# INFLUENCE OF PHOTOPERIOD AND TEMPERATURE ON THE NEOSHO MADTOM (*NOTURUS PLACIDUS*) REPRODUCTIVE CYCLE

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TABLE OF CONTENTS

LIST OF TABLES ..... iii

LIST OF ILLUSTRATIONS ..... iv

ABSTRACT ..... v

INTRODUCTION ..... 1

METHODS..... 4

Fish Maintanence

Photoperiod

Temperature

Ultrasound

External Body Measurements

Spawning

RESULTS ..... 10

Overall Fish Changes

Ultrasound

Spawns

DISCUSSION ..... 13

SUMMARY AND RESEARCH NEEDS..... 19

ACKNOWLEDGEMENTS ..... 20

BIBLIOGRAPHY ..... 21

APPENDIX

TABLES ..... 28

FIGURES ..... 34

LIST OF TABLES


| Table | | Page |
|---|---|---|
| 1. | Summary of the number of fish during each phase of collection ..... | 29 |
| 2. | Descriptions of various external body measurements taken during this study ..... | 30 |
| 3. | Summary of the four ratios obtained from exterior body measurements ..... | 31 |
| 4. | Summary of the ANOVA tests for individual ratios ..... | 32 |
| 5. | Summary of spawns that occurred during the third summer period .... | 33 |

LIST OF ILLUSTRATIONS


| Figure | | Page |
|---|---|---|
| 1. | Neosho madtom (*Noturus placidus*) ..... | 35 |
| 2. | Neosho madtom distribution ..... | 36 |
| 3. | Photoperiod changes comparing natural to simulated ..... | 37 |
| 4. | Temperature changes comparing natural to simulated ..... | 38 |
| 5. | Ultrasound picture of a female Neosho madtom during the summer .... | 39 |
| 6. | Ultrasound picture of a male Neosho madtom during the summer ..... | 40 |
| 7. | Size of Neosho madtom males and females over four years ..... | 41 |
| 8. | The weight length relationship of Neosho madtoms ..... | 42 |
| 9. | Number of eggs estimated during the two summer time periods ..... | 43 |

ABSTRACT

The key to successful fish culture is to understand the environmental cues that trigger spawning. In temperate fishes, photoperiod and temperature are important in many species including the family Ictaluridae. The object of this study was to examine whether natural photo-thermal conditions in the laboratory could stimulate the reproductive cycle of Neosho madtoms (*Noturus placidus*). For three years a small population of Neosho madtoms were maintained under natural conditions and continually sampled using ultrasound to examine interior gonad state and exterior body measurements. The purpose was to examine the secondary sexual characteristics that normally occur during the spawning period. Every year the fish cycled in and out of spawning condition, including production and reabsorbtion of eggs. The best external measurement found to distinguish between sexes was the ratio of head length to total length. Internal measurements found the average number of eggs per female increased as the fish length increased and over time but the average sizes of the eggs were constant. After years in the simulated environment 13 different fish were involved in ten spawns. The use of ultrasound to examine gonad in madtoms is promising, especially the lack of injury associated with the procedure. Overall laboratory conditions that simulated the natural photo-thermal environment, especially daily temperature fluctuations, were successful at stimulating the reproductive cycle of Neosho madtoms including egg cycling and spawning. These results show promise towards culture of madtoms especially for those species that are rare and endangered.

# INTRODUCTION

Captive propagation of threatened and endangered species can be essential to their recovery. For fishes, culturing a species increases knowledge of its reproductive ecology and perceptions of how the environment impacts its reproduction. The culture of several endangered and threatened fish species has assisted their recovery efforts (e.g., bonytail chub, *Gila elegans;* humpback chub, *Gila cypha;* razorback sucker, *Xyrauchen texanus;* Colorado squawfish/pike minnow, *Ptychocheilus lucius*; see Hamman 1982a, Hamman 1982b, Hamman 1985, Hamman 1986). An intimate understanding of these species' reproductive cycles and what environmental triggers influence these cycles has been critical to the success of these efforts.

Which and how environmental triggers stimulate reproduction in the family Ictaluridae is not well understood (Busch 1985). For example, although researchers have identified the physiological and hormonal mechanisms that control reproduction in cultured channel catfish (*Ictalurus punctatus*), how specific environmental factors trigger endocrine responses remains uncertain (Busch 1985). Consequently, most ictalurid culture has occurred under natural conditions in outdoor ponds (Huner and Dupree 1984, Busch 1985, Tucker and Robinson 1990).

For many temperate fish species, their gonadal development is triggered by seasonal photo-thermal changes (Lam 1983). Members of the family Ictaluridae are similar to other temperate species in that changes in photoperiod and temperature appear to stimulate reproduction (Huner and Dupree 1984, Busch 1985). For example, the cycle

of ictalurid spawning can be delayed through photo-thermal manipulation (Brauhn 1971, Brauhn and McCraren 1975, Vasal and Sundararaj 1976, Davis et. al 1986, Kelly and Kohler 1996). Photo-thermal manipulation also affects madtoms (Ictaluridae: *Noturus*) reproductive chronology and behavior (Stoeckel 1993, Bulger 1999). It is further suggested that photoperiod is important for madtom gonadal development (Dinkins and Shute 1996) and that spawning occurs as temperatures warm (Clark 1978, Starnes and Starnes 1985, Dinkins and Shute 1996, Burr and Stoeckel 1999). In general, photo-thermal triggers seem to influence the reproductive cycles of most ictalurids.

The Neosho madtom (*Noturus placidus*) is one of five madtoms included on the U.S. federal threatened and endangered species list (Burr and Stoeckel 1999, U. S. Fish and Wildlife Service 1991). Which and how environmental cues trigger reproduction in these species is poorly understood. Neosho madtoms (Figure 1) are small fish, reaching 35-70 mm as adults (Fuselier and Edds 1994, Edds 1995, Bulger and Edds 2001). As is common with most madtom species (Burr and Stoeckel 1999), Neosho madtom lifespan is short with most collected as one- or two-year-olds, the eldest being three years old (Moss 1981, Bulger and Edds 2001). Individuals are typically found on mainstem gravel bars in the Neosho River basin (Figure 2, Taylor 1969, Moss 1981, Moss 1983, Wilkinson and Edds 1997, Bulger 1999).

What is known about Neosho madtom reproduction is similar to that of most other madtoms. The sexes are dimorphic during the spawning season (Taylor 1969, Clark 1978, Mayden and Burr 1981) with males exhibiting enlarged cephalic epaxial head muscles and swollen lips, whereas females lack the swollen head muscles but possess extended abdomens indicative of egg production. In addition, the genital papillae

swell and, unique to Neosho madtoms, the tooth patches redden in both sexes during the spawning season (Pfingsten and Edds 1994, Bulger 1999). Together, these external characteristics can be used to sex individuals during the spawning season (Clark 1978, Mayden and Burr 1981, Bulger 1999). Spawning occurs in nests constructed under larger rocks in the gravel (Wilkinson and Edds 1997, Bulger 1999). Observations of female parental care in madtoms are rare (Burr and Stoeckel 1999); only male parental care has been observed in Neosho madtoms and lasts 8-9 d after spawning (Wilkinson and Edds 1997, Bulger 1999). The lifetime reproductive potential of Neosho madtoms is limited by their short lifespans to one or two episodes (Bulger and Edds 2001). Although Bulger (1999) determined that artificial shortening of day length decreased Neosho madtom reproductive activity, how photoperiod and temperature specifically influence reproduction has not been established for this species. Gaining a greater understanding of the reproductive cycle in the Neosho madtom will help managers understand how environmental changes impact this species and other endangered madtoms.

The purpose of the present study is to investigate whether and how simulating natural temperature and photoperiod regimes could successfully trigger reproductive maturation, secondary sexual character development, and spawning in captive Neosho madtoms. Previous efforts at captive Neosho madtom propagation have met with limited success (Pfingsten and Edds 1994, Wilkinson and Edds 1997, Bulger 1999). An important difference for this study is the precision with which natural fluctuations in the photo-thermal environment were simulated.

# METHODS

*Fish Maintenance*

The Neosho madtoms used in this study (n=58) were collected as one-year-olds from the Neosho and Cottonwood rivers (Figure 2) in the summer of 1998 (see Bulger 1999). Maintenance of the fish for this study began in January 1999 when the fish were two-year-olds, and continued until May 2001 when they approached their fourth year of life. In the laboratory, all specimens were maintained in a partitioned 720-L Frigid Units Living Stream$^{R}$ (Model LS-900) aquarium housed in an isolated temperature-controlled room. The fish were fed to satiation three times a week and excess food was removed routinely to prevent disease. Foods included: frozen brine shrimp (*Artemia salina*), frozen bloodworms (*Chironomus* sp.) and Hikari Sinking Carnivore Pellets$^{R}$.

*Photoperiod*

Photoperiods over the two+ years of maintenance were varied to simulate that of nature based on once-weekly daylength calculated from sunrise and sunset at Emporia, Kansas (N 38$^{o}$ 26 min., W 96$^{o}$ 12 min., U.S. Naval Observatory 1999-2001; Figure 3). Photoperiods varied from 8 to 16 hr of light per day; the smallest change in photoperiod was 15 minutes every two weeks, whereas the largest change was 1 hr every week. Eight GE$^{R}$ cool white 34-watt fluorescent bulbs (47" x 1.5" diameter, 22" above aquaria) provided the light; transitions between light and dark were immediate. Neosho madtoms are nocturnal (Moss 1981, Bulger 1999), and those in the lab acclimated to the point of

anticipating the lighting changes, immediately becoming active as soon as the lights were turned off.

*Temperature*

To simulate natural annual thermal cycles, weekly and/or daily temperature changes were regulated using a Frigid Units[R] 1.0 h.p. water heater/chiller (model D1-100; 3000 W heater) in the main holding aquarium, and 300 W Aquarium Systems Visi-Therm[R] aquarium heaters for individual aquaria. The temperature cycles for January 1999 through July 2001 (Figure 4) approximated those collected from January 1996 to July 1998 in the Neosho River (Bulger 1998, Wilkinson and Edds 1997). Although the river temperatures during this timeframe ranged between 0°C in January 1997 and 31°C in July 1998 (Figure 4), the laboratory thermal regime (range 9 to 27°C) was kept slightly warmer during the winter to maintain fish health and as chiller limitations were encountered. Excluding daily temperature fluctuations, the overall laboratory temperature changes varied from 1°C over two weeks to 2°C every week (Figure 4), similar to what occurred in the wild.

Once the simulated summer photoperiod and thermal maxima were reached each year, we varied the daily water temperatures between ~20 and 25°C (shown as dark areas in the temperature line, Figure 4) to more closely match natural fluctuations and as an additional spawning stimulus. In addition, during 2001 we also varied daily temperatures as the peak in the simulated spring rise was approached. As further explanation, once the weekly temperature maximum reached 22°C, it was varied daily between an evening low of 21°C and a daily high of 22°C (temperatures were decreased at 2300 hours and

increased at 1100 hours). The subsequent five weeks' daily temperature peaks increased from 22-26°C with a constant 21°C minimum (Figure 4). Thereafter, the daily temperature range was maintained between 20-25°C until the fish showed no further reproductive activity.

*Ultrasound*

To monitor the reproductive status of the fish, we used a non-invasive ultrasonic assessment method. Because fish numbers were limited and the species is threatened (U.S. Fish and Wildlife Service 1991), this approach allowed for repeated monitoring of the fish without injury or appreciable stress. The five ultrasound examinations were conducted biannually (summer and winter) between the summers of 1999 and 2001. Although individual fish were not marked, maintaining the fish in same-sex groups allowed the gender of individual group members to be known and the ultrasound results to be confirmed. Due to the random loss of several ultrasound images, together with the deaths of a few individuals due to unrelated causes, not all fish were assessed each time (Table 1).

Two ultrasound machines were used, a General Electric LOGIQ 700 EXPERT$^{R}$ set to 8 or 13 MHz (summer 1999, winter and summer 2000), and a Shimadzu SDU-400 Plus$^{R}$ with a 7.5 MHz probe (winter and summer 2001). The change in units was a consequence of availability; both produced equivalent images. For imaging, each fish was anesthetized with MS222 until they allowed themselves to be rolled belly-up in the water so a ventral picture could be acquired (Figure 5 and 6). Single ultrasound images were sufficient to visualize the entire body cavity of each fish due to their small size

(Table 1). Each image is automatically scaled; from this, average egg diameter (from all well-defined ova) and total egg mass area were subsequently measured using OPTIMAS 6.1 Image Analysis[R] software.

The volume of an egg was estimated using a cube volume equation (volume = abc, Beyer 1981). The volume of a square was used to incorporate the interstitial spaces between the eggs into the egg number estimates. The body volume was divided by the average egg volume to estimate fecundity for each female.

To estimate the number of eggs in each female from the ultrasound, an ellipse shape was used to estimate the volume of the egg mass (volume = 4/3abc, where a, b, and c were the lengths of the three respective orthogonal axes which are length, width and depth, Beyer 1981). Length and depth of the egg mass were measured internally from the ultrasound picture. During summer three, the body cavity width was measured externally (Figure 5, Table 2) with the assumption being the body wall thickness was negligible. These estimates were only calculated during the summer period when the body cavity was completely full of eggs. The body width of fish in summer one and two were estimated from a regression equation obtained using fish from summer three. During summer one and two body width was predicted using the simple linear regression equation of body width = 1.862 + 0.346 * body depth (df = 24, p-value = 0.0561, $r^2$ = 0.1560). One fish from summer three was not used in this equation due to an erroneous body width measurement; consequently this fish had body width estimated using the above equation.

During summer one, the length of nine female fish (out of 21) were estimated using the equation of total length = 80.75 + 7.551* egg mass length (df = 48, p-value =

0.0000, $r^2 = 0.4771$) which was acquired using simple linear analysis on female fish from summers one and two. To examine the relationship between egg size, egg count, and fish length over the three summers, Wilks' Lambda multiple analysis of covariance (MANCOVA) was used to test differences (SAS 1990). The number of eggs was transformed using $\log_{10}$ because of nonnormality of error variance. A Students t-test was used to examine whether the mean egg count differed from 63, the largest recorded number of eggs in any Neosho madtom egg clutch (Pfingsten and Edds 1994) or number of eggs in a preserved female specimen (Bulger 1999).

*External Body Measurements*

In addition to the internal measurements obtained from the ultrasound, complimentary external measurements were also made although not always at identical times. During 2000 and 2001, each fish was measured during the summer and winter. In the summer of 1999 a complete sample was not obtained so the external measurements were not used. One male fish was emaciated and sick throughout the three years and consequently eliminated from all analyses. These external measurements were made using calipers and included head width (hw), head length (hl), head depth (hd), total body length (tl) and weight (w) (Table 2). Previous research has suggested the ratios of different body measurements (hw:hd, hw:hl, hl:tl and hd:hl) can be easily measured in the field to identify male and female madtoms (Clark 1978, Mayden and Burr 1981). Consequently these ratios were tested to find the best overall ratio for use in field sex determination.

After calculating these ratios, differences between sexes over year and season were tested using a three-way analysis of variance (ANOVA, model df = 6, error df = 148). SAS/LAB as part of SAS (SAS 1992) was used to analyze for constancy and normality of error variance. This software tested constancy of variance of residuals from the three-way ANOVA model with two-way interactions using chi-square goodness-of-fit tests between predicted and residual values. The software then suggested appropriate transformations as necessary to obtain constant variance. Each ratio was tested separately with year, season, and sex as factors. Multiple comparisons among factors were tested using Bonferoni comparison tests for each ratio.

Length-weight relationships were calculated during 2000 and 2001 using SAS/LAB (SAS 1992) to assess age and size affects on this relationship for Neosho madtoms. This software tested constancy of variance of residuals from the simple linear regression model using chi-square goodness-of-fit tests between predicted and residual values. The software then suggested appropriate transformations as necessary to obtain constant variance. An overall test using analysis of covariance (ANCOVA) consisting of the three-way model with two-way interactions (response = weight, covariate = length, factors = year, season, and sex, model df = 7, error df = 147). If the factors did not increase the amount of variance explained in the model then a simple linear regression model containing the $\log_{10}$ transformed length and weight were used in an overall regression analysis and one equation was calculated for the entire population of sampled fish (n = 155).

*Spawning*

The spawning season of the Neosho madtom occurs during the summer months (May-August) at temperatures 25 to 30°C (Bulger and Edds 2001, Edds 1995). During the corresponding time period, the photoperiod and temperature of the laboratory environment were increased until the maximums were reached (photoperiod 16 hr and temperature 26°C) and then held constant until the fish no longer showed any spawning activity. To encourage spawning the fish were grouped into male/female pairs and isolated in seperate aquaria. Reproduction was observed using Micro Video Products MVC 2000-WP-LED underwater infrared cameras positioned in the aquaria directly above the nest. For the video-taped spawnings that occurred in the laboratory, clutch size was estimated using OPTIMAS 6.1 Image Analysis software. After the fish spawned the egg numbers were estimated off the videotape by calculating the area of each egg layer and dividing by the average individual egg area.

## RESULTS

*Overall Fish Changes*

The photo-thermal manipulation was successful at creating a yearly cycle of gonad production (Table 1) and even a cycle in the consumption of food (Figure 3 and 4). Over three years the madtoms grew considerably reaching a maximum size of 135 mm TL at four years of age. The mortalities that occurred during the three years did not appear selective thus the population was considered to be representative throughout the

three years (Figure 7). For the weight through time model, length, year/season interaction, sex, and sex/season interaction were all significant in the ANCOVA model (model df = 7, error df = 147, p-values: length = 0.0000, year/season = 0.0000, sex = 0.0004, sex/season = 0.0424, $r^2 = 0.8685$). Length accounted for 27.8% of the variation in the model whereas the next highest variable only accounted for 2.7%. Because length accounted for the vast majority of variance in the model, the other factors were removed and simple linear regression model was used to predict the relationship between Neosho madtom weight and length ($\log_{10}$ weight = -4.033 + 2.613*$\log_{10}$ length, model df = 1, error df = 153, $r^2 = 0.8213$, Figure 8). The summer of 1999 data that was eliminated from the analysis due to sampling bias did have similar values as the other two summers (male mean length = 106.5 mm, male mean weight = 14.9 g, female mean length = 92.4 mm, female mean weight = 11.2 g).

Each spring as temperatures reached 15-17°C, both males and females developed secondary sexual characteristics common to madtoms. The ratios of these external body measurements did show differences throughout the years, seasons and sexes (Table 3). The hw:hl ratio was unable to distinguish between the sexes so it was eliminated from the possibilities. For the remaining ratios when the year effect was significant it never accounted for more than 10.2% of the variation as a main effect or in any interaction (Table 4) so the criteria for selecting the "best" ratio concentrated on the differences between sex and season. Only hl:tl ratio was able to distinguish between sex and season with sex explaining 19.1% of the variation in the model. The mean hl:tl ratio for males was 0.2016 and for females 0.1992.

*Ultrasound*

Ultrasound was unable to distinguish male testes from other organs. For females, there was clear evidence of a yearly cycle of egg production in the spring and egg reabsorbtion in the winter (Table 1). The large and numerous eggs apparent on the ultrasound images allowed gravid females to be distinguished easily any time of the year. Each summer 93 to 100% of the females measured produced eggs. During winter one (January 2000, fish were 1 ½ years old) a few large eggs could be seen in one female; these were assumed to be eggs in the process of reabsorbtion. During winter two (February-March 2001) numerous small eggs were found in seven females and were assumed to represent eggs being produced for the succeeding summer (Table 1).

The mean egg size (mean = 2.5 mm, range 2.0 to 3.3 mm) was not related to year or length of female fish (model df = 3, error df = 67, year p-value = 0.2739, length p-value = 0.3475). The $\log_{10}$ of the egg count was related to year and length of fish (model df = 3, error df = 67, year p-value < 0.0001, length p-value = 0.0038, Figure 9). The average number of eggs per female was greater than previous estimates of 63 for one-year-old Neosho madtoms (year one, t-statistic = 7.37, n = 21, p-value < 0.0001; year two, t-statistic = 15.79, n = 25, p-value < 0.0001; year three, t-statistic = 11.35, n = 25, p-value < 0.0001; Pfingsten and Edds 1994).

*Spawns*

Each summer the paired fish exhibited all known reproductive behaviors, but only during summer three (2001) did any spawns occur. During summer three, ten different pairs of Neosho madtoms spawned (fish were four-years-old, Table 5) and occurred

during the daily temperature fluctuations (Figure 4). Three different males spawned with ten different females; one male spawning five times (male id #3, Table 5). Overall there was a spawn every 4-5 d. The average number of eggs in a clutch was 271.43; the average estimated number of eggs of these females prior to spawning was 250.43 (range 219.53 to 273.65). The mean egg size post spawn was 3.5 cm. After each spawn, the female was examined with ultrasound to determine whether she expelled all eggs. The first 6 females had expelled all eggs but the last four still contained eggs in the body cavity. Only one of the early spawns were confirmed fertile with the embryos lasting 3 d (Table 5). The parents consumed five egg clutches the day after the spawn and three clutches did not develop. The majority of the spawns occurred inside the nest cover (9 out of 10) with spawn #5 occurring in a depression of gravel in the back corner of the tank. During this spawn the male continually stayed by the nest cover while the female frequented the back corner of the tank.

## DISCUSSION

### *Overall Fish Changes*

Although we established culture conditions only slightly warmer than the minumum temperature in the wild (Figure 4, Bulger et al.1998, Wilkinson and Edds 1997) the fish grew to unprecedented size and age for Neosho madtoms. Ultimately reaching sizes of almost twice that attained in nature (wild caught <75mm, Moss 1981). Despite advanced age and captivity these fish continued to exhibit a yearly cycle in gonad development, reabsorbtion, and food consumption. The length and age of these fish have

exceeded all previous reports from any collections of Neosho madtoms (Moss 1981, Bulger 1999). Even with the extreme age and size of this population, the length-weight relationship is typical of madtoms (Burr and Stoeckel 1999).

Secondary sexual characteristics were evident every year in the laboratory at temperatures similar to wild fish (15-17°C, Bulger and Edds 2001). We found the best predictor to distinguish between the sexes during the winter and summer season was the ratio of hl:tl. Alternatively, Clark (1978) found the ratio of hw:hl was important for distinguishing between the sexes and seasons. Other ratios (hd:hl and hw:hd) were also different between the sexes and seasons but were affected by interactions and explained minimal amounts of variation in the model.

*Ultrasound*

Over three summers the number of eggs estimated per female increased. The summer three average estimate of eggs per female was on the high end of the range for madtoms with only the checkered (*N. flavater*, Burr and Mayden 1984) and stonecat (*N. flavus*, Walsh and Burr 1985) having similar ovarian fecundity estimates. Whereas the first two summers estimates where similar to other species of madtoms (Burr and Stoeckel 1999). The egg count for all years was greater than previous estimates of one-year-old Neosho madtoms (Pfingsten and Edds 1994, Wilkinson and Edds 1997). Egg count increased over time and length of fish. Similar to most ictalurids, fecundity increases with an increase in body size (Burr and Stoeckel 1999). The size of the eggs did not increase over the three years. The egg sizes measured using ultrasound were

similar to other prespawn female Neosho madtoms (mean chorion diameter = 2.5 mm, Bulger 1999) and other madtom species (Burr and Stoeckel 1999).

*Spawns*

The ten spawns that occurred in summer three occurred at temperatures similar but slightly lower than other Neosho madtom spawns (25-26.5°C, Pfingsten and Edds 1994, Wilkinson and Edds 1997) but similar to other madtom species (Burr and Stoeckel 1999). The photoperiod during spawning was consistent with previous Neosho madtom spawns (Wilkinson and Edds 1997) and the natural spawning season (Edds 1995, Bulger and Edds 2001). The high percentage of parental consumed egg clutches is typical of aquarium-spawned madtoms (Stoeckel and Burr 1999) where successful madtom spawns only occur sporadically.

The average size of eggs measured post spawn (3.5mm) was similar to previous Neosho madtom spawns (Pfingsten and Edds 1994, 3.1 mm, Wilkinson and Edds 1997, 3.1 mm, Bulger 1999, 3.7 mm). On the contrary, the estimate of the number of eggs per female and per spawn was over 4.5 times the number of eggs in previous spawns (Wilkinson and Edds 1997, Bulger 1999). This is most likely due to the increased age and size of the fish. This aspect of madtom life history is rarely examined do to the short life span but an increase in the number of eggs per female over the years does suggest some relationship with increased size since size is confounded with time; this has been suggested for other Ictalurids (Burr and Stoeckel 1999) with larger species having a larger mean clutch size. The highest Neosho madtom clutch during this study (417.8) is the highest clutch size ever recorded for any madtom species (Burr and Stoeckel 1999).

This could be due in part to the time at which most egg clutches are found. With the use of videotape our counts occurred almost immediately after spawning. Throughout the next few hours and days the number of eggs invariably decreased, some due to ingestion or handling by the parents. Chan (1995) also found a large reduction (up to 50%, n = 2) in the number of eggs in the first 48 hr due to natural causes. So if the egg clutch was found even a few hours after the spawn the numbers would inevitably be smaller. At the time of our clutch count, fertilization was not distinguishable so this number included infertile eggs and in some cases involved completely infertile clutches.

Through the use of ultrasound we were able to distinguish whether females had expelled all eggs. Through spawn number 6 the females had expelled all eggs, but after spawning seven females retained eggs. This would suggest that as the spawning season progresses ideal conditions deteriorate and females do not expel all the eggs. These findings could also explain in part the observation of researchers who have found females with approximately half the usual number of eggs in the ovaries (Clark 1978, Mayden and Burr 1981, Walsh and Burr 1985, Dinkins and Shute 1996).

Interestingly we observed the same males spawning with more than one female. This did not occur at the same time but consecutively and with three different males. This has been observed for only two other catfish species, the channel catfish (Clemens and Sneed 1957, Bondari 1983) and the brown bullhead (*Ameiurus nebulosus*, Breder 1935) and only reported for one species of madtom (*N. phaeus*, Chan 1995). These results are promising for madtom culture but successful fry rearing is still uncertain (Stoeckel 1993).

The ability to simulate the environment in a laboratory for a limited time to spawn madtoms is becoming more common (Burr and Stoeckel 1999). But the ability to observe yearly reproductive behaviors for the same population and to spawn them after three years in captivity in a species thought to live only two years (Moss 1981, Bulger 1999) has not been done for any madtom. Only Bowen (1980) was been able to spawn *N. miurus* after extended captivity (361-420 d). Even though it is well known that species live longer in captivity than in the wild, our population of Neosho madtoms was subjected to winter and summer periods and still lived more than double the normal life span (Fuselier and Edds 1994, Edds 1995, Bulger and Edds 2001). The ability to maintain madtoms in reproductive conditions for extended periods can also help in the future culture of madtoms.

The anal pore openings have been used extensively to determine the sex of madtoms (Burr and Stoeckel 1999) but this method has been used with only some success. The use of external measurement ratios has been suggested as a quick and objective method to determine the sex of madtoms (Clark 1978). In this study the only ratio found to determine between the sexes was head length to fish total length. This type of sex determination is fast and inexpensive and could be used in conjunction with the anal pore shape to determine the sex of the fish.

Another promising tool for noninvasive sex determination and egg evaluation is ultrasound and is an important tool for fish species of limited numbers and those not easily sexed using external characteristics, such as madtoms. Sex determination in similarly 'hard to sex species' have also shown promise. Reimers et al. (1987), Blythe et al. (1994), and Burtle et al. (1997) sucessfully used ultrasound to examine the gonads of

striped bass, salmon, and catfish, respectively. These researchers had limited success on small individuals but sex determination using ultrasound in mature fish does seem promising for multiple species.

The use of ultrasound techniques is slightly limited for sexually mature madtoms due to the inability to detect testes in male madtoms. However this result is not surprising due to the madtom testes small size, the proximity to the intestines, and its similar appearance to intestine (Clugston and Cooper 1960, Clark 1978, Stoeckel 1993, Stoeckel and Burr 1999). These characteristics of madtom testes make them difficult to detect with ultrasound even under ideal conditions. In contrast, ultrasound has promise in evaluation of madtom eggs. Throughout the year egg size and fecundity estimates can be acquired; even during winter periods eggs could be distinguished. After spawning, females with and without eggs could be easily determined with minimal stress and without sacrificing fish.

Multiple life history characteristics have been found that could assist future research and propagation of madtoms (i.e. extended life span, continued cycling of reproductive condition, and multiple spawns for males). Specifically, the extention of Neosho madtom life while in captivity is important to note. The increase of almost 2 times the normal life spawn creates numerous opportunities for life history research and culture. This could also be possible for other threatened and endangered madtoms which have short life spans and very few individuals. These aspects of Neosho madtom life history and the lack of older Neosho madtoms in the wild does suggest stressors that limit their life span in the wild. These could include natural and anthropogenic factors such as predation, habitat destruction, and pollution (U. S. Fish and Wildlife Service 1991,

Fuselier and Edds 1995, Wildhaber et al. 2000a, Wildhaber et al. 2000b). Research of these potential stressors are important in understanding the factors that affect the life of the Neosho madtom.

## SUMMARY

Overall, this study was successful through the use of manipulationg photo-thermal cues in stimulating the reproductive cycle of Neosho madtoms. This led to the spawning of ten pairs of fish during the third year in captivity. Also, through the use of ultrasound techniques, we were able to estimate fecundity of the females and, after spawning, the presence of females with and without eggs. We found that the number of eggs per female was related to the size of the fish over the years.

The applicability of these results to natural populations of Neosho madtoms and other species of madtoms can only be suggestive at the present; more research is needed. This study is also limited by the use of a single confined population of madtoms sampled over time. Even with these limitations, data like this does not exist and the results of this study are consistent with other fish populations, and they lay important groundwork for future research.

The findings of this research increases the capability of researchers to successfully propagate many threatened and endangered madtoms. The use of controlled photo-thermal cues could be used to extend the spawning season for madtoms as it has been done for channel catfish (Brauhn 1971, Stoeckel 1993). The research community is beginning to understand the reproductive cycle in madtoms and it can be advanced

greatly with the aid of ultrasonic techniques, making the sacrifice of limited fish reserves unnecessary. The expansion of ultrasound to madtom field studies could help in determining the reproductive cycle of natural madtom populations and help in understanding the factors that limit the population. The most important use of ultrasound in the field would be with fish species where sex determination is difficult. Portable ultrasound units are readily available and could be used to examine wild populations without the use of invasive collection methods. Overall the results from this study could help in the recovery of many madtom species.

## ACKNOWLEDGEMENTS

We would like to thank David R. Edds of Emporia State University, Emporia, KS and Charles F. Rabeni of the University of Missouri – Columbia MO, graduate committee members, for their guidance and patience. A special thanks goes to Dr. Jimmy Latimer and Dr. Kristi Cook of the University of Missouri – Columbia Veterinary Teaching Hospital, who offered their expertise, time, and guidance for the ultrasound. We would like to extend sincere gratitude to all the researchers and staff at the U.S. Geological Survey Columbia Environmental Research Center. Jim Randolph of the U.S. Army Corp of Engineers Tulsa District for funding opportunities.

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APPENDIX
TABLES

Table 1. Summary of the number of Neosho madtoms during each phase of collection. One sick male was deleted from all tests. Other than noted, all fish number differences were due to the loss of images.

| Date of ultrasound | Total # of fish in population | Sex | Mortality | # of measured fish | # of ultrasound fish pictures | # of female ultrasound pictures with eggs | % of females with eggs |
|---|---|---|---|---|---|---|---|
| 1/1/99 | 36 | f | | | | | |
| | 22 | m | | | | | |
| 8/17/99 [a] | 28 | f | 8 | 0 | 21 [b,c] | 21 | 100 |
| | 22 | m | 0 | 0 | 16 | | |
| 1/7/00 | 27 | f | 1 | 26 | 27 | 1 | 4 |
| | 21 | m | 1 | 19 | 12 | | |
| 5/24/00 | 27 | f | 0 | 15 | 27 [c,d] | 27 | 100 |
| | 20 | m | 1 | 14 | 17 | | |
| 2/26/01 | 27 | f | 0 | 27 | 27 | 7 | 26 |
| | 15 | m | 5 | 14 | 15 | | |
| 5/1/01 | 27 | f | 0 | 27 | 27 | 25 [e] | 93 |
| | 14 | m | 1 | 13 | 14 | | |
| Total | 286 | | 17 | 155 | 203 | 81 | |

[a] Did not use body measurements due to sampling bias.

[b] Estimated body width for egg estimate using the equation, egg mass width = 1.862 + 0.346 * egg mass depth, acquired from summer three females.

[c] Estimated total body length on nine fish using the equation, total length = 80.75 + 7.551* egg mass length, acquired from summer two and three females.

[d] Unable to aquire egg mass depth or length from two pictures that contained eggs.

[e] One female body width was estimated for egg estimate using the equation, body width = 1.862 + 0.346 * egg mass depth, acquired from summer three females.

Table 2. Descriptions of the external body measurements collected during this study. All measurements were taken on live fish while suspended in water.

**Head Width (A):** The distance directly anterior from the pectoral fins between the opercules.

**Head Length (B):** The distance from the most anterior point on the upper lip to the most distant point on the opercular membrane.

**Head Depth (C):** The distance from the dorsal side of the head to the ventral side of the mouth, measured midway between the eye and pectoral fin.

**Body Cavity Width (D):** The distance from each side of the fish without depressing the skin of the fish. Measured directly anterior to the dorsal fin.

**Total Length (E):** The distance from the most anterior point on the upper lip to the most posterior point on the caudal fin.

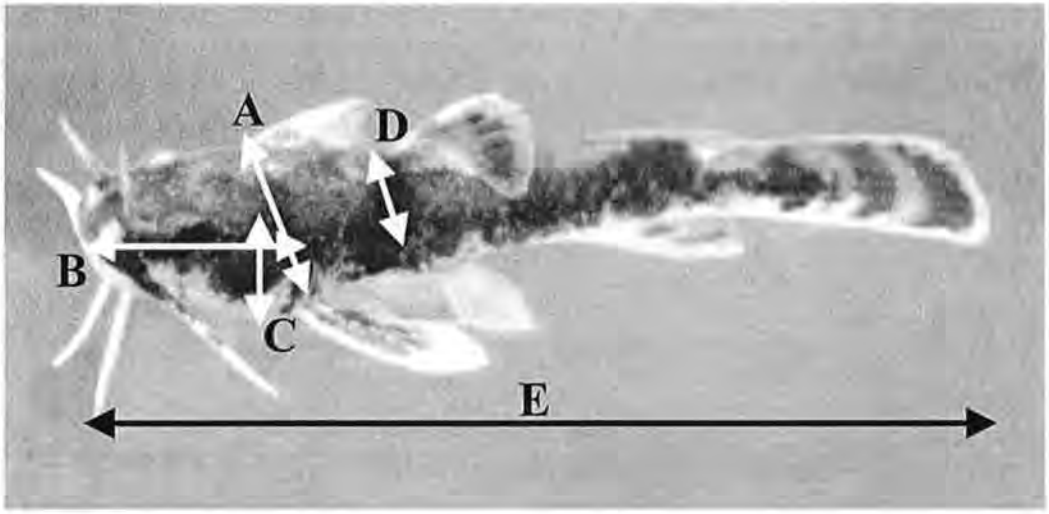

Table 3. Summary of the four ratios obtained from exterior body measurements: Head length to total length, head width to head length, head width to head depth, and head depth to head length. The observed mean, number of observations, standard deviation and groups for each ratio are given for each combination of year, season, and sex (model df = 6, error df = 148). Summer one was excluded due to sampling bias. 'Equivalent to' is the three way comparison where letters represent individual groups that are equal to each other. Bonferroni multiple comparisons were calculated using least squared means.

| | Winter | | | | Summer | | | |
|---|---|---|---|---|---|---|---|---|
| | Female | | Male | | Female | | Male | |
| | 2000 (a) | 2001 (b) | 2000 (c) | 2001 (d) | 2000 (e) | 2001 (f) | 2000 (g) | 2001 (h) |
| head length: | 0.1909 | 0.1859 | 0.2085 | 0.2017 | 0.1917 | 0.2094 | 0.2100 | 0.2179 |
| total length | (26, 0.0098) | (27, 0.0135) | (19, 0.0142) | (14, 0.0124) | (15, 0.0142) | (27, 0.0144) | (14, 0.0124) | (13, 0.0055) |
| equivalent to | b d e | a e | d f g h | a c e f g | a b d | c d g h | c d f h | c f g |
| head width: | 1.079 | 1.1184 | 1.0356 | 1.1010 | 1.0951 | 1.0231 | 1.0648 | 1.0386 |
| head length | (26, 0.0671) | (27, 0.0816) | (19, 0.0682) | (14, 0.0709) | (15, 0.0999) | (27, 0.0592) | (14, 0.0766) | (13, 0.0364) |
| equivalent to | b c d e g | a d e g | a d e g | a b c e g | a b c d g | none | a b c d e | none |
| head width: | 1.8285 | 1.9104 | 2.0 | 2.0230 | 1.7842 | 2.022 | 1.971 | 2.006 |
| head depth | (26, 0.1483) | (27, 0.1632) | (19, 0.1411) | (14, 0.2025) | (15, 0.1234) | (27, 0.1851) | (14, 0.1898) | (13, 0.1691) |
| equivalent to | b e g h | all | b d f g h | b c f g h | a b g | b c d g h | all | a b c d f g |
| head depth: | 0.5930 | 0.5881 | 0.5204 | 0.5479 | 0.6153 | 0.5096 | 0.5437 | 0.5204 |
| head length | (26, 0.0503) | (27, 0.0509) | (19, 0.0529) | (14, 0.0513) | (15, 0.0572) | (27, 0.0495) | (14, 0.0542) | (13, 0.0374) |
| equivalent to | b d e g | a d e g | d f g h | all | a b | c d g h | all | c d f g h |

Table 4. Summary of the ANOVA tests for the four different ratios of external body measurements on Neosho madtoms. For all tests, model df = 6 and error df = 148. Individual p-values and F statistics are given for each factor and interaction.

| | Ratio | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | head length:total length | | head width:head length | | head width:head depth | | head depth:head length | |
| | p | F | p | F | p | F | p | F |
| sex | < 0.0001 | 49.70 | 0.1254 | 2.38 | 0.0001 | 15.81 | < 0.0001 | 24.50 |
| season | < 0.0001 | 26.25 | 0.0173 | 5.79 | 0.7232 | 0.13 | 0.0524 | 2.36 |
| year | 0.1081 | 2.61 | 0.8907 | 0.02 | 0.0010 | 11.31 | 0.0024 | 9.54 |
| sex*season | 0.4267 | 0.64 | 0.3371 | 0.93 | 0.3074 | 1.05 | 0.1267 | 2.36 |
| sex*year | 0.2044 | 1.63 | 0.1476 | 2.12 | 0.0291 | 4.86 | 0.0017 | 10.24 |
| season*year | < 0.0001 | 22.24 | < 0.0001 | 19.35 | 0.0735 | 3.25 | < 0.0001 | 23.32 |

Table 5. Summary of spawns that occurred during the third summer period. The temperatures given are the high and lows for the day of the spawn. 'Mean egg size' was measured after the spawn. 'Estimated # of eggs in fish' are from ultrasound pictures before the spawn. Only males had multiple spawns so each female is a new fish.

| | Date | Time | Male Id | Temp | Light period (hr) | Female size (mm) | Mean egg size (cm) | Estimated clutch size | Estimated fecundity | All eggs expelled | Fertile | Outcome of eggs |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 6/16/01 | ~2000 | 4 | 21-22 | 15 | 116 | 4.019 | 313.13 | 273.65 | yes | ? | eggs ate next day |
| 2 | 6/26/01 | 0031 - 0127 | 3 | 21-24 | 15.75 | 113 | 3.405 | 417.8 | 257.69 | yes | yes | all eggs dead by 6/29/01 |
| 3 | 6/30/01 | daylight before 2059 | 3 | 21-24 | 15.75 | 105 | 4.119 | 387.81 | 219.53 | yes | ? | female ate eggs next day |
| 4 | 7/5/01 | ~1030 | 4 | 21-25 | 16 | 113 | 3.662 | 284.89 | 257.69 | yes | ? | male stopped taking care of eggs 7/7/01 |
| 5 | 7/9/01 | night? | 3 | 21-26 | 16 | 114 | ? | ? | 262.91 | yes | no | all eggs dead on 7/13/01 |
| 6 | 7/16/01 | early afternoon | 3 | 21-25 | 16 | 112 | ? | 150* | 252.58 | yes | no | all eggs dead on 7/18/01 |
| 7 | 7/22/01 | daylight before 2100 | 3 | 21-25 | 16 | 114 | 3.095 | 275.78 | 262.91 | no | no | all eggs dead on 6/26/01 |
| 8 | 7/26/01 | 1300 - 1500 | 5 | 21-25 | 16 | 113 | 3.130 | 95.13 | 257.69 | no | ? | ate next day |
| 9 | 7/30/01 | 1300 - 1430 | 5 | 21-25 | 16 | 112 | 3.589 | 125.46 | 252.58 | no | ? | male ate eggs next day |
| 10 | 8/6/01 | ~1430 | 5 | 21-25 | 16 | 105 | 3.247 | 20* | 219.53 | no | ? | male ate eggs next day |
| | | | | Mean | | 111.7 | 3.533 | 271.43 | 250.43 | | | |

* Visual estimate

APPENDIX
FIGURES

Figure 2. Neosho madtom distribution. The Neosho River basin located in Kansas, Missouri, and Oklahoma contains four large dams and 16 low-head dams (not shown). The Neosho madtom is only found in the mainstem sections of the Cottonwood, Neosho and the Spring rivers (modified from Wildhaber et al. 1999).

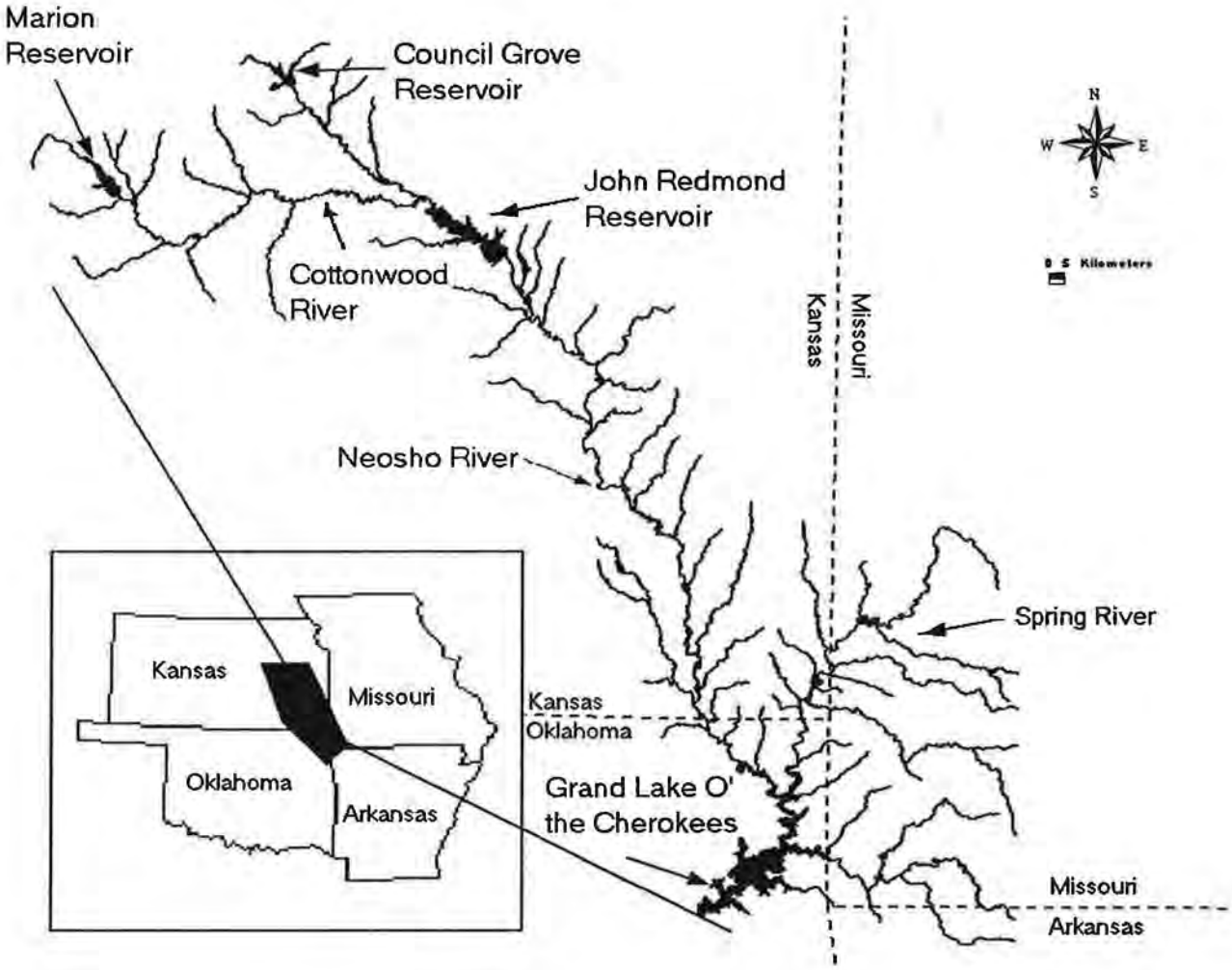

Figure 3. Photoperiod changes over three years that simulated the natural environment. The natural photoperiod is from the cooresponding longitude and latitude of Emporia, Kansas (N 38° 26 min., W 96° 12 min., U.S. Naval Observatory 1999-2001).

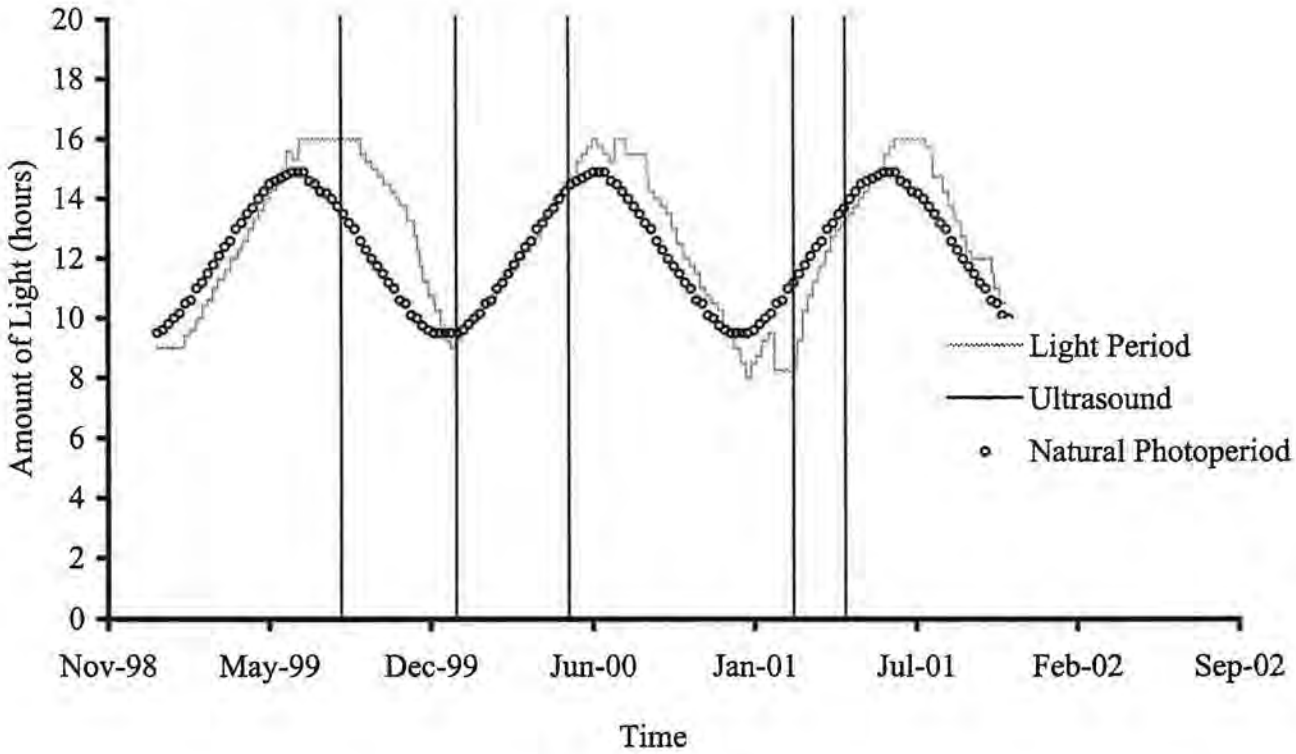

Figure 4. Temperature changes during three years that simulated the natural environment. The natural temperatures were collected during 1996-1998 from Neosho River (Bulger et al. 1998, Wilkinson and Edds 1997). Dark areas in the temperature reflect the daily high and low temperature changes. Breaks in the food line were removed due to the bias samples during the spawning season.

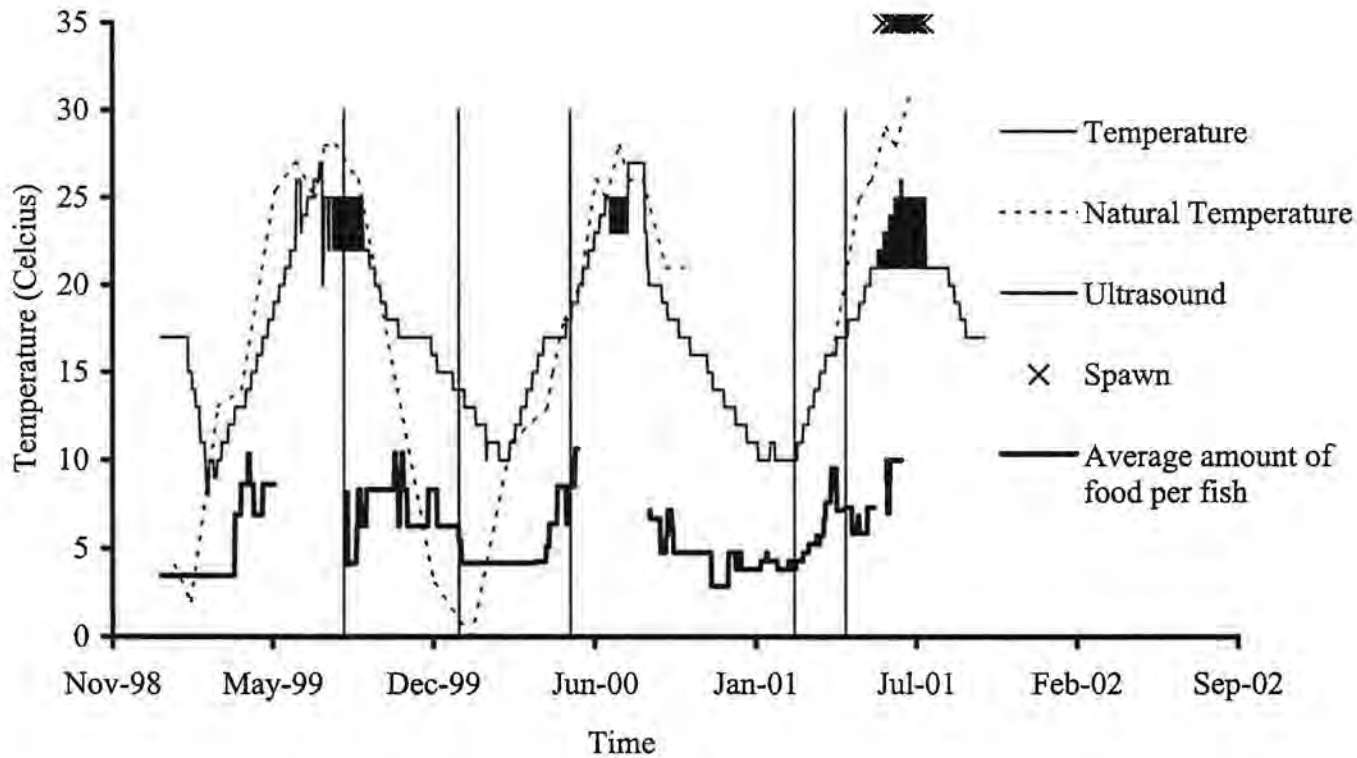

Figure 5. Ultrasound picture of a female Neosho madtom during the summer. Ultrasound was used to calculate egg size and fecundity. The fish is inverted so the ventral side of the fish is at the top of the picture. The fishes head is on the left side of the picture and the tail is on the right. Letters A,B,C are measurements of inidvidual eggs. Arrow indicates fishes pelvic fin.

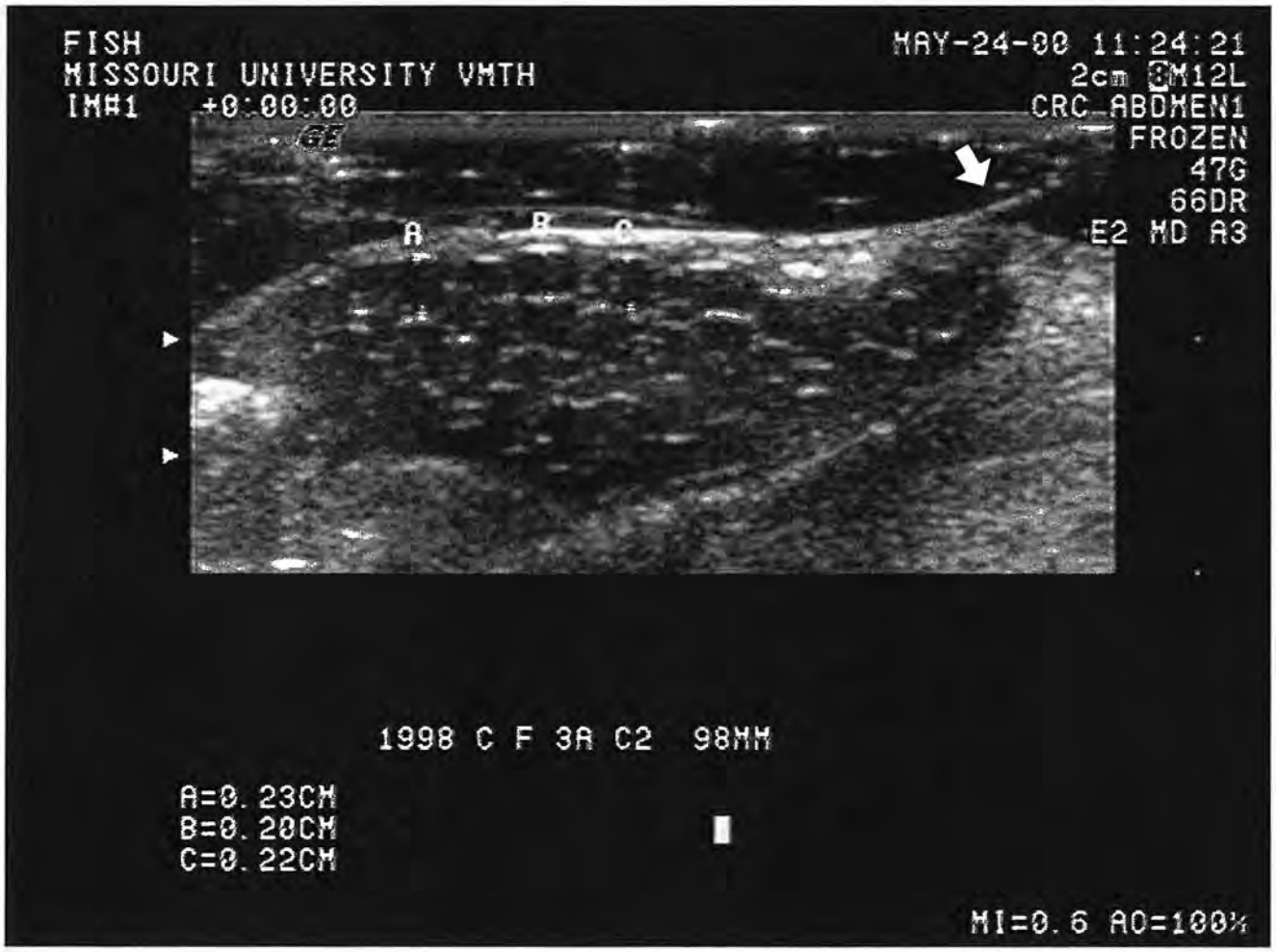

Figure 6. Ultrasound picture of a male Neosho madtom during the summer. The fish is inverted so the ventral side of the fish is at the top of the picture. The fishes head is on the left side of the picture and the tail is on the right. Note the absence of eggs in the body cavity. The one circular object in the picture is the stomach noted with the arrow.

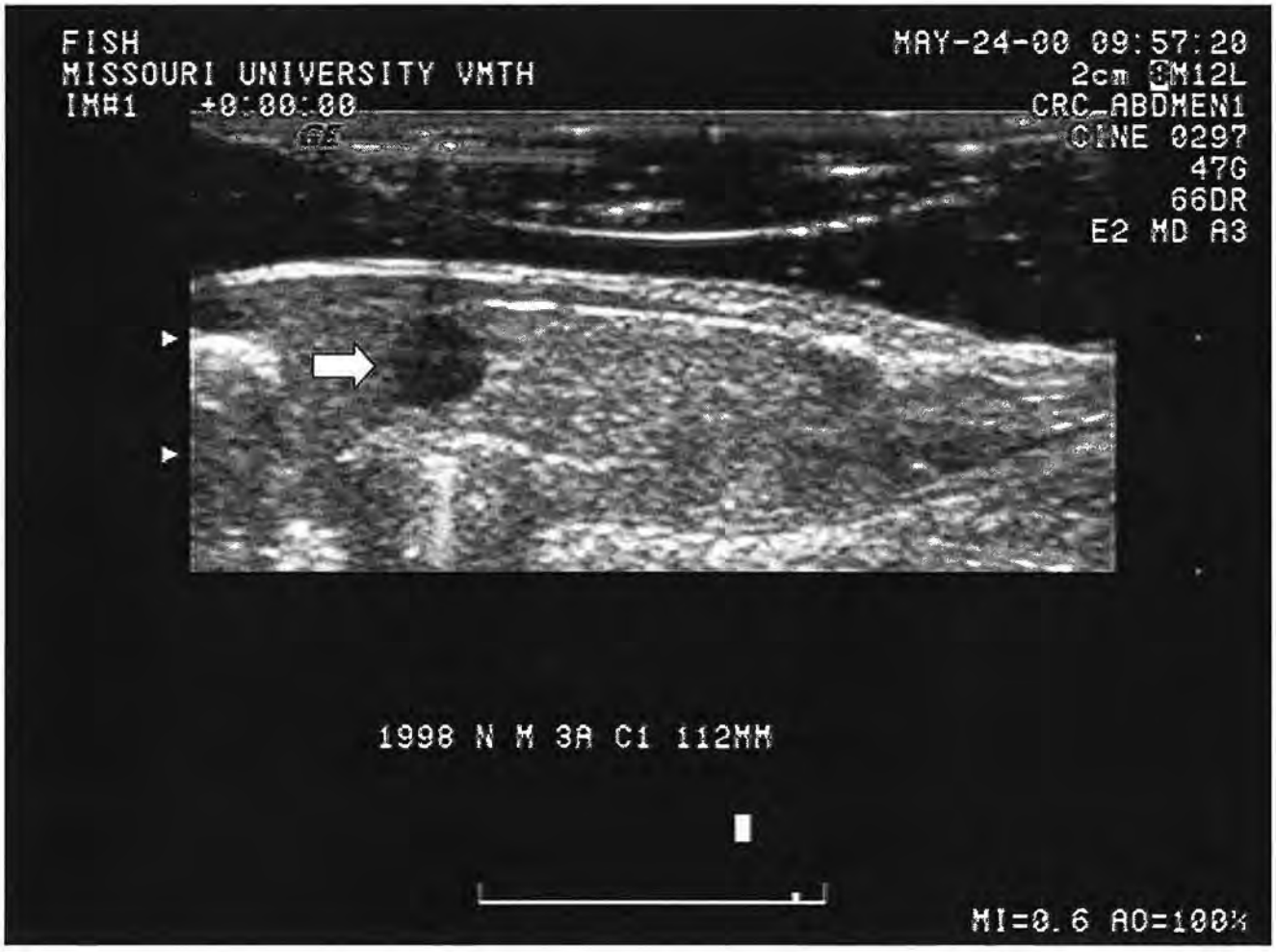

Figure 7. Growth of Neohso madtoms over five years. '--' represent ranges of yoy Neosho madtom total caught in the fall of 1996 (Wilkinson and Edds 1997). 'o' indicates males, '+' indicates females, and 'X' indicate mortalities. The summer of 1999 data was eliminated from the analysis due to sampling bias but had similar values as the other two summers (i.e. male mean length = 106.5 mm, female mean length = 92.4 mm).

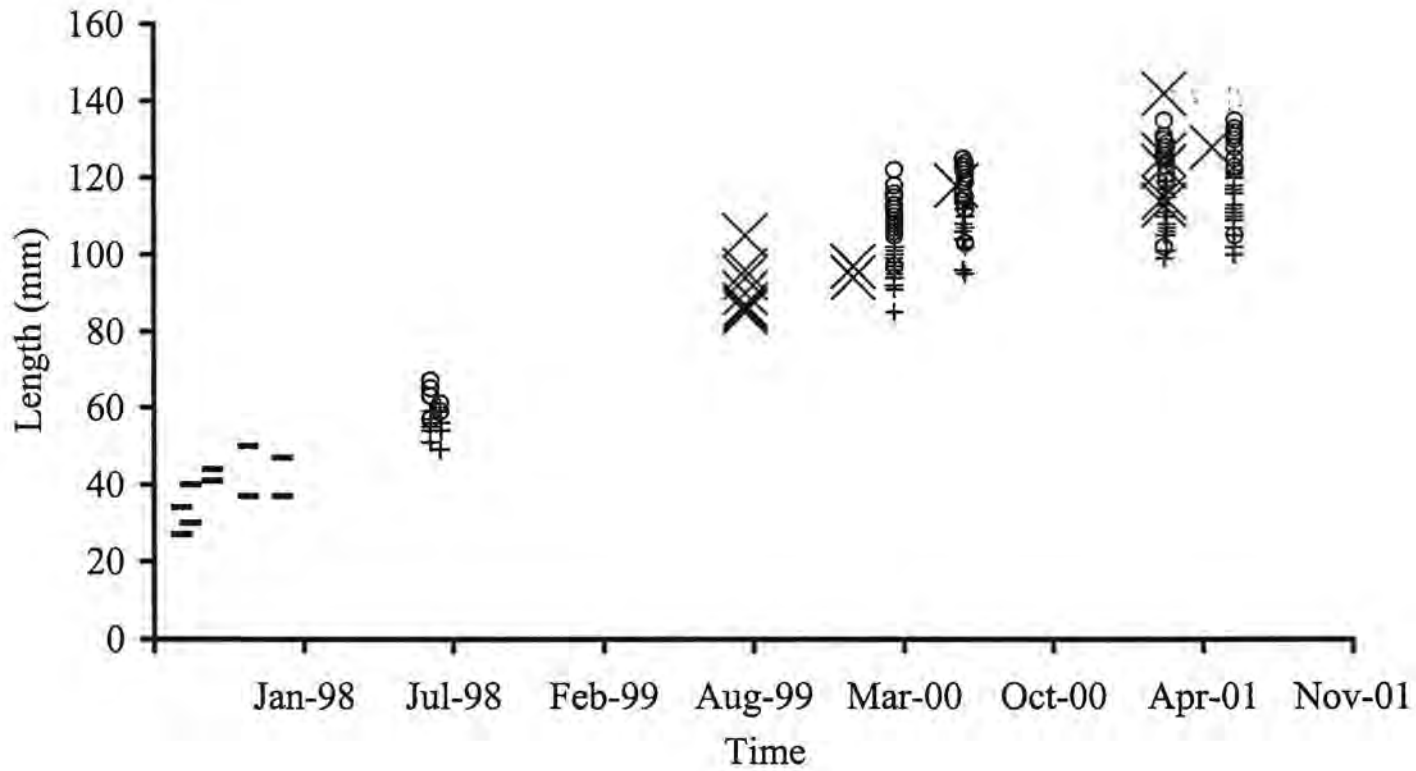

Figure 8. The weight length relationship of Neosho madtoms. Graph contains male and female fish from 2000 and 2001. Both length and weight axis are a $\log_{10}$ scale ($\log_{10}$ weight = -4.033 + 2.613*$\log_{10}$ length, $r^2 = 0.8213$, n = 155).

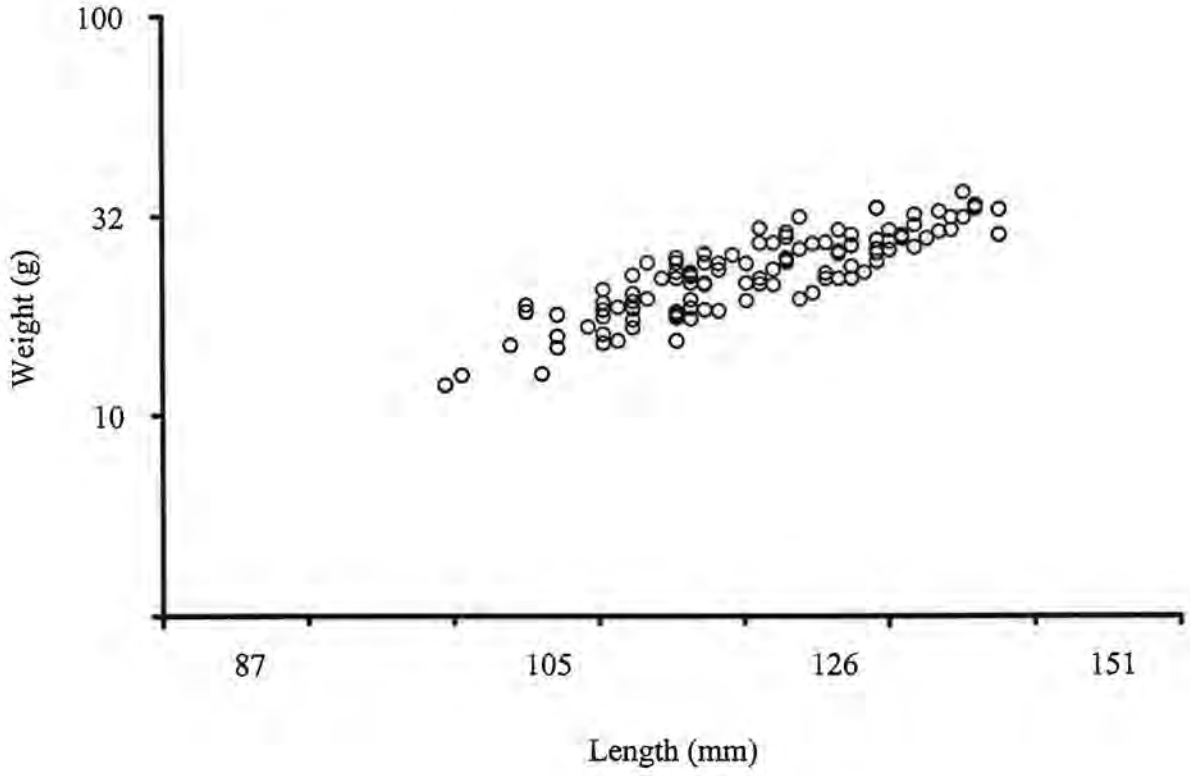
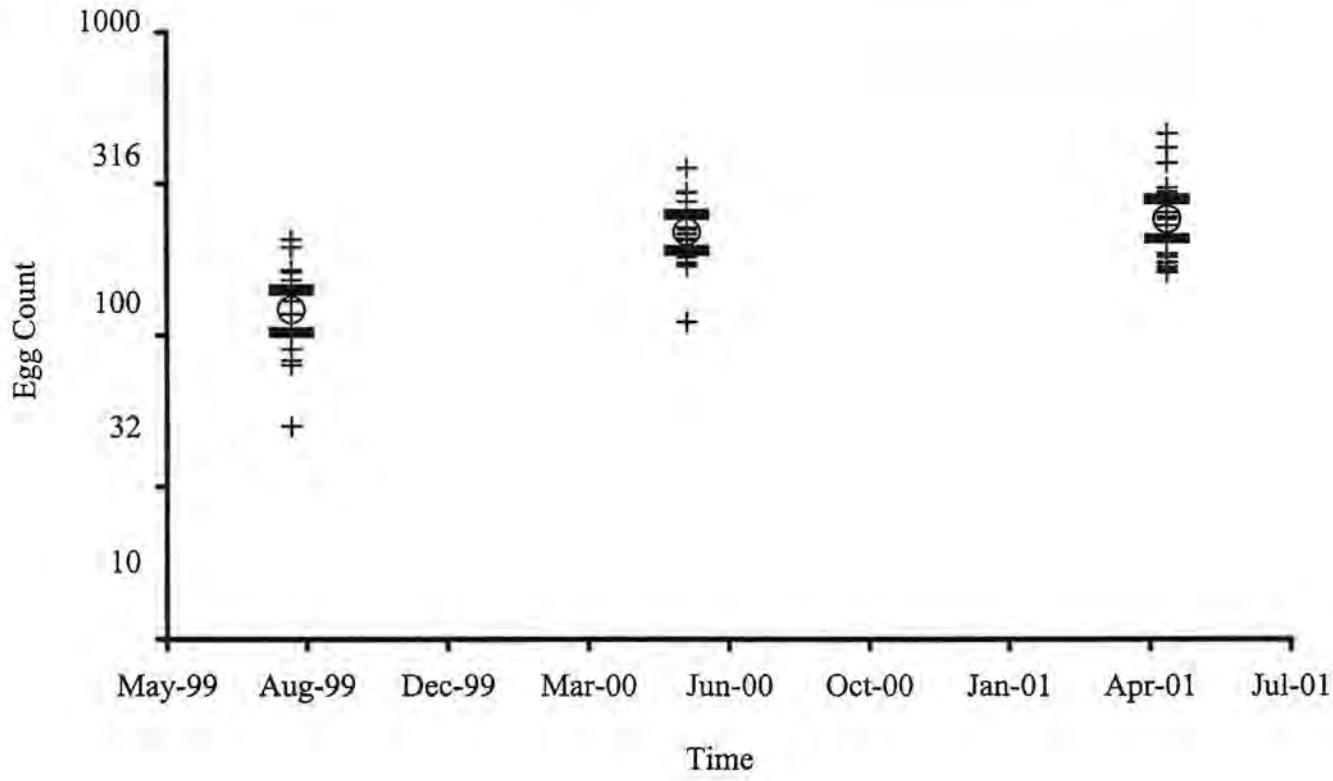
Figure 9. The number of eggs estimated in female Neosho madtoms during three summers. The $\log_{10}$ (egg count) did change with fish length over time. Summer one was significantely lower than summer two and three. Circles indicate means, lines indicate 95% confidence intervals (n = 71).