[Article]

Examining Neosho Madtom Reproductive Biology Using Ultrasound and Artificial Photothermal Cycles

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Abstract.—We examined whether extended laboratory simulation of natural photothermal conditions could stimulate reproduction in the Neosho madtom *Noturus placidus*, a federally threatened species. For 3 years, a captive population of Neosho madtoms was maintained under simulated natural conditions and monitored routinely with ultrasound for reproductive condition. Female Neosho madtoms cycled in and out of spawning condition, producing and absorbing oocytes annually. Internal measurements made by means of ultrasound indicated the summer mean oocyte size remained consistent over the years, although estimated fecundity increased with increasing fish length. In the summer of 2001, after 3 years in the simulated natural environment, 13 out of 41 fish participated in 10 spawnings. Simulation of the natural photothermal environment, coupled with within-day temperature fluctuations during the spring rise, seemed important for the spawning of captive Neosho madtoms. The use of ultrasound to assess the reproductive status in Neosho madtoms was effective and resulted in negligible stress or injury to the fish. These procedures may facilitate future culture of this species and other madtoms *Noturus* spp., especially when species are rare, threatened, or endangered.

Captive breeding programs provide knowledge of the reproductive biology of rare species and assist in the recovery of endangered and threatened fish species (e.g., bonytail chub *Gila elegans*, humpback chub *Gila cypha*, razorback sucker *Xyrauchen texanus*, and Colorado pikeminnow *Ptychocheilus lucius*; see Hamman 1982a, 1982b, 1985, 1986). To increase the knowledge base, we investigated the effects of natural photothermal cycles on the reproductive cycles of a captive population of Neosho madtoms *Noturus placidus*.

The Neosho madtom is one of five madtoms *Noturus* spp. included on the U.S. list of threatened and endangered species (USFWS 1991; Burr and

Stoeckel 1999). They are endemic to the Neosho River basin in Kansas, Missouri, and Oklahoma, and are mostly found at main-stem gravel bars (Taylor 1969; Moss 1983; Fuselier and Edds 1994; Bulger and Edds 2001).

As in other madtoms, spawning occurs May through July as temperatures approach 25°C (Pfingsten and Edds 1994; Bulger and Edds 2001; Bulger et al. 2002b). Nests are constructed under large objects in the gravel (Bulger et al. 2002a, 2002b). Observations of female parental care in madtoms are rare (Burr and Stoeckel 1999); only male parental care has been observed in Neosho madtoms and it lasts for 8–9 d after spawning (Bulger et al. 2002b). Short life spans of Neosho madtoms restrict reproduction to one or two spawning seasons (Fuselier and Edds 1994; Bulger and Edds 2001).

The use of ultrasound for sexing large common fish species is not new (Martin et al. 1983) and has recently been used on threatened and endangered species (Moghim et al. 2002; Columbo et

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al. 2004; Wildhaber et al. 2005). Ultrasound has been especially useful in identifying gravid females in fish species with nondescript sexual characteristics (Mattson 1991; Blythe et al. 1994; Karlsen and Holm 1994; Martin-Robichaud and Rommens 2001). Even though madtoms are sexually dimorphic during part of the year (Pfingsten and Edds 1994; Bulger and Edds 2001), researchers have had difficulty distinguishing madtom gender (Bulger et al. 2002a). In addition to gender identification, ultrasound techniques have been used to measure internal reproductive structures, such as oocyte diameter and gonad size (Mattson 1991; Blythe et al. 1994; Martin-Robichaud and Rommens 2001). Standard oocyte collection techniques disrupt female maturation in margined madtoms *N. insignis* (Stoeckel and Neves 2001), so alternative techniques such as ultrasound need to be examined for their potential use on madtoms. Because madtoms are difficult to sex, obtain oocyte samples, and many species are threatened or endangered (Burr and Stoeckel 1999; Bulger et al. 2002a), we employed ultrasound techniques to help us better understand the Neosho madtom reproductive cycle.

Photothermal cycles influence the reproductive chronology of madtoms (Stoeckel 1993; Burr and Stoeckel 1999). Bulger et al. (2002a) showed that greater photoperiod length increases the amount of Neosho madtom reproductive behavior. Stoeckel (1993) also found that margined madtoms exposed 1 year to a natural photothermal cycle in the laboratory had a similar gonadosomatic index pattern as wild fish. Not known is whether exposure to successive years of artificial photothermal cycles will induce yearly gonadal maturation and spawning of captive populations of Neosho madtoms. For the Neosho madtom and other endangered madtom species, knowing how environmental cues influence reproduction (and having a technique to assess reproductive status) will help propagate and manage these populations. The objectives of this study were to examine (1) the use and applicability of ultrasound to assess the reproductive status of Neosho madtoms, and (2) whether simulated natural photothermal cycles can trigger gonadal development and spawning of the Neosho madtom in successive years.

Methods

Laboratory conditions.—Neosho madtoms were collected from Kansas sections of the Neosho (*n* = 24) and Cottonwood rivers (*n* = 34) in the summer of 1998 (Bulger et al. 1998). These fish were assumed to be 1-year-olds because of their small size. They were maintained by Bulger et al. (1998) in 1998 and by us from January 1999 until August 2001. Fish were kept in a 720-L Living Stream (Frigid Units, Inc., Toledo, Ohio, Model LS-900) in a photoperiod- and temperature-controlled room. Males and females were initially sexed based on their external secondary sexual characteristics (Bulger et al. 2002b) and were separated in the Living Stream. Fish were fed to satiation 3 times/week with frozen brine shrimp *Artemia* spp., frozen bloodworms *Chironomus* spp., or Hikari Sinking Carnivore Pellets (Kyorin Company, Ltd., Himeji, Japan). Excess food was removed routinely to prevent water fouling.

Photothermal cycles.—Laboratory photoperiods mimicked the times of sunrise and sunset for Emporia, Kansas (38°26′N, 96°12′W), near the location where the fish were collected (Figure 1; U.S. Naval Observatory 1999–2001). Illumination was provided by eight General Electric (GE) Cool White 34-W fluorescent bulbs suspended 0.6–1.4 m from the water surface. Lights were controlled by a Precision time switch (Precision Multiple Controls, Inc., Midland Park, New Jersey, Model CD103).

To simulate natural thermal cycles, water temperatures in the Living Stream were regulated with a 0.746-kW water heater–chiller (Frigid Units, Model D1-100, 3000-W heater). In the individual aquaria, we used 300-W Visi-Therm aquarium heaters (Aquarium Systems, Inc., Mentor, Ohio) to regulate water temperatures. Water temperatures mimicked monthly Neosho River temperatures recorded from August 1996 to October 1997 and from April to July 1998 (Figure 1; Bulger et al. 1998), except the minimum winter temperature of 9°C (Figure 1) was warmer than the river winter low of 0°C because of chiller limitations. In 1999 and 2000, once water temperatures reached 25°C, we varied the laboratory within-day water temperatures to simulate natural daily fluctuations (Figure 1, inset). They were varied daily in 1999 between an evening low of 22°C and a daily high of 25°C (morning increase at 1100 hours and evening decrease at 2300 hours), and in 2000 they were varied between 23°C and 25°C. We also varied temperatures in 2001 during the spring rise starting at 22°C (Figure 1). During the 4-week spring rise, the respective daily temperature ranges were 21–23°C, 21–24°C, 21–25°C, and 21–26°C. After the 4th week, the temperature ranged between 21°C and 25°C. Daily temperature fluctuations were continued until fish ceased reproductive activity.
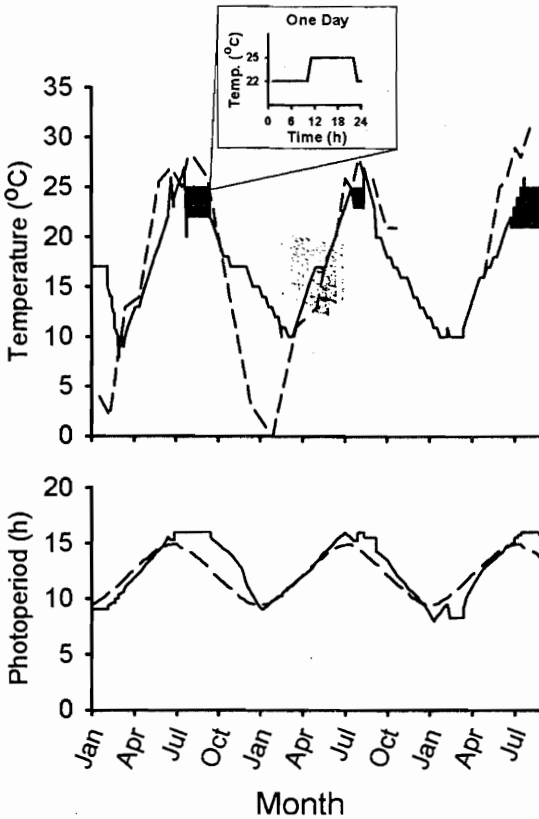
FIGURE 1.—Laboratory (solid lines) and corresponding natural (dashed lines) temperatures and photoperiods for the years 1999 through 2001 during a laboratory assessment of the reproductive behavior of Neosho madtoms from the Neosho and Cottonwood rivers in Kansas. The inset gives an example of a daily temperature fluctuation during the spawning season.

Fish spawning.—At the beginning of each laboratory spawning season, males and females that exhibited prominent secondary sexual characteristics were paired and placed into separate aquaria. Each pair was provided with an inverted half-section of polyvinyl chloride pipe (1999: 13.3 cm long × 10.2 cm wide × 5.0 cm high; 2000: 12.7 cm long × 14.0 cm wide × 6.4 cm high). Pairings were maintained until all reproductive activity ceased. If a pair spawned, the female was replaced with another gravid female. Because of the nocturnal nature of Neosho madtoms, we recorded all activities with video cameras. For each videotaped spawning, the number of eggs per clutch was determined by measuring the chorion diameters of all visible eggs in the clutch (approximately 50–75) with Optimas image analysis software (Media Cybernetics 1999). We then calculated each egg's area ($\pi[\text{diameter}/2]^2$). Clutch size was estimated by calculating the total area occupied by all layers that comprised the egg mass and dividing the total area by the mean egg area for that clutch. After spawning, females were examined with ultrasound (see Ultrasound Examinations) to determine whether or not they had expelled all eggs.

Length and weight measurements.—We assessed the size and reproductive status of the fish in our captive population of 2-year-old and older Neosho madtoms. During the summer and winter of 2000 and 2001, we measured the lengths and weights of either all fish or a random subsample (Table 1). We did not include fish measured in summer 1999 because this subsample consisted of only the largest fish (Table 1).

TABLE 1.—Number of Neosho madtoms during each length–weight examination during a laboratory assessment of their reproductive behavior. Numbers in parentheses represent numbers of fish for which data were not available.

Date	Sex	Live fish	Mortalities	Length and weight	Ovaries and fecundity	Contained eggs	
						n	%
Winter 1999	♀	36					
(start of study)	♂	22					
Summer 1999	♀	28	8	23[a] (5)	21 (7)	21	100
(17 Aug)	♂	22	0	13[a] (9)	16 (6)	0	
Winter 2000	♀	27	1	26 (1)	27	1	4
(7 Jan)	♂	21	1	19 (2)	12 (9)	0	
Summer 2000	♀	27	0	15 (12)	25 (2[b])	27	100
(24 May)	♂	20	1	14 (6)	17 (3)	0	
Winter 2001	♀	27	0	27	27	7	26
(26 Feb)	♂	15	5	14 (1)	15	0	
Summer 2001	♀	27	0	27	25 (2[c])	25	93
(1 May)	♂	14	1	13 (1)	14	0	
Total		228	17				

[a] Body measurements not used because of a size-biased sample of fish.
[b] Unable to acquire ovary depth or length because ovary periphery was outside ultrasound image.
[c] Females did not contain eggs.

Fish to be measured were anesthetized with tricaine methanesulfonate (MS-222 ; Argent Chemical Laboratories, Inc., Redmond, Washington) and placed into a transparent, water-filled pan. Total length (TL) was measured to the nearest millimeter with a ruler placed under the pan. Fish wet weight was measured to the nearest 0.1 g with a Mettler top-loading balance (Mettler Toledo, Inc., Columbus, Ohio, Model P120). We calculated the $\log_{10}$ weight: $\log_{10}$ length relationship by means of linear regression (SAS 1992).

Ultrasound examinations.—We examined fish with ultrasound to monitor fish gonadal development by means of either a GE LOGIQ 700 Expert (GE Medical Systems, Waukesha, Wisconsin) with an 8- or 13-MHz probe (summer 1999, winter and summer 2000) or a Shimadzu SDU-400 Plus (Shimadzu Corporation, Kyoto, Japan) with a 7.5-MHz probe (winter and summer 2001). We referred to Zweibel and Sohaey (1998) for correct ultrasound technique.

We examined each surviving fish each summer and winter between summer 1999 and summer 2001 (Table 1). To acquire gonad images, each fish was first anesthetized with MS-222 and then rolled belly-up in a pan of water so that the ultrasound probe could be placed against the fish's abdomen. Each ultrasound image depicted a two-dimensional longitudinal section through the sagittal plane of the body cavity (Figure 2).

Ovary and fecundity measurements.—We measured the diameter of each clearly defined oocyte in the ultrasound image with Optimas image analysis software (Media Cybernetics 1999). By measuring only clearly defined oocytes, we minimized the use of oocytes that were not scanned through their equators. Oocyte volume was estimated by means of a cubic volume equation (volume = mean diameter^3) to incorporate the interstitial spaces among the oocytes.

The estimate of a fish's ovary volume was based on the ovary pair's being ellipsoidal in shape (ellipsoid volume = $[4\pi/3]abc$, where a, b, and c are the three respective orthogonal axes [length, width, and depth]; Beyer 1981). Length and depth of the ovary were measured from the ultrasound images each year. Because the ultrasound image showed the thickness of the body wall to be negligible (Figure 2), we measured ovary width externally in summer 2001. For summers 1999 and 2000, we estimated ovary width from ovary depth with the equation, ovary width = $1.862 + 0.346 \times$ ovary depth ($n = 24$; $r^2 = 0.16$; $P = 0.0561$). The ovary width of one fish from summer 2001
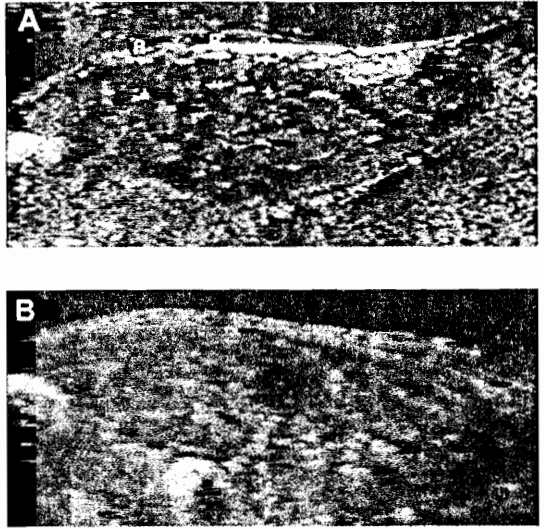

FIGURE 2.—Ultrasound images of captive female Neosho madtoms during a laboratory assessment of the reproductive status of Neosho madtoms, 1999–2001. Image A is of a gravid female during summer and image B is of a female without visible eggs during winter. The images are of the sagittal plane of the fish (anterior to the left, ventral on the top, posterior to the right, and dorsal on the bottom). Annotations A, B, and C in image A indicate oocyte diameter measurements of 2.3, 2.0, and 2.2 mm, respectively.

was also estimated this way to compensate for an erroneous body width measurement. The fecundity estimate was calculated by dividing each fish's ovary volume by its mean oocyte volume.

To test for yearly differences in mean oocyte diameter and estimated fecundity, we used a multivariate analysis of covariance (MANCOVA; model df = 3, error df = 67; SAS 1992) and examined the resulting value of Wilk's lambda, where the dependent variables were oocyte diameter and fecundity and the independent variables were year and TL. Previous research has found a positive relationship between madtom length and fecundity (Burr and Stoeckel 1999); consequently, we used length as a covariate. Fecundity data were square-root transformed to homogenize variance and normalize the distribution.

Results

Each year as spring temperatures reached 15–17°C (April–May), captive males and females developed secondary sexual characteristics typical of wild fish (Bulger et al. 2002b). Average mortality among years was similar (Table 1). Female mor-
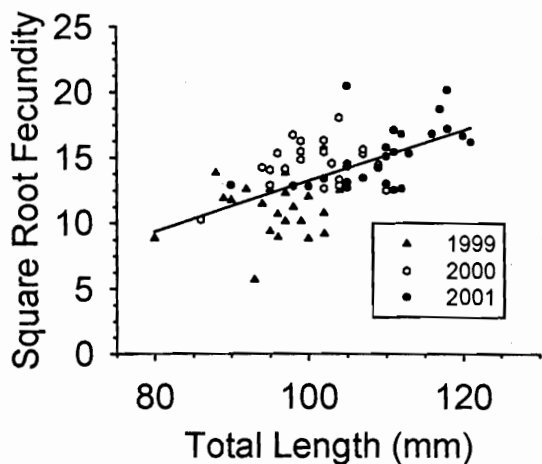
FIGURE 4.—Estimated fecundity versus total length of captive Neosho madtoms during a laboratory assessment of their reproductive behavior, 1999–2001. All years are combined in the length: fecundity equation: square root of fecundity = $-6.22 + 0.1947$ total length ($n = 71$; $r^2 = 0.37$; $P < 0.0001$).

fecundities we estimated for these females by means of the summer 2001 length: fecundity relationship (range, 208–271 eggs; mean, 246 eggs). The mean chorion diameter of the spawned eggs was 3.5 mm, which was higher than the mean pre-spawn oocyte diameter obtained from the ultrasound images of all fish (summer 2001, mean = 2.6 mm). Only the eggs of one spawn developed to the neurula stage, indicating that fertilization had occurred. These eggs died when we attempted to culture them further. For four spawnings, the eggs stopped developing at the morula stage, suggesting that false activation had occurred. For the five remaining clutches, one or both of the parents consumed the eggs the day after spawning.

Discussion

Ultrasound proved to be an effective, noninvasive tool for determining the sex, oocyte diameter, and fecundity of Neosho madtoms. The noninvasive ultrasound allowed us to perform repeated fish examinations without causing injury or appreciable stress. In addition, the technique was easy to learn. Females were easily distinguished from males during the summer, and developing oocytes could be discerned, even during late-winter periods. The application of the ultrasound is limited to females because testes cannot be distinguished from other organs. This outcome is not surprising because madtom testes are small, elongate, and closely associated with the intestines (Clugston and Cooper 1960; Stoeckel 1993; Stoeckel and Burr 1999). Sonography is a viable, noninvasive technique for ascertaining the reproductive condition of female Neosho madtoms, an important consideration for a rare species. New portable ultrasound models allow this technique to be used in the field.

Madtoms are notorious for not spawning in the laboratory (Burr and Stoeckel 1999); thus, use of daily temperature fluctuations during the prespawning and spawning seasons appears to be important for the induction of spawning. The first 2 years of study did not yield any Neosho madtom spawnings from mature gravid individuals, even when daily temperature fluctuations were incorporated into the summer spawning season. Spawning occurred only during the third year, when daily temperature fluctuations accompanied the spring and summer periods.

TABLE 2.—Summary of Neosho madtom spawning conditions in summer 2001. The temperatures given are the high and low water temperatures on the day of spawning.

Date	♀ ID	♂ ID	Time (hours)	Temperature (°C)	Photoperiod (h)	Female TL (mm)
16 Jun	1	4	~2000	21–22	15.0	116
26 Jun	2	3	0031–0127	21–24	15.8	113
30 Jun	3	3	Daylight before 2059	21–24	15.8	105
5 Jul	4	4	~1030	21–25	16.0	113
9 Jul	5	3	Night	21–26	16.0	114
16 Jul	6	3	Afternoon	21–25	16.0	112
22 Jul	7	3	Daylight before 2100	21–25	16.0	114
26 Jul	8	5	1300–1500	21–25	16.0	113
30 Jul	9	5	1300–1430	21–25	16.0	112
6 Aug	10	5	~1430	21–25	16.0	105
Mean						112

[a] Visual estimate.

TABLE 3.—Summary of Neosho madtom spawning results in summer 2001. Mean egg diameter was measured after egg deposition. Estimated fecundity was calculated from the fecundity : length relationship for the summer of 2001; nd = not determined.

Date	Mean egg chorion diameter (mm)	Estimated clutch size	Estimated fecundity	All eggs expelled	Fertile	Clutch outcome
16 Jun	4.0	313	271	Yes	nd	Consumed next day
26 Jun	3.4	418	253	Yes	Yes	Dead by 29 Jun
30 Jun	4.1	388	208	Yes	nd	Female consumed next day
5 Jul	3.7	285	253	Yes	No	Male parental care Stopped 7 Jul
9 Jul	nd	nd	259	Yes	No	Dead on 13 Jul
16 Jul	nd	150[a]	247	Yes	No	Dead on 18 Jul
22 Jul	3.1	276	259	No	No	Dead on 26 Jul
26 Jul	3.1	95	253	No	nd	Consumed next day
30 Jul	3.6	125	247	No	nd	Male consumed next day
6 Aug	3.2	20[a]	208	No	nd	Male consumed next day
Mean	3.5	230	246			

Laboratory fish exceeded the length, weight, and age maxima recorded for wild Neosho madtoms (Fuselier and Edds 1994; Bulger and Edds 2001). This was probably a result of the ideal captive conditions (i.e., abundance of food, warmer winter temperatures, lack of competitors, and absence of predators). Relative to data collected on other madtoms following similar methods, the Neosho madtom $\log_{10}$ length–weight relationship was most like that of the margined madtom ($\log_{10}$ weight = $-4.75 + 2.89 \cdot \log_{10}$ length; Clugston and Cooper 1960).

Even though the ultrasound technique yielded conservative fecundity estimates when compared with actual clutch sizes of females that expelled all eggs, fish fecundities were similar to those of other like-aged madtom species (Burr and Stoeckel 1999). The summer 2001 average fecundity was high for madtoms; only the stonecat *N. flavus* yielded higher mean fecundity estimates (Langlois 1954, 973 eggs; Walsh and Burr 1985, 278 eggs). This increase in fecundity is attributable to both the larger size and larger abdominal cavity volume of the Neosho madtom females. An increase in fecundity with increasing body size is common among ictalurid species that live longer than 2 years (Burr and Stoeckel 1999). Since wild Neosho madtoms rarely live beyond 2 years (Fuselier and Edds 1994; Bulger and Edds 2001), future propagation efforts would benefit by using conditions in this study that resulted in both an increased life span and a higher fecundity of captive Neosho madtoms.

The largest Neosho madtom clutch in this study was significantly greater than the previously reported maximum (Bulger et al. 2002b). Only the stonecat clutch size exceeds the maximum clutch size estimated in this study (500 eggs; Greeley 1929). Clutch sizes were atypically large because of two factors: the large sizes of the females involved and the time at which clutch sizes were estimated. The clutch size counts from the videotape were made almost immediately after spawning and included infertile eggs as well as eggs that died later or were ingested by the parents. For example, Chan (1995) found up to a 50% reduction in brown madtom *N. phaeus* clutch size in the first 48 h after spawning and attributed this to natural causes.

The photothermal conditions of the laboratory spawnings were comparable to the environmental conditions reported in previous Neosho madtom spawnings. The range of maximum daily temperatures on the days the fish spawned (22–26°C) was slightly lower than that recorded for previous spawnings (25–28°C; Pfingsten and Edds 1994; Bulger et al. 2002b), but was well within the range of other madtom species (Burr and Stoeckel 1999). The photoperiod during the spawnings (15–16 h) was consistent with that of the natural spawning season (Bulger and Edds 2001; Bulger et al. 2002a) and previous Neosho madtom spawnings in the laboratory (Bulger et al. 2002b).

Egg diameters of the pre- and postspawn eggs were also comparable to those found in previous studies. The prespawn oocyte diameters obtained with ultrasound were the same as those measured from a preserved prespawn Neosho madtom female (mean diameter = 2.5 mm; Bulger et al. 2002b) and were comparable to those for other madtom species (Burr and Stoeckel 1999). The postspawn mean egg chorion diameters were sim-

ilar to those obtained from previous clutches (3.1 mm, Pfingsten and Edds 1994; 3.1 and 3.7 mm, Bulger et al. 2002b). The apparent increase in egg size after spawning is probably attributable to the water absorption that occurs during water hardening (Saunders 1982; Jaffe 1985).

As in other madtom studies (Stoeckel and Burr 1999), we noted the high frequency at which the parents consumed their egg clutches after spawning. Human disturbance of the nest has been the predominant rationale offered on why the parents consume the eggs (Stoeckel and Burr 1999). However, some of the egg masses in this study were consumed even when they were not disturbed. This suggests that other reasons could cause this oophagy, including egg infertility or the postspawning confinement of parents. Use of larger tanks has been suggested as a possible solution (Stoeckel and Neves 2001) because it would allow females to leave the area of the nest.

Partial spawning is a recognized occurrence in madtoms, as several researchers have reported incomplete egg depositions for some females (Mayden and Burr 1981; Walsh and Burr 1985; Dinkins and Shute 1996; Bulger et al. 2002b). The occurrence of partially spent females, spawned clutch sizes smaller than ovarian egg numbers, or both have been used as evidence of potential polyandry in madtoms (Menzel and Raney 1973; Burr and Stoeckel 1999). However, in this study partial spawnings during the later part of the 2001 spawning season were probably a consequence of deteriorating fish condition and suboptimal spawning conditions. The last four spawnings involved male 3 (that had completed three previous spawnings) and male 5 (that had participated in incomplete spawnings with three different females). In addition, the photothermal increases had reached a plateau by this time (16 h light: 8 h darkness with daily temperature fluctuations between 21°C and 25°C).

In conclusion, this study has identified several aspects of Neosho madtom reproductive biology that could facilitate future research and propagation efforts (i.e., serial polygyny, repeated attainment of reproductive maturity in sequential years, and sex identification with ultrasound). Combined, their application may facilitate further life history research and the culture of this and other threatened and endangered madtoms.

Acknowledgments

References

Beyer, W. H. 1981. CRC standard mathematical tables, 26th edition. CRC Press, Boca Raton, Florida.

Blythe, B., L. A. Helfrich, W. E. Beal, B. Bosworth, and G. S. Libey. 1994. Determination of sex and maturational status of striped bass (*Morone saxatilis*) using ultrasonic imaging. Aquaculture 125:175–184.

Bulger, A. G., and D. R. Edds. 2001. Population structure and habitat use in Neosho madtom (*Noturus placidus*). Southwestern Naturalist 46:8–15.

Bulger, A. G., M. L. Wildhaber, and D. R. Edds. 1998. Population dynamics, reproductive biology, and behavior of the Neosho madtom, *Noturus placidus*: a state and federally listed threatened catfish. Final report submitted to the U.S. Army Corps of Engineers, Environmental Analysis and Support Branch, Tulsa District, Tulsa, Oklahoma.

Bulger, A. G., M. L. Wildhaber, and D. R. Edds. 2002a. Effects of photoperiod on behavior and courtship of the Neosho madtom (*Noturus placidus*). Journal of Freshwater Ecology 17:141–150.

Bulger, A. G., C. D. Wilkinson, D. R. Edds, and M. L. Wildhaber. 2002b. Breeding behavior and reproductive life history of the Neosho madtom, *Noturus*

placidus (Teleostei: Ictaluridae). Transactions of the Kansas Academy of Science 105:106–124.

Burr, B. M., and J. N. Stoeckel. 1999. The natural history of madtoms (genus *Noturus*), North America's diminutive catfishes. Pages 51–101 *in* E. R. Irwin, W. A. Hubert, C. F. Rabeni, H. L. Schramm, Jr., and T. Coon, editors. Catfish 2000: proceedings of the international ictalurid symposium. American Fisheries Society, Symposium 24, Bethesda, Maryland.

Chan, M. D. 1995. Life history and bioenergetics of the brown madtom, *Noturus phaeus*. Master's thesis. University of Mississippi, Oxford.

Clugston, J. P., and E. L. Cooper. 1960. Growth of the common eastern madtom, *Noturus insignis* in central Pennsylvania. Copeia 1960:9–16.

Columbo, R. E., P. S. Wills, and J. E. Garvey. 2004. Use of ultrasound imaging to determine sex of shovelnose sturgeon. North American Journal of Fisheries Management 24:322–326.

Dinkins, G. R., and P. W. Shute. 1996. Life histories of *Noturus baileyi* and *N. flavipinnis* (Pisces: Ictaluridae), two rare madtom catfishes in Citico Creek, Monroe County, Tennessee. Bulletin of the Alabama Museum of Natural History 18:43–69.

Fuselier, L. C., and E. R. Edds. 1994. Seasonal variation in habitat use by the Neosho madtom (Teleostei: Ictaluridae: *Noturus placidus*). Southwestern Naturalist 39:217–223.

Greeley, J. R. 1929. Fishes of the Erie–Niagara watershed. Pages 150–179 *in* A biological survey of the Erie–Niagara system. Supplement to the Annual Report of the New York State Conservation Department 18, Albany.

Hamman, R. L. 1982a. Induced spawning and culture of bonytail chub. Progressive Fish-Culturist 44:201–203.

Hamman, R. L. 1982b. Spawning and culture of humpback chub. Progressive Fish-Culturist 44:213–216.

Hamman, R. L. 1985. Induced spawning of hatchery-reared razorback sucker. Progressive Fish-Culturist 47:187–189.

Hamman, R. L. 1986. Induced spawning of hatchery-reared Colorado squawfish. Progressive Fish-Culturist 48:72–74.

Jaffe, L. F. 1985. Calcium explosions, waves, and pulses in activating eggs. Pages 127–165 *in* C. B. Metz and A. Monroy, editors. The fertilization of the egg, volume 3. Academic Press, New York.

Karlsen, O., and J. C. Holm. 1994. Ultrasonography, a noninvasive method for sex determination in cod (*Gadus morhua*). Journal of Fish Biology 44:965–971.

Langlois, T. H. 1954. The western end of Lake Erie and its ecology. J. W. Edwards Brothers, Ann Arbor, Michigan.

Martin, R. W., J. Meyers, S. A. Sower, D. J. Phillips, and C. McAuley. 1983. Ultrasonic imaging: a potential tool for sex determination in live fish. North American Journal of Fisheries Management 3:258–264.

Martin-Robichaud, D. J., and M. Rommens. 2001. Assessment of sex and evaluation of ovarian maturation of fish using ultrasonography. Aquaculture Research 32:113–120.

Mattson, N. S. 1991. A new method to determine sex and gonad size in live fishes by using ultrasonography. Journal of Fish Biology 39:673–677.

Mayden, R. L., and B. M. Burr. 1981. Life history of the slender madtom, *Noturus exilis*, in southern Illinois (Pisces: Ictaluridae). Occasional Papers of the Museum of Natural History, University of Kansas 93:1–64.

Media Cybernetics. 1999. Optimas image analysis software, version 6.51. Silver Spring, Maryland.

Menzel, B. W., and E. C. Raney. 1973. Hybrid madtom catfish, *Noturus gyrinus* × *Noturus miurus*, from Cayuga Lake, New York. American Midland Naturalist 90:165–176.

Moghim, M., A. R. Vajhi, A. Veshkini, and M. Masoudifard. 2002. Determination of sex and maturity in *Acipenser stellatus* by using ultrasonography. Journal of Applied Ichthyology 18:325–328.

Moss, R. E. 1983. Microhabitat selection in Neosho River riffles. Doctoral dissertation. University of Kansas, Lawrence.

Pfingsten, D. G., and D. R. Edds. 1994. Reproductive traits of the Neosho madtom (*Noturus placidus*) (Pisces: Ictaluridae). Transactions of the Kansas Academy of Science 97:82–87.

SAS. 1992. SAS/LAB software, release 6.08. SAS Institute, Cary, North Carolina.

Saunders, J. W., Jr. 1982. Developmental biology: patterns, problems, and principles. Macmillan, New York.

Stoeckel, J. N. 1993. Controlled cultivation techniques for the recovery of threatened fishes in Virginia. Doctoral dissertation. Virginia Polytechnic Institute and State University, Blacksburg.

Stoeckel, J. N., and B. M. Burr. 1999. A review of key reproductive traits and methods used to spawn ictalurids. Pages 141–159 *in* E. R. Irwin, W. A. Hubert, C. F. Rabeni, H. L. Schramm, Jr., and T. Coon, editors. Catfish 2000: proceedings of the international ictalurid symposium. American Fisheries Society, Symposium 24, Bethesda, Maryland.

Stoeckel, J. N., and R. J. Neves. 2001. Induced spawning of the margined madtom. North American Journal of Aquaculture 63:99–108.

Taylor, W. R. 1969. A revision of the catfish genus *Noturus* Rafinesque, with an analysis of higher groups in the Ictaluridae. U.S. National Museum Bulletin 282.

USFWS (U.S. Fish and Wildlife Service). 1991. Neosho madtom recovery plan. USFWS, Denver.

U.S. Naval Observatory. 1999–2001. Sunrise–sunset data. U.S. Naval Observatory, Astronomical Applications Department, Washington, D.C. Available: http://aa.usno.navy.mil/.

Walsh, S. J., and B. M. Burr. 1985. Biology of the stonecat, *Noturus flavus* (Siluriformes: Ictaluridae), in central Illinois and Missouri streams, and comparisons with Great Lakes populations and congeners. Ohio Journal of Science 85:85–96.

Wildhaber, M. L., D. M. Papoulias, A. J. DeLonay, D. E. Tillitt, J. L. Bryan, M. L. Annis, and J. A. Allert. 2005. Gender identification of shovelnose sturgeon using ultrasonic and endoscopic imagery and the application of the method to the pallid sturgeon. Journal of Fish Biology 66:1–19.

Zweibel, W. J., and R. Sohaey. 1998. Introduction to ultrasound. Saunders Company, Philadelphia.