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Development of Propagation Methods For Unionid Mussels

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Survival of juvenile unionid mussels cultured under several food and water regimes

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INTRODUCTION

Many researchers have discussed the plague of the freshwater mussels, past and present in the United States (Williams et al, 1993, Bates and Dennis, 1978, Nalepa et al, 1991) Yet, problems associated with dam construction, pollution, and general habitat loss persist. We are just now beginning to understand how these impacts are affecting our freshwater mussel species. Our understanding of mussels have advanced a great deal from the time when early researchers thought the glochidia of mussels were actually parasites on the mussel, thus they were named *Glochidium parasiticum* (Ellis, 1929). However, our knowledge of growth and survival has not yet reached a point where we can successfully achieve long term culture of freshwater mussels. Researchers from the early 1900's performed culture experiments in troughs or in ponds and met with reasonable success. These experiments usually involved collecting suitable host fishes, infecting them with glochidia, then returning them to the streams or rivers (Reuling, 1920). Other experiments collected fishes, infected them and then the fishes were held in cages with solid bottoms then suspended in lakes or ponds. Juvenile production and survival could then be monitored annually (Corwin, 1920 and 1921, Howard, 1914) However, moving the culture activities into the laboratory has met with less success, but has still provided invaluable incite into the life requirements of early life stages of freshwater mussels. (Hudson and Isom, 1984, Gatenby, Neves, and Parker, 1996)

MATERIALS AND METHODS

Mussels used in this study were obtained from the Suwannee River, Florida at Fanning Spring, Dixie County (lat, long) (*Elliptio icterina, Lampsilis straminea claibornensis, Lampsilis teres, Utterbackia imbecilis, Villosa vibex, Villosa villosa*). Gravid mussels were collected by grubbing or snorkeling and transported back to the laboratory in coolers wrapped in wet burlap sacks. Glochidia were excised from the marsupia and pipetted directly onto the gill of fish hosts. To infect smaller fish, several thousand glochidia were placed in a container of vigorously aerated water containing fish for 30-45 minutes to allow for infection. Fish were then held in 10 gallon aquarium until juvenile transformation occurred. Juveniles were siphoned from the bottom of aquarium and held in petri dishes at 22° C for 6-10 days before the start of each experiment. Each treatment, either a food source or water type, had three replicates. Each replicate consisted of 25 individuals. All test were preformed at well water temperature of 22° C. Survival was monitored every 7-10 days. Seven different food types were used in this experiment, consisting of a tri-algal mixture, sediment, cow manure, Tetramin, yeast-trout chow, and leaf detritus. Pond water and well water were used in the flow-through system. The tri-algal mix fed consisted of Selenastrum, Neochloris, and Chlorella in equal portions, at concentration of 3.0×10^5. The sediment was collected from the New River, Florida and analyzed for contaminants by Environmental Science and Engineering, Gainesville, Florida by standard EPA methodology (Table 2). This sediment was placed it the bottom of each respective container at a depth of 3 cm.

Cow manure was a commercially available brand and was also placed it the bottom of each container at a depth of 3 cm. Ten grams of Tetramin® flake food was mixed with 100 mL of water and placed in a blender for 10 minutes. This mixture was fed at a rate of 1 mL per day. Yeast-trout chow food source is comprised of yeast and trout food mixed in equal portions. Leaf detritus was collected by siphoning the bottom layer of a *Hyallela azteca* culture tank and obtaining the unused shredded leaf portions. Like the sediment and cow manure, the leaf detritus was placed on the bottom of the aquarium to a depth of 3 cm. There were two water regimes, well water and pond water. Pond water regime had an average algal cell concentration of 1 X 10^4 cells/mL from September to December. Species composing the majority of the pond water varied throughout the season but, were dominated by diatoms (*O. centrales*) and bluegreen algaes (*Microcystis incerta*). Each treatment was conducted in 20 L aquarium constructed as in figure 1. Each replicate container was a 2" diameter by 3" long PVC tube. Four equally spaced 1/4" holes were drilled around the base of each container to allow water to flow through. The water exchange holes as well as the base where covered with 105 um mesh netting. This size mesh allowed for small particles to enter each container from the substrate but was small enough to retain juveniles. All survival data were also tested for normality with the Chi-square test. Survival data then were analyzed with ANOVA, and Tukey's method of multiple comparisons to detect significant differences between treatments.

RESULTS

Elliptio icterina were exposed to pond water with and without a sediment substrate for 120 days. Results are presented in Table 1. Juveniles cultured without sediment survived longer than those with sediment. However, both treatments had a high number of predators commonly found in pond waters, such as Rotifera and protozoa. No significant differences in survival were detected. *Lampsilis claibornensis* juveniles achieved highest survival in sediment substrate, well water, and tri-algal mixture (SWA) over the 120 day period. Significant survival over all other mixtures was achieved during days 20-60 for SWA. SWA also maintained survival longer than any other food source. Algae and well water (AW) also showed significance, but only at 20 days. All other treatments presented no significant survival over the 120 day test period. *Lampsilis teres* experiments showed than none of the food sources provided the necessary requirements to support survival past 60 days and only one food source was significant, the leaf detritus and well water mixture, at 20 days. *Utterbackia imbecillis* results are also presented in Table 1. SWA combination provided for not only highest survival, but also longest survival for this species as well. The mixture of algae and well water without the presence of sediment also showed significant survival over all other food trials. For most other test trials, complete mortality was achieved by 40 or 60 days. *Villosa vibex*, however presents a different picture. Here, the SWA did not maintain juveniles past 60 days and the algae and well water alone did not maintain juveniles past 20 days. However, with algae, well water, and cow manure, survival was high and past 80 days. In fact, the cow manure-well water combination without any algae was the second highest in survival, far superior to the SWA treatment. Finally, *Villosa villosa* was tested with only the SWA treatment and survival was higher than any treatment, regardless of species. These juveniles did in fact survive far beyond the 120 days of this experiment.

DISCUSSION

Freshwater mussel juvenile survival seems to have several time periods of critical development. There is clearly a first wave that occurs between 20 and 40 days as evident by marked decreases in survival between these time periods. Many trials would have greater than 90 percent survival at 20 days only to have no survivers by day 40. Another critical time period in development may occur between 60 and 80 days. Again, a wave of mortality seems to strike as with *Lampsilis claibornensis* in the SWA treatment (Table 1) going from 69 percent survival at 60 days to only 14 percent survival at 80 days. Or as with the *U. imbecillis* in the cow manure, algae, well water treatment. Survival at 60 days was 77 percent and by 80 days survival had decreased to only 9 percent. In between these two time periods, survival appears to be somewhat stable and may provided researchers with windows for other testing.

Differences in responses to food sources varied by species. Such as the high survival of *L. claibornensis*, *U. imbecillis*, and *V. villosa* to the SWA. But, *V. villosa* showed an affinity for cow manure, algae, and well water many times greater than the SWA treatment. This response could be due to different food requirements of these species or filtration efficiencies of individual species.

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Table 1. Percent survival for Juveniles over 120 days for various food sources.

Elliptio icterina

Test Variables	Days of Survival						
	0	20	40	60	80	100	120
Sediment, Pond Water	100	45	25	20	0	-	-
Pond Water Only	100	56	29	17	12	5	0

Lampsilis claibornensis

Test Variables	Days of Survival						
	0	20	40	60	80	100	120
Sediment, Well Water, Algae	100	96*	86*	69*	14	7	7
Algae, Well Water	100	88*	13	0	-	-	-
Sediment, Pond Water	100	5	0	-	-	-	-
Pond Water Only	100	38	5	2	2	2	0
Cow Manure, Well Water	100	30	15	0	-	-	-
Cow Manure, Algae, Well Water	100	2	2	0	-	-	-

Lampsilis teres

Test Variables	Days of Survival						
	0	20	40	60	80	100	120
Sediment, Well Water, Algae	100	72	44	11	0	-	-
Cow Manure, Well Water	100	44	38	0	-	-	-
Leaf Detritus, Well Water	100	91*	64	0	-	-	-

Utterbackia imbecilis

Test Variables	Days of Survival						
	0	20	40	60	80	100	120
Sediment, Well Water, Algae	100	86	62*	62*	56*	18	0
Algae, Well Water	100	93	52*	13	7	0	-
Sediment, Pond Water	100	87	11	8	0	-	-
Pond Water	100	61	16	0	-	-	-
Cow Manure, Well Water	100	96	0	-	-	-	-
Leaf Detritus	100	91	0	-	-	-	-

Villosa vibex

Test Variables	Days of Survival						
	0	20	40	60	80	100	120
Sediment, Well Water, Algae	100	96	17	0	-	-	-
Algae, Well Water	100	99	0	-	-	-	-
Sediment, Well Water	100	100	51	11	0	-	-
Sediment, Pond Water	100	83	15	0	-	-	-
Pond Water Only	100	68	16	4	0	-	-
Cow Manure, Well Water	100	89	83*	53*	0	-	-
Cow Manure, Algae, Well Water	100	100	96*	77*	9	0	-

Table 1 cont.

Villosa villosa

Test Variables				**Days of Survival**			
	0	20	40	60	80	100	120
Sediment, Algae, Well Water	100	93	86	79	69	64	55

Tri-algal mixture in well water with sediment

Test Species				**Days of Survival**			
	0	20	40	60	80	100	120
L. claibornensis	100	96	86*	69*	14	7	7
L. teres	100	72	44	11	0	-	-
U. imbecilis	100	86	62	62*	56*	18	0
V. vibex	100	96	17	0	-	-	-
V. villosa	100	93	86*	79*	69*	64*	55*

* indicates significant difference (p=0.05)

Table 2. Chemical constituents of silt used in growth tests.

Analyte	Concentration (mg/Kg dry)
Aluminum	999
Antimony	<0.381
Arsenic	<0.318
Barium	14
Beryllium	<0.633
Cadmium	<0.633
Calcium	3190
Chromium	1.43
Cobalt	<2.53
Copper	0.870
Iron	413
Lead	2.24
Magnesium	104
Manganese	11.1
Mercury	<0.026
Nickel	<2.53
Potassium	<127
Selenium	<0.318
Silver	<0.633
Sodium	297
Thallium	<0.313
Vanadium	1.45
Zinc	<6.33

Analyte	Concentration (ug/Kg dry)
Aldrin	<0.859
BHC, A	<0.859
BHC, B	<0.859
BHC, D	<0.859
BHC, G (Lindane)	<0.859
Tech. Chlordane	<4.30
DDD, PP'	<0.859
DDE, PP'	<0.859
DDT, PP'	<0.859
Dieldrin	<0.859
Endosulfan, A	<0.859
Endosulfan, B	<0.859
Endosulfan sulfate	<0.859
Endrin	<0.859
Endrin Aldehyde	<0.859

Heptachlor	<0.859
Heptachlor Epoxide	<0.859
Methoxychlor	<0.859
Toxaphene	<85.9
PCB-1016	<17.2
PCB-1221	<17.2
PCB-1232	<17.2
PCB-1242	<17.2
PCB-1248	<17.2
PCB-1254	<17.2
PCB-1260	<17.2
Acenaphthene	273
Acenaphthylene	<121
Anthracene	500
Benzo (A) Anthracene	141
Benzo (A) Pyrene	77.7
Benzo (B) Fluoranthene	161
Benzo (GHI) Perylene	114
Benzo (K) Fluoranthene	67.8
Chrysene	130
Dibenz' (A,H) Anth'Cene	16.0
Fluoranthene	621
Fluorene	113
Indeno (1,2,3-CD) PYRN	37.3
Naphthalene	336
Phenanthrene	127
Pyrene	682
2,4-D	<2.58
2.4-DB	<2.58
2,4,5,-T	<2.58
2,4,5-TP/Silvex	<2.58
Dalapon	<2.58
Dicamba (Banvel)	<2.58
Dichloroprop	<2.58
Dinoseb	<2.58
MCPA	<51.5
MCPP	<51.5
Total Organic Carbon (%)	0.562