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

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ABSTRACT.—Reproduction of *Megalonaias 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. *Megalonaias 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 thi in this drainage. In August 1952, completion of the Wolf Creek Dam in Kentucky sig cantly altered both the flow and the temperature regime of a significant portion o Cumberland River. The dam, located at Cumberland River kilometer (CRK) 741.8, cre a reservoir with a surface area of 18,333 ha at maximum pool. Water is released fro many as six generators, with inlets located below the thermocline. As a result, the p house 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 i pounded by Cordell Hull Dam at CRK 504.5 near Carthage, Tennessee. The cold v 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 Cumbe River. Center Hill Dam releases hypolimnetic water into the Caney Fork River, which the Cumberland River just below Cordell Hull Dam. Cordell Hull Reservoir has a very hydraulic retention time, ranging from 3.2 to 11.8 d, and has little influence on v temperature (Luers, 1980). The net effect is that water temperatures in the Cumbe River near Carthage (CRK 495) rarely exceed 20 C throughout the entire year. Loc

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

Temperature activates spawning for marine bivalves (Loosanoff and Davis, 1952; Sas 1966; Young and DeMartini, 1970). Coker *et al.* (1921) reported that increasing water te peratures initiated the reproductive activities of some mussels. Matteson (1955) found t low temperatures were correlated with unsuccessful reproduction. Recent research c ducted with freshwater mussels has provided further insight into the importance of te perature in regulating spawning. In laboratory experiments, spawning of *Etheostoma c planata* was delayed 6 and 12 wk successively by manipulating water temperature and p toperiod (Bill Lellis, U.S. Geological Survey, Research and Development Laboratory, We boro, Pa., pers. comm.). Female *Hyridella australis,* normally gravid throughout the y were not gravid during periods of abnormally low water temperatures (Jones *et al.,* 198 Decreased temperatures have also been implicated as a cause for the lack of recruitme and subsequent extirpation of several mussels from other systems (Layzer *et al.,* 1993). C water, often thought to eliminate warmwater host fish, has not eliminated any of the ported hosts of *Megalonaias nervosa* in this area of the Cumberland River (TWRA, 19 Layzer *et al.* (1993) presented evidence that lack of hosts in one tailwater was not problem; instead, they argued that the low water temperatures upset the gametogenic cy of mussels.

The mainstem Cumberland River once supported at least 68 species of freshwater mus before dam construction (Starnes and Bogan, 1988). Before impoundment by the V Creek Dam, Neel and Allen (1964) reported finding at least 39 species of mussels. Gord and Layzer (1989) reported that the Cumberland River drainage historically supported species of freshwater mussels. Freshwater mussels in tributaries to the Cumberland Ri have also been adversely affected by impoundment. For instance, only 38% (23 species the historical mussel fauna remains in the Caney Fork River system (Layzer *et al.,* 19 The 37 species extirpated includes two which are now extinct and seven that are feder listed as endangered.

Several postimpoundment surveys have shown a dramatic decline in mussel diver (Stansbery, 1969; Parmalee *et al.,* 1980). Today, 38% of the historic assemblage of the Cu berland River drainage is now globally extinct or listed as endangered, and several ad tional species exist only in small nonreproducing populations (Gordon and Layzer, 198 A survey by Miller *et al.* (1984) found few individuals of only two mussel species existing the Cumberland River below the Wolf Creek Dam.

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

METHODS

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

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

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, fro all three sites, specifically to check for glochidia.

A portion of gonadal material and marsupia was removed from each individual and fixe in 10% buffered formalin. Tissues were then dehydrated in a series of aqueous ethan solutions, cleared in Hemo De clearing agent (Fisher™), infiltrated with, and then embe ded in, paraplast. Once in paraplast, serial sections 8–10 μm thick were cut and staine with hematoxylin and eosin (Humanson, 1979).

All sections were microscopically examined to determine the state of gametogenesis, an the presence of glochidia in marsupia of females. Histological sections were classified l 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 ovocytes, sex differentiated.
2. spermatids present or developing ovocytes 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 an Parada (1984). Mean water temperature, total dissolved solids and phosphorus were ca culated for the Cordell Hull and Pickwick tailwaters from data reported by the U.S. Ge logical Survey 1993, 1994, 1995.

RESULTS

Stage 1 predominated in translocated individuals after the 1st year (1994) (Table 1). E the end of the 2nd year (1995), individuals of this group reached Stage 3 of reproductiv development in August (Fig. 1A), and by October 49% of this group had mature glochidi present in their marsupia (Table 2). Females in the Kentucky Lake reference sample wer in Stage 4 of reproductive development in October 1994. In August 1995, all individua collected from Kentucky Lake were in Stage 3. In contrast, mussels collected from th 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 a male or female, they did not have the antecedent cells of either eggs or sperm present t fit the true criteria for Stage 1 (Fig. 1B). Therefore, it is unlikely that these individua would have progressed to Stage 3.

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

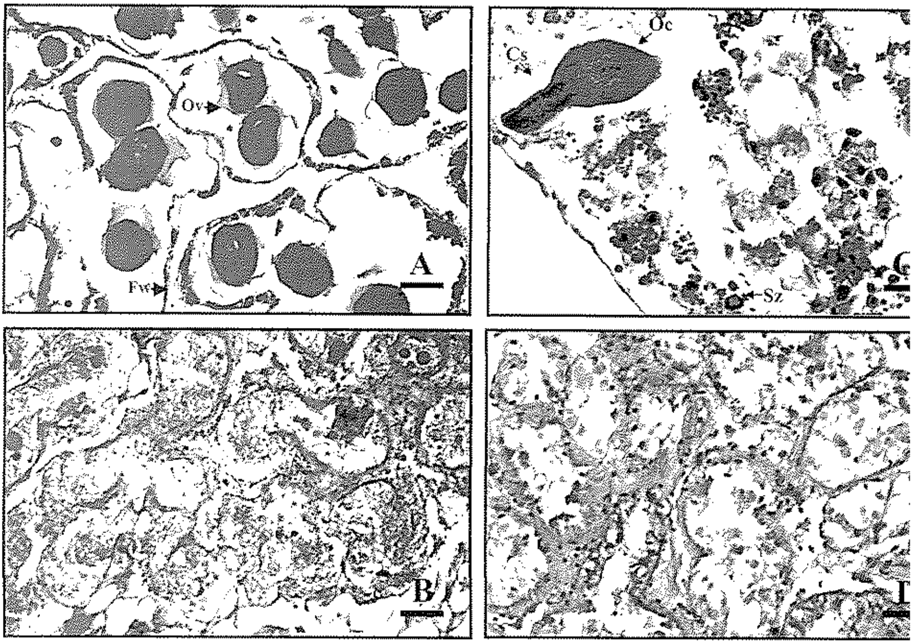

FIG. 1A.—Female gonadal tissue of *Megalonaias nervosa* from the translocated population, take August 1995, in late stages of oogenesis (100×) bar length = 100 µm. Ova (Ov) in the lume follicle. Follicle wall (Fw). B. Female gonadal tissue of *Megalonaias nervosa* from the Cumberland I population, taken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

ably, hermaphrodites were functioning successfully as females because only 20% of translocated group examined in August 1995 were females (Table 3), but 49% of the r sels checked in October were gravid (Table 2). The gender composition of the Cumberl River sample was comprised of males, females, a few hermaphrodites, and some individ that were completely undifferentiated. While undifferentiated individuals were found in translocated sample after the 1st year, none were found in the 2nd yr. Gonads of undi entiated individuals were shrunken and emaciated, which was the common appearance 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 populatio *Megalonaias 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 *Megalonaias nervosa* in 1994 and 199

Population	Date	n	Sex: Male	Female	Hermaphrodite	Undifferentiated
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 ten peratures for the Cordell Hull Dam discharge (Cumberland River) rarely exceeded 20 (in 1994 (Fig. 2). A comparison of total dissolved solids and phosphorus between Corde Hull and Pickwick (upstream boundary of Kentucky Lake) tailwaters for 3 y revealed fe differences (Table 4).

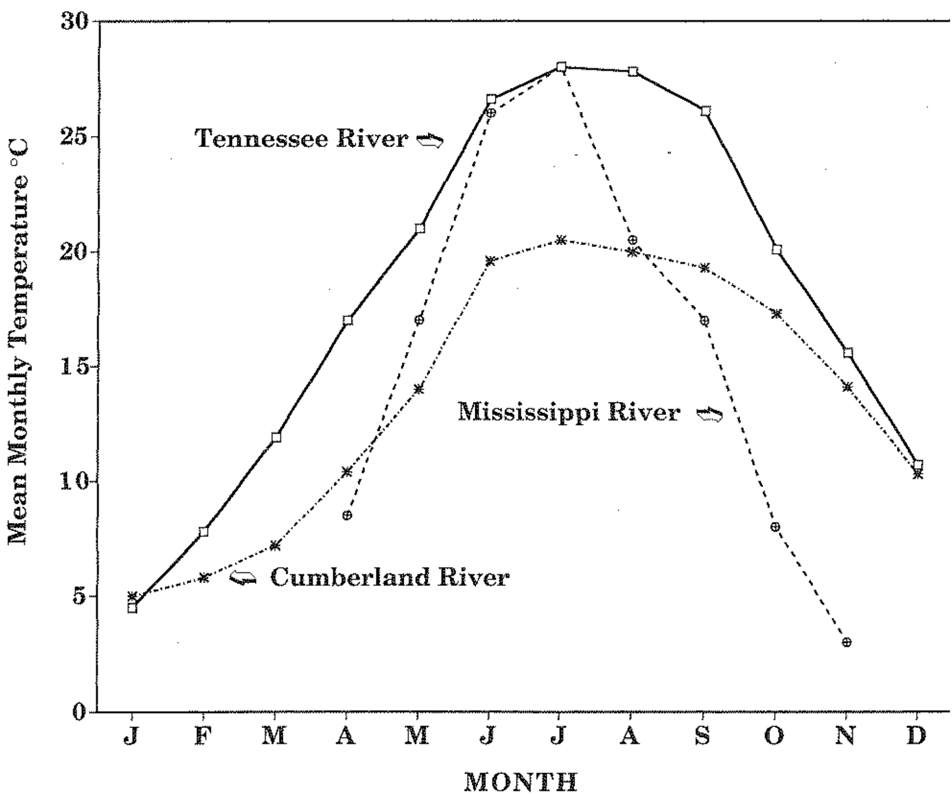

FIG. 2.—Mean monthly temperatures for 1994 for the Cordell Hull Dam discharge (Cumberlan River); Kentucky Lake (Tennessee River) and Pool 10, (Mississippi River). Records for the Mississipp River are from Woody and Holland-Bartels (1993)

TABLE 4.—Mean values and standard deviations (SD) of total dissolved solids and total phospho for the Cordell Hull Dam and Pickwick Dam tailwaters for 3 y (Data from U.S. Geological Survey, 19 1994, 1995)

Variable	Year	Cordell Hull Mean	Cordell Hull SD	Pickwick Tailwater Mean	Pickwick Tailwater 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 freshwa mussels in particular. Hermaphroditism is often considered to be accidental or devel mental, 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 c for each sex, to the predominance of one sex within an individual (Coe, 1943). The hi frequency of hermaphrodites in the translocated sample is unusual for most freshwa mussel populations thus far examined. For instance, van der Schalie (1970) examined h tological sections of 1871 mussels of 97 species and found that only four species w regularly hermaphroditic and 22 other taxa were occasionally hermaphroditic. Woody a Holland-Bartels (1993) found no hermaphrodites in 255 *Megalonaias nervosa* collect from the upper Mississippi River where water temperatures exceeded 25 C during the su mer. The hermaphrodites in our study followed a pattern similar to the varied cell dis bution within each gonad described by Coe (1943). Many individuals also possessed merous hermaphroditic follicles, a condition rarely reported in other hermaphroditic valves (Tepe, 1943). Kat (1983) found hermaphroditic follicles in *Alasmidonta undul* only in populations with low density. Bauer (1987) indicated that the frequency of h maphrodites increased at low densities in a population of *Margaritifera margaritifera*. T cause of the high incidence of hermaphroditism in the translocated mussels is unkno however, the proportion of hermaphrodites in the translocated sample was significar greater than in the Cumberland River sample ($\chi^2 = 4.29$; $P < 0.05$), which suggests t *Megalonaias nervosa* is a facultative hermaphrodite. The incidence of hermaphrodites the translocated group does not appear to be related to low density. On the contrary, th mussels were held in close proximity to >100,000 *M. nervosa*.

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

The atypical Stage 1 development and the lack of glochidia in our Cumberland Ri 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 t large-sized (>130 mm long) individuals in our samples from the Cumberland River is u usual. Perhaps these individuals were juveniles when the Wolf Creek Dam began operatic or they represent adults that have regressed. In contrast, Cumberland River mussels tra located to Kentucky Lake did reproduce; however, most *Megalonaias nervosa* required ! following translocation before they reproduced. The 1st y after translocation, only o individual in the sample was gravid and two individuals were still sexually undifferentiate but after 2 y, 49% were gravid and all were sexually differentiated. Although few stud have examined the possibility of freshwater mussels being facultative hermaphrodites, t research of Kat (1983), Bauer (1987) and ours suggests that its occurrence may be mo widespread than previously thought. Moreover, the occurrence of hermaphroditic follic suggests the possibility of self-fertilization. If indeed, other unionids are facultative h maphrodites, capable of self-fertilization, it could explain the occurrence of gravid indiv uals in low density populations.

Because nutritional requirements of freshwater mussels are not well understood, inac quate nutrition cannot be totally ruled out as a causative factor limiting reproduction. N trition is critical to successful reproduction in marine bivalves (Coe and Turner, 193ξ Total dissolved solids represent an average edaphic condition for a watershed and is close tied to biological productivity (Kemp, 1971). Total dissolved solids and phosphorus leve were similar between the Cordell Hull Dam and Pickwick Dam tailwaters suggesting th nutrients were not limiting. In contrast, water temperatures in these tailwaters differe substantially. Therefore, we believe that water temperature, or factors directly related temperature, is the causative factor limiting reproduction of *Megalonaias nervosa* in tl Cumberland River. Unless dam operations are modified, all or nearly all mussels in tl study area will be extirpated.

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

Existing populations of endangered species in the Cumberland River, although relative small, still probably consist of a few thousand individuals. Since these populations are great 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 propagation or life history studies. Taking a small number of individuals would not h any measurable effect on the existing population and again, unless dam operations changed, the point is moot. Finally, commercial harvest regulations should be reviewed consistency with any long-term plans developed. The system is in jeopardy and in dire n of a management plan to restore its former diversity.

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