

Reproduction by Individuals of a Nonreproducing Population of *Megaloniais nervosa* (Mollusca: Unionidae) Following Translocation

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ABSTRACT.—Reproduction of *Megaloniais nervosa* (Rafinesque, 1820) has not been documented for over 20 y in much of the Cumberland River, where water temperatures have decreased and flow regimes have been greatly altered by hypolimnetic discharges from impoundments. Studies in other streams have implicated low temperatures or changes in discharge patterns as causative factors inhibiting reproduction. *Megaloniais nervosa* were collected from the Cumberland River, translocated to the Tennessee River, and held in an embayment of Kentucky Lake. After the first and second y, samples of *M. nervosa* were taken from the Cumberland River, an existing population in Kentucky Lake, and the translocated group. Histological examination indicated that translocated mussels had a high incidence of hermaphroditism, and like mussels originating in Kentucky Lake, had undergone an otherwise normal reproductive development. Individuals functioning successfully as females from the translocated group had mature glochidia in their marsupia. Females from the Kentucky Lake sample also had mature glochidia present. In contrast, there was no indication of reproductive activity in gonads or marsupia of individuals collected from the Cumberland River. Our results indicate that a return to a more natural temperature regime in the Cumberland River would reinstate a normal reproductive cycle. We suspect that the altered temperature regime is also disrupting the gametogenic cycle of all mussels, including at least six federally listed endangered species occurring in the Cumberland River. These relic populations will disappear unless they are translocated or the thermal regime returned to normal.

INTRODUCTION

Impoundments on the Cumberland River and many of its tributaries in Kentucky Tennessee have profoundly impacted the diverse freshwater mussel fauna that once thrived in this drainage. In August 1952, completion of the Wolf Creek Dam in Kentucky significantly altered both the flow and the temperature regime of a significant portion of the Cumberland River. The dam, located at Cumberland River kilometer (CRK) 741.8, creates a reservoir with a surface area of 18,333 ha at maximum pool. Water is released from many as six generators, with inlets located below the thermocline. As a result, the powerhouse releases cold, hypolimnetic water during all times of the year (Miller *et al.*, 198

From Wolf Creek Dam, the river flows SW for approximately 237 km before it is impounded by Cordell Hull Dam at CRK 504.5 near Carthage, Tennessee. The cold water discharged from the Wolf Creek Dam is reinforced by the hypolimnetic releases of Hollow and Center Hill dams which are located on major tributaries to the Cumberland River. Center Hill Dam releases hypolimnetic water into the Caney Fork River, which joins the Cumberland River just below Cordell Hull Dam. Cordell Hull Reservoir has a very short hydraulic retention time, ranging from 3.2 to 11.8 d, and has little influence on water temperature (Luers, 1980). The net effect is that water temperatures in the Cumberland River near Carthage (CRK 495) rarely exceed 20 C throughout the entire year. Loca

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ologists have suggested that either the low temperatures of the Cumberland River are responsible for the lack of recruitment of *Megaloniaias nervosa*, or the mussel populations are senile and incapable of reproducing.

Temperature activates spawning for marine bivalves (Loosanoff and Davis, 1952; Sast 1966; Young and DeMartini, 1970). Coker *et al.* (1921) reported that increasing water temperatures initiated the reproductive activities of some mussels. Matteson (1955) found that low temperatures were correlated with unsuccessful reproduction. Recent research conducted with freshwater mussels has provided further insight into the importance of temperature in regulating spawning. In laboratory experiments, spawning of *Etheostoma coplanata* was delayed 6 and 12 wk successively by manipulating water temperature and photoperiod (Bill Lellis, U.S. Geological Survey, Research and Development Laboratory, Westboro, Pa., pers. comm.). Female *Hyridella australis*, normally gravid throughout the year, were not gravid during periods of abnormally low water temperatures (Jones *et al.*, 1984). Decreased temperatures have also been implicated as a cause for the lack of recruitment and subsequent extirpation of several mussels from other systems (Layzer *et al.*, 1993). Cold water, often thought to eliminate warmwater host fish, has not eliminated any of the reported hosts of *Megaloniaias nervosa* in this area of the Cumberland River (TWRA, 1999). Layzer *et al.* (1993) presented evidence that lack of hosts in one tailwater was not the problem; instead, they argued that the low water temperatures upset the gametogenic cycle of mussels.

The mainstem Cumberland River once supported at least 68 species of freshwater mussels before dam construction (Starnes and Bogan, 1988). Before impoundment by the Wolf Creek Dam, Neel and Allen (1964) reported finding at least 39 species of mussels. Gordon and Layzer (1989) reported that the Cumberland River drainage historically supported 68 species of freshwater mussels. Freshwater mussels in tributaries to the Cumberland River have also been adversely affected by impoundment. For instance, only 38% (23 species) of the historical mussel fauna remains in the Caney Fork River system (Layzer *et al.*, 1993). The 37 species extirpated includes two which are now extinct and seven that are federally listed as endangered.

Several postimpoundment surveys have shown a dramatic decline in mussel diversity (Stansbery, 1969; Parmalee *et al.*, 1980). Today, 38% of the historic assemblage of the Cumberland River drainage is now globally extinct or listed as endangered, and several additional species exist only in small nonreproducing populations (Gordon and Layzer, 1989). A survey by Miller *et al.* (1984) found few individuals of only two mussel species existing in the Cumberland River below the Wolf Creek Dam.

The objective of this study was to evaluate the reproductive competence of *Megaloniaias nervosa* from the Cumberland River after translocation to a more natural temperature regime.

METHODS

In November 1993, 100 *Megaloniaias nervosa* were collected by SCUBA diving in the Cumberland River, below Cordell Hull Dam at CRK 484.5. Mussels were placed in coolers, draped in wet burlap, and transported the following morning to an embayment of Kentucky Lake at Tennessee River km (TRK) 166. They were then held in suspended pocket nets at a pearl farm for 2 y.

Random samples of these translocated individuals were taken during August and October of 1994 and 1995 for histological examination of the gonads. Samples were also collected within 11 days from Kentucky Lake (reference sample) at TRK 164.7, and the Cumberland

TABLE 1.—Reproductive stages for three populations of *Megalonaias nervosa* in 1994 and 1995

Population	Date	n	Stages				
			0	1	2	3	4
Translocated	16 October 1994	7	2	4	0	0	1
	31 August 1995	30	0	1	0	29	0
Cumberland River	26 October 1994	6	2	4	0	0	0
	11 September 1995	30	5	23	0	2	0
Kentucky Lake	16 October 1994	4	0	1	0	0	3
	31 August 1995	11	0	0	0	11	0

River at CRK 484.5. Additional samples were taken at the end of the 2nd y in October, from all three sites, specifically to check for glochidia.

A portion of gonadal material and marsupia was removed from each individual and fixed in 10% buffered formalin. Tissues were then dehydrated in a series of aqueous ethanol solutions, cleared in Hemo De clearing agent (Fisher[®]), infiltrated with, and then embedded in, paraplast. Once in paraplast, serial sections 8–10 μm thick were cut and stained with hematoxylin and eosin (Humanson, 1979).

All sections were microscopically examined to determine the state of gametogenesis, and the presence of glochidia in marsupia of females. Histological sections were classified by the following stages adapted from Yokley (1972) and Woody and Holland-Bartels (1993):

0. gonadal tissue undifferentiated, sex undetermined.
1. some spermatogonia or oogonia and small oocytes, sex differentiated.
2. spermatids present or developing oocytes are moving into the lumina.
3. spermatozoa or mature ova fill the lumen.
4. females with marsupia filled with embryos or glochidia.

Cell types were distinguished using the descriptions of Dinamani (1974), and Peredo and Parada (1984). Mean water temperature, total dissolved solids and phosphorus were calculated for the Cordell Hull and Pickwick tailwaters from data reported by the U.S. Geological Survey 1993, 1994, 1995.

RESULTS

Stage 1 predominated in translocated individuals after the 1st year (1994) (Table 1). By the end of the 2nd year (1995), individuals of this group reached Stage 3 of reproductive development in August (Fig. 1A), and by October 49% of this group had mature glochidia present in their marsupia (Table 2). Females in the Kentucky Lake reference sample were in Stage 4 of reproductive development in October 1994. In August 1995, all individuals collected from Kentucky Lake were in Stage 3. In contrast, mussels collected from the Cumberland River in September or October each year were mainly in Stage 1 (Table 1). Although most individuals collected from the Cumberland River could be differentiated as male or female, they did not have the antecedent cells of either eggs or sperm present to fit the true criteria for Stage 1 (Fig. 1B). Therefore, it is unlikely that these individuals would have progressed to Stage 3.

Individuals collected from Kentucky Lake were distinctly either male or female (Table 3). In comparison, nearly one-half of the translocated mussels were hermaphrodites after the 2nd year. Moreover, most hermaphrodites not only possessed separate male and female follicles, but also contained follicles with both male and female gametes (Fig. 1C). Presun-

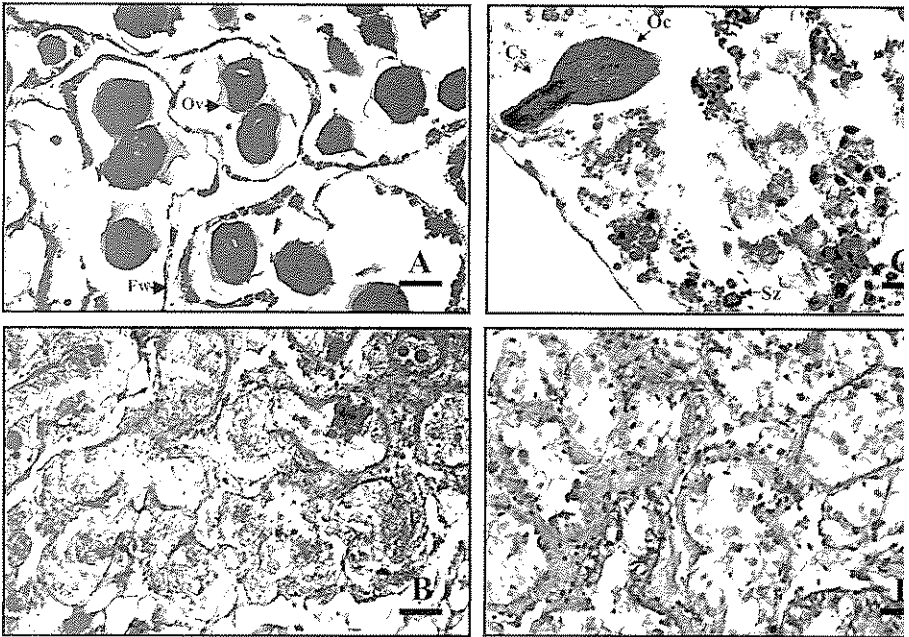


FIG. 1A.—Female gonadal tissue of *Megaloniais nervosa* from the translocated population, taken August 1995, in late stages of oogenesis (100 \times) bar length = 100 μ m. Ova (Ov) in the lumina of follicle. Follicle wall (Fw). B. Female gonadal tissue of *Megaloniais nervosa* from the Cumberland population, taken in September 1995, in atypical Stage 1 development (100 \times) bar length = 100 μ m. Oocyte (Oc) attached to a cytoplasmic stalk (Cs). Spermatozoa (Sz) associated with sperm morulae within the same follicle. C. Hermaphroditic follicle tissue of *Megaloniais nervosa* from the translocated population (400 \times) bar length = 25 μ m. Oocyte (Oc) attached to a cytoplasmic stalk (Cs). Spermatozoa (Sz) associated with sperm morulae within the same follicle. D. Undifferentiated gonadal tissue of *Megaloniais nervosa* from the Cumberland River population, taken in September 1995 (200 \times) bar length = 50 μ m.

ably, hermaphrodites were functioning successfully as females because only 20% of the translocated group examined in August 1995 were females (Table 3), but 49% of the mussels checked in October were gravid (Table 2). The gender composition of the Cumberland River sample was comprised of males, females, a few hermaphrodites, and some individuals that were completely undifferentiated. While undifferentiated individuals were found in the translocated sample after the 1st year, none were found in the 2nd yr. Gonads of undifferentiated individuals were shrunken and emaciated, which was the common appearance of all mussels taken from the Cumberland River (Fig. 1D). Glochidia were not present in

TABLE 2.—Percent frequency of glochidia in all individuals examined from three populations of *Megaloniais nervosa*

Population	Date	Percent with glochidia	Sample size
Translocated	6 October 1995	49%	49
Cumberland River	10 October 1995	0%	41
Kentucky Lake	6 October 1995	55%	40

TABLE 3.—The gender composition in three populations of *Megaloniais nervosa* in 1994 and 1995

Population	Date	n	Sex			Undifferentiated
			Male	Female	Hermaphrodite	
Translocated	16 October 1994	7	4	1	0	2
	31 August 1995	30	10	6	14	0
Cumberland River	26 October 1994	6	2	2	0	2
	11 September 1995	30	5	15	5	5
Kentucky Lake	16 October 1994	4	1	3	0	0
	31 August 1995	11	7	4	0	0

mussel collected in either year from the Cumberland River (Table 2). Mean monthly temperatures for the Cordell Hull Dam discharge (Cumberland River) rarely exceeded 20 C in 1994 (Fig. 2). A comparison of total dissolved solids and phosphorus between Cordell Hull and Pickwick (upstream boundary of Kentucky Lake) tailwaters for 3 y revealed few differences (Table 4).

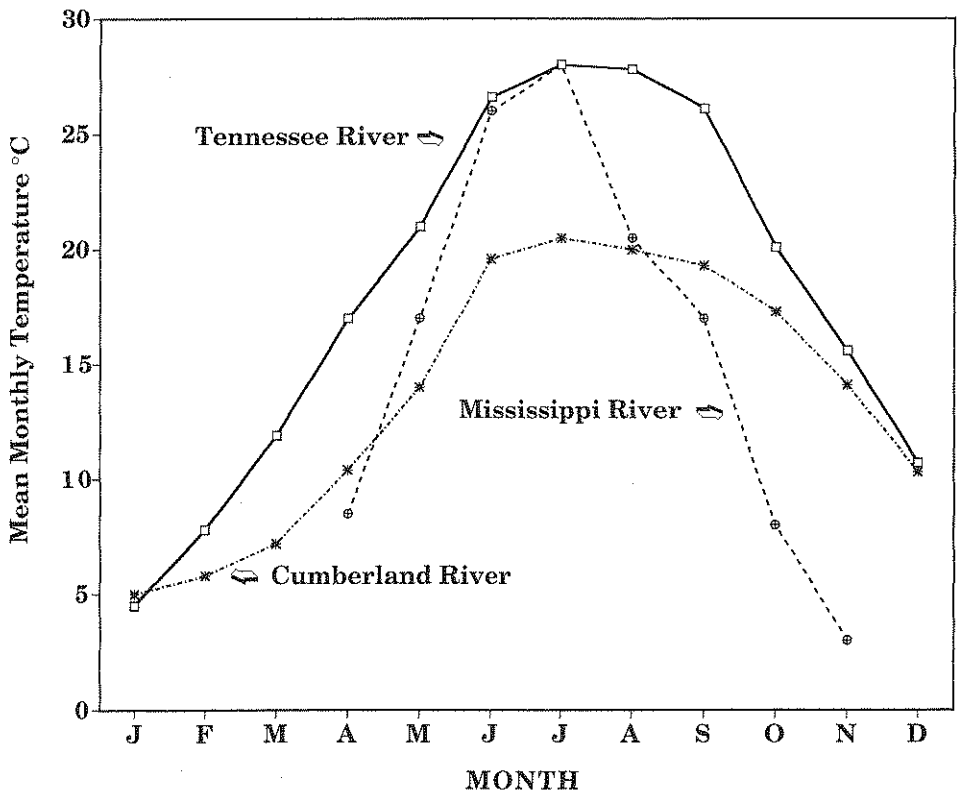


FIG. 2.—Mean monthly temperatures for 1994 for the Cordell Hull Dam discharge (Cumberland River); Kentucky Lake (Tennessee River) and Pool 10, (Mississippi River). Records for the Mississippi River are from Woody and Holland-Bartels (1993)

TABLE 4.—Mean values and standard deviations (SD) of total dissolved solids and total phosphorus for the Cordell Hull Dam and Pickwick Dam tailwaters for 3 y (Data from U.S. Geological Survey, 1994, 1995)

Variable	Year	Cordell Hull		Pickwick Tailwater	
		Mean	SD	Mean	SD
Total dissolved solids (g/liter)	1992	0.11	0.00	0.10	0.01
	1993	0.12	0.01	0.09	0.01
	1994	0.10	0.02	0.09	0.02
Total phosphorus (mg/l as P)	1992	0.070	0.092	0.040	0.018
	1993	0.030	0.019	0.060	0.008
	1994	0.040	0.024	0.050	0.022

DISCUSSION

Very little is understood about hermaphroditism in bivalves in general and freshwater mussels in particular. Hermaphroditism is often considered to be accidental or developmental, caused by deviation or failure of the sex-differentiating mechanism. This type deviation is characterized by gonadal tissues that range from equal portions of germ cells for each sex, to the predominance of one sex within an individual (Coe, 1943). The high frequency of hermaphrodites in the translocated sample is unusual for most freshwater mussel populations thus far examined. For instance, van der Schalie (1970) examined histological sections of 1871 mussels of 97 species and found that only four species were regularly hermaphroditic and 22 other taxa were occasionally hermaphroditic. Woody and Holland-Bartels (1993) found no hermaphrodites in 255 *Megaloniaias nervosa* collected from the upper Mississippi River where water temperatures exceeded 25 C during the summer. The hermaphrodites in our study followed a pattern similar to the varied cell distribution within each gonad described by Coe (1943). Many individuals also possessed numerous hermaphroditic follicles, a condition rarely reported in other hermaphroditic bivalves (Tepe, 1943). Kat (1983) found hermaphroditic follicles in *Alasmidonta undulata* only in populations with low density. Bauer (1987) indicated that the frequency of hermaphrodites increased at low densities in a population of *Margaritifera margaritifera*. The cause of the high incidence of hermaphroditism in the translocated mussels is unknown; however, the proportion of hermaphrodites in the translocated sample was significantly greater than in the Cumberland River sample ($\chi^2 = 4.29$; $P < 0.05$), which suggests that *Megaloniaias nervosa* is a facultative hermaphrodite. The incidence of hermaphrodites in the translocated group does not appear to be related to low density. On the contrary, the mussels were held in close proximity to >100,000 *M. nervosa*.

Although recruitment of *Megaloniaias nervosa* is not occurring in the Cumberland River a few individuals have been observed to abort glochidia (Don Hubbs, pers. comm.). While a gravid individual may occasionally be found, no recruitment by *M. nervosa* or any other species has been observed in the nearly 300-km, free-flowing section of the Cumberland River. Density of *M. nervosa* at our collection site in the Cumberland River was estimated to be 3.45/m² (Hubbs, 1994). Several suitable warmwater hosts were present in the system in particular, gizzard shad (*Dorosoma cepedianum*) were abundant. Consequently, the lack of recruitment cannot be attributed to an absence of hosts.

The atypical Stage 1 development and the lack of glochidia in our Cumberland River samples in both years indicates that little or no reproduction was occurring. Stage 0,

definition, is normally characteristic only of juveniles. The occurrence of Stage 0 in the large-sized (>130 mm long) individuals in our samples from the Cumberland River is unusual. Perhaps these individuals were juveniles when the Wolf Creek Dam began operation or they represent adults that have regressed. In contrast, Cumberland River mussels translocated to Kentucky Lake did reproduce; however, most *Megaloniaias nervosa* required 2 years following translocation before they reproduced. The 1st year after translocation, only one individual in the sample was gravid and two individuals were still sexually undifferentiated but after 2 years, 49% were gravid and all were sexually differentiated. Although few studies have examined the possibility of freshwater mussels being facultative hermaphrodites, the research of Kat (1983), Bauer (1987) and ours suggests that its occurrence may be more widespread than previously thought. Moreover, the occurrence of hermaphroditic follicles suggests the possibility of self-fertilization. If indeed, other unionids are facultative hermaphrodites, capable of self-fertilization, it could explain the occurrence of gravid individuals in low density populations.

Because nutritional requirements of freshwater mussels are not well understood, inadequate nutrition cannot be totally ruled out as a causative factor limiting reproduction. Nutrition is critical to successful reproduction in marine bivalves (Coe and Turner, 1938). Total dissolved solids represent an average edaphic condition for a watershed and is closely tied to biological productivity (Kemp, 1971). Total dissolved solids and phosphorus levels were similar between the Cordell Hull Dam and Pickwick Dam tailwaters suggesting that nutrients were not limiting. In contrast, water temperatures in these tailwaters differ substantially. Therefore, we believe that water temperature, or factors directly related to temperature, is the causative factor limiting reproduction of *Megaloniaias nervosa* in the Cumberland River. Unless dam operations are modified, all or nearly all mussels in the study area will be extirpated.

The results of our study clearly indicate that the population of *Megaloniaias nervosa* in the Cumberland River is capable of successfully reproducing in a suitable environment. A thorough evaluation of the number of species affected by cold water should be conducted. We suspect that other species present (including the federally endangered *Cyprogenia stuarti*, *Dromus dromas*, *Epioblasma obliquata*, *Lampsilis abrupta*, *Pleurobema plenum* or *Obovaria retusa*) are also capable of reproducing if their environment is changed. Major changes in dam operations throughout the entire Cumberland River system could return water temperatures and flow regimes to more normal conditions and likely increase existing mussel populations. In the absence of such action, we believe that the number one priority for this system is the development of a detailed management plan for all Cumberland River mussels. The plan must be decisive and specifically address the fate of the existing populations. For instance, should mussels be left in the Cumberland River, essentially keeping them in "cold storage," until techniques for long-term holding, propagation, and translocation are developed sufficiently to ensure that these individuals have a high probability of surviving and ultimately contributing to an overall mitigation effort? Alternatively, the best use of these mussels may be for research purposes and re-establishing populations in other streams. Currently, mussels are being collected from other waters in Tennessee where reproduction is occurring. These mussels are being used to establish captive populations and for translocation to other locations within and outside of Tennessee. To the extent possible we recommend that all mussels for such purposes come from nonreproducing populations such as those of the Cumberland River.

Existing populations of endangered species in the Cumberland River, although relatively small, still probably consist of a few thousand individuals. Since these populations are greatly dispersed and densities are extremely low, a massive rescue attempt would be impractic-

Perhaps the few individuals that are encountered by researchers should be retained for propagation or life history studies. Taking a small number of individuals would not have any measurable effect on the existing population and again, unless dam operations changed, the point is moot. Finally, commercial harvest regulations should be reviewed for consistency with any long-term plans developed. The system is in jeopardy and in dire need of a management plan to restore its former diversity.

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