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POPULATION DYNAMICS, REPRODUCTIVE BIOLOGY AND BEHAVIOR OF THE NEOSHO MADTOM, *NOTURUS PLACIDUS*--A STATE AND FEDERALLY LISTED THREATENED CATFISH

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Abstract

The Neosho madtom, *Noturus placidus*, is a small catfish listed as threatened by the federal government and the state of Kansas, and listed as endangered by the states of Missouri and Oklahoma. The U.S. Fish and Wildlife Service Neosho madtom recovery plan regarded understanding the habitat use, reproductive biology, and breeding behavior of the fish as critical for its recovery. However, due to high flows and turbidity during the spawning season in the rivers in which it occurs, and the secretive, nocturnal habits of the fish, little reproductive information has been gathered in the field. Additionally, attempts at captive propagation have met with limited success.

To assess Neosho madtom adult and young-of-the-year (YOY) habitat use during the spawning and non-spawning seasons, we monitored populations in the Neosho and Cottonwood rivers, Kansas, from August 1996 to October 1997 and April to July 1998. To investigate the location of adults on the gravel bar during these seasons, each study site was divided into an upper, mid and lower section. Habitat variables, including current velocity, water depth, substrate compaction and composition, and mesohabitat, were measured at the locations where fish were captured. Overall density estimates from kick-seining in the Neosho River ranged from 0.00 - 9.78 individuals per 100 m^2, and averaged 4.5/100 m^2. In six of ten samples made during the breeding season adult Neosho madtoms were found mainly on the upstream portion of the gravel bar. YOY tended to utilize areas with slower flow, lower substrate compaction, and shallower depth. In the Cottonwood River overall density estimates ranged from 0.00 - 5.80/100 m^2, and averaged 1.89/100 m^2. During spawning season, adults in the Cottonwood River did not show movement from one area of the gravel bar to the other. YOY tended to utilize areas with slower flow. Analysis of variance showed no interaction between breeding season and location of capture on the gravel bar, indicating that season had no effect on placement of adults on upper, mid, and lower sections of the gravel bar.

Length-frequency distributions revealed two obvious age classes, suggesting that most individuals spawn as Age I individuals (in their second summer) and very few, if any, survive to spawn at Age II. YOY:adult ratios ranged from 0.42 to all YOY in the Neosho River and from 1.0 to all YOY in the Cottonwood River. YOY first appeared in samples in mid to late July, ranging in size from 19 - 42 mm in the Neosho River and 19 - 40 mm in the Cottonwood River. Throughout the study adults ranged 38 - 71 mm in the Neosho River and 39 - 78 mm in the Cottonwood River. Development of genital papillae and other external morphological characteristics was observed. Fifty females exhibiting secondary sexual characteristics ranged 47 - 70 mm total length (TL); 15 males, 56 - 70 mm TL, showed secondary sexual characteristic development.

To describe breeding behaviors and investigate the effects of photoperiod on Neosho madtom breeding and activity, an isolation chamber was constructed at the Environmental and Contaminants Research Center (CERC) in Columbia, Missouri, and adult Neosho madtoms were transported there for study. The chamber was constructed to allow 24-hour observation of 12 pairs of fish with time-lapse video recording at two

photoperiods (16-h light:8-h dark and 12-h light:12-h dark). Activity levels and behaviors were monitored. Both groups showed a highly significant difference in proportion of time spent active during dark hours versus light hours, as expected for a nocturnal fish. Individuals held under longer daylight hours were more active during the daylight cycle and spent a higher proportion of time performing cavity enhancement behaviors. In addition, cavities were deeper and gravel size was smaller in cavities constructed by the longer daylight group. The two male/female pairs held under the 16:8 photoperiod performed courtship behaviors, but neither of the two male/female pairs in the 12:12 photoperiod performed courtship.

Two successful spawning events occurred in the laboratory during this study. The first took place at Emporia State University, Emporia, Kansas, on 14 July 1998 following two injections of Ovaprim, a synthetic hormone used to induce spawning in many fishes. The clutch consisted of 32 eggs ($\bar{x}$ chorion diameter = 3.7 mm) but no parental care was shown by the male, and after removal from the breeding tank only two eggs survived. The eggs hatched in eight days, individuals were 8.6 mm and 9.0 mm TL at the time of hatching, and 13.0 mm and 14.0 mm TL eight days later when yolk sacs were depleted. The second successful spawning event occurred at the CERC on 18 July 1998. Approximately 30 eggs were deposited in a slight depression in one of the study tanks. Video recording revealed the male chasing the female from the nest after she attempted to eat some of the eggs. The female was removed and the male was observed guarding or resting over the eggs. However, between the second and third day of development the eggs disappeared, and it is presumed that the male consumed the remaining eggs. Courtship behaviors similar to those seen in other madtoms, such as the "carousel" (swimming head to tail in circles) and "tail curl" (male wraps tail around head of female and both fish quiver), were observed and recorded during this spawning event.

Prior to this study, little was known of the breeding behavior of the Neosho madtom, and the effects of photoperiod on breeding had not been investigated. Adult and YOY habitat use had not been compared, differences in the genital papillae of breeding adults had not been documented, and little was known of habitat use during the breeding season. Though many questions remain, this research has increased our understanding of Neosho madtom reproductive biology, and has aided the effort to understand the life history and ecology of this species.

Introduction

Little is known about reproduction of the Neosho madtom, *Noturus placidus*, a catfish listed as threatened by the federal government and the state of Kansas, and as endangered by the states of Missouri and Oklahoma. The Neosho madtom recovery plan (U. S. Fish and Wildlife Service, 1991) regarded understanding this species' reproductive biology and habitat use as critical for its recovery. Much remains to be learned about Neosho madtom reproduction, including spawning locations, breeding behavior, and environmental cues that trigger breeding.

Due to high river turbidity and flow, field observations have been limited during the presumed spawning season, May through August (Moss, 1981); thus little is known of the habitat use of breeding adults or young-of-the-year (YOY), and no Neosho madtom spawning or nests have been observed in the wild. Edds (1995) and Edds and Wilkinson (1996) hypothesized that during spawning season Neosho madtoms utilize the head or crest of gravel bars where there is an abundance of large cobble available for nesting cover. Cochran (1996) suggested that cavity enhancement, especially during the spawning season, is a behavioral trait of many madtom species, and reports have indicated 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; *N. baileyi* and *N. flavipinnis*, Dinkins and Shute, 1996; Cochran, 1996). In Neosho madtoms, evidence of this behavior was seen by Wilkinson and Edds (1997) when a clutch of eggs was laid in a depression the fish had made in gravel under a piece of plastic PVC pipe in the lab at Emporia State University (ESU).

Attempts at captive propagation of Neosho madtoms have had limited success. Of two clutches laid in captivity, one did not develop and was likely never fertilized (Pfingsten and Edds, 1994); the other resulted in 43 surviving Neosho madtoms

(Wilkinson and Edds, 1997). Understanding environmental cues important for stimulating spawning could increase success of captive propagation, studies of which were called for in the USFWS recovery plan (1991). Photoperiod is one factor important in stimulating sexual maturation and ovulation in many fishes (Wootton, 1990; Shute et al., 1993), and it may play a role in triggering captive spawning of the Neosho madtom.

In order to understand breeding biology of the Neosho madtom, researchers must examine population dynamics of the fish, including age and sex structure. Male:female ratios could influence reproductive strategies; however, external sexing is difficult in many fishes (Moyle and Cech, 1996), including madtoms, even when secondary sexual characteristics are well developed (Burr and Mayden, 1984; Simonson and Neves, 1992). Identifying differences in external morphology of male and female Neosho madtoms is important to the study of Neosho madtom reproduction.

Our research focused on breeding biology of the Neosho madtom. Objectives were to characterize YOY and adult habitat use and distribution during the breeding season, determine spawning locations in the Neosho and Cottonwood rivers in Lyon and Chase counties, Kansas, observe reproductive characteristics of fish during the spawning season, describe Neosho madtom courtship, spawning, and rearing behavior, and investigate the effects of photoperiod on breeding behavior and activity.

Materials and Methods

Field study

Study areas were located in the Neosho River, Lyon County, Kansas (SE 1/4, Sec. 8, T19S, R12E) and Cottonwood River, Chase County, Kansas (NW 1/4, Sec. 28, T19S, R8E) (Fig. 1, Appendix 1). These areas were also used and described by Edds (1995), Edds and Wilkinson (1996), and Wilkinson and Edds (1997). A Garmin Global Positioning Unit (GPS) indicated location of the Neosho River site as N 38° 24.4 min., W 96° 06.2 min., at an elevation of 313 m and the Cottonwood River site as N 38° 22.6

min., W 96° 31.8 min., at an elevation of 327 m. A U.S. Geological Survey (USGS) ecological gauge station near Americus, Kansas, reported the Neosho River's long-term median daily flow as 0.8 m^3/s (USGS, 1998), and a gauge station on the Cottonwood River near Plymouth, Kansas, reported the river's long-term median daily flow as 3.0 m^3/s (USGS, 1998).

Sampling was performed during daylight hours using a 4.6-m by 1.8-m seine with 4.7 mm mesh (15' x 6' x 3/16"). Weather and river conditions permitting, each site was sampled once a month from August 1996 to October 1997 and April to July 1998. When possible, each site was sampled twice in May - August 1997 and May - July 1998 to search for Neosho madtom nests and to assess habitat use during the breeding season. Each site consisted of one large gravel bar, and was divided into an upper, mid, and lower section to examine Neosho madtom positioning on the gravel bar during breeding versus non-breeding season (Fig. 2, Appendix 1).

In the Neosho River, quantitative samples were performed 23 times. In 1996 the river was sampled on 6 August, 4 September, 22 October, and 7 December. We were unable to sample in November due to high flows. In 1997 samples were performed on 22 January, 12 March, 25 April, 2 May, 16 May, 5 June, 25 June, 8 July, 24 July, 5 August, 22 August, 20 September, and 11 October. High water prevented sampling in February. In 1998 samples were taken on 14 April, 18 May, 2 June, 20 June, 2 July, and 21 July. The study site encompassed approximately 120 m by 20 m, ca. 2400 m^2 of water surface area which contained a variety of mesohabitats, including pools, backwaters, and ca. 2100 m^2 of riffles and runs. Standardized sampling involved performing approximately 30 kick hauls in riffles and runs (10 each in the lower, mid, and upper sections of the gravel bar) and nine sweep hauls in pools and backwaters (three hauls in each of three areas). Sampling times varied from 70 to 160 min., depending on weather, flow conditions, and the number of Neosho madtoms captured. Kick-seining began approximately 2-m upstream from the seine, yielding 9.2 m^2 hauls. Neosho madtom density of occurrence

(species-specific density or number of Neosho madtoms per 100 m^2 in seine hauls that contained Neosho madtoms) and overall density (number of Neosho madtoms/100 m^2 in the total area sampled in riffles and runs) were calculated for each visit (Wenke et al., 1992).

In the Cottonwood River, 24 quantitative samples were taken. In 1996 samples were performed on 2 August, 30 September, and 30 October. Only one sample was taken in August, and none in November and December due to high water levels. In 1997 samples were done on 19 January, 15 February, 22 March, 26 April, 4 May, 14 May, 13 June, 26 June, 8 July, 24 July, 5 August, 29 August, 9 September, and 28 September. In 1998 samples were performed on 13 March, 18 April, 19 May, 5 June, 17 June, 2 July, and 21 July. The study site, an artificial riffle constructed in 1992 (Fuselier and Edds, 1995), encompassed approximately 150 m by 30 m, ca. 4500 m^2 of water surface area, with a variety of mesohabitats, including pools, backwaters, and ca. 3000 m^2 of riffles and runs. Standardized sampling involved performing approximately 30 seine hauls (ten each in the low, mid, and upper sections of the gravel bar) and six sweep hauls in pools and backwaters (three hauls in each of two areas). Sampling times varied from 60 to 175 min., depending on weather, flow conditions, and the number of Neosho madtoms captured. Neosho madtom density of occurrence and overall density were calculated for each visit.

At both sites the following data were collected. Water temperature was measured each sampling day. All captured Neosho madtoms were measured and examined for development of secondary sexual 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 (Moss, 1981; Pfingsten and Edds, 1994), before being released. For each site of Neosho madtom capture, location on the gravel bar was recorded (upper, mid, or lower section), and the following habitat characteristics were measured. Mesohabitat was coded as 1 for backwater, 2 for pool, 3 for run, or 4 for riffle. Depth was measured

with a meter stick. Current velocity (cm/sec) was measured at the substrate (0% depth) and at approximately 50% depth with either a Teledyne Gurley pygmy current meter no. 625, or with a Global Flow Probe model FP 101 (Global Water, Fair Oaks, California) except on two dates (2 August 1996 and 13 March 1998) when equipment failed. Substratum was sampled with a shovel (Grost et al., 1991) and visual estimates of composition were made, based on a modified Wentworth scale (Cummins, 1962): mud < 0.1 mm; sand 0.1 - 2 mm; gravel 2 - 64 mm; small cobble 64 - 256 mm; large cobble 256 - 500 mm; and boulder > 500 mm. Substrate compaction was coded 1 for loose, 2 for moderate, and 3 for compacted.

Using length-frequency-distributions (Figs. 4, 5, Appendix 1), we characterized captured individuals as adult or YOY by grouping members of common length-classes into cohorts and following them through time (Busacker et al., 1990). YOY:adult ratios were calculated using individuals characterized by this method. Habitat use by YOY and adults was described by calculating mean values for each habitat variable, weighted by the number of individuals (YOY or adult) captured at each location on the study site. Variables from adult and YOY capture locations throughout the year were compared with a Wilcoxon-Mann-Whitney two-tailed test (Zar, 1996) with a sequential Bonferroni correction (Rice, 1989).

To assess changes in placement on the gravel bar during breeding versus non-breeding seasons, breeding season was determined by the presence or absence of secondary sexual characteristics (e.g., pink tooth pads, distended abdomens, swollen genital papillae, swollen epaxial muscles). For each river, the proportion of individuals captured in each section (upper, mid, and low) during breeding and non-breeding seasons was compared using two-factor ANOVA (Zar, 1996), with section on each site as the experimental unit and the two rivers as replications.

Lab study

Columbia Environmental Research Center (CERC)

Collection of individuals

To describe courtship, spawning, and rearing behavior of the Neosho madtom, we observed individuals in the laboratory during the summers of 1997 and 1998. Eight individuals were captured in the Cottonwood River on 13 August 1996 and transported to the CERC in Columbia, Missouri, where they were kept in 59-liter holding tanks at a 13.5-h light: 10.5-h dark photoperiod at a water temperature of 21°C. Twenty-one individuals were captured in the Cottonwood River on 17 and 18 May 1997, transported to the CERC on 19 May and kept in 59-liter holding tanks at a 13.5-h light: 10.5-h dark photoperiod at water temperatures of 18°C.

Neosho madtoms used in 1998 were collected from the Cottonwood River on two occasions. Eleven individuals were captured on 23 June, and 23 on 7 July. These individuals were transported to the CERC on 7 July, paired according to secondary sexual characteristics, and kept at 16-h light: 8-h dark photoperiod in water temperatures of 25 - 28°C.

Lab set-up

Twelve 29.5-L aquaria were placed in an isolated room at the CERC. Well water (pH = 7.5) was pumped into each tank, maintained at approximately 20 cm depth, and drained at each end, creating a slight flow in each tank, with a turnover rate of approximately seven times per day. The bottom of each tank was covered approximately 4 cm deep with 2-24 mm diameter gravel. Structure was provided by cutting 12.5-cm PVC pipes (10 cm diameter) in half lengthwise, resulting in a U-shaped shelter. Use of the

PVC provided nesting structure while allowing observation from the front of the tank. An isolation chamber was built around the tanks to prevent entry of external light during the dark cycle of the photoperiod and to dampen sound disturbances. The chamber was divided down the middle with black plastic to facilitate two treatment groups (Fig. 3, Appendix 1). Photoperiod in one treatment group was held at a 16-h light:8-h dark cycle (16:8 photoperiod), and in the other group it was held at a 12-h light:12-h dark cycle (12:12 photoperiod). Water temperature was maintained at 25 - 27°C with an aquarium heater in each tank. Light was provided by fluorescent bulbs mounted in the ceiling, and regulated by a timer.

On 28 May 1997 two fish were placed in each tank (18 or 21°C, 13.5:10.5 photoperiod) within the isolation chamber. Attempts were made to determine the sex of each individual based on the development of secondary sexual characteristics, so that each tank would contain one male and one female. Individuals not placed in study tanks remained in holding tanks. Temperatures were raised 1°C every three days beginning 3 June for tanks at 18°C and 15 June for tanks at 21°C. On 24 June the temperature in all holding and study tanks was 25°C. To test the effects of photoperiod on activity, photoperiod was adjusted to treatment photoperiods (16:8 and 12:12) within the isolation chamber on 16 June. Madtoms were fed a diet of live amphipods (*Hyalella azteca*) and blackworms (*Lumbriculus* sp.) approximately every three days throughout the study.

In 1998, seven pairs of Neosho madtoms were monitored within the isolation chamber. They were held at 25°C in a 16-h light: 8-h dark photoperiod on both sides of the chamber to increase the chances of a successful spawn. Individuals were fed frozen brine shrimp (*Artemis* sp.) six days a week and live blackworms once a week.

Data collection

Three Panasonic closed circuit TV black & white cameras, model WB-BP310, were mounted on each side of the isolation chamber so that each camera recorded activity in two tanks; six pairs were monitored on each side of the chamber. American Dynamics 30-watt LED (light emitting diode) infrared illuminators, model AD 1020/30, were mounted above the tanks to illuminate and allow us to record nighttime behaviors. Neosho madtom behavior was recorded 24-hours a day using a Toshiba virtual real-time and time-lapse VCR, model KV-7168-A.

As a control, data were collected for three days (12 - 14 June 1997) while both groups had a water temperature of 21°C and a photoperiod of 13.5-h light:10.5-h dark. Experimental data were then collected during two eight-day periods while groups were held under treatment photoperiods (16:8 and 12:12). Each experimental period consisted of four consecutive days of data collection, two days of rest, and four more consecutive days of data collection. By analyzing two minutes of each hour for each tank during the control and two experimental periods, the data set consisted of approximately 456 two-minute observations for each tank; observations were not made when an observation period took place during feeding or tank maintenance. The two-minute interval for each hour was chosen randomly, beginning at 00 seconds. Each time the behavior of either individual changed during the two-minute interval, the time and new behavior were recorded. Attempts were made to record the behaviors of each individual separately, but due to the small size of the fish and the film quality this was not always possible. However, this collection method allowed us to determine the time spent performing each behavior within a tank. Behaviors recorded (Table 1, Appendix 2) were taken from an ethogram created from a combination of observations of Neosho madtoms by us, observations made during previous attempts at Neosho madtom captive breeding (Pfingsten and Edds, 1994; Wilkinson and Edds, 1997; C. Wilkinson, pers. comm.), and

from descriptions of spawning behavior of the brown madtom, *Noturus phaeus* (Chan, 1995).

On 23 June, five individuals were removed due to health problems or lack of development of secondary sexual characteristics, and replaced with individuals from the holding tanks. After a five day acclimation period for the new individuals, the first experimental period (experimental period 1) began (28 June - 1 July and 4 - 7 July). On 9 July, nine fish were removed and replaced with individuals from the holding tanks. After a five day acclimation period for the nine new individuals, the second experimental period (experimental period 2) began (14 - 17 July and 20 - 23 July).

To examine the effects of photoperiod on cavity enhancement, gravel was placed evenly in each tank at the beginning of the study and measured at the end of the study. Depth of the gravel substrate was measured under the structure and outside the structure. In addition, the diameter of three randomly chosen pieces of gravel was measured from under the structure, and three were measured from the rest of the tank. These measurements allowed us to compare the depth of and gravel size within the cavity in each tank, compared to the rest of the tank bottom.

On October 3, 1997 all fish used in the study were euthanized in MS-222, measured, and sexed internally. Internal sexing allowed us to verify the sex of each individual that had been used in the study. Pectoral spine clippings from study fish were stored in 75% EtOH + 10% EDTA for possible future genetic study.

Lab procedures were similar in 1998, except that there was no fish replacement. Fourteen individuals were paired in tanks on 7 July and observational data were recorded using the time lapse video recording apparatus described previously. Video monitoring at CERC during 1998 was to describe courtship, breeding, and parental behaviors in the case of a spawning event.

Data analysis

Each behavior was classified as either active or inactive (resting) (Table 1, Appendix 2). The proportion of time spent active in each control group was compared using a Wilcoxon-Mann-Whitney two-tailed test with the null hypothesis that there was no difference in the activity between the two groups (α=0.05 for all lab analyses). Similarly, the proportion of time spent active in each treatment group was compared, with the null hypothesis that the activity did not differ between photoperiods. The proportion of time spent active in light versus dark hours was also tested in each treatment group using a Wilcoxon-Mann-Whitney one-tailed test under the alternative hypothesis that fish activity was higher during the dark than during the light cycle of the photoperiod.

Each behavior from the ethogram was assigned one of six classifications or behavior types: courtship, cavity enhancement, aggression, swimming, resting, or feeding. Courtship behaviors included those seen in the pair that successfully bred in 1996 at ESU (Wilkinson and Edds, 1997; C. Wilkinson, pers. comm.), plus those described by Chan (1995) for the brown madtom. We compared the proportion of time spent performing each behavior type between treatment groups with a Wilcoxon-Mann-Whitney two-tailed test. Cavity enhancement was examined by performing a Wilcoxon-Mann-Whitney two-tailed test on gravel size within cavities and on depth of cavities, between treatment groups. A Pearson correlation coefficient (Zar, 1996) was calculated to examine strength of the relationship between cavity depth and gravel size within the cavity.

Emporia State University

Neosho madtoms were collected from the Neosho River on 26 April, 13 June, and 1 July, and from the Cottonwood River on 28 May and 7 July 1998, and returned to ESU. Individuals were paired according to secondary sexual characteristic development and

placed in static aquaria, keeping fish captured in the Neosho River separate from those captured in the Cottonwood River.

Seventeen pairs of Neosho madtoms were studied at ESU in 1998. Twelve pairs were placed in 38-L aquaria, one pair in a 72-L aquarium, one pair in a 102-L aquarium, one pair in a 192-L aquarium, and one pair in each side of a 205-L aquarium that had been divided down the middle by a plastic screen. The bottom of each aquarium was covered with gravel, and each tank had an airstone, a flow-through charcoal filter, and some type of structure, such as large flat rocks (10 - 25 cm length), mussel shells, half PVC pipes, hollow bones, and aluminum cans. Substrate size, depth, and type varied among the tanks. The 102-L and six of the 38-L tanks were covered; the others were not. To reduce visual disturbances eight of the 38-L aquaria were wrapped in blue plastic bags and/or black construction paper. All individuals were held at a 16-h light: 8-h dark photoperiod controlled by overhead lights set on a timer. Water temperatures were controlled in each tank with an aquarium heater, and ranged from 24 - 31°C. Neosho madtoms were not exposed to total darkness during the study because red lights (25 W) were turned on 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 that it was the lowest intensity light throughout the photoperiod.

On July 12 at 1430 (after no breeding had occurred) individuals were given a preliminary injection (1.25μl) of Ovaprim, a synthetic hormone used to induce breeding in many fishes (Syndel Laboratories, Vancouver, B.C., ca. 0.5ml/kg) (Dr. J. Stoeckel, Arkansas Tech University, pers. comm.). Four hours later they were given a full dosage of 2.5 μl each. Injections of 2.5 μl were given daily until 20 July, when the dosage was increased to 3 μl and only five pairs were injected (12 pairs were not injected). The following day, eight pairs were injected with 3 μl, only one of which had been injected the day before. On 22 and 23 July 15 pairs were injected with 5μl each, and on 24 July these were injected with 10 μl each; only 15 pairs were injected on those days because, based on

development of secondary sexual characteristics, it was determined that two of the 17 pairs were female/female pairs.

Individuals were fed frozen brine shrimp and frozen blood worms (Chironomidae) every one to three days. These foods were supplemented with aquatic insect larvae and river water from the Neosho and Cottonwood rivers weekly.

Results

Field study

Neosho River

Quantitative samples performed in the Neosho River yielded 270 Neosho madtoms; 111 YOY and 159 adults. Mean density of occurrence in the Neosho River was 19.8/100 m^2 (SE = 1.44) (Table 2, Appendix 2). Sample means ranged from zero on 22 January 1997 to 32.6/100 m^2 on 7 December 1996 (Table 2, Appendix 2). Mean overall density was 4.5/100 m^2 (SE = 0.58), ranging 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 (Table 2, Appendix 2) in riffle and run areas with loose to moderately compacted substrate consisting of mainly gravel (82.8%) (Table 3, Appendix 2). Mean water depth at capture locations was 38.4 cm, mean current speed at the substrate was 25.6 cm/sec, and mean current speed at 50% depth was 55.0 cm/sec (Table 3, Appendix 2). Habitat variables differed between locations where YOY and adults were captured (Table 3, Appendix 2). Current speed at 0% and current speed at 50% depth were lower at sites where YOY were found compared to adult capture sites (P=0.0006 and 0.0001, respectively), as were water depth and substrate compaction (P=0.0001 and 0.001, respectively) (Table 3, Appendix 2).

Length frequency distributions indicated at least two age classes (Fig. 4, Appendix 1), suggesting that most *N. placidus* bred as Age I individuals and few, if any, survived to

breed at Age II. In each year the first YOY were captured in July at total lengths ranging 19 - 41 mm. YOY captured in August ranged 27 - 45 mm, and YOY captured in September ranged 41 - 55 mm TL (Table 4, Appendix 2). After the first appearance of YOY, YOY:adult ratios ranged from 0.42 on 6 August 1996 to all YOY later in the year (Table 4, Appendix 2). Adult Neosho madtoms ranged 38 - 79 mm TL (Table 4, Appendix 2).

In the Neosho River, 51 individuals that showed development of secondary sexual characteristics were captured; 39 of those were female, nine were male, and three were not developed enough to determine their sex (Table 5, Appendix 2). Secondary sexual characteristics included slightly pink, pink, or red tooth patches in both males and females, slightly swollen or swollen epaxial muscles and elongated genital papilla in males, and rounded genital papilla, slightly gravid, gravid, or already spawned in females. Individuals showing development of secondary sexual characteristics were found from April to August (Table 5, Appendix 2). Females showing developed secondary sexual characteristics ranged 47 - 70 mm TL; developed males ranged 57 - 70 mm TL (Table 5).

Cottonwood River

In the Cottonwood River quantitative samples yielded 122 Neosho madtoms; 54 YOY and 68 adults. Mean density of occurrence in the Cottonwood River was 11.9/100 m^2 (SE = 1.23) (Table 6, Appendix 2). Sample means ranged from zero on three dates to 24.8/100 m^2 on 30 October 1996 (Table 6, Appendix 2). Mean overall density was 1.9/100 m^2 (SE = 0.34), ranging from zero on three dates to 5.8/100 m^2 on 30 October 1996.

Neosho madtoms were found in temperatures ranging from 2 to 29°C (Table 6, Appendix 2) in riffle and run areas with loose to moderately compacted substrate consisting of mostly gravel (78.0%) (Table 7, Appendix 2). Mean water depth was 55.7 cm, mean current speed at the substrate was 27.0 cm/sec and at mean current speed at

50% depth was 61.4 cm/sec (Table 7, Appendix 2). In the Cottonwood River mean current speed at 50% depth was lower where YOY were found (P=0.0001) (Table 7, Appendix 2).

Length frequency distributions were similar to those in the Neosho River (Fig. 5, Appendix 1), suggesting that most *N. placidus* bred as Age I individuals and very few, if any, survived to breed as Age II adults. YOY first appeared in samples in July at total lengths ranging 19 - 40 mm TL. In August YOY ranged 28 - 40 mm TL and in September YOY ranged 36 - 53 mm. After YOY first appeared in samples, YOY:adult ratios ranged from 1.0 in September 1996 and August 1997 to all YOY (Table 8, Appendix 2). Adult Neosho madtoms ranged 39 - 78 mm TL (Table 8, Appendix 2).

During spring and summer months, 17 individuals were captured in the Cottonwood River that showed development of secondary sexual characteristics; 11 of those were female and six were male (Table 9, Appendix 2). Individuals with developed characteristics first appeared in April (one male) and were present until August when it is presumed that the breeding season ended (Table 9, Appendix 2). Females showing development of secondary sexual characteristics ranged 60 - 69 mm TL; developed males ranged 56 - 70 mm TL (Table 9, Appendix 2).

Placement on gravel bar

In the Neosho River a higher percentage of adults was captured in the upper portion of the site, in six out of ten samples performed during the breeding season (April - August, when secondary sexual characteristics were present) (Table 10, Appendix 2). However, throughout the rest of the year adults were more often found in mid or lower portions of the site, with the exception of October 1996 and 1997 (Table 10, Appendix 2).

In the Cottonwood River, the highest percentage of individuals was captured in the mid portion of the gravel bar in June and July, and in all other months the highest percentage of individuals was captured in the upper portion of the bar (Table 11,

Appendix 2). It should be noted that only one individual was captured in September and in October (Table 11, Appendix 2). An equal percentage of individuals was captured in the upper and lower portions of the gravel bar in April.

Results from ANOVA performed on the mean number of individuals captured in each section of each site in breeding and non-breeding seasons showed no significant interaction between placement on the bar and season (F=0.62, df=2, P=0.57). Season and placement effects also were not significant (F=0.40, df=1, P=0.55 and F=2.11, df=2, P=0.20, respectively), indicating for the populations sampled the number of individuals captured was not affected by season and that there was no difference in the number of individuals captured in each section of the gravel bars.

Lab study

CERC

1997 study

Fish sexing

Throughout the collection periods at the CERC (control, experimental period 1, and experimental period 2), a total of 36 pairs of Neosho madtoms was observed within the isolation chamber. Subsequent internal sexing revealed that ten of those pairs were female/female, one was male/male, and twelve were male/female (Table 12, Appendix 2). A total of 15 fish could not be sexed due to mortality and removal of individuals that were not externally marked for identification from study tanks to holding tanks. This resulted in 13 pairs in which the sex of at least one individual was unknown (Table 12, Appendix 2). Two male/female pairs were in each group during each data collection period.

Neosho madtoms used in this study showed development of secondary sexual characteristics, including red tooth patches in males and females, swollen cephalic epaxial muscles in males, and distended abdomen in females; these were similar to characteristics previously described (Moss, 1981; Pfingsten and Edds, 1994; Edds and Wilkinson, 1996;

Wilkinson and Edds, 1997). Other secondary sexual characteristics included elongated genital papilla in males, swollen or rounded genital papilla in females (Fig. 6, Appendix 1), and a tendency for males to quickly change from medium brown to light tan when disturbed by researchers during capture for hormone injection or removal of cavity structure while searching for eggs. Neosho madtom genital papillae (Fig. 6, Appendix 1) were drawn from representative preserved individuals in the ESU teaching collection, differences depicted were often difficult to determine on live specimens.

Females held in the shorter photoperiod 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 the treatment group with longer daylight hours, four females had slightly distended abdomens and one had a fully distended abdomen on 1 August. A concomitant decrease in secondary sexual characteristics was seen in males. On 1 August only two of five males held in the 12:12 photoperiod had slightly swollen cephalic epaxial muscles, though both males held in the 16:8 photoperiod had fully swollen head musculature.

Breeding behavior

No spawning was observed during the 1997 lab study, however, based on observations of the brown madtom (Chan, 1995), speckled madtom, *N. leptacanthus* (Clark, 1978), brindled madtom, *N. miurus* (Bowen, 1980), and observations during previous Neosho madtom breeding studies (Pfingsten and Edds, 1994, Wilkinson and Edds, 1997), behaviors were seen that indicated courtship. From late June to mid July, individuals were seen "carouselling" or swimming in circles head to tail over the gravel substrate under the PVC structure (Fig. 7, Appendix 1). This behavior was usually followed by the "tail curl," in which individuals would rest head to tail with the tail of the male wrapped around the head of the female (Table 1, Appendix 2). In this position the two would quiver slightly for approximately 30 seconds to two minutes, then separate

After separation, the process was often repeated, beginning with the "carousel," or else the female was chased from under the structure. The "carousel" behavior had been seen previously in *N. placidus* (Wilkinson and Edds, 1997), and it and the "tail curl" are identical to behaviors described by Clark (1978), Bowen (1980), and Chan (1995) for other madtom species.

Cavity enhancement was also observed; depressions under structures were first apparent on 6 June. Cavity depth in this study ranged from 1 - 37 mm (Table 12, Appendix 2). Cavities deeper than 20 mm were made in four of 12 tanks (tanks 2, 3, 4, and 6), all in the 16:8 photoperiod (Table 12, Appendix 2). Mean gravel diameters within the cavities in the 16:8 photoperiod ranged from 12.0 mm (SD=3.61) to 16.7 mm (SD=2.89) ($\bar{x}$=14.5, SE=0.81); in the 12:12 photoperiod means ranged from 14.0 mm (SD=3.61) to 23.7 mm (SD=4.93) ($\bar{x}$=18.1, SE=1.14). Throughout the tank, mean rock diameter ranged from 17.7 mm (SD=5.51) to 20.7 mm (SD=4.04) in the 16:8 photoperiod ($\bar{x}$=19.1, SE=0.96), and from 17.0 mm (SD=3.00) to 22.3 mm (SD=3.06) in the 12:12 photoperiod ($\bar{x}$=19.9, SE=0.95). Comparison of cavity depth and gravel size within cavities between treatment groups showed that cavities were deeper (P=0.02) and gravel size within cavities was smaller (P=0.02) in cavities in the 16:8 photoperiod. Gravel size throughout the tank did not differ between the two treatment groups (P>0.80). Pearson's correlation coefficient comparing gravel size and cavity depth suggested a negative relationship (r=-0.55), but was not significant (P=0.07).

Both males and females were observed nudging gravel out of the cavity ("nudge") and spinning in circles in the depression ("spin"), activities that increased cavity depth. However, only males were observed removing gravel with their mouth ("rock move"). Although males were most often observed performing all three of these behaviors, tanks containing only females (Table 12, Appendix 2) also had depressions under structures, including two of the three deepest cavities (Table 12, Appendix 2).

Activity levels

Results from the control period (13.5-h light:10.5-h dark photoperiod in both groups) showed no significant difference between the two groups in the proportion of time spent active in the dark or light cycle of the photoperiod ($P>0.3$ and $P>0.5$, respectively). Analyses were performed on each experimental period separately because a two-tailed Wilcoxon-Mann-Whitney comparison of activity levels within each treatment group between experimental periods showed a significant difference in the proportion of time spent active by individuals in the 12:12 photoperiod; more activity was seen during the second experimental period than during the first in the dark cycle of the photoperiod ($P=0.02$). In addition, during experimental period 1, camera failure caused uneven sample sizes; tanks 9 and 10 (12:12 photoperiod) were not monitored.

Both treatment groups showed a highly significant difference in proportion of time spent active during dark hours versus light hours (16:8 experimental period 1, $P<0.0025$; experimental period 2, $P<0.0025$; 12:12-experimental period 1, $P=0.025$; experimental period 2, $P<0.0025$), as expected for a nocturnal fish. Comparison of the proportion of time spent active during the second experimental period showed individuals under the 16:8 photoperiod were more active during the light cycle than those under the 12:12 photoperiod ($P=0.05$); the difference was not significant during the first experimental period ($P=0.10$). During dark hours, there was no significant difference in activity levels (experimental period 1, $P>0.20$; experimental period 2, $P>0.20$).

Behavior types

The proportion of time spent performing different behavior types in light and dark hours was compared between the two treatment groups for each collection period. Cavity enhancement, aggression, swimming, resting, and feeding behaviors (Table 1, Appendix 2) were compared, but only those with significant differences ($\alpha \leq 0.05$) are reported. During experimental period 2, in the dark cycle of the photoperiod, individuals held under the

16:8 photoperiod spent a higher proportion of time performing cavity enhancement behaviors than those in the 12:12 photoperiod (P=0.05). Differences were also seen in the proportion of time spent performing resting behaviors. During experimental period 2, in the light cycle of the photoperiod, individuals held under the 12:12 photoperiod spent a higher proportion of time resting (P=0.05) than those held under the 16:8 photoperiod.

No statistical analyses were performed on courtship behaviors because of the small sample size (two male/female pairs in each treatment group). However, in each experimental period both male/female pairs held under the 16:8 photoperiod were observed performing courtship behaviors, such as the "carousel" and "tail curl" (Table 1, Appendix 2), whereas neither male/female pair held under the 12:12 photoperiod was observed performing such behaviors.

1998 study

On 18 July 1998, at 1115 hours, a clutch of approximately 30 eggs was discovered in one of the study tanks at the CERC. Water temperature in the tank at the time of discovery was 28°C. The eggs were spherical, yellow in the center surrounded by a clear chorion, and adhered to one another in one cluster, similar to clutches described by Pfingsten and Edds (1994) and Wilkinson and Edds (1997) for the Neosho madtom, and by Mayden and Burr (1981) for the slender madtom. Both the male and female were present with the egg mass under the structure, but the male was soon observed biting and chasing the female away after she attempted to eat some of the egg mass by picking off one or two eggs at a time. After observing the female eat approximately eight eggs we removed her from the tank. Following the removal of the female, the male was observed resting near, fanning, and hovering over the eggs under the structure. The eggs disappeared between the second and third day at which time the male was observed to have a distended abdomen; placement of the gravel in the tank hindered observation but it is presumed that the male ate the eggs.

Gravel pushed against the front of the tank by the fish, and the positioning of the fish in the depression under the structure, prevented detailed observation of events. We were able to see the male and female performing the "carousel" followed by the "tail curl" several times from the period shortly before the lights went off (2100) to after they came on (0500). As the night progressed, the circles made during the "carousel" behavior became smaller and the fish moved faster in a circle. The "tail curl" behavior lasted up to about seven minutes on some occasions. Beginning at approximately 0300 hours, while both fish were under the structure, we noted the tail of one flipping above the substrate from the depression under the structure. Shortly after the lights came on in the chamber, we observed the male and female "carouselling" and the tail of one fish flipping, until the female was chased from the nest. Another female was placed in the tank with the male the following day but no other clutch was subsequently observed.

ESU

On 14 July 1998, after one preliminary (1.25 µl) and two 2.5 µl injections of Ovaprim, a clutch of eggs was discovered in one of the 38-L aquaria; 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, range=3.5 - 3.8 mm, N=7), the eggs were amber in color and, except for the division of the two clusters, adhered to one another. Both the male (73 mm TL) and female (67 mm TL) were resting near the eggs in a slight depression under a large stone. After discovery of the eggs, the female was removed from the tank and frozen for possible future genetic study; dissection revealed one empty ovary and one containing approximately 23 eggs.

The 14 egg cluster was removed and placed into a hatching apparatus designed by the CERC fish culturist. The apparatus consisted of a plastic stand containing twelve 35 ml syringes with the plungers removed and a screen window on one side. The stand containing the syringes was placed in a 38-L aquarium filled with water, and an air hose

was attached to the bottom of each syringe so that air slowly bubbled in and kept the eggs suspended in water. The cluster of 18 eggs was left in the cavity depression with the male.

Development of the eggs (Fig. 8, Appendix 1) was very similar to that described by Mayden and Burr (1981) for the slender madtom and to that of the Neosho madtom described by Wilkinson and Edds (1997). At the time of discovery, the eggs were estimated to be less than 20 hours old.

On 16 July, eleven of the eggs in the hatching apparatus had broken or turned white, and the three remaining eggs were removed and placed in a glass dish with a small air stone. The cluster of 18 eggs that had been left with the male was found under the large stone, but to the side of the depression where the male rested, and fungus was apparent on the eggs. The eggs were carefully placed back in the depression with the male, and left for seven hours, by which time approximately 10 of the eggs had turned white, indicating that they were no longer developing. For this reason and because no care by the male had been observed, the remaining eight eggs were removed and placed with the cluster that had been put in the petri dish three hours earlier. The water temperature in the dish had dropped to 21°C, so it was placed in a water bath and raised to 27°C. Later that day, while observing the eggs with a dissecting microscope, it was determined that most of the eggs had stopped development. By Day 4 (17 July) only six eggs remained. Two of these eggs contained a developing embryo. The other four eggs turned white over the next two days and were removed. Beginning 18 July, the eggs were treated each day for fungus by immersing them in a solution of 1.9 ml formalin/3.79 L water for two to seven minutes.

By 0930 on July 22, Day 9 of development, in water 25.5°C, one egg had hatched and the other had its tail out of the chorion. The formalin treatment may have hardened the chorion; the individual was having trouble leaving the egg. At 1200, using a dissecting scope, probe, and forceps, we removed the second embryo from its egg. The first individual that hatched was 9.0 mm TL and the second was 8.6 mm. Both were light

yellow with black eyes and had a large yolk sac (Fig. 9, Appendix 1). The larvae were moved to a larger glass dish with small gravel (5 - 10 mm) and two large cover stones (140 and 78 mm long). Yolk sacs were depleted by 30 July, Day 17, when individuals were 13 - 14 mm TL. Larval development proceeded as described by Wilkinson and Edds (1997), with melanophores spread over the body and pelvic fins developed by the time of yolk sac depletion, eight days after hatching. However, Wilkinson and Edds (1997) reported a mean of 6.8 mm TL at hatching, which is smaller than recorded during this study.

The tank in which the clutch of eggs was discovered contained gravel ranging 7 - 17 mm ($\bar{x}$=12.3 mm, SD= 2.65, N=21); the top of the tank was uncovered, but the sides were covered by blue plastic. The eggs were in a depression under the only cover structure in the tank; a triangular shaped rock (surface area ≈ 240 cm^2).

Three times during the study it was 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. In such cases it was presumed that the male had eaten eggs that had been laid by the female.

Discussion

Field study

Mean density of occurrence (19.8/100 m^2) and overall density (4.5/100 m^2) in the Neosho River (Table 2, Appendix 2) were slightly lower than those reported for the same location (20.2/100 m^2 and 5.0/100 m^2, Edds, 1995; 25.6/100 m^2 and 5.7/100 m^2, Edds and Wilkinson, 1996; 24.0/100 m^2 and 8.3/100 m^2, Wilkinson and Edds , 1997). The mean overall density reported by Wenke et al. (1992) (6.8/100 m^2) for eight sites on the Neosho River in Kansas and Oklahoma was higher than we found, and results from nighttime electroshocking on the Neosho River near Erie, Kansas (Moss, 1983) were much higher (32.4/100 m^2 and 43.3/100 m^2), probably due to increased activity of

Neosho madtoms during nighttime hours. On the Neosho River, Eberle and Stark (1995) reported overall densities (1.8/100 m^2 - 97/100 m^2) and densities of occurrence (18.0/100 m^2 - 108.0/100 m^2) that ranged from much lower to much higher than our results.

In the Cottonwood River, mean density of occurrence (11.9/100 m^2) and overall density (1.9/100 m^2) were similar to those previously reported for the site (15.5/100 m^2 and 3.3/100 m^2, Fuselier and Edds, 1994; 12.5/100 m^2 and 2.5/100 m^2, Edds, 1995; 12.5/100 m^2 and 1.7/100 m^2, Edds and Wilkinson, 1996; 11.7/100 m^2 and 2.4/100 m^2, Wilkinson and Edds, 1997). Eberle and Stark (1995) reported densities for five sites sampled on the Cottonwood River (61.0/100 m^2 and 40.0/100 m^2, density of occurrence and overall density, respectively) that were much higher.

The differences among the densities could be due to yearly fluctuations in Neosho madtom populations, different water conditions, such as flow, at the time of sampling, seasonal differences, differences in sampling methods, gear, or efficiency, or differences in habitat quality among locations sampled. Densities reported by Wilkinson et al. (1996) for the Spring River in Missouri and Kansas, ranged from lower to higher than our results (9.1 - 30.0/100 m^2 density of occurrence and 0.9 - 4.3/100 m^2 overall density), most likely due to differences in the drainages.

In the present study, densities were higher in the Neosho River than in the Cottonwood River (19.8/100 m^2 vs. 11.9/100 m^2, density of occurrence; 4.5/100 m^2 vs. 1.9/100 m^2, overall density). Eberle and Stark (1995) reported higher densities in the Cottonwood River than in the Neosho River. However, densities on the Cottonwood River site have been consistently lower than on the Neosho River site over the past four years (Edds, 1995; Edds and Wilkinson, 1996; Wilkinson and Edds, 1997). Because the Cottonwood River site is different from the Neosho River site, including being wider (30 m vs. 20 m) and deeper ($\bar{x}$ depth=55.7 cm vs. 38.4 cm; Tables 3 and 8, Appendix 2), the latter may provide more suitable habitat for the Neosho madtom. The Cottonwood River site is also unique because the habitat there was manipulated to create an artificial

riffle (Fuselier and Edds, 1995). According to Fuselier and Edds (1995), within a year after construction, the gravel bar on the Cottonwood River site had physical features similar to those of two natural riffles, however, the consistently low densities at the site, compared to those at the Neosho River site, may indicate that the habitat is not as suitable to Neosho madtoms as the natural riffle on the Neosho River. It is also possible that Neosho madtoms were simply easier to capture at the Neosho River study site.

Neosho madtoms utilized riffle and run habitat exclusively throughout the year, as reported by others (Moss, 1981; Wenke et al., 1992; Fuselier and Edds, 1994; Edds, 1995; Edds and Wilkinson, 1996; Wilkinson and Edds, 1997). Eberle and Stark (1995) noted the absence of Neosho madtoms in riffles with bedrock or large stone substrate, and suggested that the fish's habitat would best be defined as, "..areas of loose gravel often associated with riffles." Where the majority of Neosho madtoms were captured in the present study, current speed was moderate (~26 cm/sec at the substrate), water depth was less than 1.0 m, gravel comprised more than 70% of the substrate, and substrate compaction was low. Fuselier and Edds (1994) found that the best predictors of Neosho madtom presence were current at the substrate surface, low variance of that current, shallow depth, and low substrate compaction. Loosely embedded substratum was reported as an indicator of "good" Neosho madtom habitat by Moss (1981), Wenke et al. (1992), and Eberle and Stark (1995), and may be a limiting factor to the fish because of the tendency for exposed gravel to become compacted during times of low flow; the drying of organic material in riffles during drought periods causes substrate compaction to remain high for some time following the drought, which may force Neosho madtoms into less suitable habitat (Deacon, 1961; Moss, 1981), where survival rates could be lower. Others have reported infrequent captures in pool habitat (Wenke et al., 1992; Fuselier and Edds, 1994). In this study, Neosho madtoms were captured in areas of no flow only twice, on 20 September and 11 October 1997, both in the Neosho River during a period of low discharge. However, the areas in which these six individuals were captured were

not pools, but runs with swiftly flowing water at times of normal discharge that registered no flow on our current meter at the time of capture.

Habitat use of adult and YOY Neosho madtoms differed only slightly, with both utilizing areas with abundant loose gravel. However, YOY were found in areas of slower flow, shallower depth, and lower substrate compaction than adults, presumably seeking food, refuge from swift current, and/or avoiding predators. Wilkinson and Edds (1997) noted that YOY were sometimes captured along shallower edge habitat early in their development. Although the distance from the edge of capture locations was not recorded during this study, capture of YOY near the edge is consistent with the results from the present study because current speed is often slower and water depth is usually shallower in edge habitat.

Length frequency distributions and YOY:adult ratios revealed that YOY first appeared in July, grew quickly during the first few months of life, and slowed growth thereafter. YOY:adult ratios reported by Edds (0.5 - 22.0; 1995) and Edds and Wilkinson (0.5 - 109.0; 1996) were higher than those in this study (0.42 - 15.0), but ratios from Wilkinson and Edds (0.42 - 15.0; 1997) were similar. The high ratio reported by Edds and Wilkinson (1996) could have been a result of good habitat conditions during the spawn that year, or the high number of YOY captured could have been due to chance.

The length-frequency data are consistent with the hypothesis that few individuals live to breed as Age II adults. This could be critical for the recovery of the species. Relying on one or two cohorts for reproduction is risky because ecological 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 that are not federally listed spawn up to six times during their lifetime (Simonson and Neves, 1992). Understanding how age-class structure of the

Neosho madtom relates to its breeding biology is crucial, and there is a need to age the fish in a more accurate manner, such as by examining spines or otoliths.

External sexing of individuals is difficult, at best, and nearly impossible when secondary sexual characteristics are not well developed. A success rate of only 90% can be expected under optimal conditions, when secondary sexual characteristics are well developed (B. Burr, Southern Illinois University, pers. comm.). Our study indicates that individuals began to show signs of secondary sexual characteristic development in April, were in prime breeding condition in June and July, and showed decreased secondary sexual characteristics in August. Most individuals captured with well-developed breeding characteristics were between 55 and 70 mm TL, however one 47 mm female was captured that appeared gravid, indicating females of that size can be sexually mature. The genital papillae of males and females (Fig. 6, Appendix 1) during the breeding season were similar to those illustrated for other madtom species (*N. albater*, Mayden et al. 1980; *N. exilis*, Mayden and Burr, 1981; *N. nocturnus*, Burr and Mayden, 1982; and *N. flavater*, Burr and Mayden, 1984), however it is important to note that Neosho madtom male and female genital papillae are usually not distinguishable during the non-breeding season. Burr and Mayden (1982; 1984) also had difficulty distinguishing males and females during the non-breeding season while studying freckled, slender, and checkered madtoms. Determining differences in genital papillae of live Neosho madtoms was extremely difficult, even during breeding season.

It is notable that the majority of individuals captured was female. Based on lab observations, it is possible that males are less likely to leave a spawning cavity than females, thus males may be less likely to be kicked into a seine while sampling. Clugston and Cooper (1960) also suggested that male madtoms are less likely to be captured during the spawning season because they are guarding nests. It is also possible that the populations we studied had a low male:female ratio; it has been suggested that populations of slender and least madtoms are slightly female biased (Mayden and Burr, 1981; Mayden

and Walsh, 1984). Such bias could indicate a promiscuous or polyandrous mating strategy in which females lay a clutch of eggs with two or more males each breeding season.

ANOVA results showed no significant difference in the placement of adults on the gravel bars during the breeding season versus the non-breeding season when data from both rivers were pooled. In the Neosho River, however, more individuals with developed secondary sexual characteristics were found in the upper portion of the site in May and June, during the peak of the breeding season, but not in April or July and August, when the breeding season was apparently just beginning and ending. In April, secondary sexual characteristics were developed in some fish and only beginning to develop in others, with only a slight reddening of the premaxillary tooth patches present, and in July and August many fish may have already bred but had not yet lost secondary sexual characteristics. Individuals that show secondary sexual characteristics earlier in the season could spawn earlier than those that show development of such characteristics later. Likewise, those that lose secondary sexual characteristics earlier may breed earlier, while those that lose characteristics later probably spawn later in the season. Baker and Heins (1994) showed a correlation between female size and oocyte developmental stage in least madtoms (*N. hildebrandi*), and suggested that larger females spawn before smaller ones; this could also be the case with Neosho madtoms. Thus, because different fish may spawn at different times, both early and late in the season, and because development and loss of secondary sexual characteristics is not an instantaneous process, it may be important to investigate habitat use during only May and June, when the majority of individuals could be breeding. The way breeding season was defined in this study may have masked differences in placement on the gravel bar in breeding versus non-breeding season. Or, perhaps habitat use differs by a specific habitat variable such as flow, substrate type, or substrate compaction, rather than by location on the gravel bar.

Lab study

Morphological changes

Due to inconsistencies in external sexing in 1997, internal sexing was necessary. Unfortunately there was low success in pairing males with females. However, there were four male-female pairs, and important breeding behaviors of the species were recorded and described for the first time.

Success in sexing fish was higher in 1998. We were more experienced with the species, and individuals were captured when secondary sexual development was advanced, aiding external sexing. During breeding season, differences were seen in genital pores and head shape. Males had an elongated genital papilla and no swelling of the anus, while females had a rounded, swollen anus and genital pore (Fig. 6, Appendix 1). Cephalic epaxial muscles of males became enlarged and lips were sometimes swollen, but the head of females tended to remain more conical in shape, similar to heads of both sexes during non-breeding season, and lips did not swell. Reddening of the tooth patches, although often brighter in males, occurred in both sexes. Mayden and Burr (*N. exilis*, 1981), Burr and Mayden (*N. nocturnus*, 1982), Burr and Mayden (*N. flavater*, 1984), and Mayden and Walsh (*N. hildebrandi*, 1984) described similar secondary sexual characteristics in other madtoms. No obvious color changes occurred during the breeding season, as has been reported in the slender madtom (*N. exilis*) (Burr and Mayden, 1982) and the smoky madtom (*N. baileyi*) (Dinkins and Shute, 1996). The tendency for males to quickly lighten in color when disturbed could be unrelated to breeding condition but merits further study.

Activity levels

Higher daylight activity levels in experimental period 2 fish held under a longer daylight photoperiod might be attributed to restlessness. We observed individuals in the 16:8 photoperiod swimming about or foraging for food one to two hours before the light cycle ended, but saw very little movement before the dark cycle in individuals held in the

12:12 photoperiod. No breeding behaviors and very few cavity enhancement behaviors were seen during the light cycle; most of the activity was swimming behavior. Results from the analysis of resting behavior during the day indicated that individuals in the 12:12 photoperiod spent a higher proportion of time resting than performing any other behavior, this is consistent with the results of overall activity, which indicated that individuals in the 16:8 photoperiod were more active during the day than those in the 12:12 photoperiod group.

Behavior types

Cavity enhancement behaviors were considered important because of evidence that indicates madtoms use nests during spawning (*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; *N. baileyi* and *N. flavipinnis*, Dinkins and Shute, 1996; Cochran, 1996; *N. placidus*, Wilkinson and Edds, 1997). During the second experimental period individuals held in the 16:8 photoperiod spent a higher proportion of time performing cavity enhancement behaviors at night. A difference was also seen in cavity depth and gravel size within cavities in tanks in the 16:8 photoperiod at the end of the study. It is clear that individuals held in longer daylight hours put more effort into cavity enhancement, however it should be pointed out that cavity measurements were taken only at the completion of the study, and that, because cavity enhancement was apparent in both sides of the treatment chamber within the first two weeks of the study, it is possible that individuals held in shorter daylight hours reduced the amount of energy they put into cavity enhancement. In addition, no difference was seen in the proportion of time spent performing these behaviors during the first collection period, but a significant difference was seen between the 16:8 and 12:12 groups during nighttime hours in the second experimental period. This could suggest that keeping Neosho madtoms in a 12:12

photoperiod with 25 - 27°C water temperatures over a one month period, the duration of the study, is not sufficient to encourage cavity enhancement behaviors, or that fish in the 16:8 photoperiod delayed cavity enhancement behaviors for an unknown reason.

Cavities were enhanced in tanks containing both male/female and female/female pairs. Most cavity enhancement behaviors observed were performed by males, however the presence of cavities, including the deepest, in tanks containing only females suggests 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), 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.

The two male/female pairs held under the 16:8 photoperiod performed courtship behaviors but the two male/female pairs held under the 12:12 photoperiod did not. In addition, it was observed that many of the females held at the 12:12 photoperiod had resorbed their eggs by 1 August and females held at 16:8 photoperiod had not. Likewise, swelling of cephalic epaxial muscles in males decreased in the 12:12 photoperiod before those in the 16:8 photoperiod. These observations suggest that a photoperiod similar to that in mid-July, at water temperatures of 25 - 27°C may be important to the breeding cycle of the Neosho madtom.

Breeding

The two clutches laid in captivity in 1998 were similar to each other. At the CERC, the clutch was laid under a PVC structure in a slight depression on a gravel bottom at a water temperature of 28°C. The clutch at ESU was laid in a depression that had been made in the gravel under a large flat stone at a water temperature of 27°C. In

both cases, eggs adhered together in a group but not to the substrate, as has been noted in other *N. placidus* captive spawns (Pfingsten and Edds, 1994; Wilkinson and Edds, 1997). Mean chorion diameter of the ESU eggs (3.7 mm) was larger than the mean (3.1 mm) reported by both Pfingsten and Edds (1994) and Wilkinson and Edds (1997). The size of the female, 67 mm (TL), was not likely responsible for this difference, because Pfingsten and Edds (1994) and Wilkinson and Edds (1997) reported females of 70 and 61 mm, respectively. Clutch size between the two 1998 spawns differed only slightly from each other, but were much smaller (almost half the size) than clutches laid in captivity previously. Pfingsten and Edds (1994) reported a clutch of 63 unfertilized eggs, and Wilkinson and Edds (1997) reported approximately 60 fertilized eggs. At both the CERC and ESU, it is possible that either the male or female ate some of the eggs before they were discovered. Another possibility is that the clutch was the second to be laid by the female, as these fish were collected much later in the breeding season (23 June and 7 July) than those collected in previous studies (4 May, Pfingsten and Edds, 1994; 16 May, Wilkinson and Edds, 1997; 17 and 18 May, first summer of present study). Many studies have suggested polyandry in *Noturus* (*N. exilis*, Mayden and Burr, 1981; *N. nocturnus*, Burr and Mayden , 1982; *N. hildibrandi*, Mayden and Walsh, 1984; and *N. phaeus*, Chan, 1995; *N. baileyi* and *N. flavipinnis*, Dinkins and Shute, 1996), but such a mating strategy has not been confirmed in any madtom species. Dissection of the female that spawned at ESU suggested that she had deposited eggs from only one ovary. This is consistent with the hypothesis that multiple clutches may be laid, but it is also possible that human disturbance interrupted the spawning event in this study, resulting in an incomplete clutch.

Why neither male cared for his clutch 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*, Dr. J. Stoeckel, pers. comm.). Perhaps the male at the CERC ate the eggs due to stress caused by captivity, and the male at ESU did not care for the eggs because of stress caused by either captivity or the hormone injection process. It is

also possible that the male at the CERC ate the clutch of eggs because they were not fertilized, although, based on observation, they appeared to have been. Regardless of the reasons, the behaviors seen in this study suggest that at least a portion of the egg mass should be removed from the nesting cavity and hatched separately if the goal of a study is to raise eggs to adults. Unfortunately, the success rate using this method was low, most likely due to temperature fluctuations, harsh physical conditions of the hatching apparatus, and fungus, which may be controlled in the wild by parental care (Wilkinson and Edds, 1997). Others have reported low success rates in rearing madtom eggs (Shute et al., 1993; Chan, 1995; Dr. J. Stoeckel, Arkansas Tech University, pers. comm.).

In order to accurately sex individuals for captive spawning, it is important to collect fish when secondary sexual characteristics are well developed. The problem is, however, that it is often late in the breeding season by the time such characteristics are well defined, and the researcher risks missing the crucial interval in which successful breeding in captivity may occur, with or without use of reproductive hormone. If one plans to inject individuals with hormone, we believe it is important to begin injections as early as late May; the later in the season, the higher the risk that the fish may be past the point where injections will aid captive spawning.

Research Needs

Our knowledge of *N. placidus* breeding biology and behavior is still lacking in many regards. What is the environmental "key" to promote captive breeding? Do females lay multiple clutches in one breeding season? Do males care for multiple clutches in one breeding season? What effects do high or low flows have on the breeding success of Neosho madtoms? What habitat do individuals use for breeding? Do individuals move from riffle to riffle or from riffle to pool for breeding purposes? Is there a specific habitat variable, such as current velocity, water depth, water temperature, availability of food, or substrate type, composition, or compactness, that is important in triggering the breeding

process? What type of mating system is used by the Neosho madtom? What is normal recruitment in a given year? Though many questions remain, this research increased our understanding of this fish's reproductive biology, and has aided the search for understanding of the life history and ecology of this species.

Summary

Prior to this study, little was known of Neosho madtom breeding biology or YOY habitat use. Attempts at captive propagation had met with limited success, and hormone injection to induce captive spawning had been unsuccessful.

Field monitoring at the study sites on the Neosho and Cottonwood rivers from 1996 to 1998 confirmed that adult Neosho madtoms utilize riffle and run areas with loose to moderately compacted substrate primarily consisting of gravel. Mean water depth at capture locations was 38.4 cm in the Neosho River and 55.7 cm in the Cottonwood River. Mean current velocity at 50% depth was 55.0 cm/sec in the Neosho River and 61.4 cm/sec the Cottonwood River. Comparison of YOY and adult habitat use indicated YOY used areas of slower flow, lower substrate compaction, and shallower depth. No difference was seen in placement of adults on the gravel bar (upper, mid, and lower section) in breeding versus non-breeding season. Length-frequency distributions revealed two obvious age classes, suggesting that most individuals spawn as Age I individuals (during their second summer) and very few, if any, survive to spawn at Age II. Beginning of the breeding season was indicated by the first appearance of secondary sexual characteristics in April, and the characteristics were last seen in August, suggesting the end of breeding season. YOY first appeared in samples in mid to late July and grew rapidly during the next few months (captured YOY ranged 19-42 mm in July to 37-63 mm in October) before the onset of winter.

Lab research confirmed the nocturnal nature of the Neosho madtom, as individuals spent a higher proportion of time active during dark hours versus light hours. Individuals

held under a longer daylight photoperiod spent a higher proportion of time performing cavity enhancement (nest building) behaviors than those held under a shorter daylight hour photoperiod. The two male/female pairs held under longer daylight hours were observed performing courtship behaviors, but the two in shorter daylight hours were not. The increase in cavity enhancement, which is an important part of spawning as indicated by successful lab breedings, and the higher incidence of courtship behaviors seen in the individuals held under longer daylight hours indicate the importance of a long daylight photoperiod for captive spawning.

Two successful spawning events occurred in the laboratory during this study; one at ESU in Emporia and one at the CERC in Columbia. Both occurred in July, 1998, and both clutches consisted of approximately 30 eggs. Courtship behaviors, including the "carousel" followed by the "tail curl", were observed, recorded, and documented for the first time. At the CERC, video recording revealed the male chasing the female from the nest after she attempted to eat some of the eggs. After the female was removed from the tank the male was observed resting over the eggs for approximately two days before apparently consuming them. The breeding at ESU occurred after two consecutive days of injection with Ovaprim, the first successful hormone-induced spawn for this species. Two surviving eggs hatched in eight days and yolk sacs were depleted after another eight days.

This study increased our understanding of Neosho madtom adult and YOY habitat use, habitat use during the breeding season, sexual dimorphism, the effects of photoperiod on breeding activity, hormone-induced captive spawning, and breeding behavior. Though many questions remain, this research has enhanced our knowledge of the reproductive biology of this threatened catfish, and has aided the effort to understand the life history and ecology of this species.

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Appendix 1.

Figures

Fig. 1. Location of study areas on the Neosho River, Lyon County, and Cottonwood River, Chase County, Kansas.

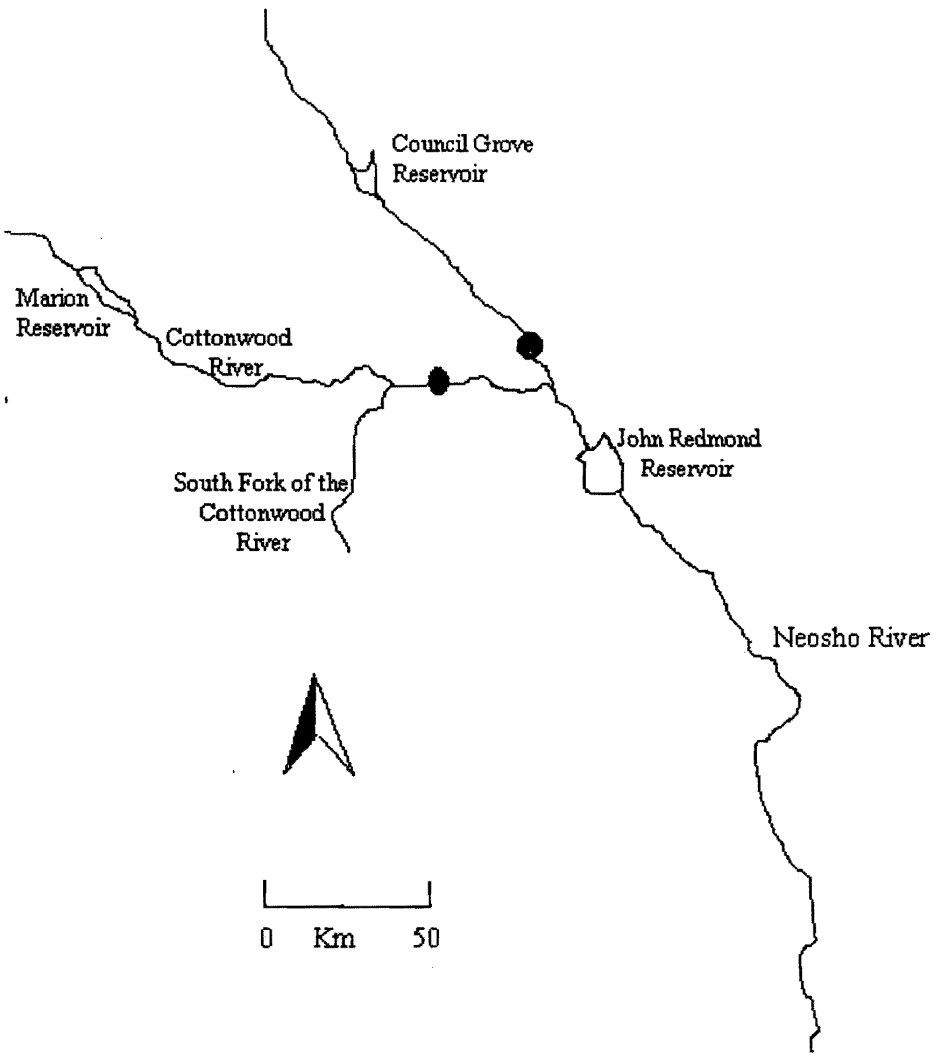


Fig. 2. Study sites on the Neosho and Cottonwood rivers, Lyon and Chase counties, Kansas, divided into upper, mid, and lower sections

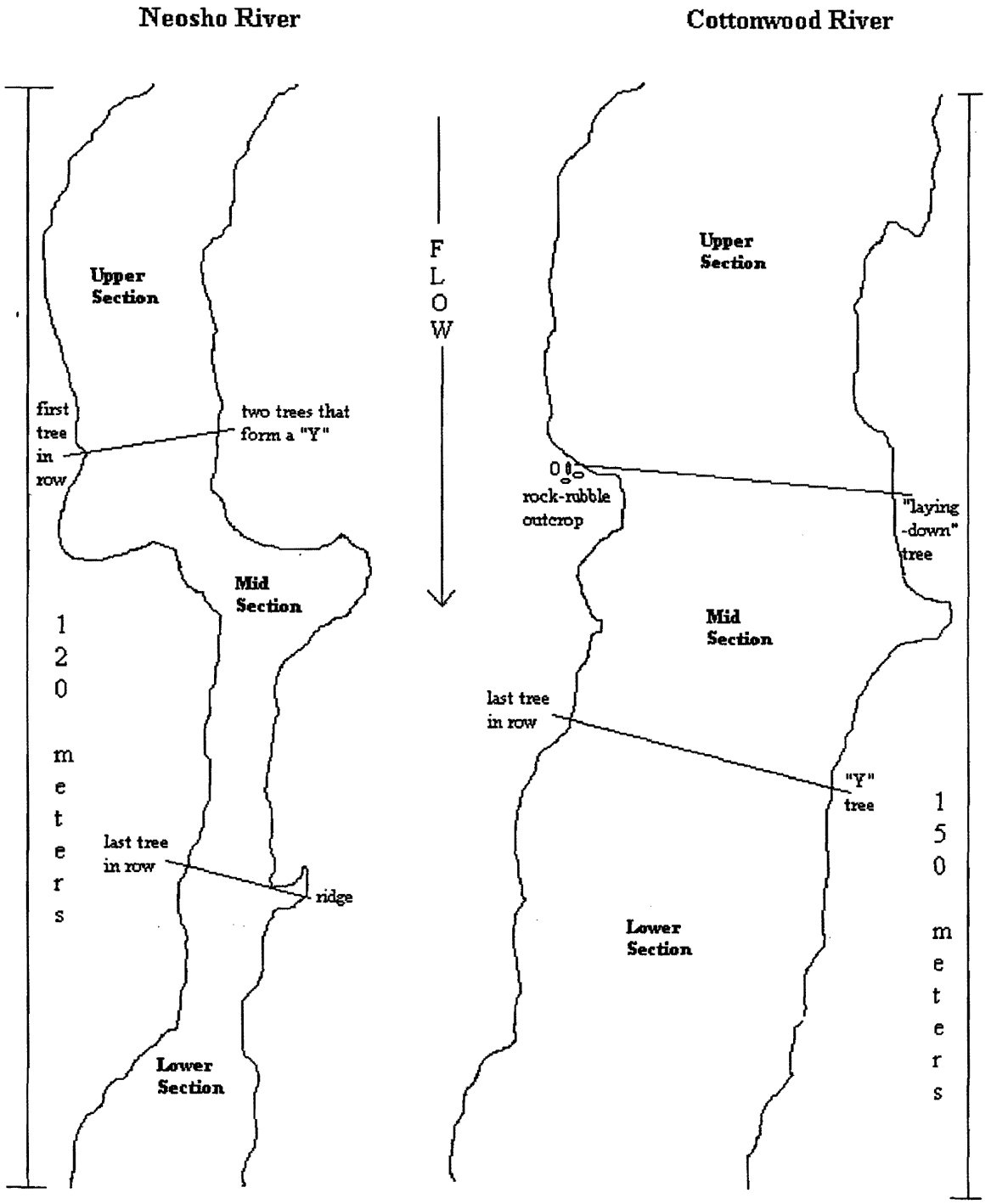
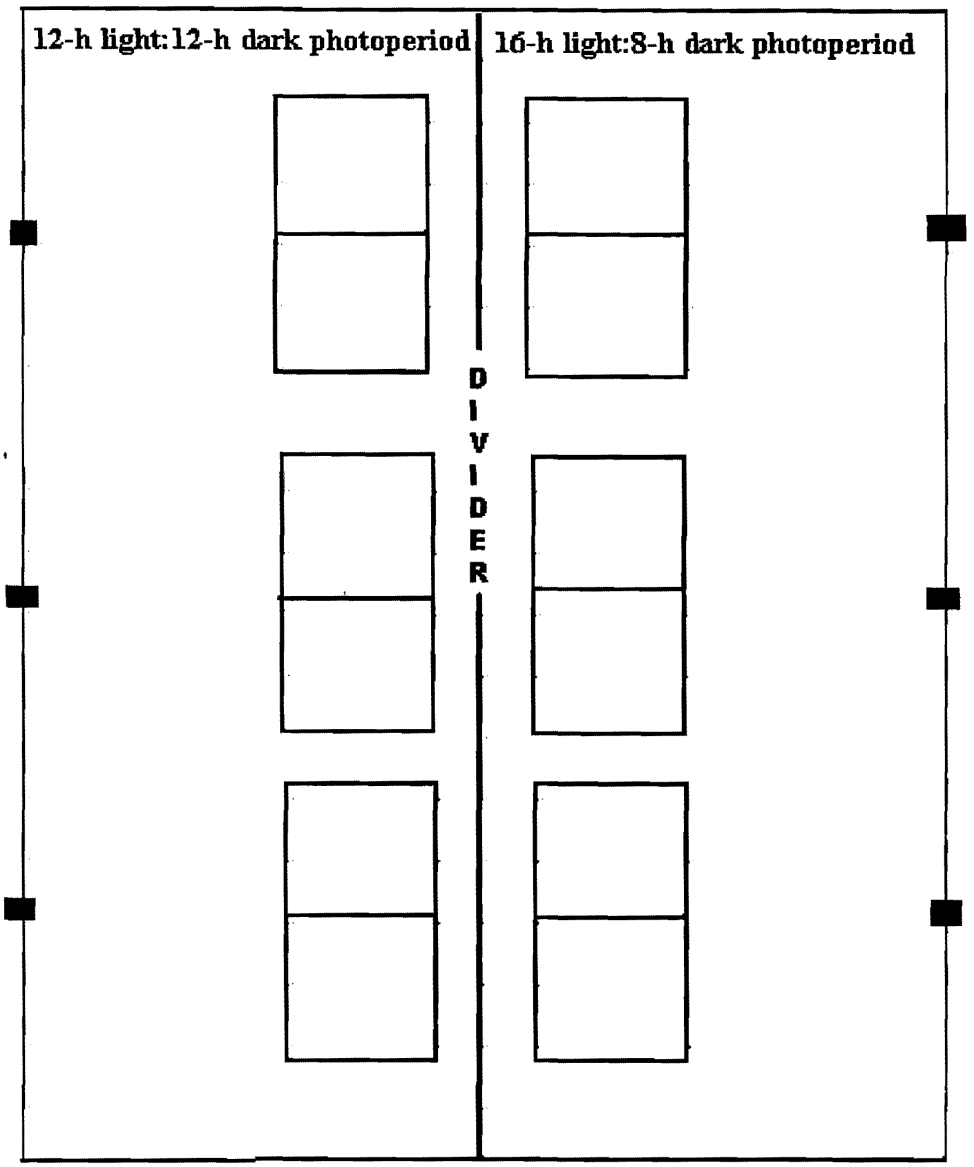


Fig. 3. Arrangement of study tanks in isolation chamber (2.79 m x 1.93 m x 0.91 m) at ECRC, Columbia, Missouri.



Key

■ = video camera

Fig. 4. Length-frequency distribution for 270 Neosho madtoms collected from the Neosho River, Lyon County, Kansas, 1996-1998.

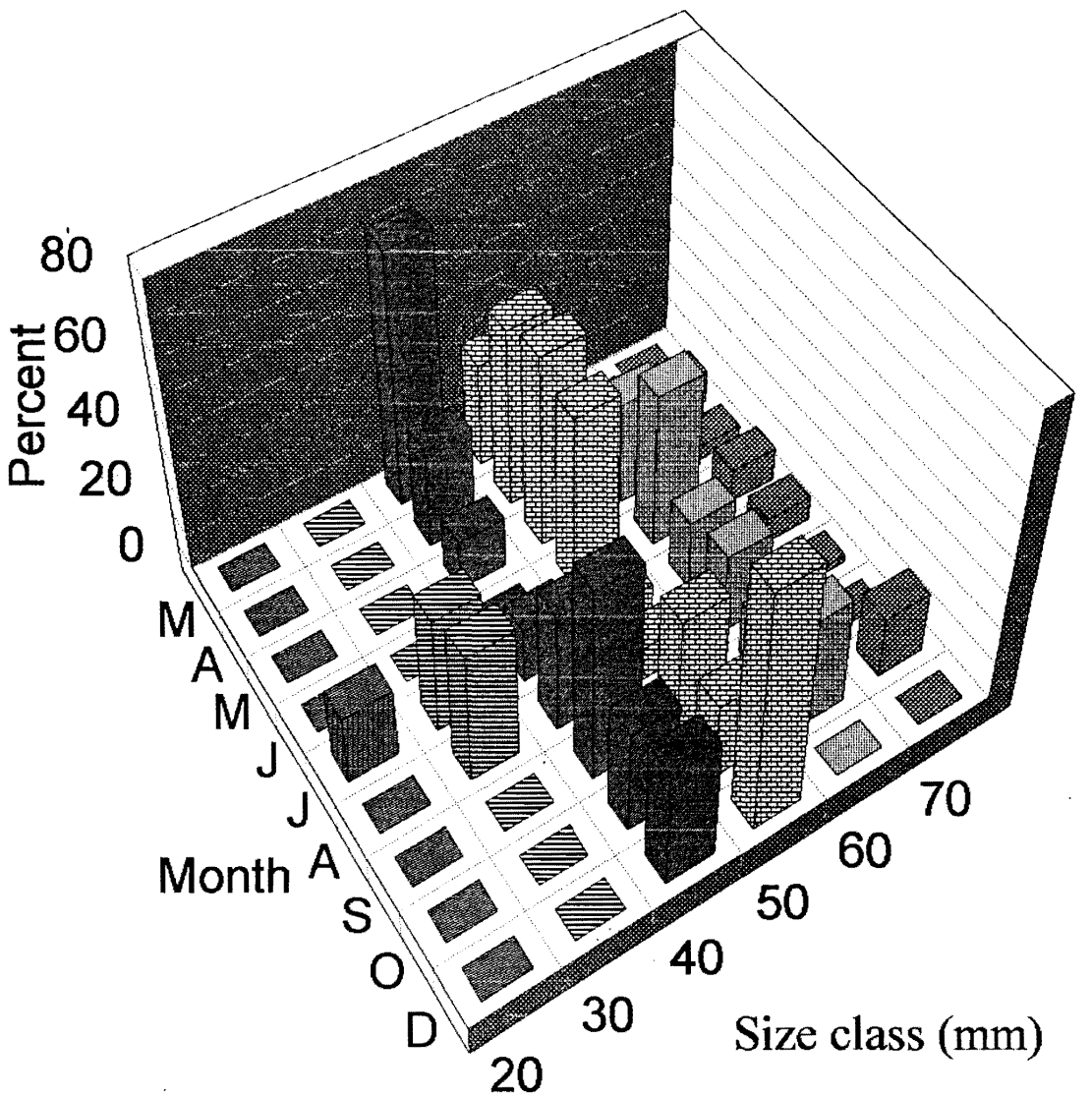


Fig. 5. Length-frequency distribution for 122 Neosho madtoms collected from the Cottonwood River, Chase County, Kansas, 1996-1998.

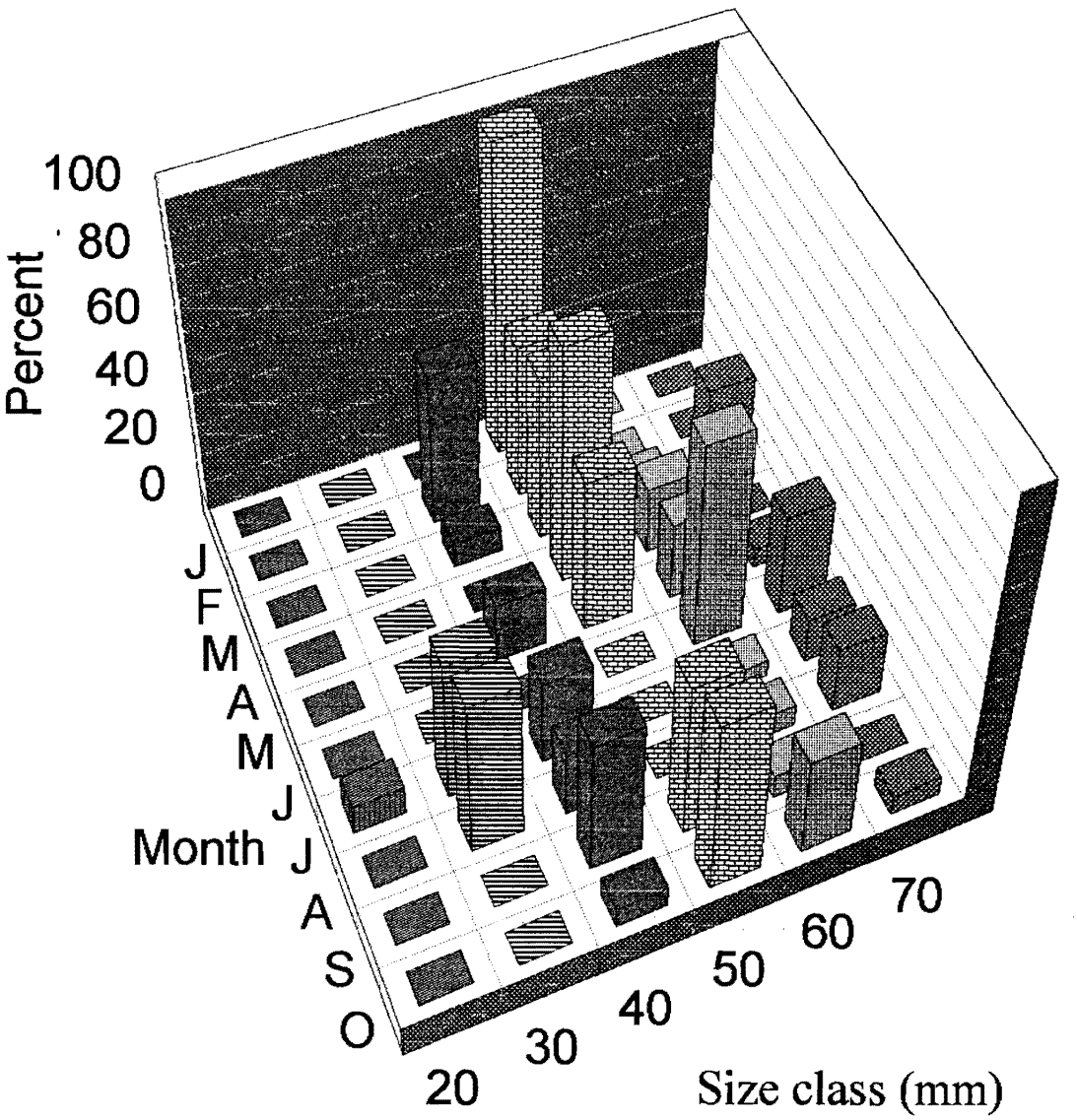
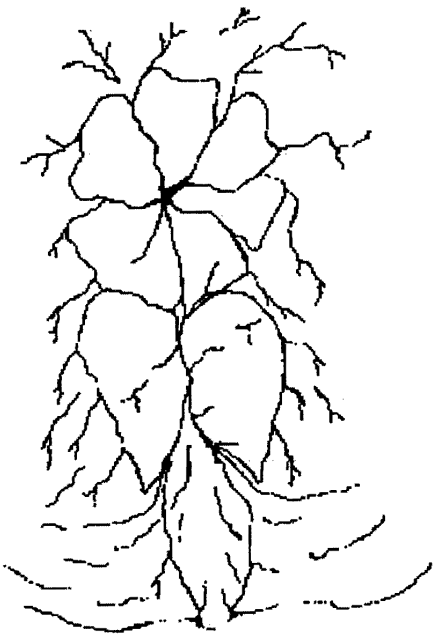


Fig. 6. Genital papillae of the Neosho madtom: A) breeding female; B) breeding male; C) non-breeding individual.

A.



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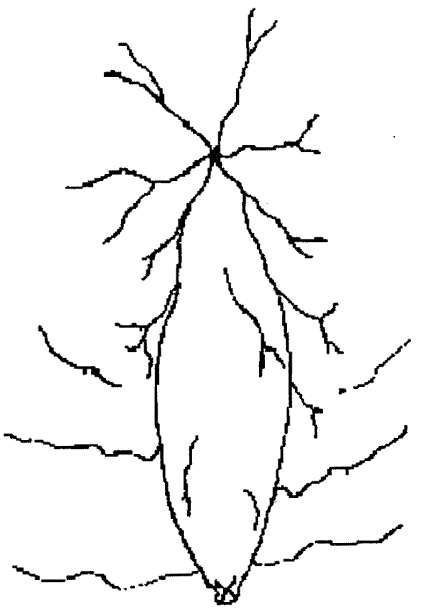


C.

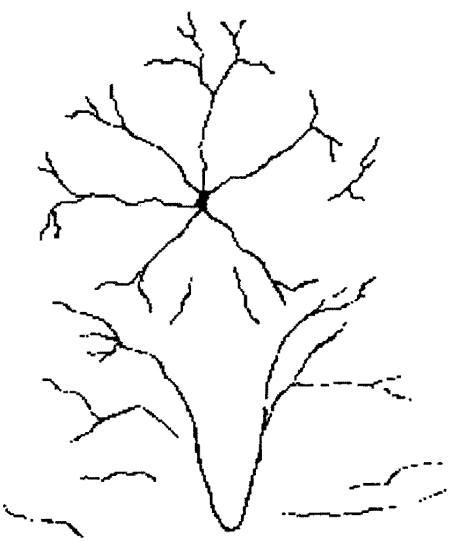


Fig. 7. The "carousel," a courtship behavior of the Neosho madtom. Male and female swim in circles head to tail near the substrate, usually followed by the "tail curl."

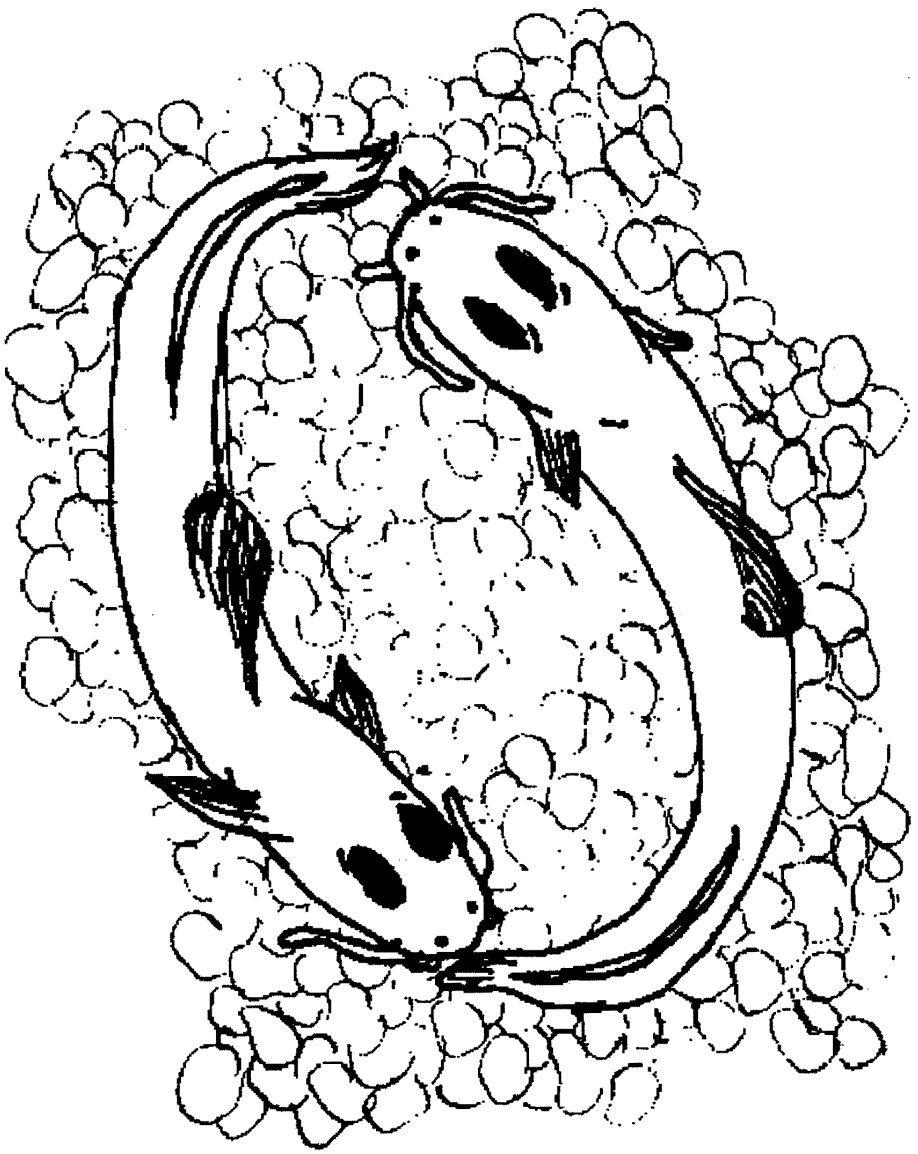
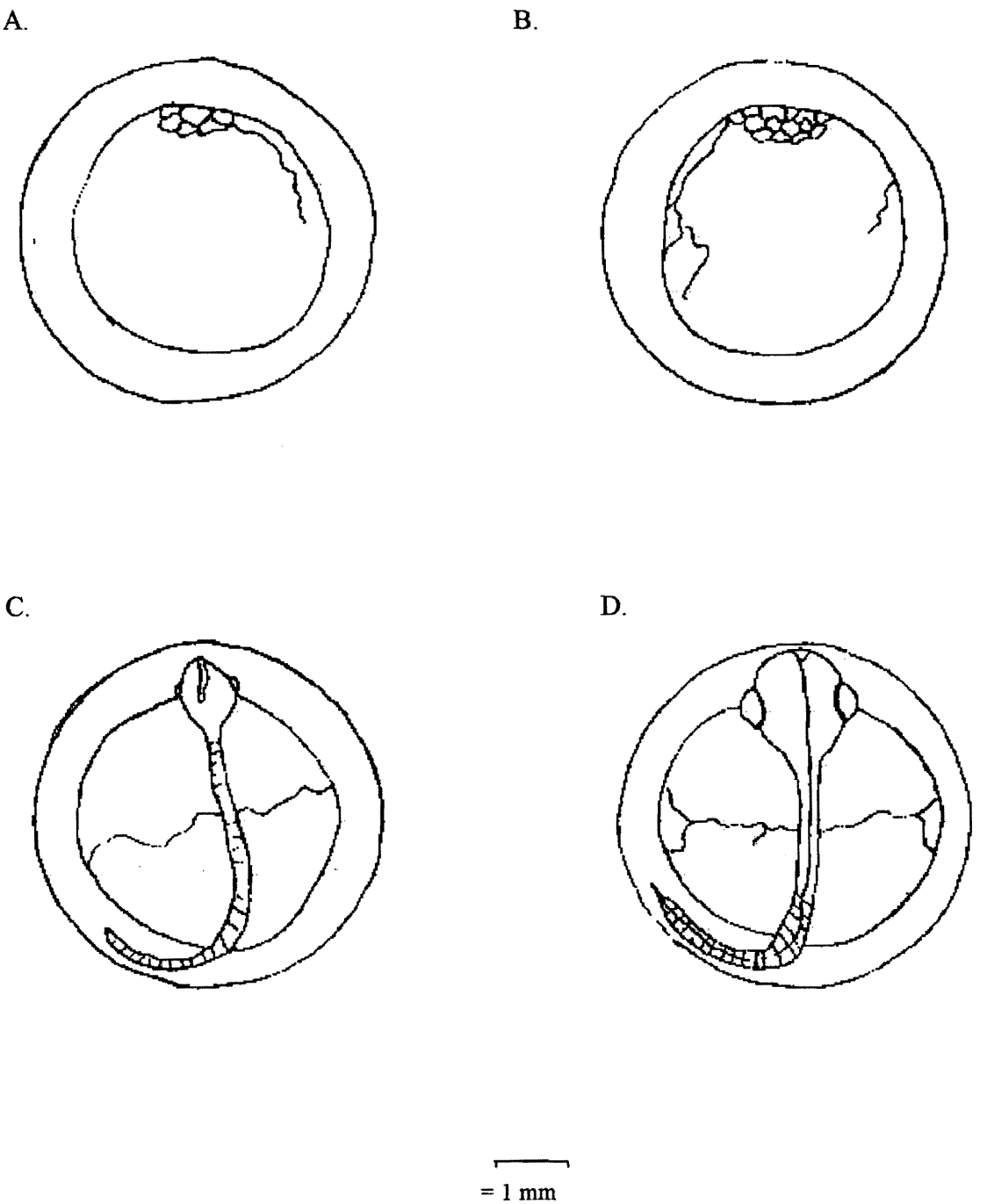


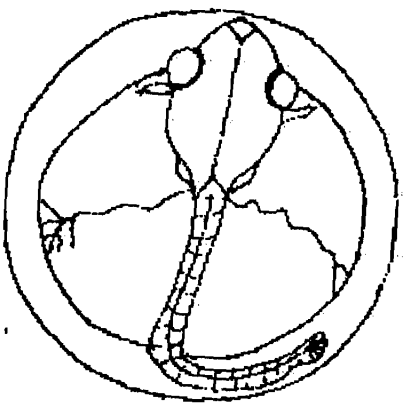
Fig. 8. Development of Neosho madtom eggs laid 14 July 1998 at ESU. A=Day 1, B=Day 2, C=Day 4, D=Day 5, E=Day 6, F=Day 7, and G=Day 8.



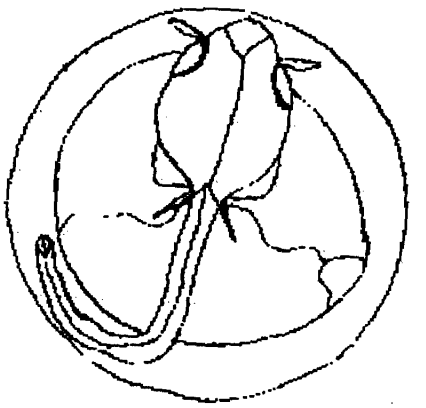
Continued

Fig. 8 (cont.)

E.



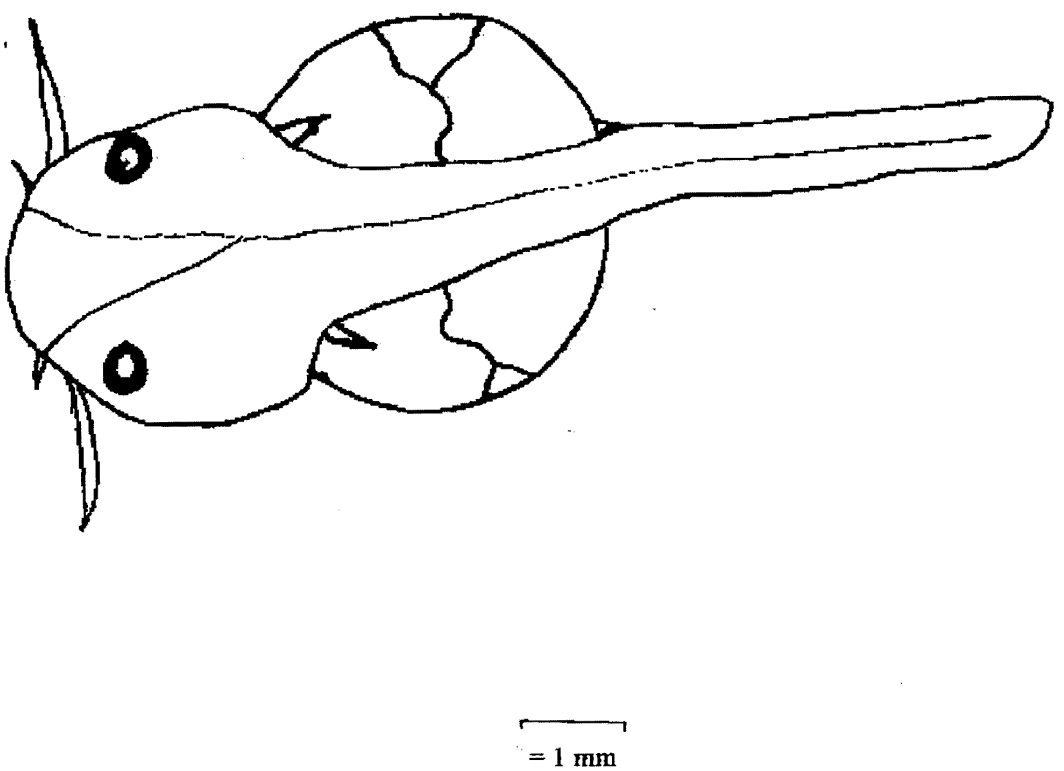
F.



G.

= 1 mm

Figure 9. Larval Neosho madtom on day of hatching (22 July 1998). Two pairs of barbels are present, pectoral spines are beginning to develop, and the vitelline vein is visible on the yolk sac. Total length = 9.0 mm.



Appendix 2.
Tables

Table 1. Ethogram of Neosho madtom behaviors performed by either or both fish during 1997 and 1998 lab studies. Each behavior is labeled as active or inactive (resting) (A=active or I=inactive), and classified as a behavior type: courtship, aggression, cavity enhancement (cav enahn), feeding, swimming, or resting.

Behavior	Description	Activity	Classification
Performed by either fish:			
Circle alone	swimming in circles against glass at front, back, or side of tank	A	swimming
Swim	swimming in no particular pattern	A	swimming
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
Feeding	feeding	A	feeding
Fanning	fanning tail while resting under structure	I	courtship
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
Performed by both fish:			
Bite	one fish bites at body of other fish	A	aggression
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
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
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

Table 2. Density of occurrence (based on 9.2 m^2 seine hauls) and overall density (based on 276 m^2 riffle/run habitat sampled) of Neosho madtoms captured during quantitative sampling in the Neosho River, Lyon County, Kansas, 1996-1998.

Date	No. Captured	Density of occurrence per 100 m^2	Overall density per 100 m^2	Water Temp (°C)
1996				
6 Aug.	17	18.5	6.2	28
4 Sept.	5	10.9	1.8	26
22 Oct.	14	25.4	5.1	14
7 Dec.	3	32.6	1.1	3
1997				
22 Jan.	0	0.0	0.0	0
12 Mar.	7	25.4	2.5	11
25 April	8	14.5	2.9	13
2 May	5	18.1	1.8	15
16 May	9	19.6	3.3	18
5 June	10	21.7	3.6	20
25 June	13	15.7	4.7	26
8 July	7	12.7	2.5	25
24 July	10	21.7	3.6	28
5 Aug.	24	26.1	8.7	26
22 Aug.	4	13.6	1.5	26
20 Sept.	8	14.5	2.9	21
11 Oct.	11	19.9	4.0	21
1998				
14 April	25	30.2	9.1	16
18 May	14	19.0	5.1	25
2 June	15	23.3	5.4	26
20 June	9	16.3	3.3	29
2 July	27	26.7	9.8	28
21 July	25	24.7	9.1	31
Total	270			
Mean densities (SE)		19.8 (1.44)	4.5 (0.58)	

Table 4. Number of Neosho madtoms captured during quantitative sampling in the Neosho River, Lyon County, Kansas, 1996-1998, with size ranges and YOY:adult ratios.

Date	No. Total	No. YOY	Size range (TL) YOY (mm)	No. Adults	Size range (TL) Adults (mm)	YOY:adult
1996						
6 Aug.	17	5	30-40	12	51-64	0.42
4 Sept.	5	4	41-44	1	65	4.00
22 Oct.	14	8	37-50	6	62-71	1.33
7 Dec.	3	3	37-47	0	--	ALL YOY
1997						
22 Jan.	0	0	--	0	--	--
12 Mar.	7	0	--	7	38-49	--
25 April	8	0	--	8	43-70	--
2 May	5	0	--	5	44-56	--
16 May	9	0	--	9	40-58	--
5 June	10	0	--	10	45-69	--
25 June	13	0	--	13	45-68	--
8 July	6	0	--	6	47-66	--
24 July	10	7	31-41	3	57-68	2.33
5 Aug.	24	22	27-45	2	57-65	11.00
22 Aug.	5	5	31-42	0	--	ALL YOY
20 Sept.	8	8	42-55	0	--	ALL YOY
11 Oct.	11	11	44-63	0	--	ALL YOY
1998						
14 April	25	0	--	25	39-70	--
18 May	14	0	--	14	45-65	--
2 June	15	0	--	15	47-67	--
20 June	9	0	--	9	50-65	--
2 July	27	16	19-3	11	49-70	1.45
21 July	25	22	20-42	3	62-69	7.33
Total	270	111		159		

Table 5. Neosho madtoms captured in the Neosho River, Lyon County, Kansas (1996 - 1998), with designation of secondary sexual characteristics: SP=slightly pink tooth patches, PP=pink tooth patches, RP=red tooth patches, SS=slightly swollen epaxial muscles, SE=swollen epaxial muscles, EP=elongated papilla, OP=rounded genital pore, SG=slightly gravid, GR=gravid, ST=spent.

Date	Male Characteristics	TL (mm)	Female Characteristics	TL (mm)
1996				
6 Aug.	--	--	GR	62
1997				
25 April	SP	70	--	--
16 May	--	--	SG, SP	50
	--	--	SG, OP	56
	--	--	SG, OP	56
5 June	--	--	GR, SP	60
	--	--	GR, SP	55
	--	--	SG	61
25 June	SE, RP	68	GR, PP	55
	--	--	SG, PP	56
	--	--	SG, PP	60
	--	--	GR, PP	53
	--	--	GR, PP	61
	--	--	SP	55
	--	--	GR, PP	53
	--	--	SG, PP	54
8 July	--	--	RP	66
	--	--	GR, PP, OP	62
	--	--	PP	55
24 July	--	--	GR, PP, OP	62
	--	--	GR	63
	--	--	SG, PP	57
5 Aug.	--	--	ST, RP	65
	--	--	SG, SP	57

Continued

Table 5 (cont.)

1998

18 May	--	--	PP	65
	--	--	GR	57
	--	--	GR, PP	60
2 June	SP, EP	60	GR	55
	SE, PP	67	GR	60
	--	--	GR, PP	61
	--	--	GR, PP	60
	--	--	GR	47
	--	--	GR	51
	--	--	GR, PP	57
20 June	SS, EP	64	GR, PP	59
	--	--	GR, PP	65
2 July	SP, EP	61	SG, PP	70
	PP, EP	57	GR, PP, OP	62
	--	--	GR, PP, OP	63
21 July	PP, SE, EP	69	PP, OP	65
	SP, SS	62	--	--

Table 6. Density of occurrence (based on 9.2 m^2 seine hauls) and overall density (based on 276 m^2 riffle/run habitat sampled) of Neosho madtoms captured during quantitative sampling in the Cottonwood River, Chase County, Kansas, 1996-1998.

Date	No. Captured	Density of occurrence per 100 m^2	Overall density per 100 m^2	Water Temp (°C)
1996				
2 Aug.	5	10.9	3.0	26
30 Sept.	2	10.9	0.7	18
30 Oct.	16	24.8	5.8	12
1997				
19 Jan.	3	10.89	1.1	2
15 Feb.	4	14.5	1.5	2
22 Mar.	13	17.7	4.7	12
26 April	5	13.6	1.8	14
4 May	6	10.9	2.2	17
14 May	4	10.9	1.5	19
13 June	0	0.0	0.0	21
26 June	0	0.0	0.0	26
8 July	0	0.0	0.0	22
24 July	3	10.9	1.1	28
5 Aug.	4	10.9	1.5	28
29 Aug.	1	10.9	0.4	27
9 Sept.	1	10.9	0.4	22
28 Sept	11	23.9	4.0	19
1998				
13 Mar.	1	10.9	0.4	3
18 April	4	10.9	1.5	15
19 May	5	13.6	1.8	25
5 June	9	16.3	3.3	22
17 June	3	10.9	1.1	23
2 July	8	14.5	2.9	29
21 July	14	15.2	5.1	29
Total	122			
Mean densities (SE)		11.9 (1.23)	1.9 (0.34)	

Table 7. Mean habitat use by total (N=122), YOY (N=53), and adult (N=69) Neosho madtoms captured during quantitative sampling in the Cottonwood River, Chase County, Kansas, 1996-1998. Significant Wilcoxon-Mann-Whitney P-value, after sequential Bonferroni correction, is indicated by an asterisk (*).

Habitat Variable	**Total Mean (SD)**	**YOY Mean (SD)**	**Adult Mean (SD)**	**P≤**
Mesohabitat	3.5 (0.50)	3.5 (0.50)	3.5 (0.50)	0.98
Water depth (cm)	55.7 (22.51)	52.5 (20.08)	57.1 (23.90)	0.44
Current speed at 0% depth (cm/sec)	27.0 (11.87)	24.9 (13.16)	28.5 (10.98)	0.04
Current speed at 50% depth (cm/sec)	61.4 (22.28)	49.1 (18.38)	67.8 (19.59)	0.0001*
% Mud	5.3 (4.25)	5.3 (4.36)	4.8 (3.23)	0.84
% Sand	6.7 (4.42)	6.9 (4.29)	6.4 (4.50)	0.046
% Gravel	78.0 (10.20)	77.2 (11.92)	79.1 (8.41)	0.83
% Small Cobble	7.9 (5.79)	7.7 (6.77)	8.2 (5.01)	0.07
% Large Cobble	1.8 (3.29)	2.5 (4.32)	1.3 (2.13)	0.74
% Boulder	0.0 (0.18)	0.1 (0.24)	0.0 (0.17)	0.43
Substrate Compaction	1.8 (0.40)	1.8 (0.36)	1.9 (0.43)	0.53

Table 8. Number of Neosho madtoms captured during quantitative sampling in the Cottonwood River, Chase County, Kansas, 1996-1998, with size ranges and YOY:adult ratios.

Date	No. Total	No. YOY	Size range (TL) YOY (mm)	No. Adults	Size range (TL) Adults (mm)	YOY:adult
1996						
2 Aug.	5	4	28-40	1	63	4.0
30 Sept.	2	1	36	1	52	1.0
30 Oct.	16	15	44-59	1	66	15.0
1997						
19 Jan.	3	0	--	3	48-51	--
15 Feb.	4	0	--	4	40-53	--
22 Mar.	13	0	--	13	39-78	--
26 April	5	0	--	5	45-56	--
4 May	6	0	--	6	43-57	--
14 May	4	0	--	4	41-69	--
13 June	0	0	--	0	--	--
26 June	0	0	--	0	--	--
8 July	0	0	--	0	--	--
24 July	3	2	36-37	1	67	2.0
5 Aug.	4	2	30-32	2	67-70	1.0
29 Aug.	1	1	31	0	--	All YOY
9 Sept.	1	1	44	0	--	All YOY
28 Sept.	11	10	40-53	1	63	10.0
1998						
13 Mar.	1	0	--	1	54	--
18 April	4	0	--	4	45-53	--
19 May	5	0	--	5	48-60	--
5 June	9	0	--	9	55-69	--
17 June	3	0	--	3	61-65	--
2 July	8	8	22-31	0	--	All YOY
21 July	14	10	19-40	4	63-68	2.5
Total	122	54		68		

Table 9. Neosho madtoms captured in the Cottonwood River, Chase County, Kansas (1996-1998), with designation of secondary sexual characteristics: SP=slightly pink tooth patches, PP=pink tooth patches, RP=red tooth patches, SS=slightly swollen epaxial muscles, SE=swollen epaxial muscles, EP=elongated papilla, OP=rounded genital pore, SG=slightly gravid, GR=gravid, ST=spent.

Date	Male Characters	TL (mm)	Female Characters	TL (mm)
1996				
2 Aug.	--	--	GR, RP	63
1997				
26 April	SP	56	--	--
14 May	--	--	GR, OP, RP	69
24 July	--	--	GR, OP, PP	67
5 Aug.	SS, RP	70	--	--
	SS, PP	67	--	--
1998				
19 May	--	--	GR	60
5 June	PP, EP	69	SG, OP	61
	SP	64	GR	62
17 June	--	--	GR	62
	--	--	GR, PP	61
	--	--	GR, PP	65
21 July	SP	67	RP	68
	--	--	SG	65

Table10. Percentage and number of adult Neosho madtoms captured each month in the upper, mid, and lower portions of the Neosho River study site, Lyon County, Kansas, 1996-1998. Values are pooled for months sampled twice (May 1997, June and July 1997-98, and August 1997).

Month	% Upper	% Mid	% Lower	Total (N)
1996				
Aug.	67	25	8	12
Sept.	0	0	100	1
Oct.	83	0	17	6
Dec.	0	0	0	0
1997				
Jan.	0	0	0	0
Mar.	86	14	0	7
April	13	13	75	8
May	79	7	14	14
June	52	30	17	23
July	33	22	44	9
Aug.	0	0	100	2
Sept.	0	0	0	0
Oct.	100	0	0	2
1998				
April	40	8	52	25
May	64	21	14	14
June	58	29	13	24
July	21	43	36	14

Table 11. Percentage and number of adult Neosho madtoms captured each month in the upper, mid, and lower portions of the Cottonwood River study site, Chase County, Kansas, 1996-1998. Values are pooled for months sampled twice (May 1997, June and July 1997-98, August and September 1997).

Month	% Upper	%Mid	%Lower	Total (N)
1996				
Aug.	100	0	0	1
Sept.	100	0	0	1
Oct.	100	0	0	1
1997				
Jan.	100	0	0	3
Feb.	100	0	0	4
Mar.	77	8	15	13
April	40	20	40	5
May	80	10	10	10
June	0	0	0	0
July	100	0	0	1
Aug.	0	50	50	2
Sept.	100	0	0	1
1998				
Mar.	0	100	0	1
April	50	0	50	4
May	100	0	0	5
June	8	67	25	12
July	25	75	0	4

Table 12. Sex of Neosho madtoms in study tanks at the ECRC during collection periods (12 June - 23 July 1997), with depth of cavity (mm) under the structure (depth of gravel outside structure - depth of gravel under structure) and mean diameter (mm) of rocks from under structure and from outside structure at end of experiment. Exp.=experimental, M=male, F=female, and ?=unknown sex.

Tank	Control Period	Exp. Period 1	Exp. Period 2	Cavity Depth	Mean Rock Diameter under (SD)	Mean Rock Diameter out (SD)
1	M/F	M/F	M/F	15	15.7 (1.2)	20.0 (2.0)
2	F/F	F/F	F/F	37	12.0 (3.6)	19.3 (7.2)
3	F/F	F/F	F/F	25	16.7 (2.9)	19.0 (4.0)
4	M/F	M/F	M/F	33	13.3 (1.5)	17.7 (5.5)
5	?/?	?/?	?/F	11	13.7 (6.8)	17.7 (3.5)
6	?/?	?/F	F/F	22	15.7 (2.1)	20.7 (4.0)
7	M/F	M/F	M/F	5	20.0 (5.0)	22.3 (3.1)
8	M/?	M/?	M/?	4	23.7 (4.9)	20.0 (5.6)
9	?/?	?/?	M/M	1	19.0 (1.0)	17.0 (3.0)
10	M/F	?/F	M/F	16	17.0 (2.6)	18.0 (1.7)
11	?/F	?/F	F/F	3	14.0 (3.6)	21.7 (5.5)
12	F/F	M/F	F/F	11	16.5 (3.5)	18.0 (1.4)