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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. These tanks were 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 September 1998, we collected mussels from the Diamond Island area of the Tennessee River, and hand-scrubbed them to remove any zebra mussels. Prior to quarantine, all fish were removed from the recirculating system, and then mussels were introduced at a density of 1 mussel/3.4 liters of water (for the entire system). Ammonia levels remained below 0.25 mg/L, and generally were undetectable. Survival of all species was $\geq 83\%$ at the end of the quarantine (Table 1). Following quarantine, these mussels were translocated to the French Broad River.

feeding?

Because the recirculating system was so successful, we built a second system. At the same time, we began culturing algae (*Bracteococcus grandis*) to feed mussels during quarantine. In October 1998, we again collected mussels near Diamond Island. We followed the same protocol used for the September quarantine with the following exceptions. Mussels were quarantined in one recirculating system at the same density (1 mussel/3.4 L) used in September; the density was doubled (1 mussel/1.7 L) in the second recirculating system. Throughout the quarantine period mussels in both recirculating systems were fed live algae at a rate of 1.4 mg (dry weight) per mussel every 2-3 days. Overall mussel survival exceeded 95% in both recirculating systems (Table 1). Following quarantine, these mussels were moved to our long-term holding facility at Center Hill Reservoir. Survival of these mussels will be monitored for the next year.

Physiological Condition

Biochemical analysis of tissue samples of *Fusconaia ebena* and *Amblema plicata* were conducted to assess physiological condition of these species in Kentucky Lake at the beginning and end of each quarantine period in 1997. Similar analyses were performed on samples of these species following quarantine. Four replicates of a composite sample of 10 individuals of each species were analyzed for percent lipid composition. In most cases, there was no apparent affect of quarantine on percent lipid composition following quarantine. Similarly, protein composition varied little among treatments; however, there was a highly significant loss in biomass during most quarantines and densities. In general, mean dry tissue weight adjusted for shell length for quarantined mussels was 11 to 32% less than for individuals of *F. ebena* not quarantined. Although dry tissue weights of *A. plicata* also decreased 5 to 40%, most of these differences were not significant. These data are being subjected to further statistical analysis.

data?

glycogen?

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. As of December 31, 1998 we have 8,057 mussels of 49 species in captivity (Table 2).

In 1996, we collected 100 mussels of 6 species from Elkhorn Creek near the Frankfort Hatchery. These mussels were transported to Elkhorn Station and placed into pocket nets suspended in an old raceway/pond. Initial (30-day) survival was 100% (Table 3). Survival after 2 years was high for most species. Moreover, some individuals of all species of *Lampsilis* became gravid while being held in this facility. In 1998, all mussels were returned to Elkhorn Creek. We believe that this facility is suitable for maintaining refuge populations of unionids should the need arise.

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 have stocked 400 of these cultured *Lampsilis cardium* (1½ to 2 yr old) into the creek. The remaining individuals (>400) are in captivity and will be stocked in different locations to enhance or reestablish mussel populations. Thus,

this facility is well-suited not only to maintain refuge populations of mussels, but also for the propagation and culture of mussels.

Survival of mussels at the Normandy Hatchery has been excellent. In addition to common species, we are holding (under the necessary Federal Endangered Species Permits) 87 mussels of 7 endangered species (Table 1). This facility has a lot of potential for the propagation and culture of endangered species. In 1995, we propagated *Lampsilis fasciola* in the raceway and have continued culturing this cohort. In September 1998, we discovered that these juveniles had spawned and became gravid. To the best of our knowledge, this represents the only instance of culturing mussels through their entire life cycle since Howard (1916).

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 during a 30-day quarantine in recirculating systems in 1998.

Month Collected	Quarantine Density (liters of water per mussel)	Species and Percent Survival		
		<i>Cyclonaias tuberculata</i>	<i>Quadrula metanevra</i>	<i>Quadrula pustulosa</i>
September	3.4/1	98.9	100	83.9
October	3.4/1	99.5	100	92.2
October	1.7/1	99.5	100	95.1

Table 2. Species and numbers of mussels in captivity as of December 31, 1998.

Species	Facility		
	Normandy Hatchery	Minor Clark Hatchery	Center Hill Reservoir
<i>Actinonaias ligamentina</i>	---	142	---
<i>Actinonaias pectorosa</i>	23	---	---
<i>Amblema plicata</i>	---	---	479
<i>Arcidens confragosus</i>	---	---	9
<i>Cyclonaias tuberculata</i>	---	---	905
<i>Cyprogenia stegaria</i>	1	---	---
<i>Elliptio crassidens</i>	---	---	133
<i>Elliptio dilatata</i>	---	240	---
<i>Ellipsaria lineolata</i>	---	---	237
<i>Epioblasma capsaeformis</i>	20	---	---
<i>Fusconaia ebena</i>	---	---	2255
<i>Fusconaia flava</i>	---	---	126
<i>Fusconaia subrotunda</i>	---	3	---
<i>Lampsilis abrupta</i>	37	---	---
<i>Lampsilis cardium</i>	---	478	---
<i>Lampsilis fasciola</i>	106	---	---
<i>Lampsilis ovata</i>	12	---	---
<i>Lasmigona costata</i>	---	5	---
<i>Lemiox rimosus</i>	10	---	---
<i>Leptodea fragilis</i>	---	---	1
<i>Ligumia recta</i>	62	---	6
<i>Medionidus conradicus</i>	23	---	---
<i>Megalonaias nervosa</i>	---	---	159
<i>Obliquaria reflexa</i>	---	---	34
<i>Obovaria olivaria</i>	---	---	32
<i>Plethobasus cicatricosus</i>	4	---	---
<i>Plethobasus cooperianus</i>	14	---	---
<i>Pleurobema cordatum</i>	13	158	71
<i>Pleurobema rubrum</i>	---	8	---
<i>Pleurobema sintoxia</i>	---	27	---
<i>Potamilus alatus</i>	---	---	10
<i>Pyganodon grandis</i>	---	1	---
<i>Ptychobranthus fasciolaris</i>	---	106	1
<i>Ptychobranthus subtentum</i>	14	---	---
<i>Quadrula apiculata</i>	---	---	1

Table 2 (continued).

Species	Facility		
	Normandy Hatchery	Minor Clark Hatchery	Center Hill Reservoir
<i>Quadrula cylindrica</i>	8	---	---
<i>Quadrula metanevra</i>	---	---	721
<i>Quadrula nodulata</i>	---	---	59
<i>Quadrula pustulosa</i>	---	---	1197
<i>Quadrula quadrula</i>	---	---	16
<i>Tritogonia verrucosa</i>	---	50	3
<i>Truncilla truncata</i>	---	---	2
<i>Utterbackia imbecillis</i>	---	1	---
<i>Villosa iris</i>	30	---	---
<i>Villosa taeniata</i>	---	1	---
<i>Villosa trabalis</i>	1	---	---
<i>Villosa vanuxemensis</i>	3	---	---
Total	381	1219	6457

Table 3. Numbers and percent survival of mussels collected July 9, 1996 from Elkhorn Creek and held at Elkhorn Station, KY.

Species	Number Held	Percent Survival		
		30-day	1-year	2-year
<i>Amblema plicata</i>	28	100	96	79
<i>Elliptio dilatata</i>	44	100	98	84
<i>Lampsilis cardium</i>	5	100	100	60
<i>Lampsilis fasciola</i>	2	100	100	100
<i>Lampsilis siliquoidea</i>	15	100	87	80
<i>Lasmigona costata</i>	6	100	83	50