Waller 1988

First draft raw data!!

Culture of Freshwater Unionid Mussels

Work Unit 966.02

Annual Report

FY 1988

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November 1988

INTRODUCTION

In FY 1987 an intermittent flow-through system was tested for its suitability in a juvenile culture system. Three different food sources (phytoplankton, alfalfa/trout chow blend, and fish culture water) were tested using three Lampsilis mussel species in this system. Although initial growth occurred under all conditions, survival was near 0% in the non-living food sources by day 14, and in the phytoplankton by day 28.

Study Objectives

I. Evaluate different culture systems, including static and no-flow situations, for their suitability in an operational culture system and for their effect on the growth and survival of juvenile mussels. Evaluate the effect of silt on the growth and survival of juvenile mussels. Develop histological indices for evaluation of the condition of juveniles in various culture conditions.

II. Test outdoor culture methods for use on an operational basis.

III. Examine the role of external versus internal food sources in the first days after release from the fish.

I. EVALUATION OF CULTURE SYSTEMS AND THE EFFECT OF SILT ON JUVENILE GROWTH AND SURVIVAL

Introduction

The habitat and food requirements of the juvenile freshwater mussel, including those of the Lampsilis genus, have not been well documented. Juveniles are rarely collected in surveys, consequently the conditions of their natural habitat remain largely unknown. Adults of the endangered mussel Lampsilis higginsi and its congener, L. ventricosa, are found in main channel to main channel border habitats in average currents of 0.6-0.7 ft/sec and fine- to medium-size sediment (Holland-Bartels and Waller 1987). However, it is not known whether the juveniles also require the same sediment type and flow regime or some completely different set of environmental conditions.

Culture systems for juvenile mussels have been designed to reflect the adult habitat of the mussel or have been modified from proven marine bivalve methods. Hudson and Isom (1984) tested a mixture of flow types and chambers for their use in juvenile mussel culture including static, ascending flow, descending flow, and riffle flow systems. They were able to rear juveniles of Dysonmia triquetra and Anodonta inbecilis up to 5 mm for a maximum of 74 days in a static system. In the static system, juveniles were placed in Nalgene containers and received a mixed phytoplankton food source, silt suspension, and daily water change. The flowing systems failed to maintain living juveniles for more than a couple of weeks with the exception of the ascending flow chamber. Hudson and Isom attributed the varied success to the larger volume of flow (>1 L/min) in the ascending flow chambers; the other designs had lower flow volumes and were not tested at higher flow rates. No

provisions were made for the addition of silt to any of the flowing systems such as that made into the static containers.

The role of silt in juvenile growth has not been clearly defined. Churchill and Lewis (1924) reported finding silt and sand in the stomach and feces of juvenile and adult mussels and Coker et al. (1921) suggested that the supply of minerals was the limiting factor in growth of the shell. Hudson and Isom (1984) found juveniles in a static culture system did not survive past 2 to 3 weeks without silt additions. Growth enhancement by silt addition has also been reported in the marine species Mytilus edulis and Spisula subruncata. Porter (1972) reported juveniles of grow four times faster in troughs supplied with a sand substrate. The question of whether inorganic materials are necessary for shell formation, more efficient processing of organic materials, or simply for burrowing has not been examined.

There is no histological information on the normal development of healthy juveniles immediately after they drop from the host. Although measurements of survival and growth do provide some information on the effectiveness of a culture system, histological data would show whether a food source was being assimilated by the juveniles or merely sustaining them at a baseline survival level. It would also indicate critical periods of growth when juveniles may be switching from an internal to an external food source or increasing their metabolic requirements. Histological grading systems for assessing the effects of starvation on fish larvae are well developed (O'Connell 1976, Theilacker 1978) and could be similarly applied to juvenile mussels.

In FY 1987, we tested an intermittent flow-through system for its use in an operational culture system. The system did not produce significantly greater results in growth and survival of juveniles of three Lampsilis species

over that of the static system modified from Hudson and Isom (1984). In FY 1988, we tested a continuous flow-through system similar to the ascending and descending flow chambers tested by Hudson and Isom (1984), but with higher flow velocities and with the addition of silt into the system. Lampsilis ventriocosa (pocketbook) juveniles from one flow trial were also preserved for histological examination at five different sampling periods. Based on the results of our flow-through system tests, we compared the effect of a suspension of silt versus a silt layer on growth and survival of juveniles. We used the two Lampsilis species, L. ventricosa, the pocketbook, and L. radiata siliquoidea, the fat mucket, to test our culture systems and for histological examination. These species are relatively easy to produce on host fish, but they are slow growing and difficult to rear in a laboratory setting.

System Development

Our initial system was designed for ascending flow through the culture chambers since Hudson and Isom (1984) reported that it produced the best results in their flow-through tests. The chambers were constructed from 6" x 10" PVC pipe. Nylon netting (150 μm) was secured to the bottom of the pipe using PVC glue and a 6" hose clamp. When used as an ascending flow chamber, an additional 4" piece of pipe, with four 1/4" holes drilled in the bottom periphery, was clamped below the netting. The chambers sat on the bottom of the tanks when using ascending flow and were suspended from wire when using descending flow. A hose connector at the side of the pipe, about 2" from the top, and tygon tubing connected the chamber to a submersible Little Giant two-way pump. The pumps were kept in a flow-through water bath to keep the

recirculating water at a constant temperature. A T-bar, with holes drilled in each arm and the center, was connected to the top of the chamber to distribute the flow of water more evenly. The velocity of flow was controlled by connecting the pumps to a rheostat.

The chambers were placed into one of two 29 gal circular tanks. The water in each tank was recirculated between all the chambers in the tank. ~~In the descending flow tests, water was drawn from the bottom of the tank and~~ pumped into the top of each chamber. Water was changed in the tanks every other day.

Following preliminary tests using an ascending flow, the system was modified for descending flow because juveniles were pulled out of the chambers in the former design even at very low flow velocities. Descending flows greater than 3 L/min were eliminated when it became apparent that the nylon netting would clog and the chambers would overflow at velocities above this value. Flow velocities through each chamber were slightly different. Average flow velocities (Table 9) were ~~measured~~ calculated by measuring the output of water to each chamber.

Suspended silt tests were run in conjunction with the flow tests. Turbidity and suspended solid measurements were taken before tests began in order to establish a decay curve for the amount of silt in suspension over time [illegible]. Silt [illegible] was added to the tanks every other day. On alternate days, the water in the tanks was changed, resuspending the silt. We did not attempt to keep a constant suspension of silt in the chambers but instead tried to produce the same level of suspension in each chamber regardless of flow, and measure the average level of suspended silt during a day. A second complication of the silt additions was the clumping effect of

silt to other silt particles and to algae causing retention of silt on the screens. The quantity of silt retained on the screens of each chamber was not measured.

The flow/silt system was tested in two separate trials using juveniles of the pocketbook, L. ventricosa. Host fish, walleye (Stizostedion vitreum vitreum) and largemouth bass (Micropterus salmoides) were infected with glochidia from 2-3 different females for each trial. After about 3 1/2 weeks at 20-22°C, juveniles ~~holding the fish mussels~~ were collected from the tanks of the infected fish. One to four days after collection the mussels were placed into the culture system. There were four treatments with two chambers in each treatment: (1) no flow/no silt, (2) flow/no silt, (3) no flow/silt, and (4) flow/silt. The four no silt chambers were placed into the tank receiving only algae while the four silt chambers were placed into the tank receiving 80 mg/L of silt every other day. No flow chambers were not connected to the pumps but were of the same design as the flow chambers.

Measurements of length and height were taken on a subsample (about 10%) of the juveniles at the start of the test and at each subsequent sampling date. In trial 1, juveniles were examined approximately every 2 weeks.

In trial 2 ~~of the flow/silt system,~~ subsamples (about 25 from each treatment) ~~of L. ventricosa~~ juveniles were processed for histological examination at 0, 5, 10, 15, and 20 days of culture. Juveniles were fixed in 10% neutral buffered formalin and embedded in JB4. Thick sections were cut, mounted on glass slides, and stained.

Juveniles were recovered from the chambers by inverting the chamber over a large beaker and rinsing the screen with a water bottle. The rinse was examined under a dissecting microscope. Measurements of length and height.

were made under a dissecting microscope using a stage micrometer. The percent survival was calculated as follows:

$$\frac{\text{Number of juveniles alive}}{\text{Number of juveniles recovered}} \times 100$$

The percent recovery was calculated as follows:

$$\frac{\text{Number of juveniles alive at previous sampling date}}{\text{Number of dead and alive juveniles found on present sampling date}} \times 100$$

The silt test in a static system was tested in two trials using fat mucket juveniles in the first and pocketbook juveniles in the second. Juveniles were produced via host fish using yellow perch for the fat mucket glochidia and were collected in the same manner as in the flow/silt test. Two treatments were tested with three replicates of each treatment: no silt and silt substrate. The no silt chambers received only algae water and the silt chambers received 9 g of silt in a layer. The silt was dried and placed in a graded series of seives for shaking. Only silt particles <75 μm were used so it would not interfere with recovery of juveniles. The silt was only changed on sampling days.

Juveniles were examined about every 2 weeks. They were recovered from the chambers by rinsing the contents through a 150-μm mesh screen and rinsing the material retained on the screen into a beaker.

Methods for measuring and calculating survival and recovery percentages are the same as those in the flow/silt study.

Physical parameters, including algal cell counts, temperature, pH, dissolved oxygen, and conductivity, were taken in each tank or chamber. The algal species present in the phytoplankton cultures varied in each trial; dominant species are indicated for each (Table 9).

Results

Evaluation of flow

Survival of pocketbook juveniles was greatest in the no-flow chambers in both trials (Tables 1 and 3). In trial 1, survival was greater on all samples dates in the no-flow chambers in comparison to the flow chambers. Live juveniles were found in all of the no-flow chambers at 61 and 62 days and in one chamber at 77 days. No juveniles were found surviving in the flow chambers after 38 days (Table 1). Differences in survival were not as pronounced between flow and no-flow chambers in trial 2, however, there was slightly greater survival in the no-flow chambers after 10 days (Table 3). Overall survival of juveniles in trial 2 was much poorer than that in trial 1, possibly due to a shift in the density and composition of the food source. Only three juveniles had survived in the no flow and none in the flow-through chambers on 37 days in trial 2.

Growth of the juveniles was also affected by flow, with greater mean lengths and heights occurring in no-flow chambers at all sample periods in both trials (Tables 2 and 4). The mean lengths and heights were similar between trials on comparable sample dates. On sample dates 11-13 in trial 1, the mean lengths in the flow chambers were 298 and 302 μm compared to means lengths on day 10 in trial 2 of 293 and 298 μm. The no-flow mean values were slightly higher in trial 1 compared to trial 2 which may have been an indication of the subsequent poor survival in the latter.

In trial 1, differences in chamber means within a treatment were relatively small (Appendix A); the differences in differences in flow velocities between chambers within a treatment in the silt and no silt tanks

did not seem to cause differences in growth between chambers (Table 9 and Appendix A). In trial 2, the within treatment differences were more pronounced (Appendix B). Means of length and height were larger in one no flow/ no silt chamber at day 20. Since this difference is not apparent until day 20, it may be a reflection of the increasing variation in size as individuals get larger. In the flow/no silt chambers, the mean lengths and heights were consistently larger on every sample date in one chamber, although the flow velocities in these chambers were similar (Table 9 and Appendix B). The same trend occurred in the flow/silt chambers. The flow velocity differences were much greater in this case, 2.83 L/min vs 1.04 L/min, with greater growth occurring in the chamber with 2.83 L/min flow.

A comparison of individual chamber means and treatment means did not change the ranking of the treatments. The largest means for length and height still occurred in the no-flow chambers with one exception; in trial 2, on day 20, the mean length and height in the slower no-flow/no-silt chamber were 320 μm (S.D. 18.6) and 302 μm (S.D. 14.2) compared to 338 μm (S.D. 26.6) and 322 μm (S.D. 21.5).

Histological Examination

Silt

The silt suspensions used in the flow-through chambers did not appear to have a significantly beneficial effect on the growth and survival of the juveniles. In the flow tests, the silt chambers had greater mean heights only at days 61-62 in trial 1. In trial 2, larger mean values in one no-flow/no-silt chamber actually indicated growth was better in no-silt conditions.

The layer of silt used in the static tests did have a more significant effect on survival of the juveniles. In static trial 1 using fat muckets, survival was greater in the silt containers on all sample days in comparison to the no-silt containers (Table 5). After 27 days, there were none left in the no-silt containers. One silt chamber retained live juveniles after day 39. In static trial 2 using pocketbook, one silt chamber was lost after day 16 due to a technical error. There was much more variation in survival within a treatment (Table 7). On day 16, the silt chambers had greater survival; only one no-silt chamber had enough surviving juveniles to continue the test. However, on 29 day, the silt chambers had survivals of only 6.7% and 95.2% while the no silt chamber had 75.9%. Only one silt chamber was left by day 43 but survival was very high in it. On day 60, juveniles in both chambers were dead; however, this may have been due to the phytoplankton or water since 2-week old juveniles in another test experienced 100% mortality at this same time.

The two static trials gave somewhat different results on growth which may indicate species differences. In trial 1, there was similar mean lengths and heights in the silt and no silt chambers at sample days 13 and 27 (Table 6). There was no growth data for any no silt treatment beyond this day. The silt chamber which showed the greatest early growth suffered the greatest early mortality, whereas, the chamber which showed the slowest early growth produced the longest-lived individuals. On day 68, the dead individuals were measured and the mean length and height were similar to that of the day 53 means.

The overall treatment means and chamber means indicated greater growth in silt at all sample periods in trial 2 (Table 8). Although juveniles continued to survive in only one chamber in each treatment after 29 days,

those in the silt chamber showed significantly greater growth (more than 100 μm greater in mean length) than those in the no silt chambers. On day 60, only dead juveniles were measured, and mean length and height in the no-silt chamber indicated very little to no growth had occurred since day 43. In the silt chamber, mean length and height were greater indicating growth continued to occur.

Discussion

Flow does not appear to be necessary for culture of juvenile mussels in a laboratory system. Higher survival and a greater rate of growth were seen in the no-flow chambers. Hudson and Isom (1984) thought flows below 1 L/min did not provide enough food to the juveniles in their ascending and riffle flow systems and led to high mortality. However, we used flow velocities greater than 1 L/min in our ascending flow chambers and still experienced relatively high mortality. The flow may cause turbulence which disrupted the feeding of the juveniles. Differences in flow velocities within a treatment did not appear to be the cause of growth differences; growth differences occurred in the no flow chambers and between chambers that had small differences in flow velocities.

Suspended silt did not appear to benefit juveniles, with the exception of juveniles at day 60 in trial 1. In fact, silt in a suspension may interfere with feeding. Bricelj et al. (1984) found that Merceneria mercenaria showed no significant growth differences in suspended sediment loads of 0-25 mg/L but growth was reduced in 44 mg/L. In contrast, a layer of silt appeared to benefit growth of juveniles significantly. Perhaps the

silt layer is necessary for burrowing or as a medium for bacterial growth. The role of silt is still not understood.

The results of this study indicate Lampsilis juveniles may survive and grow in a static system provided with silt and a phytoplankton food source for about 60 days. A narrower range of culture conditions may have to be defined to have successful survival beyond this period. Perhaps the composition and density of the phytoplankton food source or the treatment of the silt substrate will need to be more specific.

Table 1. Flow/Silt test I--Comparison of survival of pocketbook juveniles in flow (+) or no flow (-) conditions in the presence (+) or absence (-) of suspended silt. Percents are given for the two chambers in each treatment.

Days in culture	Percent survival (percent recovery)			
	-Flow/-silt	-Flow/+silt	+Flow/-silt	+Flow/+silt
11-13	96.0, 97.5 (86.5, 79.0)	96.0, 95.3 (88.3, 85.0)	78.1, 67.9 (77.5, 78.0)	84.3, 81.2 (63.5, 85.0)
24-25	83.3, 84.4 (86.9, 89.6)	82.0, 96.0 (97.0, 66.7)	12.6, 11.1 (85.1, 34.0)	24.3, 42.4 (65.4, 47.8)
36-38	83.6, 90.8 (96.7, 100.0)	82.6, 90.0 (87.1, 76.9)	11.1 (69.2)	12.5, 41.7 (94.1, 85.7)
46-47	73.4, 100.0 (96.9, 98.3)	69.9, 87.5 (87.4, 88.9)	- -	- -
61-62	32.8, 38.9 (92.8, 93.1)	12.0, 16.0 (86.2, 89.3)	- -	- -
77	0.0 (95.2)	10.0 (90.0)	- -	- -

Table 2. Mean lengths (L) and heights (H) of pocketbook juveniles in flow (+) and no flow (-) conditions in the presence (+) or absence (-) of suspended silt (Test 1).

Days in culture	Mean length and height (std. dev.) (μm)							
	-Flow/-silt		-Flow/+silt		+Flow/-silt		+Flow/+silt	
	L	H	L	H	L	H	L	H
0	228 (18.6)	256 (9.4)	230 (17.1)	254 (8.0)	221 (11.8)	254 (5.5)	221 (11.8)	254 (5.5)
11-13	347 (20.1)	318 (10.3)	334 (18.9)	310 (12.5)	298 (27.1)	293 (21.8)	302 (19.3)	298 (14.9)
24-25	361 (26.4)	333 (22.1)	356 (27.2)	319 (15.6)	317 (29.6)	313 (22.8)	307 (25.8)	302 (19.9)
35-38	371 (35.2)	339 (24.3)	356 (31.2)	332 (25.1)	324 --	360 --	307 (18.6)	309 (16.2)
46-47	396 (50.2)	358 (32.8)	395 (36.5)	358 (19.3)	-- --	-- --	-- --	-- --
61-62	408 (47.0)	378 (37.9)	389 (21.4)	347 (12.7)	-- --	-- --	-- --	-- --

Table 3. Flow/Silt test 2--Comparisons of the survival of pocketbook juveniles in-flow (+) and no-flow (-) conditions and in the presence (+) and absence (-) of suspended silt. Percents are given for the two chambers in each treatment.

Days in culture	Percent survival (percent recovery)			
	-Flow/-silt	-Flow/+silt	+Flow/-silt	+Flow/+silt
5	91.4, 97.9 (98.5, 95.3)	95.2, 96.6 (93.5, 96.7)	85.5, 91.6 (87.3, 89.0)	90.4, 95.8 (76.4, 94.7)
10	97.3, 98.1 (96.6, 91.8)	96.6, 99.2 (98.9, 95.1)	78.7, 85.5 (87.6, 86.2)	82.6, 90.9 (98.7, 80.9)
15	96.8, 99.1 (88.1, 94.9)	95.0, 98.1 (92.8, 95.7)	72.2, 81.6 (63.6, 92.0)	83.0, 91.0 (41.6, 74.1)
20	82.0, 89.6 (91.0, 93.8)	93.8, 95.1 (90.8, 94.8)	65.7, 71.4 (69.8, 94.6)	78.5, 89.3 (77.5, 96.6)
37	0.0, 0.7 (98.8, 79.2)	0.0, 1.8 (97.3, 86.0)	0.0 (75.4)	0.0 (55.7)

Table 4. Mean lengths (L) and heights (H) of pocketbook juveniles in flow (+) and no-flow (-) conditions and in the presence (+) and absence (-) of suspended silt (Test 2).

Days in culture	Mean length and height (std. dev.) (μm)							
	-Flow/-silt		-Flow/+silt		+Flow/-silt		+Flow/+silt	
	L	H	L	H	L	H	L	H
0	262 (19.5)	267 (12.8)	262 (19.5)	267 (12.8)	262 (19.5)	267 (12.8)	262 (19.5)	267 (12.8)
5	308 (21.)	301 (19.5)	305 (23.4)	295 (18.1)	293 (27.9)	291 (21.5)	289 (24.4)	284 (15.4)
10	329 (26.7)	316 (18.0)	315 (18.9)	308 (12.9)	293 (26.0)	289 (18.9)	298 (17.0)	286 (12.9)
15	338 (22.3)	316 (19.8)	323 (20.6)	307 (14.9)	308 (26.7)	298 (23.0)	308 (24.0)	297 (16.0)
20	338 (25.2)	311 (17.6)	333 (25.1)	305 (13.7)	315 (35.3)	307 (23.7)	292 (27.1)	285 (16.8)

Table 5. Silt test 1--Mean percent of survival of ~~fat~~ *L. ornata* mucket juveniles in the presence (+) and absence (-) of a silt substrate.

	- Silt		+ Silt	
Days in culture	% survival (std. dev.)	% recovery	% survival (std. dev.)	% recovery
13	65.2 (10.4)	87.5	92.3 (1.2)	93.0
27	2.7 (2.4)	85.5	43.8 (22.5)	91.2
39	-- --	--	22.3 (31.2)	93.4
53	-- --	--	66.7 --	87.9
68	-- --	--	0.0 --	82.4

Table 6. Mean lengths and heights of fat mucket juveniles in the (+) presence and absence (-) of a silt substrate (Test 1).

Days in culture	- Silt (μm)		+ Silt (μm)	
	Length (std.)	Height (std. dev.)	Length (std.)	Height (std. dev.)
0	249 (20.9)	273 (14.4)	283 (34.8)	290 (24.0)
13	367 (34.0)	340 (29.0)	371 (47.6)	344 (34.0)
27	428 (30.7)	369 (10.4)	411 (40.4)	365 (32.4)
39	-- --	-- --	445 (91.1)	404 (64.9)
54	-- --	-- --	632 (161.5)	513 (146.3)
68	-- --	-- --	558[a] (113.0)	481[a] (42.5)

[a]Dead juveniles.

Table 7. Silt test 2--Mean percent survival of pocketbook juveniles in the presence (+) and absence (-) of a silt substrate.

Days in culture	- Silt		+ Silt	
	% survival (std. dev.)	% recovery	% survival (std. dev.)	% recovery
16	30.4 (37.6)	76.2	84.0 (15.6)	88.8
29	75.9 *	93.9	6.7, 95.2	85.0
43	57.1	59.8	91.9	87.2
60	0.0	--	0.0	--

Table 8. Mean lengths and heights of pocketbook juveniles in the presence (+) and absence (-) of silt substrate (Test 2).

Days in culture	- Silt		+ Silt	
	Length	Height	Length	Height
0	238 (18.5)	254 (17.0)	238 (18.5)	254 (17.0)
16	346 (26.6)	326 (22.5)	411 (55.2)	361 (33.3)
29	401 (56.3)	351 (37.2)	506 (105.6)	425 (74.0)
43	403 (42.6)	369 (34.2)	626 (87.3)	524 (60.3)
60	432 -- (95.6)	394 -- (64.1)	749 (136.3)	556 (52.3)

Table 9. Average temperature, algal concentration, D.O., conductivity, and turbidity of laboratory culture systems.

Trial	Chamber	Temperature °C	D.O. mg/L	pH units	Conduct. us/cm	Algae conc. cell/mL x 10^5	Dominant algae sp.	Flow velocity L/min
Flow 1	no silt	19.0	9.2	7.7	410	2.4-8.6	Scenedesmus	1.52
							Selenastrum	0.78
							Closterium	
							Anabaena	
							Ulothrix	
							Pediastrum	
	silt	18.8	8.8	7.6	408	2.5-7.5	Scenedesmus	1.11
							Selenastrum	1.45
							Closterium	
							Anabaena	
							Ulothrix	
							Pediastrum	
Flow 2	no silt	21.2	8.9	7.5	361	0.18-2.4	Scenedesmus	1.35
							Selenastrum	1.27
							Ulothrix	
	silt	20.9	8.5	7.4	361	1.8-2.5	Scenedesmus	1.04
							Selenastrum	2.83
							Ulothrix	
Silt 1	no silt	20.4	8.2	8.2	322	2.9-4.2	Ulothrix	--
Fat mucket	silt	20.9	8.1	7.8	112	1.7-4.2	Ulothrix	--
Silt 2	no silt	21.9	8.0	7.3	--	1.9-3.8	Ulothrix	--
Pocketbook	silt	21.9	8.3	7.2	208	1.9-3.8	Ulothrix	--

III. OUTDOOR CULTURE

Introduction

The culture of juvenile freshwater mussels on a large scale comparable to that of today's modern fish hatcheries and marine bivalve culture facilities, is the goal of operational culture efforts. An outdoor culture phase would be less labor intensive, require less laboratory space, and reduce the need for simultaneous phytoplankton culture in the laboratory. Hudson and Isom (1984) did not attempt to place any of their laboratory reared juveniles in the field to monitor their survival. In fact, since the early 1900's few investigators have attempted outdoor culture of juvenile mussels. Coker et al. (1921) describes various systems considered by researchers at the Fairport Biological Station including (1) floating crates, (2) bottom crates, (3) concrete and earthen ponds, (4) troughs, and (5) aquaria. Infected fish were placed directly into the chambers and the juveniles were collected at later dates. The greatest rate of growth and survival was reported for Lampsilis radiata siliquoidea (fat mucket) in floating crates suspended in the river and in troughs with a fine sand substrate, perhaps, because it is a lake mussel and may be tolerant of more variable environmental conditions. Thicker shelled, more riverine-type species did not survive. The success rate of these cultures was not determined since the investigators did not report the number of juveniles initially placed in a chamber. In addition, the concentration and composition of the food sources were not reported, and other physical parameters, such as current and water temperature, were not measured.

In this study, we tested several different chamber designs for holding large numbers of juveniles, ease of recovery of juveniles, and ease of placement in the outdoor system. The chambers were tested in two different

pond types. We chose to use ponds rather than raceways because we wanted an elevated temperature and a phytoplankton bloom, both of which would be difficult in a well water supplied raceway.

Methods

Our efforts at outdoor culture of juveniles were limited to the use of earthen and cement bottom ponds. The earthen pond was a 1/10 acre and had a dirt and gravel bottom with a moderate amount of vegetation. It was filled with well water (14-15°C) and 200 mL of liquid fertilizer were added (10-34-0, Fish Cycle, Boat Cycle Manufacturing Company) on May 11, 2 days before the benthic chamber was placed in it. The water flow was reduced after filling to allow the water temperature to increase. An algal bloom was evident in the pond within 3 days after fertilization followed by a zooplankton bloom and algal crash. Small common carp (12-1" and 14-3") and 200 mL of liquid fertilizer were then added to the pond on June 6 to smooth out the cyclical bloom and crash of the pond and provide a more constant supply of plankton for the mussels. On June 16, the pond was again fertilized with 3 lbs of 10-50-0.

The cement ponds were 0.01 acre and had been fertilized randomly with 50 mL of liquid fertilizer (10-34-0) and 1 lb of dehydrated alfalfa at three different times throughout the summer. As a result, they already contained an algal bloom when the chambers were placed in them. The ponds were also stocked with goldfish which were fed 2-3 times weekly floating trout feed (30% protein). The chambers were rotated between the ponds as the algal populations cycled, in an attempt to provide mussels with a high concentration of phytoplankton. The predominant algal species in the ponds was Ulothrix.

Several different chambers were used in the ponds for mussel rearing; one was designed to sit on the bottom on the earthen pond so that the mussels could be in contact with the substrate, and another was suspended above the substrate. The stationary chamber consisted of a 1 x 1/2 x 1/2 m bag of 150 μm nylon mesh. The corner and bottom seams were reinforced with canvas. The bag was secured to a PVC pipe frame of the same dimensions using grommets and nylon rope. The entire chamber was anchored to the bottom. The suspended chambers were the same as those used in the laboratory flow tests (Section I.). They were suspended from an angle iron in the middle of the pond.

Suspended chambers were tested in the cement ponds. The tops of the chambers were covered with nylon mesh in these tests to prevent entrance of debris, outside organisms, and provide some shading.

~~The group of~~ juveniles in the earthen pond ~~was~~ were placed immediately after dropping from the fish, averaging 225 μm x 270 μm. ~~The group~~ Juveniles were placed in the cement ponds ~~was placed~~ after a short laboratory culture phase of about 3 weeks and averaged 303 μm x 295 μm. ~~About 600~~ Approximately 100 juveniles were placed in each chamber. In the earthen chamber and 245 in the suspended chamber.

Results

Earthen pond

Approximately 1,100 juveniles of ~~Lampsilis~~ ventricosa were placed in the pond in the mesh cage on May 13. The plankton population in the pond was low at this time since the pond had been fertilized on May 11. The water temperature was also relatively low (15.1°C) since the pond had only recently been filled. Water temperatures and the phytoplankton density increased steadily (Table 11) during the course of the next 2 weeks. ~~However,~~ At 11 days, a subsample of 100 juveniles were counted and measured. Survival was

estimated at 11.5% with an average size of 337.5 x 324 μm. At 33 days, no live juveniles were recovered from the chamber. It was, however, a very suitable pond and chamber for the proliferation of oligochaetes, rotifers, daphnia, and ostracods.

The suspended chamber in the earthen pond proved even less successful. Juveniles were placed in the chambers May 18 and at 15 days none of the 245 individuals were found alive. The increasing temperatures (Table 11) experienced during this 2-week period and the rise and fall of the water level in the pond may have contributed to the mortality rate in these chambers at this time.

Similarly, the 3-week old juveniles in the suspended chambers in the cement ponds fared no better than the smaller juveniles in the earthen pond. None were found surviving after only 2 weeks. No physical measurements of pond temperature, D.O., pH, conductivity, or algal density were available for these ponds.

Discussion

Culture attempts in the outdoor ponds were unsuccessful. We did not succeed in rearing juveniles for more than 2 weeks in any of the chambers and ponds. Placing juveniles of a larger size in the ponds did not increase our success. Three-week juveniles appeared to fare no better than those 1-3 days old.

The poor success rate of the ponds was probably partially attributable to the limited control over several external factors in the outdoor system. (1) Juveniles are subject to predation from a very large and diverse number of other organisms. This appeared to have been the major cause for mortality

in these tests. The eggs, larvae, and spores of many organisms can easily get into the chambers even when covered on all sides by the 150 μm mesh. (2) Water temperature was more variable in the ponds than in a laboratory setting. When the earthen pond was filled, water temperature was 15°C. However, the extreme heat experienced in May and June raised the temperature above 26°C. This may have been a contributing factor in some trials. (3) The composition and concentration of phytoplankton was difficult to control. In the laboratory cultures, alternate vats of phytoplankton were used to feed the juveniles as the populations cycled. In the earthen pond, we were working with one community of phytoplankton which experienced blooms and crashes, a cycle difficult to control even with the addition of fish to the pond. (4) Finally, with the exception of the benthic chamber, no silt or substrate was provided to the juveniles in the chambers, a factor which may have contributed to early mortality.

In the future, if an outdoor culture system for juveniles is going to be operational, it may be necessary to consider system designs in which some external variables can be more controlled. A source pond of phytoplankton may be used to pulse in a constant source of food rather than relying on the natural cycling of the pond. Water temperature may be more controlled in this manner as well. Predators are difficult to eliminate from any outdoor system without also decreasing the juveniles' access to food. ~~Larger-sized~~ Juveniles larger than the 3-week olds tested in this study, may be less subject to predation by other organisms. Juveniles may not be able to survive satisfactorily in an outdoor, less controlled system until a longer initial period in a controlled laboratory setting.

Table 10. Outdoor culture tests. Survival and growth of pocketbook juveniles (*L. ventricosa*) in outdoor pond tests. L=mean lenth, H=mean height.

Pond/Chamber	L (mm)	S.D.	H (mm)	S.D.	Survival (%)
Earthen/Benthic					
0 days (5/13)	225	9.5	270	9.5	--
11 days (5/23)	337.5	21.0	324		11.5
33 days (6/16)	*no measurements taken on dead individuals				0.0
Earthen/Suspended, open					
0 days (5/18)	225	9.5	270	9.5	--
15 days (6/02)	*no measurements taken on dead individuals				0.0
Cement/Suspended, covered--1					
0 days	303	36.7	296	32.8	--
14 days	*no measurements taken on dead individuals				0.0
Cement/Suspended, covered--2					
0 days	303	30.5	295	24.4	--
14 days	*no measurements taken on dead individuals				0.0

Table 11. Outdoor pond tests. Temperature, D.O., pH, conductivity, and plankton composition of the earthen pond.

Date	Temperature °C	D.O. mg/L	pH units	Conduct. (μs) (μS/cm)	Phytoplankton cells/mL	Algal types	Zooplankton
5/16	15.1	9.0	7.6	306		Scenedesmus	Rotifers
5/17	16.7	14.5	8.0	287		Gleocystis	Euglena
5/18	19.0	18.0	8.2	268		Volvacales	Paramecia
5/19	20.1	17.9	8.8	252		Gonium	Ostracod
5/23	22.5	14.3	8.8	213	3.9×10^5	Sphaerocystis	Daphnia
5/25	20.6	9.6	8.0	212		Closterium	
5/31	25.7	7.3	7.8	263		Schroedria	
6/06	26.5	13.6	8.0	268		Naviculae	
6/16	22.4	10.1	7.9	242		Chodetella	

IV. INITIAL GROWTH IN STARVED AND FED JUVENILES

Introduction

Juveniles of freshwater mussels are produced at the La Crosse National Fisheries Research Center by collecting them from the tanks of host fish after a brief parasitic period on the gills. During the period of this mussel culture study, it became obvious that the juveniles are capable of surviving for a period of time in the fish tank without the addition of any phytoplankton or commercial food. A study of fed and starved juveniles was conducted to determine whether the juveniles have an internal food source after dropping from the fish, how long it sustains them after transformation, and to what extent an external food source plays a role in their survival in the first days of life.

Methods

In the first phase of the study, the growth and survival of Lampsilis ventricosa juveniles were compared under starving and laboratory culture conditions. L. ventricosa juveniles were obtained 1-2 days after metamorphosis on the host fish (walleye). A subsample of 10-15 were measured, length and height, and the remaining were placed in one of the two treatments. In the fed treatment, juveniles received a mixed phytoplankton culture (about 1.5-2.0 x 10^5 cells/mL). In the starved treatment, juveniles received only well water. Juveniles were held in 500 mL cylindrical, plastic containers with an air stone. After approximately 1 week, a subsample was measured and survival was counted. The test was replicated three times using three different groups of L. ventricosa juveniles.

In the second phase of the study, histological condition of juveniles was compared under starving and laboratory culture conditions in the first 3 days after sloughing from the host fish. Approximately 300 juveniles of L. ventricosa and L. radiata siliquoidea were collected within 12 hours after sloughing from the host fish and placed into static chambers containing a phytoplankton food source or well water. Survival counts and measurements were taken after 24, 48 and 72 hours in each treatment. Subsamples (n=25) for histological processing were taken at 0, 24, 48 and 72 hours. Juveniles were fixed in Karnovship fixative at 4°C, washed in 0.2µM cacodylate buffer and post-fixed in 1% osmium tetraoxide. Samples were then dehydrated in a graded acetone series and embedded in resin. Thick sections, about 5 µm, were cut on a glass knife, mounted on slides, and stained with toluidine blue.

Results

Growth and Survival

In the first replication, there was a significant difference in the length and height of juveniles in the starved and culture conditions (Table 12). Juveniles in the culture conditions had greater mean length and height although there was no significant difference in survival. In replicates 2 and 3, there was no difference in growth within replicates (Table 12). In replicate 2, overall growth and survival of juveniles in both treatments were poor for some unknown reason. In replicate 3, the growth of juveniles in starved conditions was, in fact, slightly greater than those in the culture conditions.

Following the results of these tests, we examined juveniles histologically to determine if they may have an internal food reserve which

supplements growth from external food sources in the first few days after metamorphosis. This work is still in progress but preliminary work indicates some significant differences between the condition of juveniles in the two treatments. Starved juveniles at 72 hours showed more emaciation than fed juveniles from the ~~sample~~ sample period.

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Table 12. Histological examination of juveniles. L = Length, H = Height

	L (mm)	H (mm)	L (mm)	H (mm)	Survival
~~Report~~ Trial 1 n=100	0 days		6 days		
Fed	244.8	253.8	301.2	294.0	%
Starved	244.8	253.8	255.6	271.2	100.0
					98.2
~~Report~~ Trial 2 n=35	0 days		9 days		
Fed	226.8 (12.6)	255.6 (7.6)	279.0 (23.2)	283.5 (30.7)	Survival 13.8 --
Starved	244.8 (21.3)	254.4 (11.5)	271.8 (26.1)	270.0 (22.4)	44.8 --
~~Report~~ Trial 3 n=28	0 days		8 days		
Fed	230.4 (16.5)	257.4 (12.1)	297.0 (36.3)	286.2 (24.7)	Survival 86.4 --
Starved	226.8 (12.6)	257.4 (8.7)	306.0 (20.8)	295.2 (15.2)	78.3 --