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**DEVELOPING TECHNOLOGY FOR LONG-TERM HOLDING
OF MUSSELS IN CAPTIVITY**

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Quarantine Experiments

In 1996 and 1997, mussels were quarantined under static conditions in 1,136-liter stock tanks equipped with aerators (1/20 hp). After quarantine, all water in the tanks was treated with calcium hypochlorite for >5 hours to kill all organisms, including any zebra mussels. Following this treatment all tanks were hand scrubbed and rinsed several times with tap water prior to use for another quarantine. Thus, this treatment likely eliminated most bacteria. During each quarantine, total ammonia began to increase immediately following introduction of mussels. Typically, maximum concentrations of 0.5 to 3.2 mg/L were reached in 3 to 17 days. Ammonia levels then declined gradually and remained low for the remainder of the quarantine. We attributed this decline in ammonia to the build-up of denitrifying bacteria on the tank surfaces as well as on the mussel shells. Moreover, maximum ammonia concentrations seemed to be negatively related to mussel survival during quarantine and survival during the following 12 months at the permanent holding facility.

In 1998, we designed and constructed a two-tier recirculating system for quarantining mussels. The system includes two of the stock tanks used in previous years, a sump, and a biofilter. Water is pumped from the lower tank up to the sump. An overflow pipe in the sump drains into the biofilter. The water is then gravity-fed into the upper tank, and then down into the lower tank. About two weeks before quarantining mussels, we inoculated the biofilter with filter material from another recirculating system used for fish. At the same time, we introduced about 50 fish into the quarantine tanks to maintain the bacterial culture.

In October 1998, we collected mussels from the Diamond Island area of the Tennessee River, and hand-scrubbed them to remove any zebra mussels. Prior to introducing mussels, we removed all fish from the recirculating system. Throughout the quarantine period mussels in both recirculating systems were fed live algae (*Bracteococcus grandis*) at a rate of 1.4 mg (dry weight) per mussel every 2-3 days. Ammonia levels remained below 0.25 mg/L, and generally were undetectable. At the end of the 30-day quarantine, survival ranged from 94 to 100 % among species (Table 1). Following quarantine, these mussels were moved to our long-term holding facility at Center Hill Reservoir. In May 1999, a subsample of 100 individuals of each species was retained to monitor long-term survival; the remaining mussels were translocated to the French Broad River. After 1 year at Center Hill Reservoir, survival of *Cyclonaias tuberculata* and *Quadrula metanevra* was > 98 % but only 82 % of the *Quadrula pustulosa* survived (Table 1).

In 1999, we collected a total of 7,399 mussels from the Tennessee River near Diamond Island. All of these mussels were quarantined in the recirculating systems using the same protocol we developed in 1998. Many of the mussels we quarantined during 1999 were eventually translocated to the French Broad River. Subsamples from each collection were retained to continue monitoring their post-quarantine survival.

At the end of the quarantine, survival of all species collected in May was > 95 %. Similarly most species collected in July and September had high survival rates during quarantine; however, only 50 % of the *Ellipsaria lineolata* collected were still alive 4 months later (Table 1). Since we first begin quarantining mussels in 1995, we have found that some species such as *E. lineolata* and *Obliquaria reflexa* typically have the lowest survival rates during and after quarantine.

Captive Populations

During 1998, we consolidated our captive mussel populations into three facilities: Minor E. Clark Hatchery, KY; the Normandy Hatchery, TN; and Center Hill Reservoir. Mussels previously held at the Laurel Hill Wildlife Management Area, Elkhorn Station, and the Birdsong Marina (American Pearl Farm) were either moved to one of these facilities or were returned to their natal stream. Additionally, some captive mussels were translocated to the French Broad River. In 1999, we continued returning mussels to their natal stream and translocating others to the French Broad River. As of December 31, 1999 we have 4,633 mussels of 38 species in captivity (Table 2).

In 1994, we collected 272 mussels of 7 species from the Licking River and placed them into a raceway at the Minor E. Clark Hatchery. Additional mussels were introduced in 1995, 1996, and 1998. Most of the species became gravid in the year following their introduction, as well as in subsequent years. As part of another study on mussel propagation, juvenile *Lampsilis cardium*, produced in one of the raceways, reached a mean length of 21.5 mm after one growing season and 54.5 mm after the second growing season, and about 75 mm after the third season. Because the parental stock (4 gravid females) came from Buck Creek, KY, we stocked 400 of these cultured *Lampsilis cardium* (1½ to 2 years old) into the creek. In April 1999, we translocated 100 individuals to Horse Lick Creek. The remaining *Lampsilis cardium* (>400) are still in captivity and average about 85 mm in length. This cohort (propagated in 1996 and cultured in the raceway) matured and were gravid adults in September 1999. To the best of our knowledge, this represents only the second instance of culturing mussels throughout their entire life cycle since Howard (1916). The first instance was our report last year of mature *Lampsilis fasciola* cultured at the Normandy Hatchery. In 1999, the *Lampsilis fasciola* spawned for a second year; 70 of these mature mussels were translocated to the French Broad River. Survival of adult mussels at the Normandy Hatchery continues to be good. We have removed most common species of mussels and now use this facility mainly for holding endangered species and culturing juveniles. As of December 31, 1999 we are holding (under the necessary Federal Endangered Species Permits) 61 individuals of 7 endangered species (Table 2). Thirty-five individuals of six endangered species included in our 1998 report were returned alive to their natal stream. Each year, the endangered *Lampsilis abrupta* has spawned and became gravid in the raceway.

Final Disposition of Captive Mussels

In 2000, we plan to return most common species to their natal stream or use them for

reestablishing mussel populations in the French Broad River. Endangered species and those species being considered for listing e.g. *Lexingtonia dolabelloides*, will remain in captivity and will be used for propagation.

Reference

Howard, A.D. 1916. A second generation of artificially reared fresh-water mussels. Transactions of the American Fisheries Society 46:93-100.

Table 1. Survival of mussels quarantined in October 1998 and in 1999.

Month and Year Collected	Species	Survival (%)			
		Number Quarantined	End of Quarantine	4-6 Months	1 Year
October 1998					
	<i>Cyclonaias tuberculata</i>	566	99.5	--	98.5
	<i>Quadrula metanevra</i>	412	99.8	--	99.8
	<i>Quadrula pustulosa</i>	820	94.4	--	82.1
May 1999					
	<i>Amblema plicata</i>	21	95	--	--
	<i>Cyclonaias tuberculata</i>	610	99.9	99	--
	<i>Obliquaria reflexa</i>	34	100	85	--
	<i>Quadrula metanevra</i>	425	100	99	--
	<i>Quadrula pustulosa</i>	1,045	95	91	--
	<i>Tritogonia verrucosa</i>	6	100	--	--
July 1999					
	<i>Amblema plicata</i>	29	100	88	--
	<i>Cyclonaias tuberculata</i>	296	98	96	--
	<i>Ellipsaria lineolata</i>	24	75	50	--
	<i>Fusconaia ebena</i>	1,176	87	77	--
	<i>Megalonaias nervosa</i>	3	100	100	--
	<i>Obliquaria reflexa</i>	24	79	71	--
	<i>Quadrula metanevra</i>	228	99.5	98	--
	<i>Quadrula pustulosa</i>	450	84	81	--
	<i>Tritogonia verrucosa</i>	3	100	100	--
September 1999					
	<i>Cyclonaias tuberculata</i>	536	97	--	--
	<i>Fusconaia ebena</i>	1,740	97	--	--
	<i>Quadrula metanevra</i>	257	100	--	--
	<i>Quadrula pustulosa</i>	492	83	--	--

Table 2. Species and numbers of mussels in captivity as of December 31, 1999.

Species	Facility		
	Normandy Hatchery	Minor Clark Hatchery	Center Hill Reservoir
<i>Actinonaias ligamentina</i>	--	116	--
<i>Actinonaias pectorosa</i>	20	--	--
<i>Amblema plicata</i>	--	--	479
<i>Arcidens confragosus</i>	--	--	9
<i>Cyclonaias tuberculata</i>	--	--	615
<i>Cyprogenia stegaria</i>	3	--	--
<i>Elliptio crassidens</i>	--	--	115
<i>Ellipsaria lineolata</i>	--	--	215
<i>Epioblasma brevidens</i>	9	--	--
<i>Epioblasma capsaeformis</i>	5	--	--
<i>Fusconaia ebena</i>	--	--	955
<i>Fusconaia flava</i>	--	--	60
<i>Lampsilis abrupta</i>	28	--	--
<i>Lampsilis cardium</i>	--	404	--
<i>Lampsilis fasciola</i>	36	--	--
<i>Lampsilis ovata</i>	10	--	--
<i>Leptodea fragilis</i>	--	--	1
<i>Lexingtonia dolabelloides</i>	12	--	--
<i>Ligumia recta</i>	--	--	6
<i>Megalonaias nervosa</i>	--	--	162
<i>Obliquaria reflexa</i>	--	--	47
<i>Obovaria olivaria</i>	--	--	32
<i>Plethobasus cicatricosus</i>	7	--	--
<i>Plethobasus cooperianus</i>	16	--	--
<i>Plethobasus cyphys</i>	1	--	--
<i>Pleurobema cordatum</i> (and <i>P. sintoxia</i>)	1	152	71
<i>Pleurobema plenum</i>	3	--	--
<i>Pleurobema rubrum</i>	9	--	--
<i>Ptychobranthus fasciolaris</i>	--	86	1
<i>Quadrula cylindrica</i>	8	--	--
<i>Quadrula metanevra</i>	--	--	430

Table 2 (continued).

Species	Facility		
	Normandy Hatchery	Minor Clark Hatchery	Center Hill Reservoir
<i>Quadrula nodulata</i>	--	--	49
<i>Quadrula pustulosa</i>	--	--	431
<i>Tritogonia verrucosa</i>	--	--	6
<i>Truncilla truncata</i>	--	--	2
<i>Villosa iris</i>	30	--	--
<i>Villosa vanuxemensis</i>	3	--	--
Total	201	758	3,686