31 (0.91; 1.14)	0.34 (1.14; 0.80)
Late summer				
Dark	0.69 (0.92; 1.08)	0.81 (0.95; 1.05)	1.00 (1.00; 1.00)	0.05* (1.33; 0.67)
Light	0.17 (1.23; 0.77)	1.00 (1.00; 1.00)	1.00 (1.00; 1.00)	0.34 (1.14; 0.86)

*=16 L, 8 D > 12 L, 12 D

Figure 3.

The 'headstand,' a cavity enhancement behavior of the Neosho madtom. Individual hovers at approximately 45° and nudges rocks with head.



(Table 1), including the 'carousel' (88 times), the 'tail curl' (36 times), the 'jostle' (4 times), and the 'fan' (once), whereas these behaviors were never observed during data collection in male/female pairs held in 12 L, 12 D.

Females held in 12 L, 12 D showed signs of egg resorption in late July when most abdomens were no longer as distended as they had previously been; by 1 August only two of the six females had distended abdomens. Of the nine females in 16 L, 8 D, four had slightly distended abdomens and one had a fully distended abdomen on 1 August. A concomitant decrease in secondary sex characteristics was seen in males; on 1 August only two of five males held in 12 L, 12 D had slightly swollen cephalic epaxial muscles, though both males held in 16 L, 8 D had fully swollen head musculature.

Depressions under structures, which indicated cavity enhancement, were first apparent on 6 June. Cavity depth ranged from 1 to 37 mm (Table 2). Cavities deeper than 20 mm were made in four of 12 tanks, all in 16 L, 8 D (Table 2). In addition, the deepest cavity was constructed in 16 L, 8 D, in a tank that contained only females (Table 2). In 16 L, 8 D cavities were deeper ($Z=-2.32$, $P=0.02$) and gravel size within cavities smaller ($Z=1.79$, $P=0.02$) than in 12 L, 12 D. Mean gravel diameters within cavities in 16 L, 8 D ranged from 12.0 mm to 16.7 mm ($\bar{x}=14.5$, SD=3.42) and in 12 L, 12 D means ranged from 14.0 mm to 23.7 mm ($\bar{x}=18.1$, SD=4.84). Throughout the tanks, mean rock diameter ranged from 17.7 mm to 20.7 mm in 16 L, 8 D ($\bar{x}=19.1$, SD=4.09), and from 17.0 mm to 22.3 mm in 12 L, 12 D ($\bar{x}=19.9$, SD=4.02); gravel size throughout the tank did not differ between treatment groups ($Z=-0.49$, $P=0.62$). Pearson's correlation coefficient between gravel size and cavity depth suggested a negative relationship ($r=-0.55$), but was not significant ($P=0.07$).

DISCUSSION--Internal sexing of fish at the completion of the study revealed poor success in external sexing. External sexing of individuals is difficult, at best, and nearly impossible when secondary sex characteristics are poorly developed, with a success rate of only about 90% under favorable conditions, when secondary sex characteristics are well developed (B. Burr, Southern Illinois University, pers. comm.). Others have reported difficulty sexing madtoms externally (Burr & Mayden 1984; Simonson & Neves 1992). Bulger (Chapter 3) depicted differences in the genital papillae of male and female Neosho madtoms during spawning season. In the present study poor success in sexing reduced the chances of a successful spawn, and prevented statistical analysis of courtship behaviors due to small sample size.

Reasons for differences in activity between the early and late experimental periods are unknown. Perhaps the 5-day acclimation period was not long enough for fish to experience photoperiod effects. Results indicated higher activity in the dark than during the light cycle, as expected for a nocturnal fish. Higher activity levels during the light cycle in late summer in 16 L, 8 D could be attributed to restlessness; individuals were more active just prior to the onset of the dark cycle. No breeding behaviors and very few cavity enhancement behaviors were seen during the light cycle; most activity was swimming behavior.

Analysis of swimming, feeding, and aggression indicated no significant differences between the two treatment groups, which suggests that photoperiod does not play a role in these behaviors. Madtoms may limit or refrain from feeding during spawning season, especially males while caring for eggs or larvae (Clark, 1978; Mayden & Burr 1981; Burr & Mayden 1982; Dinkins & Shute 1996). Further investigation of feeding behavior while nesting is recommended.

Cavity enhancement behaviors are considered important because of evidence that indicates madtoms use nests during spawning (*N. albater*, Mayden et al. 1980; *N. elegans*, Burr & Dimmick 1981; *N. miurus* & *N. nocturnus*, Fitzpatrick 1981; *N. nocturnus*, Burr

& Mayden 1982; *N. flavater*, Burr & Mayden 1984; *N. hildebrandi*, Mayden & Walsh 1984; *N. eleutherus*, Starnes and Starnes 1985; *N. phaeus*, Chan 1995; *N. baileyi* & *N. flavipinnis*, Dinkins & Shute 1996; Cochran 1996; *N. placidus*, Wilkinson & Edds 1997). During the late summer period individuals held in 16 L, 8 D spent a higher proportion of time performing cavity enhancement behaviors in the dark than fish in 12 L, 12 D and, although differences were not significant, the proportion was consistently higher in 16 L, 8 D throughout the study. A difference was also seen in cavity depth and gravel size within cavities in tanks in 16 L, 8 D at the end of the study. Individuals held in 16 L, 8 D probably put more effort into cavity enhancement; however, cavity measurements were taken only at the completion of the study, and because cavity enhancement was apparent in both sides of the treatment chamber within the first two weeks of the study, possibly individuals held in short simulated daylight hours reduced the amount of energy they put into cavity enhancement in response to reduced day-length (13.5 L, 10.5 D in holding tanks, 12 L, 12 D in treatment tanks). No photoperiod effect was seen in the proportion of time spent performing cavity enhancement during early summer, but a significant difference was seen between treatments during dark hours in late summer. This suggested that keeping Neosho madtoms in 12 L, 12 D with 25 to 27°C water temperatures over a 1-month period, the duration of my study, was not sufficient to encourage cavity enhancement behaviors.

Cavities were enhanced in tanks containing both male/female and female/female pairs. Most cavity enhancement behaviors observed were performed by males; however, females were observed performing the 'headstand' and the 'spin', and the presence of cavities, including the deepest, in tanks containing only females suggested that females also play a role in cavity enhancement. Wilkinson and Edds (1997) also observed both male and female Neosho madtoms nudging rocks out of cavities to create depressions in the substrate. Female brown madtoms initiated nest construction and aided nest maintenance before breeding (Chan 1995) and both male and female freckled madtoms participated in nest construction prior to spawning (Fitzpatrick 1981), but Mayden and Burr (1981) reported that males were solely responsible for nest construction in the slender madtom. In most other *Noturus* species it is unknown whether nest construction is performed by males, females, or both. Cochran (1996) also observed stonecats (*N. flavus*), tadpole madtoms (*N. gyrinus*), and a black madtom (*N. funebris*) enlarging cavities by moving rocks in their mouths, and Fitzpatrick (1981) observed a male brindled madtom remove gravel from a can by taking it into his mouth and dropping it outside the can opening. No other accounts of behaviors used to enhance cavities have been reported for *Noturus* species, however brown bullheads (*Amieurus nebulosus*) and flathead catfish (*Pylodictis olivaris*) have also been observed moving rocks in their mouth for nest construction (Breder & Rosen 1966).

The male/female pairs held in 16 L, 8 D performed courtship behaviors, specifically the 'carousel,' 'tail curl,' 'jostle,' and 'fan,' a total of 129 times, but those held in 12 L, 12 D did not perform such behaviors. Östlund and Ahnesjö (1998) reported that male courtship displays, such as fanning and body shakes, influenced female mate choice and hatching success in fifteen-spined sticklebacks (*Spinachia spinachia*). Thus, the increase of such courtship displays by individuals held in 16 L, 8 D could indicate the importance of photoperiod in stimulating such behavior. Two of six females held at 12 L, 12 D had not resorbed their eggs by 1 August in contrast to six of nine females held at 16 L, 8 D that had not. Likewise, swelling of cephalic epaxial muscles in males decreased in 12 L, 12 D before those exposed to 16 L, 8 D. These observations suggested that a photoperiod similar to that in mid-July, at water temperatures of 25 to 27°C, may be important to the breeding cycle of the Neosho madtom. Sundararaj and Sehgal (1970) found a long photoperiod to be important in stimulating the ovarian cycle of a seasonally breeding catfish, *Heteropneustes fossilis*, which occurs where the seasonal day length varies by only 4 h. Likewise, de Vlaming (1972) reported photoperiod to be a major environmental cue triggering reproductive cycles of salmonids and gasterosteids. The importance of photoperiod to stimulating the breeding cycle is most likely related to the benefits of timing the spawn to coincide with juvenile food availability, which may optimize survival of offspring (Jobling 1995).

Literature on the effects of photoperiod on madtoms is scarce and mostly anecdotal. My study provided the first ethogram and quantitative observations of the effects of photoperiod on Neosho madtom behavior including resting, swimming, feeding, aggression, cavity enhancement, and courtship. The proportion of time spent performing cavity enhancement behaviors was higher in fish held under the long photoperiod, and more courtship behaviors were observed in those individuals. Results of my study were consistent with the hypothesis that photoperiod plays a role in the breeding cycle of this fish.

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Chapter 3

Breeding Behavior and Reproductive Life History of the Neosho Madtom, *Noturus placidus* (Teleostei: Ictaluridae)

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ABSTRACT--The Neosho madtom, *Noturus placidus*, is a small catfish listed as threatened by the U. S. Fish and Wildlife Service. Little is known of its breeding biology and behavior because high turbidity and flow during its spawning season prevent direct observation in the field, and captive propagation has met with limited success. I held Neosho madtoms in laboratory aquaria to study courtship behavior, egg and clutch size, embryological and larval development, and sexual dimorphism during breeding season, and to attempt to induce spawning. Two clutches were discovered, both in nest cavities that had been excavated under a large structure; each consisted of approximately 30 eggs. Male parental care was observed, but one clutch was eventually eaten by the male. Courtship behaviors were recorded on video tape, including "carousel" and "tail curl" displays in which the fish spun in circles head to tail then quivered, with the male's tail wrapped around the female's head. The other spawn occurred after two days of injection with synthetic hormone, Ovaprim®. Mean chorion diameter of eggs was 3.7 mm; eggs hatched after nine days and yolk-sacs were fully depleted nine days later. Dissection of one breeding female revealed 17 white, previtellogenic eggs in the lumen of one ovary and 13 in the other, with a mean chorion diameter of 0.8 mm; the other female had one empty ovary and one containing 23 previtellogenic eggs with a mean chorion diameter of 0.9 mm. Swollen lips of males, distended abdomen of females, and differences in head shape, premaxillary tooth patch coloring, and genital papillae of breeding males and females were documented.

INTRODUCTION--Madtoms (genus *Noturus*) are a group of small North American catfishes (family Ictaluridae). Information about madtom life histories was scarce until concern for these declining catfishes prompted numerous ecological studies in the 1980s (see review in Dinkins and Shute, 1996). No direct observations of spawning behavior have been made in the wild, thus descriptions of breeding behavior within the group are limited. However clutch size, mean chorion diameter, embryonic development, time to hatching, and larval development and growth have been investigated by transferring clutches from the field to the lab for study, or via captive propagation (Clark, 1978; Bowen, 1980; Fitzpatrick, 1981; Mayden et al., 1980; Burr and Dimmick, 1981; Mayden and Burr, 1981; Burr and Mayden, 1982, 1984; Mayden and Walsh, 1984; Starnes and Starnes, 1985; Vives, 1987; Baker and Heins, 1994; Pfingsten and Edds, 1994; Chan, 1995; Dinkins and Shute, 1996). Although captive propagation has had limited success (Shute et al., 1993), breeding behavior has been described for the brindled madtom, *N. miurus* (Bowen, 1980; Fitzpatrick, 1981), freckled madtom, *N. nocturnus* (Fitzpatrick, 1981), slender madtom, *N. exilis* (Fitzpatrick, 1981), and the brown madtom, *N. phaeus* (Chan, 1995), during laboratory spawns.

Little is known about breeding biology of the Neosho madtom, *N. placidus*. The species is found only in the Neosho, Cottonwood, and Spring rivers of Kansas, Oklahoma, and Missouri, and is listed by the U. S. Fish and Wildlife Service (USFWS, 1991) as threatened (55 FR 21148). Understanding breeding biology and behavior of the Neosho madtom is critical to its recovery; however high turbidity and flow during its spawning season prevent field observations. Moss (1981) examined museum specimens and characterized the species' spawning season as beginning in March with egg development and continuing through July when young-of-year first appear in samples. Sexual dimorphism is present during spawning season; characteristics include reddening of the premaxillary tooth patch and swelling of the genital papilla of males and females, swelling of lips and cephalic epaxial muscles of males, and distention of the abdomen of females

(Moss, 1981; Pfingsten and Edds, 1994; Edds and Wilkinson, 1996; Wilkinson and Edds, 1997). However, determining Neosho madtom sex by using external characteristics is difficult even when secondary sex characteristics are well developed, as in many madtom species (Burr and Mayden, 1984; Simonson and Neves, 1992; Chapter 2). There is need to be able to more accurately determine the sex of fish to assess sex ratios or differences in habitat use between the sexes during spawning season and to pair individuals for captive propagation.

Previous attempts at Neosho madtom captive spawning have led to discovery of two clutches. Pfingsten and Edds (1994) found one clutch of 63 eggs deposited under a cinder block in a flowing aquarium; however, these eggs did not develop and may not have been fertilized. Wilkinson and Edds (1997) described a clutch of approximately 60 eggs laid in a static aquarium; male parental care was observed and embryonic and larval development and growth rates were noted for the first time in this species. Although important life history information was recorded in these studies, the success rate of captive propagation has been low and spawning behaviors have not been observed. I held Neosho madtoms in aquaria at the Columbia Environmental Research Center (CERC), Columbia, Missouri, and Emporia State University (ESU), Emporia, Kansas, to observe spawning behavior and parental care, to document clutch size, egg size, and embryonic and larval development and growth, to clarify characteristics of sexual dimorphism during spawning season, and to investigate use of synthetic hormone to induce spawning.

METHODS AND MATERIALS

CERC--I collected Neosho madtoms ranging from 51 to 67 mm total length (TL) from the Cottonwood River, Lyon and Chase counties, Kansas, on 23 June and 7 July 1998 and transported them to the CERC. Individuals were sexed according to development of secondary sex characteristics and seven male/female pairs were placed in separate 29.5-L aquaria, which were housed in an isolation chamber. The chamber prevented entry of external overhead light during the dark cycle of the photoperiod and dampened sound disturbances (Chapter 2). Each aquarium had an airstone, a standing drain-pipe to maintain approximately 20 cm water depth, and inflow from a well, which created a turnover rate of ca. 8.6 L h^{-1}. Gravel (2 to 24 mm diameter) was placed on the bottom of each aquarium (30 to 37 mm depth) and a 12.5-cm PVC pipe (10 cm diameter, cut in half lengthwise) was provided for shelter. I held water temperatures between 24 and 28°C using aquarium heaters and photoperiod at 16 L, 8 D using timed fluorescent lights mounted in the chamber. I fed fish frozen brine shrimp (*Artemis* sp.) six days a week and live blackworms (*Lumbeiculus* sp.) once a week. Infrared lights and time-lapse video equipment were used to monitor fish behavior 24-hours a day (Chapter 2).

ESU--I collected Neosho madtoms ranging from 52 to 73 mm TL from the Neosho and Cottonwood rivers, Lyon and Chase counties, Kansas, from 26 April to 7 July 1998 and transported them to the lab at ESU. Thirty Neosho madtoms were sexed, paired, and placed in static aquaria ranging from 38-L to 192-L. The bottom of each aquarium was covered (30 to 100 mm deep) with gravel (2 to 64 mm). Each aquarium had an airstone, a flow-through charcoal filter, and structure in the form of large flat cobble (10 to 25 cm), mussel shells, half PVC pipes, and/or aluminum cans. Fish were held in water temperatures ranging from 24 to 31°C regulated with aquarium heaters in a 16 L, 8 D photoperiod controlled by overhead lights on a timer. I fed fish frozen brine shrimp and bloodworms (Chironomidae) every 1 to 3 d and added aquatic insect larvae and river water weekly. Red lights (25 W) were illuminated prior to the dark cycle to allow for nighttime behavioral observations; Boujard et al. (1992) demonstrated that other catfishes displayed normal nocturnal activity under red light exposure, provided it was the lowest intensity light throughout the photoperiod.

On July 12 (after no breeding had occurred) all individuals were given an intraperitoneal 1.25μl preliminary injection of Ovaprim®, a synthetic hormone used to induce breeding in many fishes (Syndel Laboratories, Vancouver, B.C., ca. 0.5 ml kg^{-1}) (J. Stoeckel, Arkansas Tech Univ., pers. comm.); 4 h later each was given a full dose of 2.5 μl. I administered injections of 2.5 μl daily until 20 July, when dosage was increased to 3 μl; dosage was increased on 22 and 23 July to 5 μl each and on 24 July to 10 μl.

RESULTS

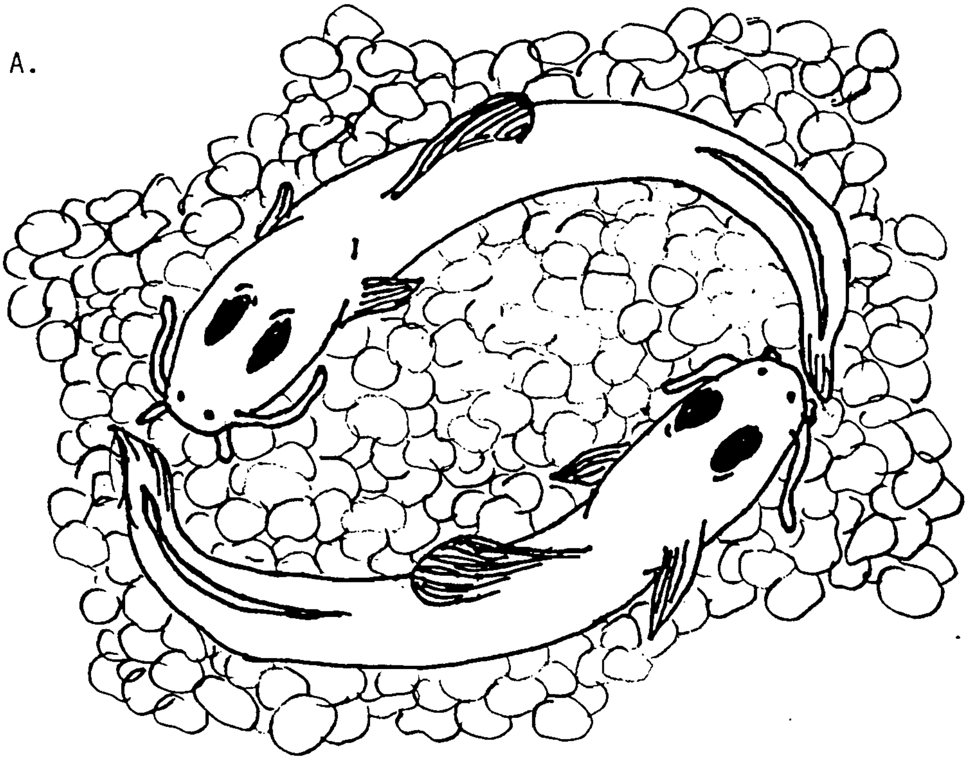
CERC--At CERC, from late June to mid July, individuals were observed performing the "carousel," or swimming in circles head to tail over the gravel substrate under the PVC structure (Fig. 1A). This behavior was typically followed by the "tail curl," in which individuals lay with the male's tail wrapped around the female's head (Fig. 1B). In this position the two quivered slightly for approximately 30 sec to 2 min, then separated. After separation, the process was often repeated, beginning with the "carousel," though occasionally the female was chased from under the structure. Cavity enhancement behaviors (Chapter 2) were also observed; individuals nudged rocks with their head, removed rocks in their mouth, and spun in circles alone over the gravel substrate under the PVC structure.

On 18 July, a clutch of approximately 30 eggs was discovered in a tank containing a male (61 mm TL) and female (59 mm TL) that had been captured on 23 June; water temperature was 28°C (Table 1). Eggs were spherical with a yellow yolk in the center surrounded by a clear chorion, and adhered to one another in a cluster. Both the male and female were present with the egg mass under the PVC structure at the time of discovery, but the female was removed from the tank when the male was observed biting and chasing her away after she ate approximately eight eggs. Following removal of the female, the male rested near the eggs, hovered over them, and fanned them with his tail. The eggs

Figure 1.

A. The "carousel," a courtship behavior of the Neosho madtom. Male and female swim in circles head to tail near the substrate. B. The "tail curl," a courtship behavior of the Neosho madtom. Male and female lay above substrate with tail of male wrapped around head of female while the two quiver.

A.



B.

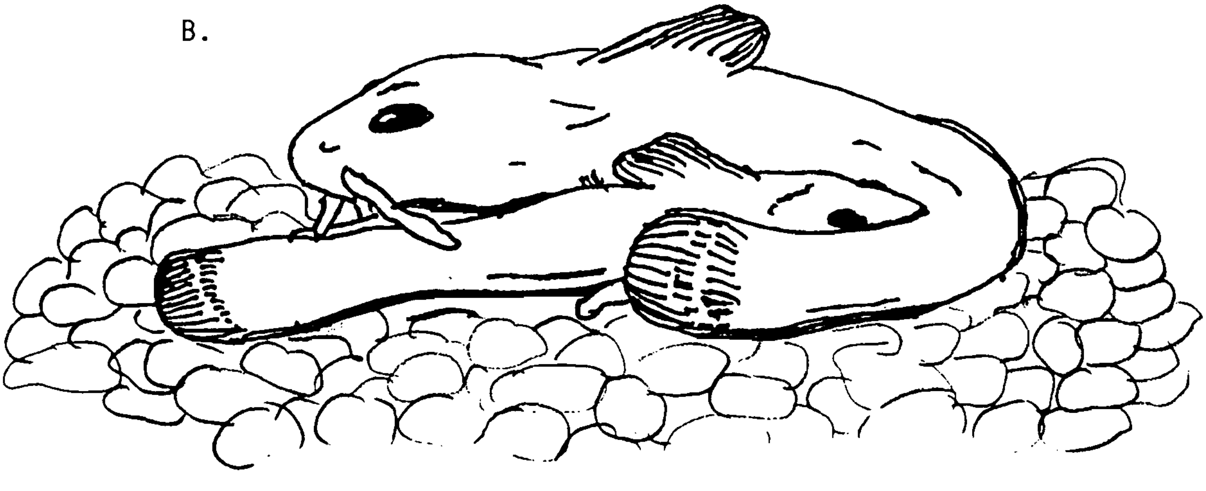


Table 1. Characteristics of two *N. placidus* clutches from this study compared with data from Pfingsten and Edds (1994) (P&E) and Wilkinson and Edds (1997) (W&E).

Clutch	Clutch size	$\bar{x}$ chorion diameter (SD, N)	Time to hatching, $\bar{x}$ TL (SD, N)	Time to yolk absorption, $\bar{x}$ TL (SD, N)	Water temp. ^{o} C
CERC	≅ 30	--	--	--	28.0
ESU	32	3.7 mm (0.10, 7)	9 d 8.8 mm (0.20, 2)	9 d 13.0 mm (0.00, 2)	25.0
P&E	63	3.1 mm (0.20, 10)	--	--	26.5
W&E	≅ 60	3.1 mm (0.15, 3)	≅ 8 - 9 d 6.8 mm (0.27, 4)	7 d 13.3 mm (0.94, 3)	25.0

disappeared between the second and third day, at which time the male had a distended abdomen; presumably, he ate the eggs.

Gravel pushed against the front of the tank by the fish, and positioning of the fish in a depression (nest) under the structure prevented detailed observation of spawning. Unlike other *N. placidus* observed in captivity (Chapter 2), the pair spent the majority of the day prior to the spawning event in the nest together and were active, performing the "carousel" followed by the "tail curl," which lasted from 1 to 7 min, several times throughout the day. When resting, the two usually lay side by side, often touching. After dark (2122 h), one individual, presumably the male, remained in the nest and the other, presumably the female, was in and out of the nest several times. As night progressed, that individual left the nest less frequently and circles made during the "carousel" became smaller and faster. Beginning at approximately 0300 hours, while both fish were under the structure performing the "carousel" and resting, the tail of one was seen flipping above the substrate from the nest. This was observed until the female was chased from the nest shortly after the lights came on in the chamber at 0524 h. Several times prior to discovery of the clutch the female returned to the nest and was chased away by the male. Dissection of the female revealed two 9.0 mm long, slightly pink ovaries, which, after preservation in 40 % ethanol, contained 17 and 13 white, previtelline eggs with a mean chorion diameter of 0.8 mm (SD = 0.13, N = 12) (Table 2).

ESU--At ESU, on 14 July, after one preliminary and two full dosage injections of Ovaprim®, a clutch of eggs was discovered in a 38-L aquarium; water temperature was 27°C. The clutch consisted of two clusters of eggs, one of 18 and one of 14. Mean chorion diameter was 3.7 mm (SD = 0.10, N = 7) (Table 1); perivitelline space was approximately 0.4 mm. Eggs were spherical, with a yellow yolk in the center surrounded by a clear chorion and, except for division of the two clusters, adhered to one another. Both the male (73 mm TL) and female (67 mm TL) had been captured on 7 July. They

Table 2. Characteristics of ovaries (R/L) and eggs (R/L) from reproductive female *N. placidus*. CERC and ESU individuals had bred, eggs in ovaries were previtelline. Specimen from ESU teaching collection was a gravid female, and eggs contained yolk.

Specimen	Total Length (mm)	Ovary length (mm)	Number of eggs	$\bar{x}$ chorion diameter (SD, N)
CERC	59	9.0, 9.0	17, 13	0.8 (0.13, 12)
ESU	67	12.0, --	21, 23	0.9 (0.11, 10)
Teaching collection	68	16.0, 16.0	39, 40	2.5 (0.19, 10)

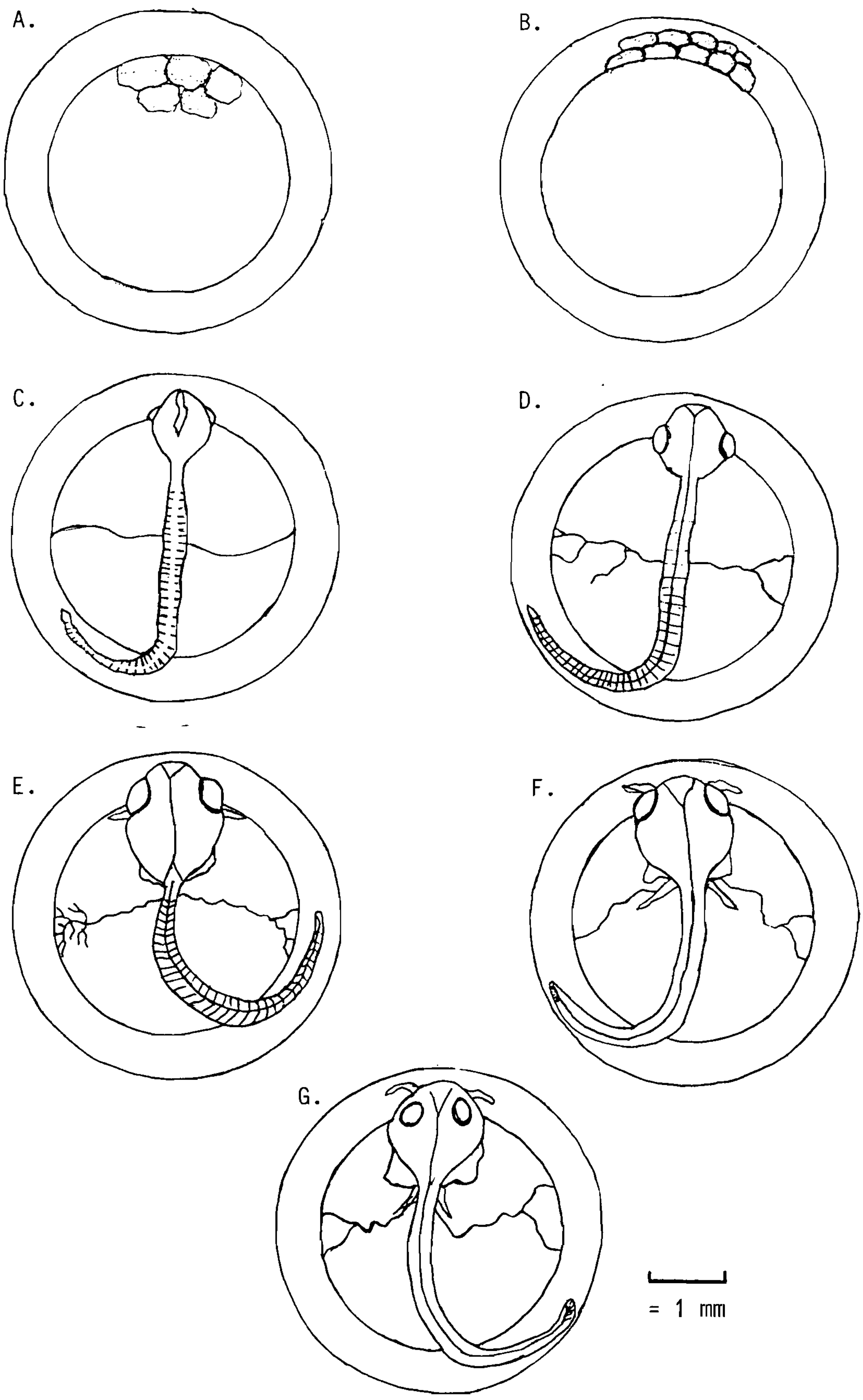
were resting near the eggs in a slight cavity or depression (nest) in the gravel under the only cover in the tank, a flat rock (8 x 14 cm). After discovery of the eggs, the female was removed and examined. Her urogenital pore was brown with vestigal blood, rather than white as before, and her abdomen was not as distended as it had been. Dissection of her left ovary revealed 23 white, previtelline eggs with a mean chorion diameter of 0.9 mm (SD = 0.11, N = 10) (Table 2). Dissection of the other ovary, after preservation in 40% ethanol, revealed 21 white, previtelline eggs.

The 14-egg cluster was removed and placed in a hatching apparatus; the 18-egg cluster was left in the cavity with the male. By Day 3, however, it was apparent he was not caring for them; eight eggs had clouded, indicating they were no longer developing. By Day 3, nine eggs in the hatching apparatus had broken or clouded, so all remaining eggs were removed and placed in a watch glass with an air stone; temperature varied from 21 to 27°C. By Day 4 only two eggs contained a developing embryo. Beginning on Day 5, these eggs were treated for fungus daily by immersing them in 0.5 % formalin for 2 to 7 min (Wilkinson and Edds, 1997).

Based on development of *N. exilis* (Mayden and Burr, 1981), and previous observations of *N. placidus* (Wilkinson and Edds, 1997), the eggs were estimated to be less than 10 h old at the time of discovery. Cleavage was in progress, the animal pole consisted of approximately 16 cells (blastomeres) in a small cluster (Fig. 2A), and the chorion was clear with a rough surface. By approximately 24 h post-fertilization, blastulation had begun (Fig. 2B); approximately 36 blastomeres at the animal pole had extended into the perivitelline space. At approximately 92 h post-fertilization organogenesis had begun. A head, with pronounced eye cups, was present and partially separated from the yolk; the tail was also separated from the yolk and whipped back and forth in the perivitelline space. Somites were differentiated from just posterior of the head to the tip of the tail, and vitelline veins extended on the yolk from either side of the fish (Fig. 2C). Approximately 120 h post-fertilization the head was larger, eye lenses were

Figure 2.

Development of Neosho madtom eggs laid 14 July 1998. A and B are lateral views, C through G are dorsal views. A = Day 1 (≅ 10 h), B = Day 2 (≅ 24 h), C = Day 4 (≅ 92 h), D = Day 5 (≅ 120 h), E = Day 6 (≅ 140 h), F = Day 7 (≅ 165 h), and G = Day 8 (≅ 190 h).


A.
B.
C.
D.
E.
F.
G.
= 1 mm

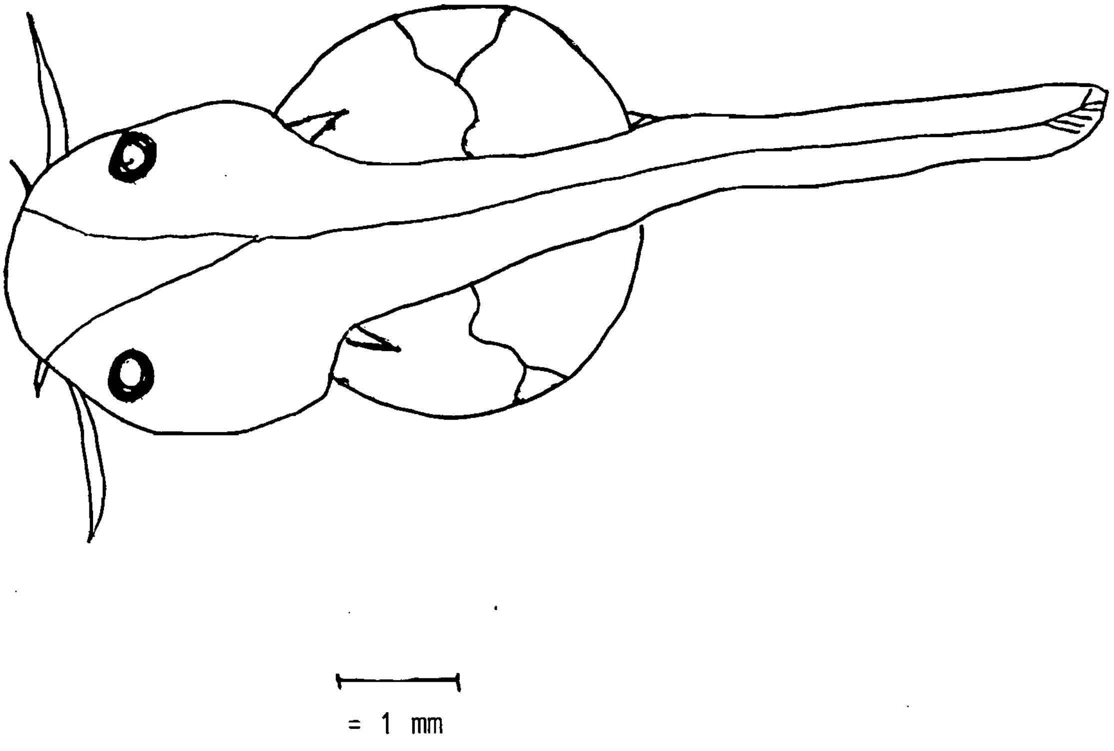
developed, and the spinal cord was visible from the head to the tip of the tail (Fig. 2D). Twenty hours later opercles were beginning to form at the base of the head, as were barbels near the mouth (Fig. 2E). At approximately 165 h, somite differentiation throughout the length of the body was no longer as distinct, opercles were better defined, pectoral spines were developing at the base of the head, and fin-forming caudal ray primordia were present at the tip of the tail (Fig. 2F). Approximately 190 h post-fertilization (estimated 10 h prior to hatching) barbels were well developed, eyes were more dorsally situated on the head, and pectoral fins were better defined (Fig. 2G), however eggs were covered with fungus by this time, which made observation difficult. On the morning of 22 July (approximately 200 hours post-fertilization, Day 9), in water 25.5°C, both eggs hatched. One individual was 9.0 mm TL and the other was 8.6 mm TL. Both were light yellow with black eyes and had a large yolk sac (Fig. 3). Following hatching, larvae sought cover in gravel and stones added to the dish. Yolk sacs were depleted by 30 July, when individuals were 13.0 mm TL. Stellate melanophores were spread over the body and pelvic fins were completely developed by the time of yolk sac depletion, 9 d after hatching.

Three times, on 14 and 15 July, in three seperate pairs, I observed that the female of a pair was spent, her belly no longer distended, as it had been the day before, her pore was red or brown rather than white as before, and that the male had a large, full belly. Presumably, the males had eaten eggs laid by the females.

Sex determination--Neosho madtoms at CERC and ESU developed secondary sex characteristics. Distinct differences were observed in the heads of breeding individuals; males developed swollen cephalic epaxial muscles on broad, flat heads with swollen lips, whereas the heads of females remained conical in shape and lips did not swell, similar to those of non-breeding individuals. Red or pink premaxillary tooth patches were present in both sexes, but were often brighter red in males. Gravid females had distended abdomens.

Figure 3.

Larval Neosho madtom on day of hatching, 22 July 1998; TL = 9.0 mm. Two pairs of barbels are present, pectoral spines are beginning to develop, and vitelline veins are visible on the yolk sac.


= 1 mm

Males were observed to quickly change color from medium brown to light tan when disturbed by observers. The genital papilla in males became elongated and swollen, and tissues adjacent to the genital papilla in females swelled and the anus became swollen and rounded (Fig. 4). Neosho madtom genital papillae (Fig. 4) were drawn from representative preserved individuals in the ESU teaching collection; differences depicted were often difficult to determine on live specimens. Dissection of five preserved specimens (56, 58, 59, 65, and 68 mm TL), which appeared gravid, revealed enlarged ovaries (x̄ length = 14.9 mm, SD = 1.20, N = 10), containing amber-colored eggs. The dissected left ovary (16 mm in length) of the 68 mm TL individual contained 39 yellow eggs and the right ovary (16.0 mm in length) contained 40 yellow eggs with a mean chorion diameter of 2.5 mm (SD = 0.19, N = 10) (Table 2).

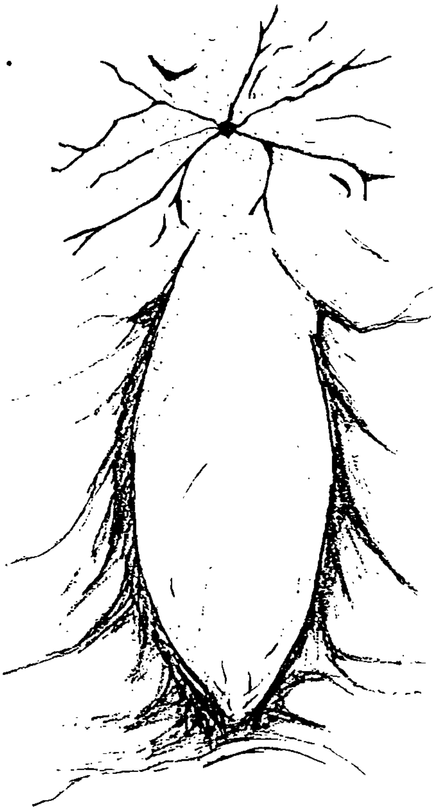
DISCUSSION--There is a paucity of information about courtship behaviors among *Noturus* species. Behaviors may, however, be similar due to evolutionary constraints within the lineage (Mayden and Walsh, 1984). The "carousel" and "tail curl" observed in my study were identical to behaviors described for the brindled madtom (Bowen, 1980; Fitzpatrick, 1981) and the brown madtom (Chan, 1995). The "tail curl" observed in my study differed slightly from that reported by Fitzpatrick (1981) for the slender and freckled madtoms, in which both fish wrapped their tail around the others head. Other ictalurids, such as the channel catfish, *Ictalurus punctatus*, flathead catfish, *Pylodictis olivaris*, and brown bullhead, *Ameiurus nebulosus*, have breeding behaviors similar to those observed in *N. placidus* (Breder and Rosen, 1966). Breeding behavior described by Breder (1935) for the brown bullhead was nearly identical to that observed in *N. placidus*; in each the "carousel" and "tail curl" behaviors were repeated several times prior to spawning.

Figure 4.

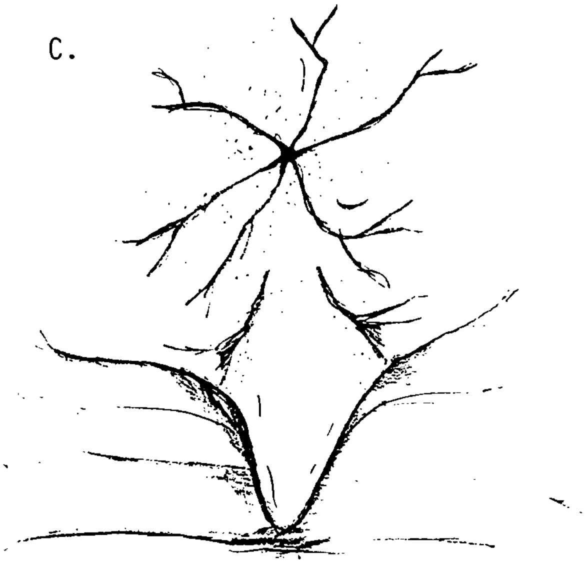
Genital papillae of Neosho madtoms: A) breeding female; B) breeding male; C) non-breeding individual.

A.

B.



C.



In both clutches laid during my study, eggs adhered together in a group but not to the substrate, similar to clutches described by Pfingsten and Edds (1994) and Wilkinson and Edds (1997) for *N. placidus*, and by Mayden and Burr (1981) for *N. exilis*, the slender madtom. Clutch size (30 to 32 eggs) differed only slightly between the two spawns in this study, but were much smaller than the 63 and ca. 60 reported by Pfingsten and Edds (1994) and Wilkinson and Edds (1997), respectively. Other *Noturus* species have been reported to have clutch sizes ranging from 14 (*N. leptacanthus*, the speckled madtom; Clark, 1978) to 124 (*N. exilis*; Burr and Mayden, 1984). Mean chorion diameter of eggs (3.7 mm) was slightly larger than that of 3.1 mm reported by Pfingsten and Edds (1994) and Wilkinson and Edds (1997). Because clutch size and egg size are inversely related (Jobling, 1995), larger egg size in my study could be associated with smaller clutches. However, it is possible that the male or female ate some eggs before they were discovered. Another possibility is that the clutches were not the first to be laid by these females that year, as these fish were collected much later in the breeding season (23 June and 7 July) than those collected by Pfingsten and Edds (1994) and Wilkinson and Edds (1997), who collected fish in May.

Several studies have suggested polyandry in *Noturus* species (*N. exilis*, Mayden and Burr, 1981; *N. nocturnus*, the freckled madtom, Burr and Mayden, 1982; *N. hildebrandi*, the least madtom, Mayden and Walsh, 1984; *N. phaeus*, Chan, 1995; *N. baileyi*, the smoky madtom, and *N. flavipinnis*, the yellowfin madtom, Dinkins and Shute, 1996), but this mating strategy has not been confirmed. In my study, eggs remaining in the ovaries of both females were much smaller than fertilized eggs or those observed in gravid museum specimens, and had not yet undergone vitellogenesis. The presence of less developed eggs in the ovaries of females is consistent with the hypothesis that multiple clutches may be laid in one season (Mayden and Burr, 1981). This points to the possibility of either sequential monogamy or polygamy, which could include polyandry. Alternatively, these eggs could be laid the following year (Baker and Heins, 1994); however field data suggest most Neosho madtoms do not live to Age 2 (Edds and Wilkinson, 1996; Wilkinson and Edds, 1997; Chapter 1).

Wilkinson and Edds (1997) observed a breeding male Neosho madtom caring for a clutch of eggs, including rubbing them with his head and belly and fanning them with his tail; these behaviors were seen until hatching, at which time the larvae remained in the nest with the male hovering over them until yolk sacs were depleted. Rubbing, fanning, and hovering behaviors have also been noted in male brown madtoms (Chan, 1995), slender madtoms (Mayden and Burr, 1981), channel catfish, flathead catfish, and brown bullheads (Breder and Rosen, 1966). The reason for lack of male parental care at ESU and consumption of eggs by the male at CERC is unknown; however, males of other madtom species have also eaten egg masses in captivity (*N. leptacanthus*, Clark, 1978; *N. miurus*, Bowen, 1980; *N. insignis*, the margined madtom, J. Stoeckel, pers. comm.), as four are believed to have done in my study. Perhaps males ate the eggs due to stress caused by captivity or by the hormone injection process. Possibly the male at the CERC ate the clutch of eggs because they were not fertilized, although they appeared to have been. Possibly the fish were simply hungry; however food was readily available in all aquaria. Regardless of the reasons, the behaviors seen in my study suggest that in efforts at captive propagation at least a portion of the egg mass should be removed from the nesting cavity and hatched separately. Unfortunately, the success rate using this method was low, most likely due to excessive agitation in the hatching apparatus, temperature fluctuation, and fungus, which may be controlled in the wild by parental care (Breder, 1935; Fontaine, 1944; Wilkinson and Edds, 1997). Others have also reported low success rates in rearing madtom eggs (*N. baileyi*, Shute et al., 1993; *N. phaeus*, Chan, 1995; J. Stoeckel, pers. comm.).

Wilkinson and Edds (1997) removed the breeding female *N. placidus* after the male was observed chasing her from the nest. In my study, the female at the CERC was removed from the tank after she was observed eating eggs and being chased from the nest by the male. Removal of the female after spawning is recommended for survival of the clutch in other ictalurids (Breder and Rosen, 1966), however it is not known whether the female plays a role in survival of the eggs early in their development. Mayden and Burr (1981) noted that female slender madtoms remain in the nests from between 12 to 22 hours after spawning, and Wilkinson and Edds (1997) did not remove the female from the spawning aquarium until approximately 72 h post-spawning and found higher success hatching eggs, compared to my study.

Egg development, hatching, and yolk resorption proceeded much as described previously by Wilkinson and Edds (1997) for the Neosho madtom and for other madtoms (*N. exilis*, Mayden and Burr, 1981; *N. hildebrandi*, Mayden and Walsh, 1984; *N. eleutherus*, the mountain madtom, Starnes and Starnes, 1985; *N. baileyi* and *N. flavipinnis*, Dinkins and Shute, 1996). One striking difference in my study was the earlier appearance of the vitelline veins on developing embryos. Wilkinson and Edds (1997) reported development of vitelline veins on day six (ca. 144 h) post-fertilization, but in my study vitelline veins were visible approximately 92 h post-fertilization. Mayden and Burr (1981) reported the appearance of vitelline veins 102 to 104 h after fertilization in *N. exilis*, and Mayden and Walsh (1984) observed their formation by 130 h post-fertilization in *N. hildebrandi*.

During breeding season, secondary sex characteristics were similar to those previously described for the Neosho madtom (Moss, 1981; Pfingsten and Edds, 1994; Edds and Wilkinson, 1996; Wilkinson and Edds, 1997). Differences between sexes were seen in head shape, lip size, redness of tooth patches, abdomen distention, and genital papillae. Other authors have described similar secondary sex characteristics in madtom species (*N. exilis*, Mayden and Burr, 1981; *N. nocturnus*, Burr and Mayden, 1982; *N. flavater*, the checkered madtom, Burr and Mayden, 1984; *N. hildebrandi*, Mayden and Walsh, 1984). Burr and Mayden (1982) and Dinkins and Shute (1996) reported color change in males during the breeding season in *N. exilis* and *N. baileyi*, respectively. In my study, no obvious color changes occurred in *N. placidus* during the breeding season. The tendency for males to quickly lighten in color when disturbed could be unrelated to breeding condition but merits further study.

Accurate sexing of *N. placidus* is easier when secondary sex characteristics are well developed, usually in late May through mid July (Bulger et al., 1998). However, for investigators planning to induce madtom spawning with hormone, injection should occur before ovulation (J. Stoeckel, pers. comm.). Success rate using hormone injection was low (4 of 15), even when dosage was increased and injections were administered over a 12 d period. Sneed and Clemens (1959) indicated that one to seven injections of human chorionic gonadotrophin are required to stimulate spawning in channel catfish. At ESU one successful spawn and three suspected spawning events occurred after three to four days of injection with synthetic hormone. However, those injections were administered late (12 July), probably after the peak of spawning season.

My research has increased our knowledge of *N. placidus* reproductive biology and behavior, but many questions still remain. The role of the female in parental care is unknown, as is mating strategy; is it monogamous or polygamous? If it is polygamous, is it polyandrous or polygynous? Breeding habitat remains to be discovered, and environmental variables that trigger breeding are unknown, as is the number of clutches laid in a lifetime. Although observation of *N. placidus* spawning behavior in my study occurred in captivity, such courtship behavior is likely representative of behavior in natural conditions; Porterfield (1998) reported similar spawning behavior in lab and natural settings for eight species of darters (genus *Etheostoma*). Lab investigations of small secretive fishes found in turbid water can be important to understanding their breeding biology and behavior.

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