RICHARD J. NEVES

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memorandum

DATE: November 17, 1986

REPLY TO ATTN OF: Director, National Fisheries Research Center, La Crosse, Wisconsin

SUBJECT: Annual Recovery Plan Implementation Report

TO: Chief, Division of Fisheries and Wetlands Research, Washington, D.C. (DFWR)

Enclosed is the report "Endangered Species Annual Summary Report *Lampsilis higginsi*" outlining activities of the NFRC-La Crosse during 1986.

Detailed questions on this project can be directed to Dr. Leslie Holland, FTS 364-3210.

Fred P. Meyer

Enclosure

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Endangered Species Progress Report: *Lampsilis higginsi*

FY 1986

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Life history and reproductive information on the endangered mussel Lampsilis higginsi is necessary for implementation of a recovery plan for this species. Our efforts have focused on the early life stages of the mussel including, the glochidium, parasitism of the host fish, and the juvenile and on identification of physical parameters associated with the habitat of the species. Field observations during summers 1984-1985 indicated that the glochidia are released during early summer, about June through early July. Artificial infection experiments have verified eight species of fish as hosts for L. higginsi including northern pike (Esox lucius), bluegills (Lepomis macrochirus), green sunfish (L. cyanellus), smallmouth bass (Micropterus dolomieui), largemouth bass (M. salmoides), white bass (Morone chrysops), walleye (Stizostedion vitreum), and yellow perch (Perca flavescens). Verification of the use of these species by L. higginsi in the field has not been possible, however. The glochidia of L. higginsi were found to be indistinguisable from those of the related species--L. radiata siliquoidea, L. ventricosa, and Ligumia recta, except by scanning electron microscopy.

The present report details our continued efforts to provide some insight into the life history of L. higginsi. Hosts for the glochidia of L. higginsi and attempts to culture the glochidia and juveniles of this species are described. In addition, the host quality of three species in the family Centrarchidae is discussed. Methods are reported for determining the effects of current on juvenile mussels transplanted onto different substrate types. Data collections to quantitatively identify the relationship of the species' distribution in the field to current and substrate characteristics are examined.

HOST SPECIFICITY

I. Host Identification

The following fishes were found to be suitable hosts for the glochidia of L. higginsi in summer 1985: northern pike, bluegills, green sunfish, largemouth bass, smallmouth bass, white bass, yellow perch, and walleyes. Results suggested that many species in higher order families such as Centrarchidae and Percidae, were suitable as hosts for this mussel. However, many of these species frequent backwaters and are not common in the main channel habitat of L. higginsi. Since verification of the fish species used by L. higginsi in the field has not been possible, more information was needed on the breadth of its host range. In this study, we tested additional species of percids (log perch [Percina caprodes], sand darter [Ammocrypta clara], and Johnny darter [Etheostoma nigrum]), an additional cyprinid species (emerald shiner [Notropis atherinoides]), and two species from previously untested families--lower order family: Lepisosteidae, longnose gar (Lepisosteus osseus), higher order family: Gasterosteidae, brook stickleback (Culaea inconstans). Northern pike was also retested since it was found to be a marginal host in a previous study (Waller and Holland, in review).

Materials and Methods

The following fishes were infected in two separate trials with glochidia from an individual L. higginsi: emerald shiner, sand darter, log perch, Johnny darter, brook stickleback, northern pike, and longnose gar. All fish were young-of-the-year and were either hatchery-reared or field collected (Table 1).

Table 1. Artificial infection of seven species of fish with the glochidia of *L. higginsi*.

Species	Source	Maximum length of infection	Juveniles produced	Number of fish
Longnose gar (*Lepisosteus osseus*)	Field	24 hours	None	2
Northern pike (*Esox lucius*)	Hatchery	15 days	None	7
Emerald shiner (*Notropis atherinoides*)	Field	24 hours	None	20
Brook stickleback (*Culaea inconstans*)	Hatchery	20 days 19 days	12 63	10 9
Sand darter (*Ammocrypta clara*)	Field	48 hours 48 hours	None None	20 16
Log perch (*Percina caprodes*)	Field	48 hours	None	15
Johnny darter (*Etheostoma nigrum*)	Field	8 days	None	15

A gravid female *L. higginsi* was collected from Pool 10 of the upper Mississippi River near Prairie du Chien, Wisconsin. Glochidia were flushed from the marsupia of the female with water injected via a hypodermic syringe and needle. Glochidia were checked for viability by placing a 1-2 mL suspension in a 1% NaCl solution. Those capable of attaching to fish responded by snapping shut.

Fish were exposed to glochidia by pipetting a drop of glochidia into one branchial cavity. Each species of fish was held separately in a 38-L

flow-through aquaria. The temperature averaged 22.2°C in the first and 21.9°C in the second trial with fluctuations ± 3°C. Fish were examined 1-hour after infection to assess the success of initial attachment and every 2-3 days thereafter. Juveniles were recovered by siphoning the tank bottom daily with a polyurethane hose through a 150-µm mesh screen. Metamorphosis to the juvenile stage was defined by movement of the foot, opening and closing of valves, and separation of two adductor muscles.

In Trial 1, three groups of fishes were tested: brook stickleback, emerald shiner, and sand darter. In Trial 2, brook sticklebacks were retested; they maintained attachments for the duration of Trial 1 but only 12 juveniles were recovered. Also, sand darters were retested because they were field collected and a question existed about possible immune development. In addition, longnose gar, northern pike, log perch, and Johnny darter were examined.

Results

The brook stickleback was the only species that served as a suitable host for the glochidia of L. higginsi in this study. This species produced 12 juveniles in the first and 63 juveniles in the second trial. The apparent difference in number of juveniles transforming on brook stickleback between the two trails was likely related to poor siphoning of the tank during Trial 1; early sloughing of glochidia was not observed in Trial 1 and greater care was taken in Trial 2. Juveniles were recovered from day 18-20 and day 15-19, respectively in the two trials. Several other species held infections for prolonged periods but produced no

juveniles including, northern pike (infected 15 days) and Johnny darters (infected 8 days). Emerald shiners, sand darters, log perch, and longnose gar lost infections within 48 hours after the start of the study.

Discussion and Conclusion

Waller and Holland (in review) found that some members of two higher order families, Centrarchidae and Percidae, were hosts for L. higginsi, whereas many species of tested lower families were not (e.g., Cyprinidae, Ictaluridae, Catostomidae). The present study found a member of an additional higher order family, the brook stickleback in the Gasterosteidae, to be a suitable host and found other members of the Percidae as well as the tested gar and shiner to be unsuitable. Since the percids and longnose gar were field collected, we cannot rule out the possibility that they had been previously infected and acquired an immunity. However, the prolonged attachment time on the Johnny darters (8 days) suggests that they were not immune since sloughing of glochidia occurs within 1-3 days in immune fish (Arey 1932). In addition, the development of immunity generally requires more than one exposure to glochidia (Arey 1932; Reuling 1918; Waller 1985). Since these were young-of-the-year fish, it is unlikely that they had been infected more than once this season. Lastly, reports of metamorphosis on darters are rare (Fuller 1974) so our results are not unusual. The nonsusceptibility of longnose gar to L. higginsi glochidia supports earlier suggestions that lower order familes are not hosts.

The graded response of fishes within the Percidae to infection with L. higginsi glochidia is similar to that within the Centrarchids (Host Quality, this report). Members of the family demonstrate different levels of susceptibility: (1) an immediate sloughing of glochidia (sand darter and log perch), (2) a lengthly shedding period (Johnny darter), (3) a sloughing of some glochidia but also with some production of juveniles (yellow perch), and (4) a production of many juveniles (walleye) (Waller and Holland, in review). The apparent use of fishes of higher order families as hosts and then only certain species within those family by L. higginsi may indicate coevolution of the mussel and fish or may reflect physiological or biochemical similarities between these fishes.

II. Host Quality

The quality of fishes as hosts for the glochidia of L. higginsi was found to vary among the three centrarchid species, the bluegill, green sunfish, and largemouth bass in experiments conducted by Waller and Holland (in review). However, these tests were run with tanks of fish as was the previously described study and no quantitative measure of the transformation percentages were possible. In this study, we determined the percent of glochidia that transformed successfully per fish in order to rank each species for host quality.

Materials and Methods

One gravid female L. higginsi was collected from Pool 10 of the UMR near Prairie du Chien, Wisconsin. Glochidia were flushed from the

marsupia of the female with water injected with a hypodermic needle and syringe. Glochidia were checked for viability by placing a 1-2 mL suspension in a 1% NaCl solution. Those capable of attaching to fish responded by snapping shut.

Ten fish of each species--largemouth bass, bluegill, and green sunfish--were infected by pipetting one drop of glochidia into the right branchial cavity. Fish were then held separately in 3.4-L jars at 20-21°C. The total number of sloughed glochidia and metamorphosed juveniles produced per fish was determined by dumping the contents of each jar every other day through a 150-µm mesh screen and examining the contents under a dissecting microscope. The gills of each fish were also checked every 2-3 days to follow the infection. Metamorphosis to the juvenile was defined by movement of the foot, opening and closing of valves and separation of two adductor muscles.

Results

Largemouth bass ranked highest for host quality followed by green sunfish and bluegills (Table 2). The percent juveniles produced per fish was greatest in largemouth bass (7.68%) and lowest in bluegills (0.00038%) with transformation intermediate in green sunfish (2.43%). A significant difference in percent transformation existed between that found for largemouth bass and values for the other two species ($P = 0.0005$). However, no significant differences existed between bluegills and green sunfish. The greatest sloughing of glochidia occurred in the first 3 days of the test. Consequently, the number of glochidia still attached to the

Table 2. Transformation percentages of bluegills, green sunfish, and largemouth bass infected with the glochidia of L. higginsi. [a]Percent transformation was calculated based on numbers of glochidia initially attached and again based on numbers of glochida present after the major 3-day drop-off period.

	Maximum attachment number (approximately)			% juveniles produced[a]			% juveniles after 3-day drop-off adjustment[a]		
Replicate	BG	GS	LMB	BG	GS	LMB	BG	GS	LMB
1	0	12	20	0.0	0.7	14.5	0.0	3.4	23.9
2	0	23	6	0.0	5.1	4.9	0.0	24.5	15.8
3	0	20	12	0.0	1.4	4.0	0.0	8.8	13.3
4	15	20	2	*	13.7	6.3	**	34.2	16.7
5	0	15	14	0.0	1.0	9.9	0.0	5.3	50.0
6	4	30	6	0.0	1.5	0.0	0.0	5.1	0.0
7	9	24	3	0.0	0.0	6.7	0.0	0.0	18.2
8	4	8	11	0.0	0.0	12.3	0.0	0.0	65.2
9	10	20	14	0.0	0.0	3.0	0.0	0.0	14.3
10	5	30	14	0.0	0.9	18.2	0.0	2.7	25.8
			$\bar{x}$ =	0	2.4	8.0	0.0	8.4	24.3

BG = bluegills; GS = green sunfish; and LMB = largemouth bass.

gills after day 3 is a better indicator of the relative number of glochidia that attached and encysted than counts done earlier. Percentages of juveniles produced per fish were higher when the initial 3-day drop-off was excluded but did not change the ranking order of the

species. Again, the largemouth bass showed a significant difference in numbers of juveniles produced ($P < 0.001$) compared to bluegills and green sunfish.

Juveniles were recovered from day 18 to 25 in jars containing largemouth bass. Partially transformed juveniles appeared from day 11 to 15 in the study on green sunfish, indicating sloughing before complete transformation. Juveniles were found on days 16 to 18. Only one partially transformed juvenile (day 15) and one completely transformed individual (day 18) were recovered from the bluegill study.

Discussion and Conclusion

Susceptibility to infection with the glochidia of L. higginsi is definitely graded in the three species of centrarchids tested in this study and may reflect a phylogenetic trend. Using the results of this study, we would rank the species as follows: (1) largemouth bass, (2) green sunfish, and (3) bluegill. Although there was not a statistically significant difference between the green sunfish and bluegill, the former is ranked higher because of differences in the course of infection. Bluegills sloughed most glochidia the first 1-3 days of the infection, whereas green sunfish retained glochidia up to a few days before complete transformation. Premature drop-off greatly decreased the transformation percentage of green sunfish.

L. higginsi is a member of the subfamily Lampsilinae, considered one of the most specialized of freshwater mussels because of their parasitic modifications. The use of fish from higher order families as glochidial

hosts by species of Lampsilis implies coevolution of mussel and fish. Similarly, the greater susceptibility of certain fishes within a family to infection may indicate phylogenetic relationships within the family and reflect the development of physiological and biochemical similarities in higher fishes of different families (e.g., walleye and largemouth bass).

This study points out the need for careful examination of the fish host literature and for testing of hosts in the laboratory before recovery plans for mussel populations are implemented. The host quality of a species should be considered before infected fishes are transplanted to the field with hopes of repopulating a mussel bed.

LABORATORY CULTURE OF L. HIGGINSI JUVENILES

Our previous attempts at in vitro culture of L. higginsi have not been successful because of problems with contamination and humidity. Similarly, juveniles survived only 2 to 3 weeks in the culture because of incidence of disease and starvation (Waller 1985). Modifications were made in the culture techniques used to rear the juveniles of the three mussels--L. higginsi, L. ventricosa, and L. radiata siliquoidea at the suggestion of Dr. B. Isom, Tennessee Valley Authority in FY 1986.

Materials and Methods

In vitro culture methods were modified from techniques of Isom and Hudson (1982). One drop of suspension containing glochidia of L. higginsi (200-1,000) was placed into a 15 mm x 60 mm petri plate containing 2 mL of the artificial media and 1 mL of plasma from common carp. The antibiotics Rifadin, Achromycin, Geopen, Garamycin, and Fungizone were also added in

amounts of 0.05 mL/plate. Fifteen plates were initially inculated with L. higginsi glochidia. A count of the number of glochidia that closed in the media of each plate gave an indication of the viability of the glochidia. Plates were placed in a CO_2 incubator at 100% humidity and 23°C and were examined daily to follow development of juveniles and checked for signs of contamination. Once a week, 0.05 mL of antibiotic/plate was added. Fungal colonies were removed with sterile forceps. When mussels appeared fully transformed, they were transferred to sterile water and observed for valve and foot movement.

Juvenile mussels of the species--L. higginsi, L. ventricosa, and L. radiata siliquoidea--were also obtained by artificial infection of host fish. They were recovered within 2 days after dropping from the fish by siphoning the tank bottom with a polyurethane hose through a 150-μm mesh screen. We were able to obtain approximately 50 L. higginsi, 500 L. ventricosa, and 35 L. radiata siliquoidea juveniles with this method.

Juvenile culture procedures were modified from those reported by Hudson and Isom (1984). When full metamorphosis of juveniles, produced either by in vitro culture or fish infection, was demonstrated, individuals of each species were transferred to Nalgene containers. Containers were filled with a suspension of algae and sediment, and then aerated with air stones. Sediment was collected from Lake Onalaska and was filtered through a 5-μm bag filter. Juveniles were washed daily in a 150-μm mesh screen, a subsample was examined to assess their health and they were returned to the Nalgene containers with fresh algae and sediment.

Results

A total of 15 L. higginsi juveniles were produced by in vitro transformation of glochidia. On day 18 of the culture, juveniles were found in three separate plates with 2, 5, and 8 juveniles, respectively. These individuals demonstrated metamorphosis by opening of valves and movement the foot when placed in water.

Laboratory culture of juveniles of all three species--L. higginsi, L. ventricosa, and L. radiata siliquoidea--has been successful for over a month. Individuals have undergone substantial growth, increasing in width and length by approximately 90 and 40 µm, respectively, in 3-4 weeks. The appearance of algae in the intestinal tract indicates the juveniles are utilizing it as a food source. A variety of other invertebrates have also invaded the culture containers including Vorticella, Rotifers, Nematodes, and most abundantly, Turbellarians. Attempts are made to keep numbers of these organisms low by decanting water from the Nalgene containers to remove the floating and less dense organisms. Flatworms have been most effectively reduced by examining culture contents under the microscope and removing worms with a pipette.

Discussion and Conclusion

Isom and Hudson (1982) reported glochidial transformation rates of 30-80% and juvenile yields after 60 days of culture of 50-80%. Based on their calculations, a single female mussel, starting with 2,000 glochidia could produce 300-1,280 juveniles in a laboratory culture (Hudson and Isom 1985). Our in vitro success rate was much lower than that reported by

Isom and Hudson (1982), 0.31, 0.77, and 2.3% per plate. However, we feel that our percentage rate will continue to increase with further familiarization with the methods. The percent yield of juveniles from our cultures after 30 days are within the expected 50-80% reported by Isom and Hudson.

The ability to rear juvenile mussels in the laboratory is a significant step toward conservation of mussel populations and toward more detailed study of the early life stages of the mussel. We will continue to use juveniles raised in our laboratory for studies on the identification of juveniles, habitat of young mussels, optimum transplant sites, predators, and optimum food types.

HABITAT REQUIREMENTS

I General Distribution: Current/Substrate

Identification of the parameters that delineate the critical habitat of Lampsilis higginsi is difficult, at best, because the species is distributed in a swift current, large river environment that is inherently difficult to sample. Techniques applied in habitat identification of small stream or lake species (e.g. I.D. by sight, hand collections) cannot be used in the mainstem of the upper Mississippi River. Therefore, to date, the habitat of the species has been described only in qualitative terms. The specific quantitative characterization of current, substrate, etc. which is needed for proper delineation of primary and secondary habitat has not occurred. However, this quantified sampling can only be accomplish with the use of divers and is extremely expensive and labor

intensive. Effort was made in our study of the key habitat characteristics of the species to minimize the amount of new quantitative data collection needed.

In FY 1985 and FY 1986, one of the best quantitative data bases for mussels in Pool 10 (Duncan and Thiel 1983) was obtained and computerized. In addition, data from the Army Corps of Engineers and private researchers were solicited to create an quantitative distributional picture of the species in Pool 10. This pool is the site of a large clamming industry and has a high diversity and abundance of mussels. Data from over 52 transects throughout the pool (Fig. 1a) representing 310 quadrats and 300 brail runs were computerized. A selected number of these sites will be examined for sediment characteristics and temporal patterns of current in FY 1987.

In FY 1986, 200 sites along 44 transects (Fig. 1b) were analyzed for complete sediment composition. In addition, mussels from 190 1/4-m^2 quadrats were taken along 14 of these transects. Samples were taken at 50-ft intervals out from the east shore to 400 ft in duplicate. All mussels were identified and measured. L. higginsi collected were returned by diver to their original site. Twenty-seven species were identified from the 1,923 individuals collected. L. higginsi were collected at seven sites (Fig. 1b) with maximum and average density of 2 and 0.04, respectively (Table 3). About 200 additional quadrats will be collected along other transects to further define the relationships of sediment type and mussel community structure to L. higginsi distribution.

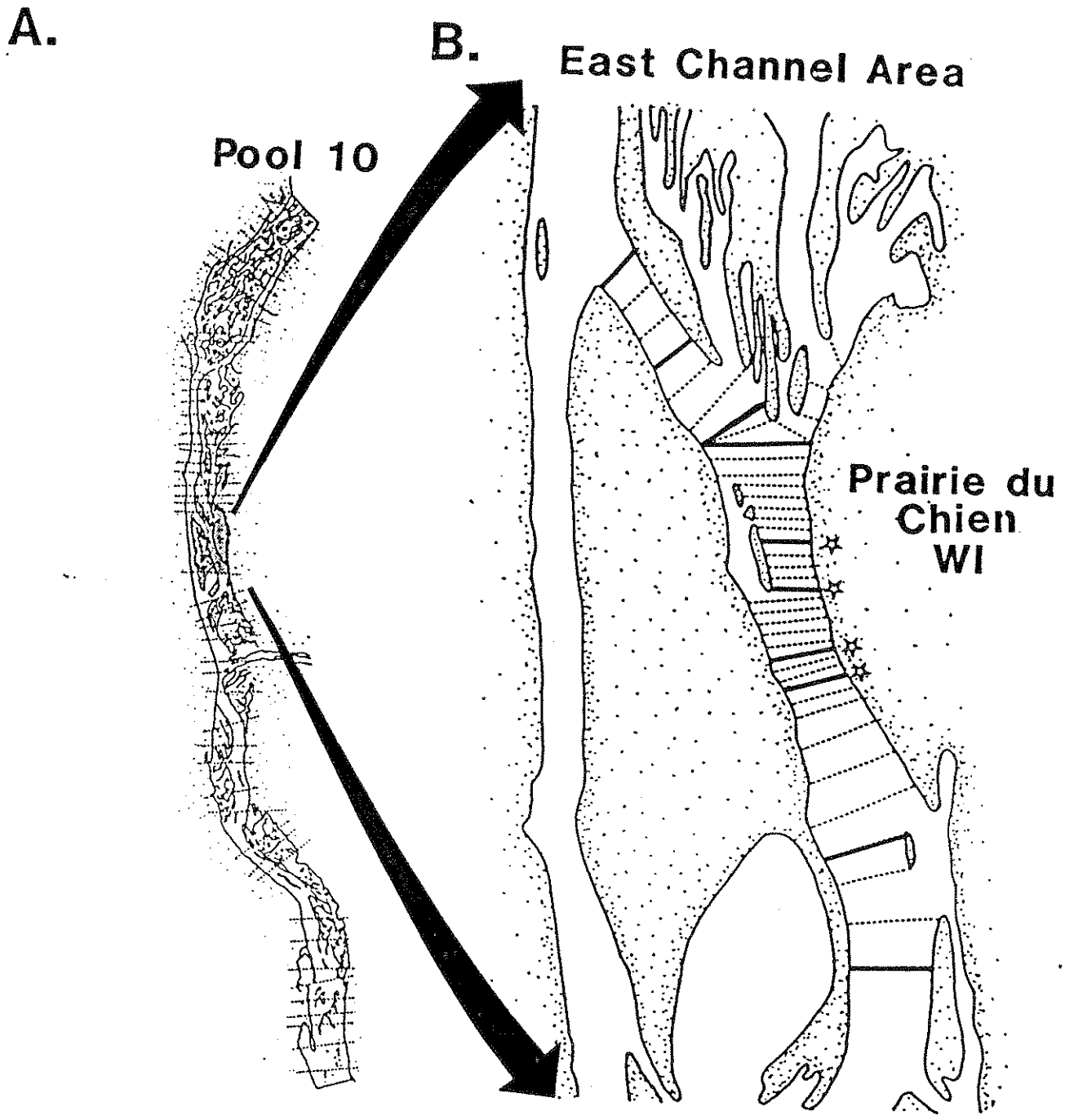


Figure 1. (A) Locations of quantitative mussel collections made by Duncan and Thiel (1983) and entered into the computer for analysis, and (B) transects were sediment analyses were completed (dashed) and quantitative mussel collections were made (solid). Transects were L. higginsi were collected are marked by stars.

Table 3. Relative abundance (#/1/4 m^2) of species of mussels collected from the East Channel, Prairie du Chien, Pool 10, upper Mississippi River (n = 190).

Species	Rank	Maximum	Mean	Standard deviation
Threeridge	1	28	5.13	5.96
Deertoe	2	11	0.91	1.74
Fragile papershell	3	7	0.64	1.27
Washboard	4	7	0.57	1.28
Pink Heelsplitter	5	6	0.35	0.82
Pig toe	6	4	0.32	0.73
Threehorn	7	5	0.31	0.68
Pimpleback	8	5	0.29	0.82
Fawnsfoot	8	5	0.29	0.72
Mapleleaf	9	4	0.26	0.60
Pocketbook	9	3	0.26	0.67
Hickorynut	10	2	0.16	0.42
Black sandshell	11	3	0.13	0.47
Spike	12	2	0.08	0.32
Wartyback	13	1	0.07	0.25
Strange floater	14	2	0.06	0.26
Higgins" Eye	15	2	0.04	0.23
Rockshell	15	1	0.04	0.19
Pink papershell	15	1	0.04	0.19
Lilliput	15	2	0.04	0.23
Monkeyface	16	2	0.03	0.19
White heelsplitter	17	1	0.02	0.14
Butterfly	17	2	0.02	0.18
Paper floater	17	1	0.02	0.12
Giant floater	18	1	0.01	0.10
Yellow sandshell	18	1	0.01	0.10
Mucket	18	1	0.01	0.10

Current direction and velocity significantly affect sediment composition, but can also be critical habitat descriptors in themselves. A survey of current direction and velocity at sites identified by Duncan and Thiel (1983) and our own quadrat sites was designed. However, unusually high and variable river discharge conditions have postponed the start of this project until reasonable river conditions re-establish.

II. Substrate Current Preference of *L. ventricosa* Juveniles

In vitro transformation of glochidia and the rearing of juvenile freshwater mussels in the laboratory represent significant tools to mitigate damage to mussel populations. Juveniles of endangered species can now be grown to a size that will increase their ability to survive in their natural environment (Isom and Hudson 1982; Hudson and Isom 1984). Unfortunately, since juveniles are seldom collected in surveys, very little information is known about the habitat and survivability of this life stage. The goal of this study was to design an apparatus for testing the effect of different current velocities on juvenile mussels transplanted onto various substrate types. Using this study design, we hope to be able to choose the most suitable substrate and current velocity for optimum survival of transplanted *Lampsilis* sp. juveniles.

Material and Methods

L. ventricosa juveniles were obtained from artificially infected largemouth bass. Juveniles were approximately 7 days old and averaged 0.272 µm x 0.292 µm. All mussels used in testing were in good health as demonstrated by active movement of the foot, response to touch and evidence of food in the intestinal tract.

An artificial stream was constructed from a fiberglass raceway with a drain at one end. Water was maintained in a closed system by connecting a recirculating pump between the drain and water inlet. Dislodged mussels were collected in a 150-µm mesh bag that was secured to the inlet hose of the pump. To vary the current in the trough, the volume of the water

circulating through the system was changed. Before testing began, the current velocity at various water levels in the trough was estimated. We chose to use current velocities that were somewhat representative of high and low water flows in the main channel of the upper Mississippi River. Current levels tested were: (1) near-no-flow--0.00269 m/s, (2) low flow--0.08217 m/s, and (3) high flow--0.1180 m/s. The substrate was placed on a 150-µm screen in the bottom of the trough. The thickness of the substrate layer was also varied to include thickness of about 2.0 mm and 5.0 mm. The substrates tested included sand, identified in the field as highly suitable for mussel habitat, and silt, relatively poor mussel habitat.

A known number of juveniles (n = 25 or 50) were pipetted slowly onto a given substrate type a marked distance from the drain (15-16 cm) prior to the start of the experiment. A near-no-flow current velocity (Table 3) was maintained for 10 minutes as a control. The mesh bag was then changed and the current was increased to a low-flow velocity and held for 10 minutes and again to a high-flow velocity for 10 minutes. Each bag was then examined for juveniles and the original substrate sample was checked for juveniles that had not been dislodged.

The success of a given trial was measured by the percent of juveniles recovered after examination of the three bags and the remaining substrate.

Results

Recovery was greatest in trials using the least amount of substrate and 25 juveniles (Table 4), however, we did not recover 100% of the juveniles in any trial. Confidence in our numbers was greatest for samples that had little to no substrate in the mesh bag[a]. Less reliable values are given for samples in which a relatively large amount of substrate had to be examined to locate juveniles[b]. At near-no-flow

Table 4. Current-substrate effects on transplanted L. ventricosa juveniles.

Substrate (thickness)	Original number of juveniles (n)	Percent of juveniles recovered				
		No flow	Low flow	High flow	Recovered from substrate	Total recovery for test
Sand (0.5 cm)	50	0[b]	0[a]	18[b]	18[b]	36%
Sand (0.2 cm)	25	0[a]	12[a]	91[a]	0[b]	92%
Sand (0.2 cm)	25	0[a]	12[a]	18[a]	64[b]	92%
Silt (0.5 cm)	50	0[a]	10[a]	40[b]	24[b]	50%
Silt (0.2 cm)	25	0[a]	8[a]	43[a]	32[b]	80%

[a]High confidence in value.

[b]Low confidence in value.

levels, juveniles were not dislodged from the substrate in any trial. At low flow, 0-14% of the juveniles were dislodged. High flow velocities were sufficient to remove a greater percentage of the remaining juveniles (18-91%). Our results appeared affected by substrate type but were very inconsistent; higher recovery efficiency and more trials are required to compare the effects of substrate type an habitat value for juveniles.

Discussion and Conclusion

The apparatus used in this study will be useful to evaluate current-substrate effects on transplanted juvenile mussels if the thickness of the test substrate is limited, the sample size of juveniles is increased (50) and the number of trials is increased. With large amounts of substrate, it becomes much too difficult to locate the juveniles in the substrate particles. However, when the substrate sample is limited to a narrow strip and is approximately 2 mm thick, recovery is near 90%. The number of juveniles used per trial had to be decreased towards the end of our study due to a limited supply. Larger sample numbers would have been more desirable and given greater confidence in our data. The small artificial stream system and experimental design used do not facilitate the use of a flow meter to directly measure current, so values must be estimated and maintained by control of the volume. An increase in the number of trials at each current velocity will add confidence to our average values.

LITERATURE CITED

Arey, L. B. 1932. A microscopial study of glochidial immunity. Journal of Morphology 53:367-379.

Duncan, R., and P. Thiel. 1983. A survey of the mussel densities in Pool 10 of the upper Mississippi River. Technical Bulletin No. 139. Wisconsin Department of Natural Resources. 14 pp.

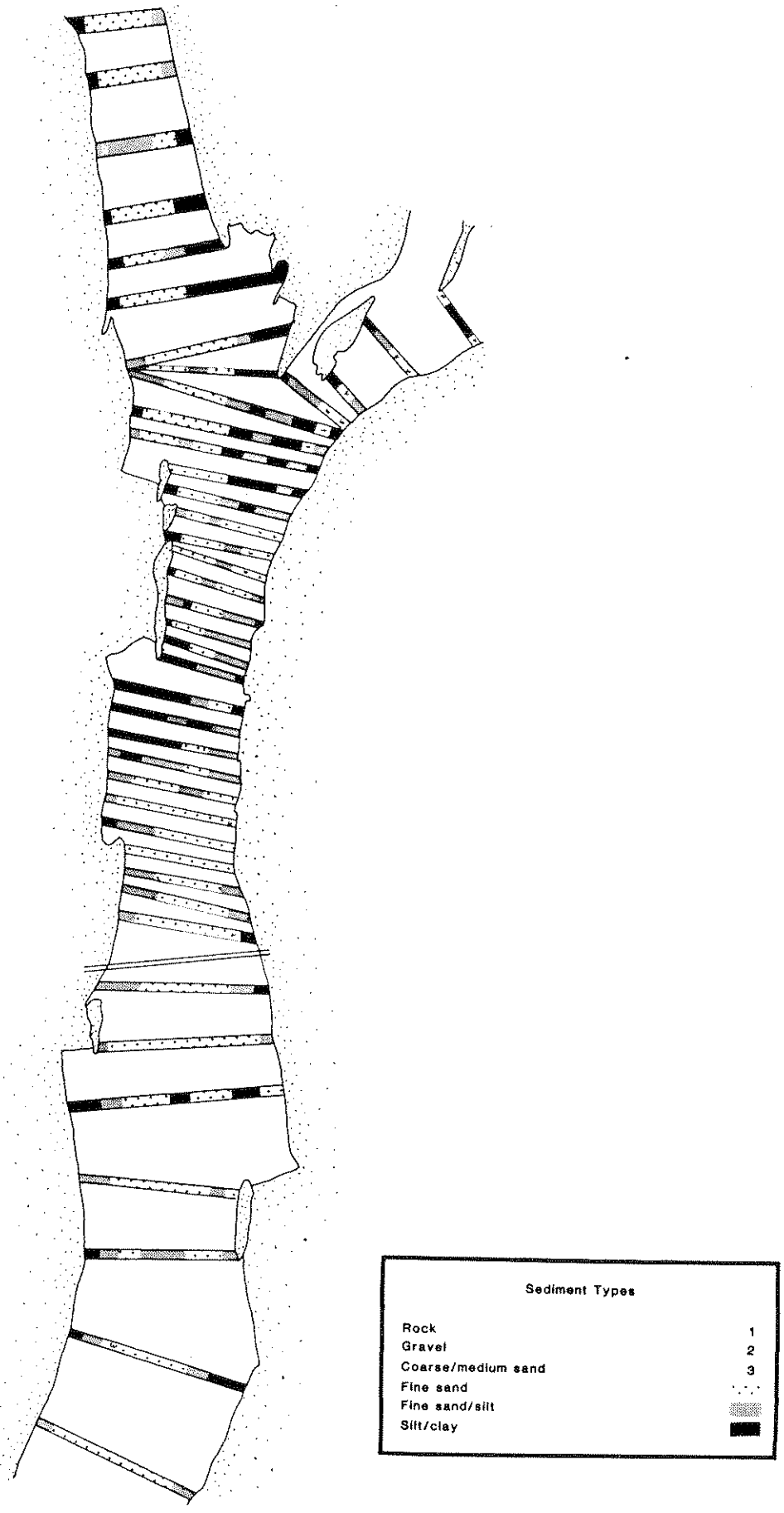
Hudson, R. G., and B. G. Isom. 1984. Rearing juveniles of the freshwater mussels (Unionidae) in a laboratory setting. Nautilus 98:129-135.

Hudson, R. G., and B. G. Isom. 1985. Field transplanting of laboratory transformed and reared juvenile mussels. FAEB 0040C. Tennessee Valley Authority.

Isom, B. G., and R. G. Hudson. 1982. In vitro culture of parasitic freshwater mussel glochidia. Nautilus 96:147-151.

Waller, D. 1985. Life history and habitat requirements of Lampsilis higginsi. Endangered species summary report, FY 1985. Completion report, National Fishery Research Laboratory, La Crosse, Wisconsin. 33 pp.

Waller, D., and L. Holland. 198_. Fish hosts for glochidia of the endangered freshwater mussel Lampsilis higginsi (Pelecypoda: Unionidae). Journal of the North American Benthological Society. (In review).


Sediment Types
Rock 1
Gravel 2
Coarse/medium sand 3
Fine sand
Fine sand/silt
Silt/clay