JOURNAL OF THE KENTUCKY ACADEMY OF SCIENCE
ISSN 1098-7096
Continuation of
Transactions of the Kentucky Academy of Science
Volume 60 Fall 1999 Number 2

J. Ky. Acad. Sci. 60(2):67–72. 1999.

Possible Decline in Reproduction in a Freshwater Unionid (Mollusca: Bivalvia) Community in the Licking River at Butler, Kentucky

Stephen E. McMurray,[1] Guenter A. Schuster, and Barbara A. Ramey
Department of Biological Sciences, Eastern Kentucky University, Richmond, Kentucky 40475

ABSTRACT

In the Licking River at Butler, Pendleton County, Kentucky, glochidia, fishes, and unionids were collected to analyze recruitment in an historically diverse unionid community. Only 14 unionid glochidia and 50 juvenile *Corbicula fluminea*, were collected with drift nets. No fishes collected had any evidence of glochidial infestation. A small percentage of the unionids collected (13.5%) had gills modified as marsupia. Sex ratios, stage of gametogenesis, and marsupial contents of two target species (*Actinonaias ligamentina* and *Elliptio dilatata*) were determined in the laboratory. *Actinonaias ligamentina* was found to exhibit a 1:1 male-to-female ratio; *E. dilatata* had a ratio statistically different from 1:1. Causal factors for this possible decline in reproduction were unclear.

INTRODUCTION

North America's rich unionacean (mussel) (Bivalvia: Unionidae) fauna (297 taxa) has disproportionately more endangered, threatened, and special concern taxa than all the groups of terrestrial organisms in North America combined. Only 70 of the unionid taxa known from the United States are considered stable (Williams et al. 1993). Human activities in Kentucky have severely impacted unionid populations during the last 200 years, making this group of organisms the most endangered in the state (Cicerello et al. 1991). One of the most severe and perplexing problems facing freshwater mussels is the documented loss of recruitment (reproduction) in unionid communities previously thought to be healthy.

The objective of our study was to analyze reproduction in a diverse unionid community in the Licking River at Butler, Pendleton County, Kentucky. Originally, the data were to be compared to those from another community in the Licking River where it was hypothesized recruitment had ceased or been dramatically decreased due to the release of hypolimnionic water from an upstream reservoir (McMurray 1997).

METHODOLOGY

Study Area

The Licking River originates on the unglaciated Allegheny Plateau in the Appalachian Province of eastern Kentucky and is a sixth-order tributary to the Ohio River. The river flows northwesterly through the extremely variable topography of the Bluegrass region of the state for 496 km (Burr and Warren 1986; Hannan et al. 1982; Harker et al. 1979). This drainage covers all or a portion of 21 counties and encompasses ca. 10% of the state (9601 km^2) (Harker et al. 1979). The drainage has a diverse unionid fauna with over half of Ken-

[1] Present address: Kentucky Division of Water, Water Quality Branch, Nonpoint Source Section, 14 Reilly Road, Frankfort, KY 40601.

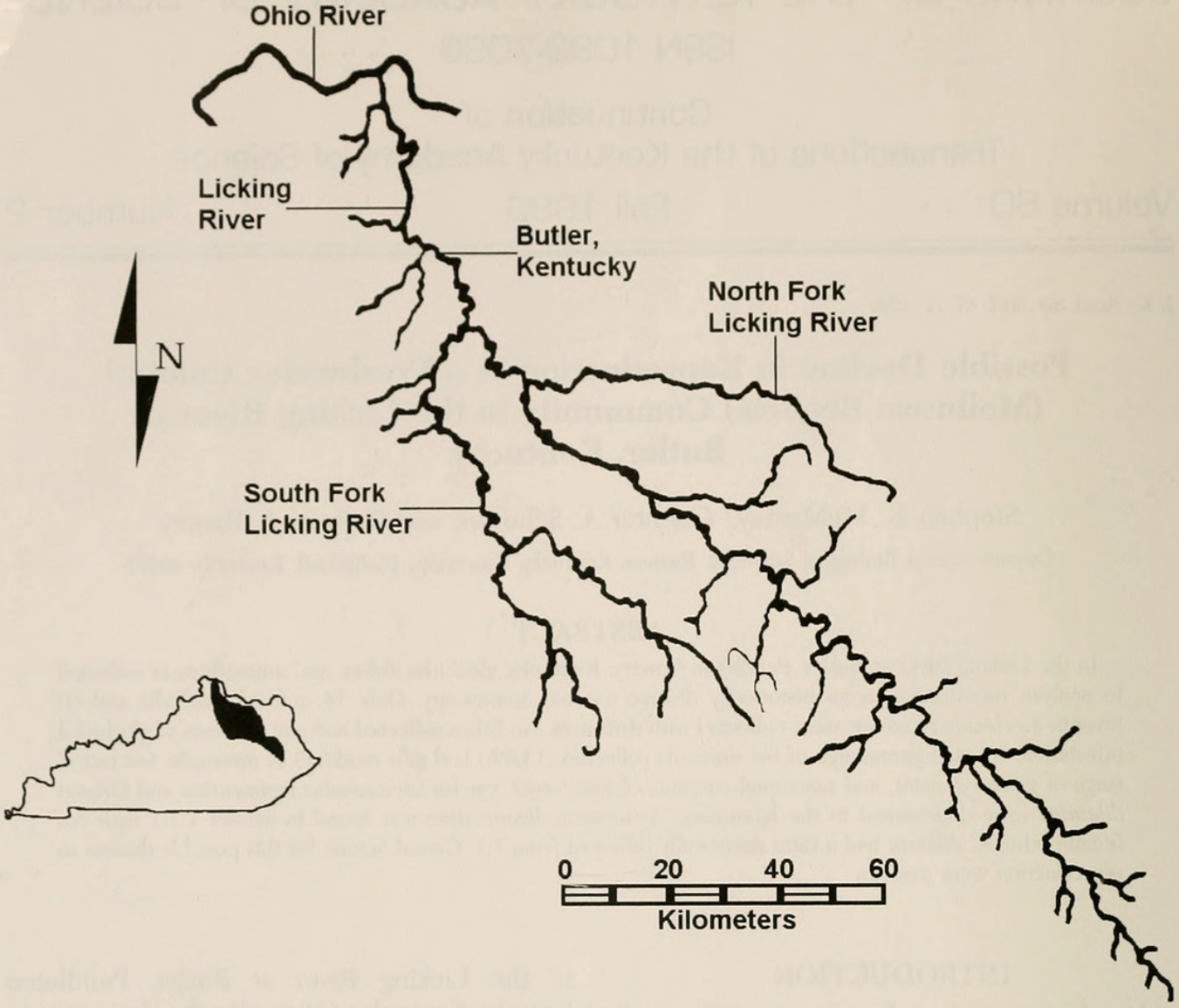

Figure 1. Location of the Butler, Pendleton County, Kentucky, unionid community. Inset shows the location of the Licking River drainage in Kentucky.

tucky's native mussel fauna, 53 taxa, historically existing in the drainage (Cicerello et al. 1991). A recent unionid survey by Laudermilk (1993) indicated that 50 taxa still reside in that portion of the river below Cave Run Reservoir.

The unionid community for our study was located just downstream from the SR 177 bridge at Butler (Figure 1). The watershed at this site is utilized mainly for agriculture, but there is some urbanization of the surrounding area. Substrate consisted mainly of cobble and boulder, with large amounts of gravel and sand intermixed in the riffle areas and along the stream margins. This site has had a diverse unionid community with 35 historical taxa (Laudermilk 1993).

Sampling and Laboratory Procedures

Five collections of glochidia, fishes, and unionids were made from July through October 1995. Unfortunately, high water conditions prevented collections in spring 1996. For each collection period a drift net was randomly placed in the bed to collect glochidia. After 1 hour, the contents of the drift net were preserved in 70% ethanol and returned to the laboratory. Drift net collections were randomly examined using cross-polarized light microscopy techniques (Johnson 1995) at 10–20× magnification. Glochidia were counted and removed along with any juvenile *Corbicula fluminea*.

Fishes were collected for 1 hour using a common sense minnow seine. All fishes re-

tained were initially preserved in 10% formalin and then transferred to 70% ethanol in the laboratory for final preservation. Following sorting and identification, the fins and scales of each individual were examined under a dissecting microscope (10–30×) for attached glochidia. The opercular flaps were removed, and each gill arch was carefully examined under a dissecting microscope for attached glochidia (Bruenderman and Neves 1993).

Unionids were randomly collected for 1 hour by snorkeling or with the use of water scopes. After identification, the shell of each unionid was carefully opened with a small screwdriver and the gills were examined for signs of gravidity. Notes were made of the species collected and the condition of the gills in each individual. All unionids, except for individuals of two target species retained for histological examination, were returned to the river. Two common unionid species, *Actinonaias ligamentina* and *Elliptio dilatata*, were chosen for histological examination. These species represented both breeding regimes of freshwater mussels (bradytictic and tachytictic, respectively), both are commonly encountered throughout their respective ranges (Oesch 1995), and neither has any federal or state protection status (KSNPC 1996). More individuals for histological examination were also collected by the previously described method if enough individuals for analysis were not obtained in the original search. In most cases individuals, and the respective data, for field and laboratory analyses were kept separate.

If possible, three to five individuals of the two target species were retained for histological examination from each collecting period. These individuals were placed into a 10% formalin solution and were then transferred to 70% ethanol in the laboratory for final preservation. The valves were opened by cutting the adductor muscles, and portions of the gonadal and gill tissues were removed and placed into either 70% ethanol or Bouins fixative. These were then dehydrated through a series of alcohols and embedded in paraffin (Humason 1967). Sections were made at a thickness of 10 μm using an American Optical 820 Microtome and were mounted with Permount. The slides were stained with Ehrlich's hematoxylin and counter stained with eosin (Humason 1967). The sections were then examined under a compound microscope (400–430×) to determine a sex ratio for both species, to determine if gametogenesis was occurring, and to determine the contents of the marsupia. All drift net, fish, and unionid collections were deposited in the Branley A. Branson Museum of Zoology, Eastern Kentucky University (EKU).

Five cell types of spermatogenesis (Garner 1993) were used to determine the stage of gametogenesis in males of both target species. Stage 1 males were those that had only spermatogonia present in their acini; Stage 5 males had mature spermatozoa present. Stages 2, 3, and 4 corresponded respectively to sperm morulae, spermatocytes, and spermatids being present in the acini. Three cell types of oogenesis in *Elliptio dilatata* (McMurray 1997) were used to determine the stage of gametogenesis in females of that target species. A similar analysis of female *Actinonaias ligamentina* was not performed due to a lack of this type of classification for females of that species (McMurray 1997). Stage 1 females were those with oogonia as the dominant cell type in their alveoli; Stage 2 were those with oocytes dominant; and Stage 3 were those with mature ova dominant. Marsupia of both target species were classified according to their contents as being empty (EM) or containing mature glochidia (MG), early embryos (EE), or advanced embryos (AE) (Garner 1993). In the case of known females that did not have their gill tissues examined, the marsupia were considered to be empty since sections were made of any gill that showed signs of gravidity.

RESULTS

Only 14 unionid glochidia and 50 juvenile *Corbicula fluminea* were collected with drift nets. A total of 307 fishes was collected; none of these had any attached glochidia. Only 26 of the 193 unionids (13.5%) (Table 1) observed in the field had their gills modified as marsupia. This represented, based only on field observations of the gill condition, a 6.42:1 male-to-female ratio. A total of 17 *Actinonaias ligamentina* and 22 *Elliptio dilatata* were returned to the laboratory. Histological examination of these individuals revealed that the male-to-female ratio for *A. ligamentina* was statistically 1:1, and for *E. dilatata* was statistically 1:2.7 ($\chi^2 = 4.5455$, $\alpha = 0.05$).

Table 1. Field observations of unionids with and without modified gills from the Licking River at Butler, Kentucky.

Taxa	With modified gills	Without modified gills	Totals
Actinonaias ligamentina (Lamarck, 1819)	0	1	1
Alasmidonta marginata Say, 1818	0	1	1
Amblema plicata (Say, 1817)	1	52	53
Cyclonaias tuberculata (Rafinesque, 1820)	0	9	9
Elliptio dilatata (Rafinesque, 1820)	1	14	15
Fusconaia flava (Rafinesque, 1820)	0	1	1
Lampsilis cardium Rafinesque, 1820	3	15	18
Lasmigona complanata (Barnes, 1823)	0	3	3
Lasmigona costata (Rafinesque, 1820)	16	24	40
Megalonaias nervosa (Rafinesque, 1820)	0	10	10
Obliquaria reflexa Rafinesque, 1820	0	2	2
Potamilus alatus (Say, 1817)	4	18	22
Ptychobranchus fasciolaris (Rafinesque, 1820)	1	7	8
Quadrula metanevra (Rafinesque, 1820)	0	1	1
Quadrula nodulata (Rafinesque, 1820)	0	2	2
Quadrula pustulosa (Lea, 1831)	0	2	2
Quadrula quadrula (Rafinesque, 1820)	0	3	3
Tritogonia verrucosa (Rafinesque, 1820)	0	2	2
Totals:	26	167	193

Most of the males of the two target species had more than one stage of spermatogenesis present in their gonads, but usually the most advanced stage present dominated the acini of the testes (Table 2). Spermatids and spermatozoa were the only cell types observed in male *Elliptio dilatata*, with spermatids being the most common. The same was observed in male *Actinonaias ligamentina*, with the exception of one male that had spermatocytes present.

The most advanced stage of gametogenesis present did not always dominate the alveoli of the ovaries in the female *Elliptio dilatata*, as was observed in the testes of the males. Most of the female *E. dilatata* were in the first (eight individuals) or second stages (seven individuals) of oogenesis with oogonia and oocytes, respectively, dominating the alveoli. Even though all of the females had mature ova present in their alveoli, none had this as the dominant cell type. One female was categorized as unknown because the stage of oogenesis could not be determined due to technical difficulties. Mature glochidia were present in the marsupia of most of the female *Actinonaias ligamentina* (57.14%). In *E. dilatata* most of the females (81.25%) had empty marsupia (Table 3).

DISCUSSION

All drift net collections were made between 1000 and 1700 (EST), which corresponds to the period when glochidial densities should have been at their highest (Kitchell 1985). Bradytictic freshwater mussel species, such as *Actinonaias ligamentina*, tend to release their glochidia from ca. August to May; tachytictic species, such as *Elliptio dilatata*, tend to release their glochidia from ca. May to August (Oesch 1995). It should reasonably be expect-

Table 2. Stages of spermatogenesis observed in male *Elliptio dilatata* and *Actinonaias ligamentina* from the Licking River at Butler, Kentucky. Determined from the most advanced cell type present where stage 1 = spermatogonia, 2 = sperm morulae, 3 = spermatocytes, 4 = spermatids, 5 = spermatozoa.

	Stage of spermatogenesis					
Taxa	1	2	3	4	5	Totals
Actinonaias ligamentina	0	0	1	5	4	10
Elliptio dilatata	0	0	0	4	2	6

Table 3. Marsupial contents observed in female *Elliptio dilatata* and *Actinonaias ligamentina* from the Licking River at Butler, Kentucky. Abbreviations are as follows: EE = early embryo; AE = advanced embryo; MG = mature glochidia; EM = empty marsupia.

	Marsupial contents				
Taxa	EE	AE	MG	EM	Totals
Actinonaias ligamentina	0	0	4	3	7
Elliptio dilatata	1	1	1	13	16

ed then, that even through our study occurred only in the summer to late fall, the glochidia of bradytictic mussel species would be collected with drift nets. The relatively large number of juvenile *Corbicula fluminea* collected (when compared to the number of glochidia collected) may impact any juvenile unionids present in the bed through resource competition (Neves and Widlak 1987).

One of the most important factors determining the success of reproduction in unionids is the presence of a suitable host. Only 25.7% of the fishes collected were suitable hosts for unionids known from the bed (Watters 1994); none of these had glochidia attached. The reason for the lack of infested fishes is unknown. The attachment of glochidia to their hosts is dependent upon several factors such as infestations of hosts by copepod parasites (Wilson 1916), age of the host, immunity caused by previous infestations (Parker et al. 1984), and water temperature (Matteson 1948).

The determination of a male-to-female ratio from field observations is probably not a true representation of the actual ratio since most unionids are not sexually dimorphic (McMahon 1991). The only way to determine the sex of an individual without using standard histological techniques is to examine the gills for signs of gravidity in the field. Since several species of unionids usually maintain a 1:1 male-to-female ratio (Jirka and Neves 1992), the 6.42:1 ratio from the field observations may indicate a problem. The male-to-female ratio based only on these field observations indicated that less than 25% of the unionids in this community were females. The cause of the 1:2.7 ratio in *Elliptio dilatata* based on histological analysis in the laboratory was not known. Small sample size alone was probably not the reason because sample size did not differ greatly among the two target species.

The lack of earlier stages of spermatogenesis in males of both species is thought to be related to a normal temperature regimen at Butler. Water temperature is believed to be the most important exogenous factor controlling reproduction in unionids (Matteson 1948; Tedla and Fernando 1969; Zale and Neves 1982) and is not regulated at this location by the releases of hypolimnionic water from an upstream reservoir as it was found to be at another site farther upstream and closer to the reservoir (McMurray 1997).

Further study in this and in other freshwater mussel communities needs to be completed to assess the current health of these historically diverse and successful communities that were previously thought to be healthy. For example, the qualitative methods used to collect unionids are not sufficient to search for juveniles. Juveniles need to be extensively searched for in the community at Butler to affirm that recruitment is occurring. Also, a search for possible upstream causes is necessary to try and ameliorate the impacts to this community.

ACKNOWLEDGMENTS

We thank C. Abbruzzese, J. S. Board, M. C. Compton, M. D. Moeykens, A. R. T. Nix, T. E. Oliver, M. A. Patterson, and D. Vey for assistance in the field and laboratory. R. R. Cicerello (KSNPC) and P. A. Ceas (EKU) assisted with fish identification. G. T. Watters (OSU) provided helpful hints on the use of cross-polarized light microscopy. D. L. Batch (EKU) served on the first author's thesis committee. Two anonymous reviewers provided helpful comments. This research was funded by a grant from the Kentucky Department of Fish and Wildlife Resources (Project No. E-2-9).

LITERATURE CITED

Bruenderman, S. A., and R. J. Neves. 1993. Life history of the endangered fine-rayed pigtoe *Fusconaia cuneolus* (Bivalvia: Unionidae) in the Clinch River, Virginia. Am. Malacol. Bull. 10(1):83–91.

Burr, B. M., and M. L. Warren, Jr. 1986. A distributional atlas of Kentucky fishes. Kentucky Nature Preserves Comm. Sci. Techn. Ser. 4.

Cicerello, R. R., M. L. Warren, Jr., and G. A. Schuster. 1991. A distributional checklist of the freshwater unionids (Bivalvia: Unionoidea) of Kentucky. Am. Malacol. Bull. 8(2):113–129.

Garner, J. T. 1993. Reproductive cycle of *Quadrula metanevra* (Unionidae) in Kentucky Reservoir, Tennessee River, Hardin Co., Tennessee. M.S. Thesis. University of Alabama, Huntsville, AL.

Hannan, R. R., M. L. Warren, Jr., K. E. Camburn, and R. R. Cicerello. 1982. Recommendations for Kentucky's outstanding water classifications with water quality criteria for protection. Kentucky State Nature Preserves Comm. Techn. Rep., Frankfort, KY.

Harker, D. F., S. M. Call, M. L. Warren, Jr., K. E. Camburn, and P. Wigley. 1979. Aquatic biota and water

quality survey of the Appalachian Province, eastern Kentucky. Kentucky Nature Preserves Comm. Techn. Rep., Frankfort, KY.

Humason, G. L. 1967. Animal tissue techniques, 2nd ed. W.H. Freeman and Company, San Francisco, California.

Jirka, K. J., and R. J. Neves. 1992. Reproductive biology of four species of freshwater mussels (Mollusca: Unionidae) in the New River, Virginia and West Virginia. J. Freshwater Ecol. 7:35–44.

Johnson, L. E. 1995. Enhanced early detection and enumeration of zebra mussel (*Dreissena* spp.) veligers using cross-polarized light microscopy. Hydrobiologia 312: 139–146.

[KSNPC] Kentucky State Nature Preserves Commission. 1996. Rare and extirpated plants and animals of Kentucky. Trans. Kentucky Acad. Sci. 57:69–91.

Kitchell, H. E. 1985. Life history of the endangered shiny pigtoe pearly mussel *Fusconaia edgariana* in the North Fork Holston River, Virginia. M.S. Thesis. Virginia Polytechnic Institute and State University, Blacksburg, VA.

Laudermilk, E. L. 1993. A survey of the unionids (Bivalvia: Unionidae) of the mainstem Licking River and selected tributaries below Cave Run Reservoir, Kentucky. M.S. Thesis. Eastern Kentucky University, Richmond, KY.

Matteson, M. R. 1948. Life history of *Elliptio complanatus* (Dillwyn, 1817). Am. Midl. Naturalist 40:690–723.

McMahon, R. F. 1991. Mollusca: Bivalvia. Pages 315–399 in J. H. Thorp and A. P. Covich (eds). Ecology and classification of North American freshwater invertebrates. Academic Press, San Diego, CA.

McMurray, S. E. 1997. Reproduction in a freshwater mussel (Bivalvia: Unionidae) community downstream of Cave Run Reservoir in the Licking River at Moores Ferry, Kentucky. M.S. Thesis. Eastern Kentucky University, Richmond, KY.

Neves, R. J., and J. C. Widlak. 1987. Habitat ecology of juvenile freshwater mussels (Bivalvia: Unionidae) in a headwater stream in Virginia. Am. Malacol. Bull. 5(1): 1–7.

Oesch, R. D. 1995. Missouri naiades: a guide to the mussels of Missouri. Missouri Department of Conservation, Jefferson City, MO.

Parker, R. S., C. T. Hackney, and M. F. Vidrine. 1984. Ecology and reproductive strategy of a south Louisiana freshwater mussel, *Glebula rotundata* (Lamarck) (Unionidae: Lampsilini). Freshwater Invertebr. Biol. 3(2):53–58.

Tedla, S., and C. H. Fernando. 1969. Observation on the glochidia of *Lampsilis radiata* (Gmelin) infesting yellow perch, *Perca flavescens* (Mitchell) in the Bay of Quinte, Lake Ontario. Canad. J. Zool. 47:705–712.

Watters, G. T. 1994. An annotated bibliography of the reproduction and propagation of the Unionoidea (primarily of North America). Ohio Biol. Surv. Misc. Contr. 1.

Williams, J. D., M. L. Warren, K. S. Cummings, J. L. Harris, and R. J. Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18(9):6–22.

Wilson, C. B. 1916. Copepod parasites of fresh-water fishes and their economic relations to mussel glochidia. Bull. U.S. Bur. Fish. 34:331–374.

Zale, A. V., and R. J. Neves. 1982. Fish hosts of four species of lampsiline mussels (Mollusca: Unionidae) in Big Moccasin Creek, Virginia. Canad. J. Zool. 60:2535–2542.