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

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The Neosho madtom, *Noturus placidus,* is a small catfish listed by the U. S. Fish and Wildlife Service as threatened. Little is known of its breeding biology and behavior because high turbidity and flow during its spawning season prevent direct observation in the field, and captive propagation has met with limited success. We held Neosho madtoms in laboratory aquaria in 1996 and 1998 to study sexual dimorphism during breeding season, courtship and nesting behavior, egg and clutch size, and embryological and larval development. We also attempted to induce spawning. Courtship behaviors were recorded on videotape, including ''carousel'' and ''tail curl'' displays in which the fish spun in circles, head to tail, then quivered, with the male's tail wrapped around the female's head. Three clutches were observed, all in nest cavities that had been excavated by the fish under a structure; one clutch (1996) consisted of approximately 60 eggs, with a mean chorion diameter of 3.1 mm, and two (1998) consisted of approximately 30 eggs, with mean diameter of 3.7 mm. In all situations, eggs hatched after eight or nine days, and yolk-sacs were fully depleted seven days later. One spawn (1998) occurred after two days of injection with synthetic hormone. Male parental care of eggs and larvae was observed in 1996. Larvae remained in the nest until yolk-sacs were absorbed, after which they dispersed throughout the tank. Dissection of two females that laid clutches in this study revealed previtellogenic eggs in the lumen of ovaries, with a mean chorion diameter of 0.9 mm. Swollen lips of males, distended abdomen of females, and differences in head shape, premaxillary tooth patch coloring, and genital papillae of breeding males and females were documented during spawning periods.

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INTRODUCTION

Madtoms (*Noturus*) are a group of small North American catfishes (Ictaluridae). Information about madtom life histories was scarce until concern for these catfishes, several of which are protected, prompted numerous ecological studies in the 1980s (see reviews in Dinkins and Shute, 1996 and Burr and Stoeckel, 1999). No direct observations of spawning behavior have been made in the wild, thus descriptions of breeding behavior within the group are limited. However, clutch size, mean chorion diameter, embryonic development, time to hatching, and larval development and growth have been investigated by transferring clutches from the field to the lab for study or via captive propagation (Clark, 1978; Bowen, 1980; Mayden, Burr, and Dewey, 1980; Burr and Dimmick, 1981; Mayden and Burr, 1981; Burr and Mayden, 1982, 1984; Mayden and Walsh, 1984; Starnes and Starnes, 1985; Vives, 1987; Baker and Heins, 1994; Pfingsten and Edds, 1994; Chan, 1995; Dinkins and Shute, 1996). Although captive propagation has had limited success (Shute, Shute, and Rakes, 1993), it has allowed breeding behavior to be described for the brindled madtom, *N. miurus* (Bowen, 1980), and the brown madtom, *N. phaeus* (Chan, 1995), during laboratory spawns.

Little is known about breeding biology of the Neosho madtom, *N. placidus.* The species occurs only in the Neosho, Cottonwood, and Spring rivers of Kansas, Oklahoma, and Missouri, and it was listed by the U. S. Fish and Wildlife Service (USFWS) as threatened on 22 May 1990 (55 F.R. 21148). Understanding breeding biology and behavior of the Neosho madtom is critical to its recovery (USFWS, 1991); however, high turbidity and flow during its spawning season prevent field observations. Moss (1981) examined museum specimens and characterized the spawning season of this species as beginning in March with egg development and continuing through July, when young-of-year first appear in samples. Sexual dimorphism is present during spawning season; characteristics include reddening of the premaxillary tooth patch and swelling of the genital papilla of males and females, swelling of lips and cephalic epaxial muscles of males, and distention of the abdomen of females (Moss, 1981; Pfingsten and Edds, 1994; Edds and Wilkinson, 1996; Wilkinson and Edds, 1997). However, determining Neosho madtom sex using external characteristics is difficult, even when secondary sex characteristics are well developed (Bulger and Edds, 2001), as in many other madtom species (Burr and Mayden, 1984; Simonson and Neves, 1992). Improved ability to sex Neosho madtoms is necessary to determine sex ratios, to evaluate differences in habitat use between the sexes during spawning season, and to pair individuals for captive propagation.

Previous attempts to induce spawning in captive Neosho madtoms led to discovery of one clutch of 63 eggs deposited under a cinder block in a flowing aquarium; however, these eggs did not develop and may not have been fertilized (Pfingsten and Edds, 1994). We held Neosho madtoms in aquaria at the Columbia Environmental Research Center (CERC), Columbia, Missouri, and Emporia State University (ESU), Emporia, Kansas, to observe spawning behavior, nesting, parental care, clutch size, egg size, and embryonic and larval development and growth, to define characteristics of sexual dimorphism during spawning season, and to investigate use of synthetic hormone to induce spawning.

MATERIALS AND METHODS

1996 ESU

On 16 May 1996, we collected 10 adult Neosho madtoms from the Neosho River, Lyon County, Kansas, and brought them into the lab at ESU. One male and one female were placed into each of three 38-L static aquaria, and two males and two females were placed in a 700-L static aquarium. The bottom of each aquarium was covered with river gravel, and two 13-cm lengths of 10.5-cm diameter PVC pipe, cut in half lengthwise, were added to each aquarium to provide cover, while allowing visual observation. We used aquarium heaters to slowly raise water temperature from 21.5 to 25°C between 23 May and 13 June, and photoperiods were held at a 13.5-h light: 10.5-h dark cycle using timed fluorescent lights. Red lights (25 W) were illuminated prior to the dark cycle to allow for nighttime behavioral observations; Boujard, Morean, and Lugnet (1992) demonstrated that other catfishes displayed normal nocturnal activity under exposure to red light if it was the lowest intensity light throughout the photoperiod. We periodically recombined individuals in an attempt to match males and females that, based on development of secondary sex characteristics, seemed to have the greatest potential to breed. Fish were fed frozen brine shrimp and wild-caught chironomid larvae.

1998 CERC

We collected adult Neosho madtoms from the Cottonwood River, Lyon and Chase counties, Kansas, on 23 June and 7 July 1998 and transported them to the CERC. Individuals were sexed according to development of secondary sex characteristics, and seven male/female pairs were placed in separate 29.5-L aquaria housed in an isolation chamber (Bulger, Wildhaber, and Edds, 2002). The chamber prevented entry of external light and dampened sound disturbances. Each aquarium had an airstone, a standing drainpipe to maintain approximately 20-cm water depth, and inflow from a well, creating a turnover rate of ca. 8.6 L h^{-1}. Gravel was placed on the bottom of each aquarium and a 12.5-cm PVC pipe (10-cm diameter, cut in half lengthwise) was provided for shelter. We held water temperatures between 24 and 28°C using aquarium heaters, and photoperiod was a 16-h light: 8-h dark cycle using timed fluorescent lights mounted in the chamber. Fish were fed frozen brine shrimp six days a week and live blackworms once a week. Infra-red lights and time-lapse video equipment were used to continuously monitor fish behavior (Bulger, Wildhaber, and Edds, 2002). Cavity enhancement and courtship behaviors were defined using the ethogram established by Bulger, Wildhaber, and Edds (2002).

1998 ESU

We collected adult Neosho madtoms from the Neosho and Cottonwood rivers, Lyon and Chase counties, Kansas, from 26 April to 7 July 1998 and transported them to the lab at ESU. Individuals were sexed, paired, and placed in static aquaria ranging from 38-L to 192-L. The bottom of each aquarium was covered with gravel. Each aquarium had an airstone, a flow-through charcoal filter, and structure in the form of large, flat cobble (10 to 25 cm), freshwater mussel halves, half PVC pipes, aluminum cans or combinations of these structures. Fish were held in water temperatures ranging from 24 to 31°C, controlled with aquarium heaters, and in a 16-h light: 8-h dark photoperiod, controlled by overhead lights on a timer. Fish were fed frozen brine shrimp and wild-caught chironomids every 1 to 3 days and, once each week, aquatic insect larvae collected from nearby rivers. Red lights (25 W) were illuminated prior to the dark cycle to allow for nighttime behavioral observations.

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

Sex determination

In addition to recording sexual dimorphism in the Neosho madtoms used during this study, we examined preserved individuals from the ESU study collection for development of secondary sex characteristics, especially the genital opening, then dissected them to determine sex internally. We described and compared differences between males and females with well-developed secondary sex characteristics, as well as individuals without.

RESULTS

1996 ESU

On 8 July 1996, a clutch of approximately 60 viable eggs and eight yolkless membranes was discovered in a 38-L static aquarium with a 61-mm

Table 1. Characteristics of three *N. placidus* clutches from this study compared with data from a previous study (Pfingsten and Edds, 1994).

Clutch	Clutch size	$\bar{x}$ chorion diameter (*SD*, *n*)	Time to hatching, $\bar{x}$ TL (*SD*, *n*)	Time to yolk absorption, $\bar{x}$ TL (*SD*, *n*)	Water temp. °C
ESU 1996	~60	3.1 mm (0.15, 3)	~8 to 9 d 6.8 mm (0.27, 4)	7 d 13.3 mm (0.94, 3)	25.0
ESU 1998	~32	3.7 mm (0.10, 7)	9 d 8.8 mm (0.20, 2)	9 d 13.0 mm (0, 2)	25.0
CERC	~30	—	—	—	28.0
Pfingsten and Edds (1994)	63	3.1 mm (0.20, 10)	—	—	26.5

total length (TL) female and a 78-mm TL male. Water temperature in the tank was 25°C at the time the eggs were discovered. The eggs had been laid in a gravel depression under the half PVC pipe. Gravel had been manipulated by one or both adults to form a depression to the glass tank bottom and was piled against the open ends of the PVC pipe, almost entirely blocking the entrances to the nest. After the male was observed chasing the female from the nest, she was removed from the tank. The male remained in the nest with the clutch and was observed fanning the eggs with his tail and rubbing them vigorously with his mouth and body.

Three eggs were removed from the tank and placed in a 1.9-L glass aquarium with an airstone. At the time of discovery, the eggs had chorion diameters of 3.3 mm, 3.0 mm, and 3.1 mm (Table 1), and the embryos already were undergoing organogenesis, with their tails separated from their yolk sacs and moving regularly from side to side. Rudimentary optic and otic vesicles were observed in the head of embryos and myomeres were visible in the tail. Based on embryonic development in *Noturus exilis* (Mayden and Burr, 1981) and *N. hildebrandi* (Mayden and Walsh, 1984), the embryos appeared to be approximately 72 hours old at the time of discovery.

During the next four days, we video recorded daily observations of the three isolated eggs under a dissecting microscope. Development was rapid; on 10 July (Day 6 embryo) eyes had developed further and vitelline vessels were present, but the eggs had become infected with fungus. We treated the eggs by immersing them in a solution of 1.9 ml formalin/3.79 L water for five minutes. After treatment, eggs were suspended in a small plastic basket over the airstone to receive constant aeration. On the morning of 11 July (Day 7 embryo) distinct pupils in the eyes, pectoral fin buds, rudimentary barbels, and rhythmic opercular movements were observed in all embryos. In addition, tail movements were less regular and much slower. On 12 July (Day 8 embryo), the embryos appeared crowded in their eggs and exhibited little movement except for periodic shuddering, during which they flexed their tails back and forth vigorously. Opercular movements and blood flow in the vitelline veins were continuous. Two of these three eggs hatched on 12 July. The eggs that had developed in the nest with the male began hatching on 13 July, and all had hatched by the morning of 14 July. We suspect that handling of the three eggs used for observation resulted in premature hatching of those eggs (Burr and Dimmick, 1981).

We recorded the hatching events on video and made measurements of the yolk-sac larvae at time of hatching, using National Institutes of Health imaging software. Total lengths of four larvae at hatching were 6.41 mm, 6.92 mm, 6.96 mm, and 6.99 mm ($\bar{x}$ = 6.82 mm, *SD* = 0.27), and few melanophores were visible on the heads. Growth and development of larvae were rapid. On 15 July (Day 3 larva), two larvae had total lengths of 11.4 mm and 12.8 mm. They had melanophores over the front half of their body, pelvic fins were beginning to form, and their yolk sacs were noticeably reduced in size. On 20 July (Day 7 larva), larvae had a mean TL of 13.3 mm (*SD* = 0.94, *n* = 3), their dorsal side was covered with melanophores, and the yolk sac was entirely depleted in most individuals.

Approximately half of the larvae were kept in a separate 38-L static aquarium (‘‘nursery tank’’). The larvae in this tank were observed first eating recently hatched brine shrimp nine days after hatching. They were subsequently fed brine shrimp and crushed frozen chironomid larvae twice daily for about 30 days. Larvae left in the breeding tank were fed brine shrimp and chironomid larvae sporadically, but we made periodic introductions of river water and insects into the tank to provide a supplemental food source. After hatching, the yolk-sac larvae left in the breeding tank remained in the nest, forming a loose aggregation. Although we did not observe the male fanning the larvae as he had the eggs, he often was observed hovering over them. Approximately 8 to 10 days after hatching (21 July to 23 July), yolk-sacs were absorbed, and larvae dispersed throughout the tank and burrowed into the gravel.

Growth rates we observed in the lab may not be typical, because different growth rates occurred in the two tanks (Wilkinson and Edds, 1997). Larvae kept in the nursery tank were fed brine shrimp and chironomid larvae more often and grew more rapidly than those left in the breeding tank. On 31 July, mean TL of larvae in the nursery tank was greater than that in the nesting tank ($F_{1,32}$ = 18.7, P = 0.0001).

1998 CERC

At CERC, from late June to mid July, male and female pairs were observed via time-lapse video ‘‘carouselling,’’ or swimming in circles head to tail over the gravel substrate under the PVC structure (Fig. 1A). This behavior was followed typically by the ‘‘tail curl,’’ in which individuals lay

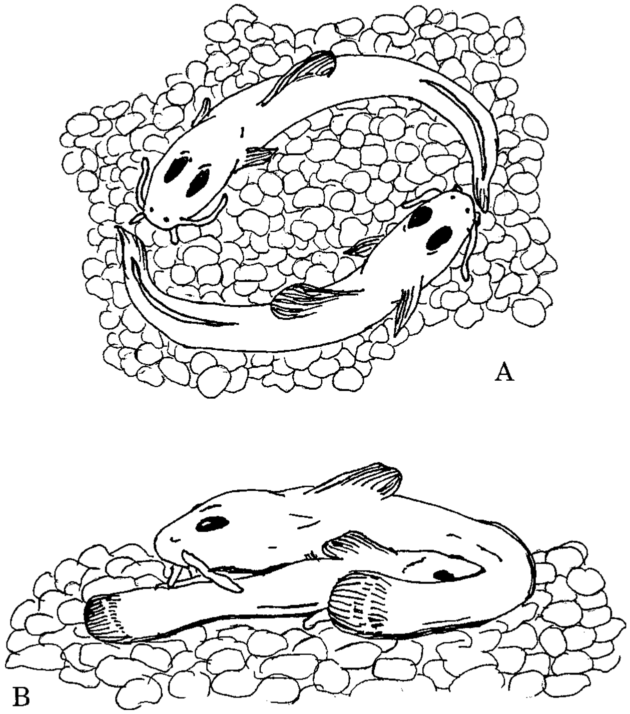

Figure 1. A. ‘‘Carousel,’’ courtship behavior of Neosho madtom. Male and female swim in circles head to tail near substrate. B. ‘‘Tail curl,’’ courtship behavior of Neosho madtom. Male and female lie above substrate with tail of male wrapped around head of female while both quiver.

with the male’s tail wrapped around the female’s head (Fig. 1B). In this position the two would quiver slightly then separate. After separation, the process was repeated frequently, beginning with the ‘‘carousel,’’ although occasionally the female was chased from under the structure. Cavity enhancement behaviors (Bulger, Wildhaber, and Edds, 2002) also were observed: males and females nudged rocks with their heads, removed rocks in their mouths, and spun in circles alone over the gravel substrate under the PVC structure. Specific details of cavity enhancement, including depth of cavity and mean rock size within cavities, were described by Bulger, Wildhaber, and Edds (2002).

On 18 July, a clutch of approximately 30 eggs was discovered in a tank containing a male (61 mm TL) and female (59 mm TL) that had been captured on 23 June; water temperature was 28°C (Table 1). Eggs were spherical, with a yellow yolk in the center surrounded by a clear chorion, and they were adhered to one another in a cluster. Both male and female

Table 2. Ovary length and number of eggs in three reproductive female *N. placidus.* CERC and ESU individuals had bred and eggs in ovaries were previtelline. Specimen from ESU teaching collection was a gravid female, and eggs contained yolk.

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

were present with the egg mass under the PVC structure at the time of discovery, but the female was removed from the tank when the male was observed biting and chasing her away after she ate approximately eight eggs. Following removal of the female, the male rested near the eggs, hovered over them, and fanned them with his tail. The eggs disappeared between the second and third day, at which time the male had a distended abdomen; it is presumed he ate the eggs.

Gravel pushed against the front of the tank by the fish and the position of the fish in a depression (nest) under the structure prevented detailed observation of spawning. Unlike other *N. placidus* observed in captivity (Bulger, Wildhaber, and Edds, 2002), video record showed that the pair spent the majority of that day in the nest together and were active, performing the ‘‘carousel’’ followed by the ‘‘tail curl,’’ which lasted from 1 to 7 min, several times throughout the day. When resting, the two usually lay side by side, often touching. After dark (2122 h), one individual, presumably the male, remained in the nest and the other, presumably the female, left and returned to the nest several times. As night progressed, that individual left the nest less frequently, and circles made during the ‘‘carousel’’ became smaller and faster. Beginning at approximately 0300 hours, while both fish were under the structure ‘‘carouselling’’ and resting, the tail of one was seen flipping above the substrate of the nest. This was observed until the female was chased from the nest shortly after the lights came on in the chamber at 0524 h. Several times prior to discovery of the clutch, the female returned to the nest and was chased away by the male. Dissection of the female revealed two, 9.0-mm long, slightly pink ovaries containing 13 and 17 white, previtelline eggs, with a mean chorion diameter of 0.8 mm (Table 2).

1998 ESU

At ESU, on 14 July, after one preliminary and two full dosage injections of Ovaprim®, a clutch of eggs was discovered in a 38-L aquarium; water temperature was 27°C. The clutch consisted of two clusters of eggs, one of 14 and one of 18. Mean chorion diameter was 3.7 mm (*SD* = 0.10, *n* = 7) (Table 1); perivitelline space was approximately 0.4 mm. Eggs were spherical, with a yellow yolk in the center surrounded by a clear chorion and, except for division of the two clusters, adhered to one another. Both the male (73 mm TL) and female (67 mm TL) had been captured on 7 July. They were resting near the eggs in a slight cavity or depression (nest) in the gravel under the only cover in the tank, a flat rock (8 × 14 cm). After discovery of the eggs, the female was removed and examined. Her urogenital pore was brown with coagulated blood, rather than white, as before, and her abdomen was not as distended as it had been; dissection revealed ovaries that contained 21 and 23 white, previtelline eggs, with a mean chorion diameter of 0.9 mm (*SD* = 0.11, *n* = 10) (Table 2).

The 14-egg cluster was removed and placed in a hatching apparatus; the 18-egg cluster was left in the nest with the male. By Day 3, however, it was apparent the eggs in the nest were not being cared for; eight eggs had clouded, indicating embryonic development had ceased. By Day 3, nine eggs in the hatching apparatus had broken or clouded, so all remaining eggs were removed and placed in a watch glass with an airstone; temperature varied from 21 to 27°C. By Day 4, only two eggs contained a developing embryo. Beginning on Day 5, these eggs were treated for fungus daily by immersing them in 0.5% formalin for 2 to 7 min.

Based on development of *N. exilis* (Mayden and Burr, 1981) and previous observations of *N. placidus* from 1996, the eggs were estimated to be less than 10 h old at the time of discovery. Cleavage was in progress, the animal pole consisted of approximately 18 cells (blastomeres) in a small cluster (Fig. 2A), and the chorion was clear, with a rough surface. By approximately 24 h post-fertilization, approximately 35 blastomeres at the animal pole had extended into the perivitelline space (Fig. 2B). At approximately 92 h post-fertilization, organogenesis had begun. A head, with pronounced eye cups, was present and partially separated from the yolk; the tail also was separated from the yolk, and it whipped back and forth in the perivitelline space. Somites were differentiated from just posterior of the head to the tip of the tail, and vitelline veins extended on the yolk from either side of the fish (Fig. 2C). Approximately 120 h post-fertilization, the head was larger, eye lenses were developed, and the spinal cord was visible from the head to the tip of the tail (Fig. 2D). At approximately 140 h, opercles were beginning to form at the base of the head, as were barbels near the mouth (Fig. 2E). At approximately 165 h, somite differentiation throughout the length of the body was no longer as distinct, opercles were better defined, pectoral spines were developing at the base of the head, and fin-forming caudal ray primordia were present at the tip of the tail (Fig. 2F). Approximately 190 h post-fertilization (estimated 10 h prior to hatching), barbels were well de-

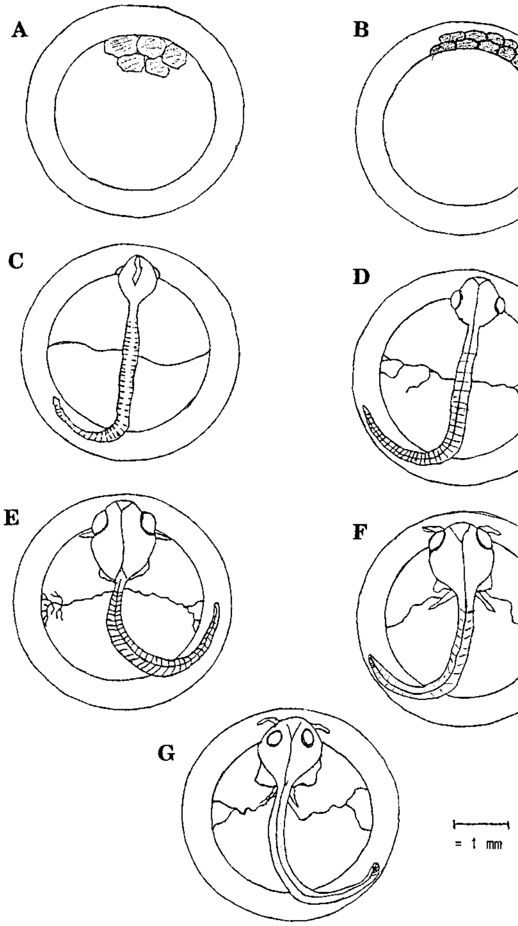

Figure 2. Development of Neosho madtom eggs laid 14 July 1998. A and B are lateral views, C through G are dorsal views. A = Day 1 (10 h), B = Day 2 (24 h), C = Day 4 (92 h), D = Day 5 (120 h), E = Day 6 (140 h), F = Day 7 (165 h), G = Day 8 (190 h).

veloped, eyes were more dorsally situated on the head, and pectoral fins were better defined (Fig. 2G); however, eggs were covered with fungus by this time, making observation difficult. On the morning of 22 July (approximately 200 h post-fertilization, Day 9), in water 25.5°C, both eggs hatched. One individual was 9.0 mm TL and the other was 8.6 mm TL. Both were light yellow with black eyes and had a well-developed yolk sac (Fig. 3). Following hatching, larvae sought cover in gravel added to the dish. Yolk sacs were depleted by 30 July, when individuals were 13.0 mm TL. Stellate melanophores were spread over the body, and pelvic fins were completely developed by the time of yolk sac depletion, 9 days after hatching.

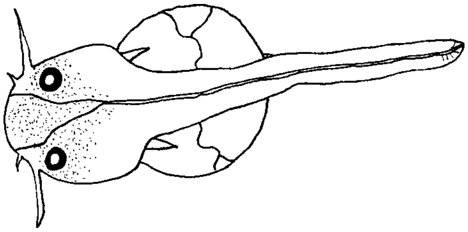

Figure 3. Larval Neosho madtom on day of hatching, 22 July 1998; TL = 9.0 mm. Two pairs of barbels are present, pectoral spines are beginning to develop, and vitelline veins are visible on yolk sac.

Twice on 14 July and once on 15 July, it was observed that the female of a breeding pair was spent, her belly no longer distended, as it had been the day before, her pore was red or brown, rather than white, and that the male had a large, full belly. In such situations, it was presumed the male had eaten all of the eggs laid by the female, although the female also might have eaten some eggs.

Sex determination

Neosho madtoms at CERC and ESU developed secondary sex characteristics. Distinct differences were observed in the heads of breeding individuals; males developed swollen cephalic epaxial muscles on broad, flat heads with swollen lips, whereas the heads of females remained conical in shape and lips did not swell, similar to those of nonbreeding individuals. Red or pink premaxillary tooth patches were present in both sexes, but usually were brighter red in males. Gravid females had distended abdomens. The genital papilla in males became elongated and swollen. Tissues adjacent to the genital papilla in females swelled, and the vent became swollen and rounded (Fig. 4).

Neosho madtom genital papillae (Fig. 4) were drawn from representative preserved individuals in the ESU teaching collection; differences depicted generally were difficult to determine on live specimens. Dissection of five preserved specimens (56, 58, 59, 65, and 68 mm TL) that appeared gravid revealed enlarged ovaries ($\bar{x}$ ovary length = 14.9 mm, *SD* = 1.20, *n* = 10), containing amber-colored eggs. The ovaries of two of these individuals were dissected and inspected for eggs. The left and right ovaries, both 16 mm in length, of the 68 mm TL individual contained 39 and 40 yellow eggs, respectively, with a mean chorion diameter of 2.5 mm (*SD* = 0.19, *n* = 10) (Table 2). One other individual (65 mm TL), had a 16-mm ovary containing eggs with a mean chorion diameter of 2.6 mm (*SD* = 0.12, *n* = 3). The number of these larger eggs could not be determined because they fell apart when disturbed within the ovary. Approximately 31 small, white eggs, with

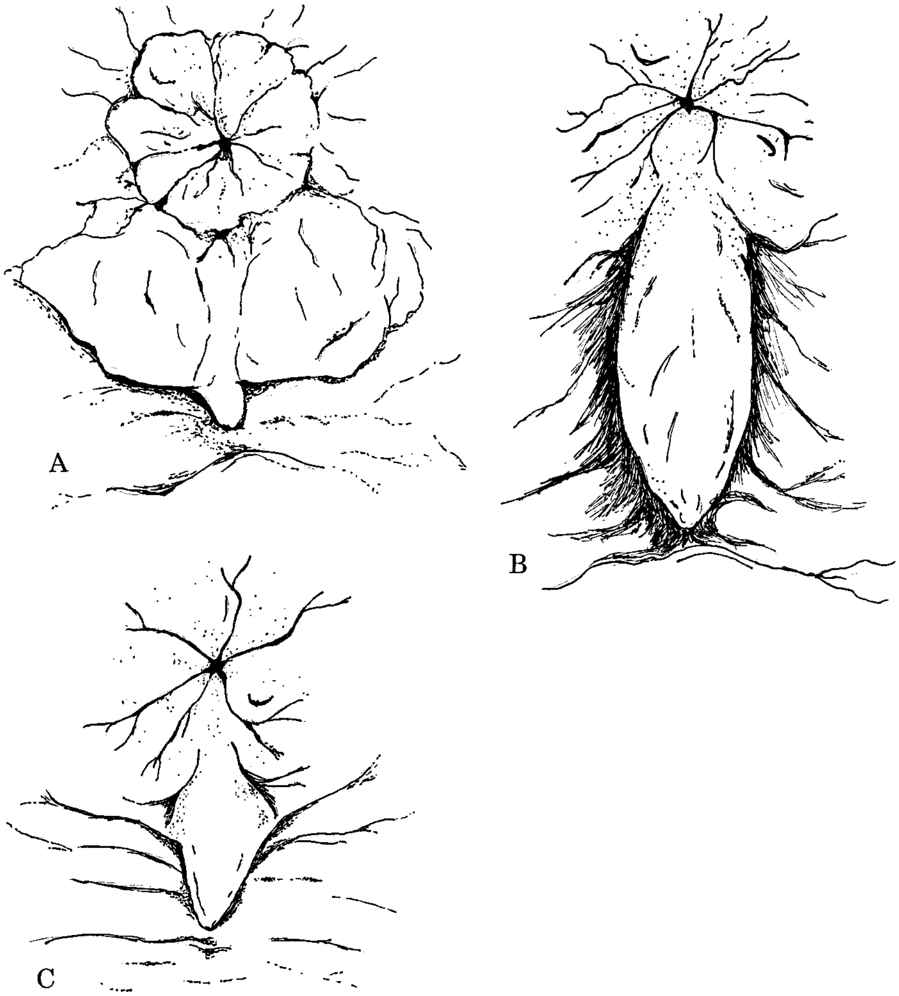

Figure 4. Genital papillae of preserved Neosho madtoms: A, breeding female; B, breeding male; C, nonbreeding individual.

mean diameter of 0.7 mm (*SD* = 0.13, *n* = 10), were observed in the lining of the ovary. The other ovary (12 mm) contained only small, white eggs in the lining; mean chorion diameter was 0.8 mm (*SD* = 0.08, *n* = 13).

DISCUSSION

There is a paucity of information about courtship behaviors among *Noturus* species. They, however, may be similar as a result of evolutionary constraints within the lineage (Mayden and Walsh, 1984; Burr and Stoeckel, 1999). The successful spawning of a single pair of Neosho madtoms in the ESU lab during the summer of 1996 allowed the first direct observation of reproduction in the species. In 1998, at CERC, courtship behaviors were recorded and, at ESU, two more clutches were observed. In general, the results of our studies indicate that *N. placidus*, similar to other madtom species, is a cavity spawner, with male parental care of eggs and fry and rapid development of embryos and larvae. After hatching, we observed larvae in the nest, and they formed a loose aggregation until yolk sacs were depleted (13 mm TL). Burr and Stoeckel (1999) reported that, within the genus *Noturus*, most young are guarded in the nest until they are 12–15 mm TL. We also observed both male and female *N. placidus* performing nest building or cavity enhancement activities, behaviors that have been observed in other madtom species as well.

The ‘‘carousel’’ and ‘‘tail curl’’ observed in this study were identical to behaviors described for the brindled madtom (Bowen, 1980) and the brown madtom (Chan, 1995) and have been referred to by other researchers as ‘‘mutual caudal embrace’’ and ‘‘male-only caudal embrace,’’ respectively (Burr and Stoeckel, 1999). Other ictalurids, such as the channel catfish (*Ictalurus punctatus*), brown bullhead (*Ameiurus nebulosus*), and flathead catfish (*Pylodictis olivaris*), have breeding behaviors similar to those observed in *N. placidus* (Breder and Rosen, 1966). Breeding behavior described by Breder (1935) for the brown bullhead was similar to that observed in *N. placidus*; in each, the ‘‘carousel’’ and ‘‘tail curl’’ behaviors were repeated several times prior to spawning.

The three clutches laid in captivity in 1996 and 1998 were similar to each other in appearance. At ESU in 1996, the clutch was laid in a depression that had been made in gravel under a PVC structure at a water temperature of 25°C. The clutch at ESU in 1998 was laid in a depression that had been made in the gravel under a large flat stone at a water temperature of 27°C. At the CERC the clutch was laid under a PVC structure in a slight depression on a gravel bottom at 28°C. In all examples, eggs adhered to each other but not to the substrate, similar to clutches described by Pfingsten and Edds (1994) for *N. placidus* and by Mayden and Burr (1981) for *N. exilis*, the slender madtom. Clutch size (30 to 32 eggs) differed only slightly between the two spawns in 1998, but were smaller than the ca. 60 observed in 1996 and the 63 reported by Pfingsten and Edds (1994). Other *Noturus* species have been reported to have clutch sizes ranging from 14 (*N. leptacanthus*, the speckled madtom; Clark, 1978) to 124 (*N. exilis*; Burr and Mayden, 1984; Burr and Stoeckel, 1999). Mean chorion diameter of eggs in 1998 (3.7 mm) was slightly larger than that of 3.1 mm observed in 1996 and that reported by Pfingsten and Edds (1994). Because clutch size and egg size in fish are related inversely (Jobling, 1995), larger egg size in 1998 could be associated with smaller clutches. However, it is possible that the male or female ate some eggs before they were discovered. Another possibility is that the clutches were not the first to be laid by these females that year, as these fish were collected later in the breeding season (23 June and 7 July) than those collected in May 1996 and by Pfingsten and Edds (1994), who also collected fish in May. Mean chorion diameters observed in our study (3.1 and 3.7 mm) are consistent with that reported by Burr and Stoeckel (1999) for *Noturus* species (3.6 mm).

Several studies have suggested polyandry in *Noturus* species (*N. exilis*, Mayden and Burr, 1981; *N. nocturnus*, the freckled madtom, Burr and Mayden, 1982; *N. hildebrandi*, the least madtom, Mayden and Walsh, 1984; *N. phaeus*, Chan, 1995; *N. baileyi*, the smoky madtom, and *N. flavipinnis*, the yellowfin madtom, Dinkins and Shute, 1996), but this mating strategy has not been confirmed. In our study, eggs remaining in the ovaries of both females were smaller than fertilized eggs or those observed in gravid museum specimens, and had not yet undergone vitellogenesis. The presence of less developed eggs in the ovaries of females is consistent with the hypothesis that multiple clutches may be laid in one season (Mayden and Burr, 1981) and consistent with a hypothesis of a polygamous mating strategy, which could include polyandry, as suggested by Burr and Stoeckel (1999). Alternatively, these eggs could be laid the following year (Baker and Heins, 1994); however, field data suggest most Neosho madtoms do not live to Age 2 (Edds and Wilkinson, 1996; Bulger and Edds, 2001).

In 1996, the male Neosho madtom was observed caring for the clutch of eggs, including rubbing them with his head and belly and fanning them with his tail. These behaviors were seen until hatching, at which time the larvae remained in the nest with the male hovering over them until yolk sacs were depleted (13 mm TL). Rubbing, fanning, and hovering behaviors also have been noted in male brown madtoms (Chan, 1995), slender madtoms (Mayden and Burr, 1981), additional madtom species (Burr and Stoeckel, 1999), and other ictalurid species (Breder and Rosen, 1966). The clean and healthy appearance of the eggs that were cared for by the male, relative to those that were kept isolated, suggests that the attention provided by the male kept them from becoming fouled or infected with fugus. It is not clear whether the male consumed any of the eggs in 1996. The male was not observed taking eggs into his mouth, but we counted 15 to 20 eggs fewer at the time of hatching, which suggests that he might have eaten some. During the time the male was guarding the nest, we saw him eat on only one occasion, when some frozen brine shrimp came to rest at the edge of the nest. Although we continued to introduce food into the nesting tank, the male did not regularly leave the nest to feed while he was guarding the eggs or larvae until about one week after the larvae left the nest.

The reason for lack of male parental care in 1998 and consumption of eggs by the male at CERC is unknown; however, males of other madtom species have eaten egg masses in captivity (e.g., *N. leptacanthus,* Clark, 1978; *N. miurus,* Bowen, 1980; *N. insignis,* the margined madtom, J. Stoeckel, pers. comm.), as four males are believed to have done in this study. Perhaps males ate the eggs because of stress caused by captivity or, in 1998, by the hormone injection. It also is possible that the male at the CERC ate the clutch of eggs because they were not fertilized, although they appeared to have been. It is possible that the fish were simply hungry; however, food was readily available in all aquaria. Regardless of the reason or reasons, the behaviors seen in this study suggest that, in efforts at captive propagation, at least a portion of the egg mass should be removed from the nesting cavity and hatched separately, as was done in our study. Success rate using this method was low in 1998, most likely because of excessive agitation in the hatching apparatus, temperature fluctuation, and fungal infection. Others have reported low success rates in rearing madtom eggs (*N. baileyi,* Shute, Shute, and Rakes, 1993; *N. phaeus,* Chan, 1995; J. Stoeckel, pers. comm.). It has been suggested that loss of eggs from fungus is controlled in the wild by parental care (Breder, 1935; Fontaine, 1944). Our efforts in the lab, however, indicated that parental care in captivity is generally lacking and consumption rates are high.

In 1996, the breeding female *N. placidus* was removed from the breeding tank after the male was observed chasing her from the nest. In 1998, the female at the CERC was removed from the tank after she was observed eating eggs and being chased from the nest by the male. Removal of the female after spawning is recommended for survival of the clutch in other ictalurids (Breder and Rosen, 1966); however, it is not known whether the female plays a role in survival of the eggs early in their development. Mayden and Burr (1981) noted that female slender madtoms remain in the nests from between 12 to 22 hours after spawning, and in 1996 we did not remove the female from the spawning aquarium until approximately 72 h post-spawning and had higher success hatching eggs, compared to attempts in 1998.

Egg development, hatching, and yolk resorption proceeded much as described previously for other madtoms (*N. exilis,* Mayden and Burr, 1981; *N. hildebrandi,* Mayden and Walsh, 1984; *N. eleutherus,* the mountain madtom, Starnes and Starnes, 1985; *N. baileyi* and *N. flavipinnis,* Dinkins and Shute, 1996; Burr and Stoeckel, 1999). One difference was the earlier appearance of the vitelline veins in 1998. During the 1996 breeding study, development of vitelline veins was observed on Day 6 (ca. 144 h post-fertilization), but, in 1998, vitelline veins were visible approximately 92 h post-fertilization. Mayden and Burr (1981) reported the appearance of vitelline veins 102 to 104 h after fertilization in *N. exilis,* and Mayden and Walsh (1984) observed their formation by 130 h post-fertilization in *N. hildebrandi.* The slight differences in development rates observed in our study could be attributed to higher temperatures in 1998 (28°C) than in 1996 (25°C). In addition, larval Neosho madtoms were observed breaking through the chorion head first in contrast to observations of other madtoms hatching tail first (Burr and Stoeckel, 1999). The behavior of hatchling Neosho madtoms was consistent with that of other madtoms in that they schooled in the nest until their yolk sacs were absorbed, and they exhibited negative phototaxis and positive geotropism (Burr and Stoeckel, 1999). On Day 10 post-hatching, when their yolk sacs were absorbed completely, juveniles dispersed throughout the gravel in the aquarium. This dispersal behavior seems to be an important life history trait, also documented by field observations that young-of-year Neosho madtoms tend to favor loosely compacted gravel substrate (Bulger and Edds, 2001), within which they presumably find refuge. However, behavior and distribution of young after leaving the nest is a topic that requires further study for *N. placidus* and other madtoms (Burr and Stoeckel, 1999).

Accurate sexing of *N. placidus* is easier when secondary sex characteristics are well developed, usually in late May through mid July (Bulger and Edds, 2001). During breeding season, secondary sex characteristics were similar to those previously described for the Neosho madtom (Moss, 1981; Pfingsten and Edds, 1994; Edds and Wilkinson, 1996). Differences between sexes were seen in head shape, lip size, redness of tooth patches, abdomen distention, and genital papillae. Other authors have described similar secondary sex characteristics in madtom species (*N. exilis,* Mayden and Burr, 1981; *N. nocturnus,* Burr and Mayden, 1982; *N. flavater,* the checkered madtom, Burr and Mayden, 1984; *N. hildebrandi,* Mayden and Walsh, 1984), and these are consistent with those reported by Burr and Stoeckel (1999). Burr and Mayden (1982) and Dinkins and Shute (1996) reported color change in males during the breeding season in *N. exilis* and *N. baileyi,* respectively. In our study, no obvious color changes occurred in *N. placidus* during breeding season.

Sneed and Clemens (1959) indicated that one to seven injections of human chorionic gonadotrophin are required to stimulate spawning in channel catfish. At ESU, in 1998, one successful spawn and three suspected spawning events occurred after three or four days of injection with Ovaprim®. Success rate using hormone injection was low (4 out of 15), even when dosage was increased and injections were administered over a 12-d period. It should be noted, however, that injections were administered late (12 July), probably after the peak of spawning season. It is important to inject before ovulation, because later hormone injections will not be as effective (J. Stoeckel, pers. comm.).

Our observations of *N. placidus* reproductive behavior are consistent with the conclusion of Burr and Stoeckel (1999) that madtoms exhibit a suite of ancestral behaviors common among ictalurids and exhibit other behaviors that have evolved independently in the madtom and flathead catfish clade described by Lundberg (1992). Our research has added to the knowledge of *N. placidus* reproductive biology and behavior, but many questions remain. The role of the female in parental care is unknown, as is mating strategy; if polygamous, is it polyandrous or polygynous? Breeding habitat in nature remains to be discovered, and environmental variables that trigger breeding are unknown, as is the number of clutches laid in a lifetime. Although observation of *N. placidus* spawning behavior in our study occurred in captivity, such courtship behavior is likely representative of behavior in natural conditions; Porterfield (1998) reported similar spawning behavior in lab and natural settings for eight species of darters (*Etheostoma*). Lab investigations of small secretive fishes in turbid water can be important to understanding their breeding biology and behavior.

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