Evaluation of Various Holding Facilities for Maintaining Freshwater Mussels in Captivity

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Abstract. Between September 1993 and September 1994, we collected 1,651 mussels representing 20 species from the Cumberland, Licking, and Tennessee rivers, and Elkhorn Creek. Mussels were brought to one of four facilities: a hatchery pond, raceway, farm pond, or an embayment of Kentucky Lake. We broadcast mussels throughout the raceway and within 15 hours, most individuals burrowed into the sand-gravel substrate. At the other three locations, mussels were placed into pocket nets and suspended 0.6 m below the water's surface. After more than 1 year in captivity, survival has ranged from 85% to 100% for most species at three of the facilities; however, at the fourth location (a hatchery pond) none of six species survived, and survival was low for the other four species held there. At all facilities, most mortality occurred within the first 30 to 60 days of captivity. Although most of the mainstem of the Tennessee River no longer supports reproducing populations of *Actinonaias ligamentina* and *Elliptio dilatata*, survival of these species was 86% to 95% in pocket nets suspended in Kentucky Lake.

Introduction

Freshwater mussels are the most imperiled faunal group in North America. In this century, about 23% of the 297 recognized taxa of mussels have either gone extinct or are federally listed as endangered. Populations of only 70 species are considered to be stable (Williams et al. 1993). Habitat loss and degradation from anthropogenic effects, such as impoundments and poor land-use practices, continue to be major causes of mussel declines (Layzer et al. 1993); pollution, commercial navigation, and overharvest of mussels have also contributed to freshwater mussel declines in the 20th century (Ortmann 1909; Neves 1993).

Populations of some species facing extinction consist only of old, nonreproducing individuals (Parmalee and Klippel 1982; Bogan 1993). Although factors controlling gametogenesis in mussels are poorly understood, the lack of reproduction in these populations does not necessarily reflect senescence. Instead, reproduction may be suppressed by environmental conditions, such as the discharge of unnaturally cold water from dams. Heinricher and Layzer (these proceedings) demonstrated that gametogenesis and successful spawning could be reinitiated in nonreproducing individuals following translocation from a cold-water habitat to a warmer one. Such translocations may be practical for reestablishing populations in some streams. Unfortunately, populations of some species, such as Dromus dromas, in the Cumberland River are so low

that it is doubtful that a sufficient number of individuals could be collected and translocated to establish a population in another stream. Alternatively, if such species could be held in captivity and propagated, a sufficient number of juvenile mussels may be produced to reestablish populations.

Despite some early success in propagating mussels (e.g., Corwin 1921), there has been little interest in artificial culture of mussels until recently (Isom and Hudson 1982). The introduction of the exotic zebra mussel (Dreissena polymorpha) into the Laurentian Great Lakes in the mid-1980s (Griffiths 1993) intensified the renewed interest in maintaining and propagating mussels in captivity. Following the discovery of zebra mussels in Lake St. Clair in 1988, unionid populations have been nearly eliminated (Riessen et al. 1993). Zebra mussels are expected ultimately to invade most waters in the United States and southern Canada (Strayer 1991). This invasion now threatens to eliminate many of the remaining populations of native mussels, particularly those living in large rivers.

Establishing and maintaining captive populations of native mussels could provide a refugia from zebra mussels and a source of brood stock for propagation. If the threat from zebra mussels declined, artificially propagated juveniles could be used to reestablish natural populations. Moreover, establishing captive populations of those species that currently exist only as nonreproducing popula-

tions may be effective in reinstating their gametogenic cycle, and thus provide a source of brood stock for the most critically endangered species.

The objectives of our study were to evaluate the use of several facilities for maintaining native mussels in captivity and to compare survival of mussels kept out of water for various time intervals and transported by two different methods.

Materials and Methods

Unionids were collected in September 1993 and from June to September 1994 from four streams: the Licking River and Elkhorn Creek in Kentucky, and the Tennessee and Cumberland rivers in Tennessee. Mussels were handpicked from the substrate with the aid of clear-bottomed view buckets in shallow water or by SCUBA diving in deeper water. Mussels were kept submerged in mesh dive bags at the collection sites to prevent desiccation while data were recorded. Individuals were identified to species and their length (greatest distance parallel to the hinge) was measured to the nearest 1 millimeter with dial calipers. Unionids collected in or near waters where zebra mussels had been reported were hand scrubbed with wire brushes at the collection site before being transported. All mussels were thoroughly rinsed before transport and before being placed into a holding facility. Generally, mussels were transported on the day of collection in coolers with a covering of wet burlap to keep them moist. Most individuals of a species that were held at the same facility were collected at the same time; however, some were collected from the same location but on different dates and are treated as separate groups in this paper. Transport time for all mussels was less than 5 hours.

Mussels were held at four locations: the Frankfort and Minor Clark fish hatcheries in Kentucky, the Laurel Hill Wildlife Management Area in Tennessee, and an embayment of Kentucky Lake in Tennessee. At the Frankfort Fish Hatchery, mussels were held in a 0.6-ha lined earthen pond that received water directly from Elkhorn Creek. Mussels were placed in pocket nets (30 mm mesh) that measured 0.60 m x 0.43 m. These nets were suspended from floating PVC pipe (10 cm diameter) approximately 0.6 m below the water's surface. Suspended pocket nets were also used to hold mussels in an embayment of Kentucky Lake and in a pond located in the Laurel Hill Wildlife Management Area. This unlined, 0.4-ha earthen pond was spring fed and approximately 2 meters deep.

Unionids taken to the Minor Clark Fish Hatchery were held in a flow-through raceway that received its water from Cave Run Lake, an impoundment on the Licking River. The raceway measured 30.5 m x 2.5 m and contained a sandgravel substrate approximately 18 cm deep. Mussels were marked with a dremel tool with one, two, or three short lines on the shell and placed in one of three corresponding sections of the raceway to monitor mussel movement. Monthly monitoring consisted of confirming mussel survival and measuring alkalinity, hardness, pH, and temperature of the water at all facilities.

To compare survival of mussels held out of water for varying time intervals and transported by two methods, we collected Fusconaia ebena on 17 June 1994 from Kentucky Lake and separated them into five groups of 50 similar-sized individuals. One group was placed in a cooler, covered with wet burlap, and held out of water for 9 hours before being placed into a holding facility. The four remaining groups were packed in ice and held out of water for 4, 9, 24, and 48 hours. One group of Ellipsaria lineolata was also packed in ice for 48 hours. The coolers were drained periodically to avoid submerging the mussels. On 28 July 1994, we collected 200 F. ebena from the same location in Kentucky Lake. Two groups of 50 mussels were placed in individual coolers and covered with wet burlap. One group was held out of water for 24 hours and the other group for 48 hours. The other two groups of 50 mussels were placed in individual coolers, covered with wet burlap, and a 7.6 cm-deep layer of ice was placed over the burlap. One group was held out of water for 24 hours and the other group for 48 hours. The 4-hour group collected on 17 June was transported to the embayment of Kentucky Lake where other mussels were held; all other groups were transported to the Frankfort Fish Hatchery in an air-conditioned vehicle. Before relocation, the 24-hour and 48-hour groups were kept in an air-conditioned room at an ambient temperature of about 21°C.

Results

A total of 1,651 mussels representing 20 species were held in captivity (Table 1). Survival was highly variable among species and holding facilities. Survival was highest for mussels held at the Minor Clark Fish Hatchery. After mussels were marked and broadcast throughout the raceway, most individuals burrowed into the sand-gravel substrate

Table 1. Numbers of individuals of each species and source of unionids held at four facilities in Tennessee and Kentucky. All mussels were collected in 1994 except for the *Cyclonaias tuberculata* collected in 1993. Numbers immediately following species names indicate groups treated separately.

				Num	Numbers of Individuals and Holding Facility			
Species	·····	Source	Month Collected	Kentucky Lake	Frankfort Fish Hatchery	Minor Clark Fish Hatchery	Laurel Hill Managemen Area	
Actinonaias ligamentina		Licking R., KY	July	50	50	50		
Amblema plicata		Elkhorn Ck., KY	August	Mile Maryana	31	MARKALAN.	-	
Cyclonaias tuberculata		Tenn. R., TN	September	101				
Ellipsaria lineolata	(1)	Tenn. R., TN	June		40		*****	
	(2)	Cumberland R., TN	September				14	
Elliptio dilatata	(1)	Elkhorn Ck., KY	June	********	102			
	(2)		August		18			
	(3)		September		50			
	(4)	Licking R., KY	July	41		104	<u></u>	
	(5)	Ü	August	60	***************************************		-	
Fusconaia ebena	(1)	Tenn. R., TN	June	50	200			
	(2)		July	war passan	200			
Lampsilis cardium	(1)	Elkhorn Ck., KY	July		1			
,	(2)	Licking R., KY	August		***************************************	4	errorenamen.	
Lampsilis fasciola	` ′	Elkhorn Ck., KY	August	· ·	3		****	
Lampsilis siliquoidea	(1)	Elkhorn Ck., KY	June		11			
, ,	(2)	,	August		43			
Lasmigona costata	(1)	Elkhorn Ck., KY	June		9	***************************************	-	
Ü	(2)	Licking R., KY	August	8	13		***************************************	
Megalonaias nervosa	(1)	Elkhorn Ck., KY	August		2	attropy to the same of the sam		
O .	(2)	Cumberland R., TN	September				4	
Pleurobema coccineum	(1)	Licking R., KY	July		********	20		
	(2)	. 0 . ,	August	13		6	Particol 1	
Pleurobema cordatum	(/	Cumberland R., TN	September		***************************************		175	
Potamilus alatus		Elkhorn Ck., KY	August		2	-		
Ptychobranchus fasciolar	is (1)	Licking R., KY	July	8		41		
 	(2)		August	51		9		
	(3)	Cumberland R., TN	September		********		1	
Quadrula metanevra	(2)	Cumberland R., TN	September	h-17	-		5	
Quadrula nodulata		Cumberland R., TN	September				1	
Quadrula pustulosa		Cumberland R., TN	September		-		2	
Quadrula quadrula		Cumberland R., TN	September		-		20	
Tritogonia verrucosa	(1)	Licking R., KY	July			27		
2,115,011111 00111100011	(2)	~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~~	August			11		
Γotals	\ /			382	775	272	222	

within 15 hours. Initially some mussels moved a few meters in a seemingly random manner; however, little or no movement between sections occurred. There was no mortality among three of the six species held in the raceway and survival of the other three species was greater than 80% (Table 2). In contrast, there was 100% mortality of six species and low survival of the four other species held at the Frankfort Fish Hatchery (Table 3). Initial mortality of three species collected on more than one occasion from Elkhorn Creek and held at the Frankfort Fish Hatchery seemed to be related to collection date. After 4 months in captivity, only 9% of the Elliptio dilatata collected in June 1994 were alive; however, 95% to 100% of the E. dilatata collected in August and September 1994 were still alive after 6 months (Figure 1). However, few *E. dilatata* of any group were alive after 13 months. Similar differences in initial survival occurred between groups of

Lasmigona costata and Lampsilis siliquoidea collected in June and August 1994 (Figures 2 and 3).

Although alkalinity and hardness were relatively low in the spring-fed pond at the Laurel Hill Wildlife Management Area (Table 4), overall survival was high for mussels held in pocket nets (Table 5). Survival was also high for mussels held in the embayment of Kentucky Lake. In particular, only 1 of 101 Cyclonaias tuberculata died during 24 months of captivity (Table 6). In contrast to the Frankfort Fish Hatchery, survival of Actinonaias ligamentina and E. dilatata held in Kentucky Lake was greater than 85%. Moreover, there was little variability in survival between groups of the same species collected on different dates and held at Kentucky Lake.

After 4 months in captivity, there was 100% survival of the *Fusconaia ebena* that had been packed in ice for 4 hours (Table 7). Although we did not use a 4-hour treatment for mussels covered with wet

Table 2. Numbers and percent survival of mussels held in a raceway at the Minor Clark Fish Hatchery. Numbers in parentheses indicate specific groups of mussels identified in Table 1.

Species	Number Held	% Survival	Months Held
Actinonaias ligamentina	50	94	14
Elliptio dilatata (4)	104	93	14
Lampsilis cardium (2)	4	100	14
Pleurobema coccineum (1)	20	100	14
Pleurobema coccineum (2)	6	100	13
Ptychobranchus fasciolaris (1)) 41	81	14
Ptychobranchus fasciolaris (2)) 9	89	13
Tritogonia verrucosa (1)	27	100	14
Tritogonia verrucosa (2)	11	100	13

Table 3. Numbers and percent survival of mussels held in a pond at the Frankfort Fish Hatchery. Numbers in parentheses indicate specific groups of mussels identified in Table 1.

Species	Number Held	% Survival	Months Held
Actinonaias ligamentina	50	0	14
Amblema plicata	31	52	13
Ellipsaria lineolata (1)	40	0	12
Elliptio dilatata (1)	102	1	15
Elliptio dilatata (2)	18	17	13
Elliptio dilatata (3)	50	4	13
Lampsilis cardium (1)	1	0	13
Lampsilis fasciola	3	0	11
Lampsilis siliquoidea (1)	11	46	15
Lampsilis siliquoidea (2)	43	89	13
Lasmigona costata (1)	9	23	15
Lasmigona costata (1)	13	47	13
Megalonaias nervosa (1)	2	0	13
Potamilus alatus	2	0	13

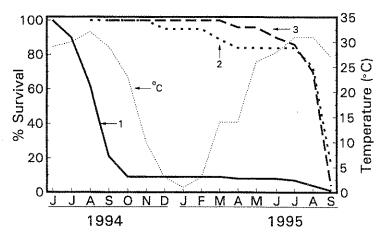


Figure 1. Monthly water temperature (°C) and percent survival of three groups of *Elliptio dilatata* collected on different dates in 1994 (1=June; 2=August; 3=September) and held at the Frankfort Fish Hatchery.

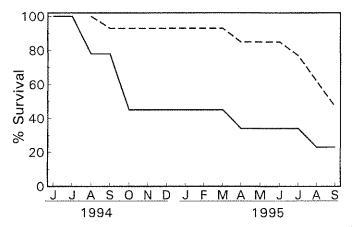


Figure 2. Percent survival of two groups of *Lasmigona costata* collected in June (solid line) and August (dashed line) 1994 and held at the Frankfort Fish Hatchery.

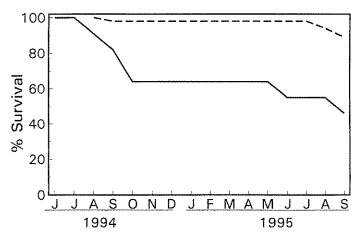


Figure 3. Percent survival for two groups of *Lampsilis siliquoidea* collected in June (solid line) and August (dashed line) 1994 and held at the Frankfort Fish Hatchery.

Table 4. Maximum water temperatures and mean values (±S.D.) of water chemistry variables for the four holding sites.

Location	Maximum Temperature (°C)	Alkalinity (mg/l CaCO3)	Hardness (mg/CaCO3)	рН
Frankfort Fish Hatchery	32	131 (<u>+</u> 23.8)	176 (±14.6)	8
Minor Clark Fish Hatchery	28	35 (±6.3)	52 (±8.9)	6.7
Laurel Hill Wildlife Management Are	a 30	$20 (\pm 1.5)$	17 (<u>+</u> 1)	7.3
Kentucky Lake	34	39 (<u>+</u> 6.3)	49 (±7)	7.6

Table 5. Numbers and percent survival of mussels held in a pond at the Laurel Hill Wildlife Management Area. Numbers in parenthesis indicate specific groups of mussels identified in Table 1.

Species	Number Held	% Survival	Months Held
Ellipsaria lineolata (2)	14	100	12
Megalonaias nervosa (2)	4	25	12
Pleurobema cordatum	175	94	12
Ptychobranchus fasciolaris (3)	1	100	12
Quadrula metanevra	5	100	12
Quadrula nodulata	1	100	12
Quadrula pustulosa	2	50	12
Quadrula quadrula	20	75	12

Table 6. Numbers and percent survival of mussels held in an embayment of Kentucky Lake, Tennessee. Numbers in parenthesis indicate specific groups of mussels identified in Table 1.

Species	Number Held	% Survival	Months Held	
Actinonaias ligamentina	50	88	14	
Cyclonaias tuberculata	101	99	24	
Elliptio dilatata (4)	41	86	14	
Elliptio dilatata (5)	60	95	13	
Lasmigona costata (2)	8	100	13	
Pleurobema coccineum (2)	13	85	13	
Ptychobranchus fasciolaris (1	.) 8	63	14	
Ptychobranchus fasciolaris (2		75	13	

Table 7. Percent survival of *Fusconaia ebena* after 4 months of captivity at the Frankfort Fish Hatchery. Mussels were held out of water for various time intervals and packed on ice or covered with wet burlap. Each treatment group consisted of 50 individuals.

	Date Collected and Transport Metho				
		1994	July 1994		
Time (hours)	Ice	Burlap	Ice	Burlap	
4	100%				
9	72%	78%	******		
24	80%		78%	94%	
48	18%	******	10%	90%	

burlap, survival was greater than 90% for mussels covered with wet burlap for 24 and 48 hours. In contrast, only 10% to 18% of the *F. ebena* packed in ice for 48 hours were alive after 4 months in captivity. There was little additional mortality after the first four months for any treatments until the following June (Figures 4 and 5). Only 30% of the *Ellipsaria lineolata* were alive after being held 1 month at the Frankfort Fish Hatchery; this group of mussels was also packed in ice and held for 48 hours.

Discussion

Survival of mussels held in captivity was high at three of the four facilities used in this study. The cause of the high mortality of mussels at the Frankfort Fish Hatchery is unknown but it was likely related to a water quality variable that was not measured. Except for Actinonaias ligamentina, Ellipsaria lineolata, and Fusconaia ebena, all mussels held at the Frankfort Fish Hatchery were collected from Elkhorn Creek. Untreated water from Elkhorn Creek is pumped directly into the pond used to hold mussels. We did not observe any recent mortality of mussels in Elkhorn Creek between June and September 1994 when we were collecting mussels. Consequently, it seems that the water quality problem was confined to the pond itself and not due to the water source. Periodically, supersaturation of dissolved oxygen occurs in the pond (M. Larimore, pers. comm.). Supersaturation of atmospheric gases can be lethal to marine bivalves (Malouf et al. 1972; Goldberg 1978); however, we are unaware of any studies implicating supersaturation of oxygen alone as the causative factor in bivalve deaths. We suspect

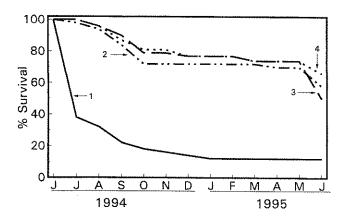


Figure 4. Percent survival of *Fusconaia ebena* collected in June and held out of water for various time intervals and packed on ice or covered with burlap (1=48h ice; 2=9h ice; 3=9h wet burlap; 4=24h ice).

that supersaturation of oxygen resulted from photosynthetic activity and was not responsible for the high mortality. It is possible that supersaturation of atmospheric gases, and hence oxygen, could have resulted from air entrainment in the pumping system. Since gaseous nitrogen was not measured, we cannot be sure of the source of supersaturated oxygen levels in the Frankfort Fish Hatchery pond.

Although most mussels were suspended in the water column, the water quality problem was not restricted to this area; one pocket net of A. ligamentina dropped to the pond bottom where it remained until the pond was drained in the fall of 1995 at which time all mussels in the net were dead. However, there was little or no mortality of largemouth bass (Micropterus salmoides) that were being raised in the pond at the same time mussels were dying. Mortality of mussels occurred almost entirely during the warmest months each year; however, the maximum water temperature we recorded at the Frankfort Fish Hatchery was 32°C but water temperatures reached 34°C in the embayment of Kentucky Lake and yet little mortality of mussels occurred there.

Although mortality occurred throughout each summer at the Frankfort Fish Hatchery, it was likely related to conditions in the pond during late May and June. Mortality was high during the first 4 months in captivity for mussels introduced in June. In contrast, mortality was low during the first 4 months for other groups of the same species introduced from July through September 1994; however, mortality of all groups of mussels increased greatly between May and June 1995. Apparently, conditions in the pond during May and June were not immediately lethal to mussels. Instead, these conditions may have severely stressed the mussels,

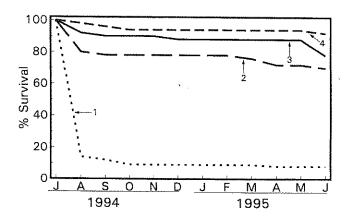


Figure 5. Percent survival of *Fusconaia ebena* collected in July and out of water for various time intervals and packed on ice or covered with burlap (1=48h ice; 2=24h ice; 3=48h wet burlap; 4=24h wet burlap).

and along with the high water temperatures resulted in delayed mortality. There was no evidence that mussels from Elkhorn Creek were already stressed or unsuitable for maintaining in ponds. On the contrary, the source and species of mussels mattered little. For instance, A. ligamentina was collected from the Licking River and held at the Minor Clark Fish Hatchery, the Frankfort Fish Hatchery, and Kentucky Lake, but mortality was high only at Frankfort. Although collection locations differed for several other species that were held at two or more locations, mortality was highest at the Frankfort Fish Hatchery. Handling and transport time were not factors causing the higher mortality because most mussels were handled in a similar manner. In fact, because Elkhorn Creek adjoined the Frankfort Fish Hatchery, mussels collected from the creek were out of water for less than 1 hour before being placed in the pond. The results of testing transport method and length of time that F. ebena were kept out of water are somewhat confounded by the water quality problem that occurred at the Frankfort Fish Hatchery. Nonetheless, results from the July experiment clearly indicate a high survival rate for mussels covered only with wet burlap and held at an ambient temperature of 21°C for up to 48 hours.

We anticipated a higher survival of mussels at the Minor Clark Fish Hatchery because the raceway conditions were more similar to a lotic environment; however, survival was also high in the spring-fed pond at the Laurel Hill Wildlife Management Area and in the embayment of Kentucky Lake. In particular, the high survival of *A. ligamentina* and *E. dilatata* held in Kentucky Lake was unexpected because these species have been nearly extirpated from the mainstem of the Tennessee River. Apparently water quality conditions in Kentucky Lake, at least within the water column, are suitable for these species.

The results of our study indicate that it is feasible to maintain riverine species of mussels in captivity but survival can be highly variable among holding facilities and species. Variation in survival among species and holding facilities is not unique to our study; Burress and Neves (1995) reported highly variable survival rates among species and holding locations in Virginia. Much additional research is needed to determine conditions conducive to high survival of all species of mussels held in captivity. Unfortunately, there may not be sufficient time to determine these conditions for all species because some are on the brink of extinction. Consequently, we recommend a two-pronged approach to establishing and maintaining captive populations. First, rigorously designed experiments are needed to elucidate the conditions necessary to promote high

survival. This approach would provide the greatest long-term benefits for most species; however, it could take several years to complete. Simultaneously, a trial-by-fire approach also should be conducted. Specifically, this approach should consist of attempting to hold a wide variety of species in as many locations as possible. This second approach might identify those locations where the most critically threatened species could be held successfully, at least for the short term.

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Initiatives for the Future



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