Experiments to Culture Juvenile Freshwater Mussels in Small Tanks, Floating Containers and Sediment Beds

by

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(ABSTRACT)

Freshwater mussel culture has become an attractive enterprise because of its application for restoring depleted freshwater mussel populations, for rearing adults to support shell and cultured pearl industries, and for environmental monitoring and assessment. This study focused on testing indoor and outdoor culture techniques to rear newly metamorphosed juveniles of the rainbow mussel (*Villosa iris*). Tanks and floating containers were used outdoors, and sediment beds and floating containers were used in indoor culture experiments.

Culture experiments with indoor sediment beds produced modest growth rates and variable survival rates for up to 4 months. Shell lengths of juveniles increased from 0.38 - 0.41 mm to 0.93 - 3.22 mm, 1.45 - 7.05 times original size; survival rates ranged from 1.27 % to 51.0 %. Experiments with indoor floating containers resulted in an increase of juvenile shell lengths from 0.38 - 0.40 mm to 0.86 - 2.07 mm, an 86 - 207 % increase; survival rates were 23.3 % to 27.0 % after 3 months. All indoor culture experiments combined water flow, inoculation of algae, fertilization, lighting and a pre-operation phase. Experimental results suggested that indoor floating container culture was a useful method to rear newly metamorphosed juvenile mussels, and the indoor sediment bed culture technique may also be suitable with modification, especially

if filamentous algae can be controlled. Both outdoor tank and floating container culture experiments yielded poor results, which were probably caused by unsuitable culture conditions.

Differences in juvenile growth rates ($P < 0.001$) and survival ($P < 0.001$) between floating container experiments I and II, which used the same culture techniques, were attributed to differences in culture conditions; water temperature ($P < 0.001$), dissolved oxygen ($P < 0.01$) and hardness ($P < 0.01$). Water temperature also contributed to a higher growth ($P < 0.001$) and survival rate ($P < 0.001$) in sediment bed culture II. The extensive occurrence of filamentous algae on sediments affected results and caused lower growth ($P < 0.001$) and survival rates ($P < 0.001$) of juveniles in the sediment bed culture experiment III, IV and V. Finally, absence of water flow may have influenced growth and survival of juvenile *V. iris*, a naturally riverine species. Lack of currents near the bottom of tank and floating containers where juveniles resided likely contributed to complete mortality of juveniles in the outdoor culture experiments.

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INTRODUCTION

The globally distributed superfamilies Unionacea (freshwater mussels) and Corbiculacea (freshwater clams) are the major taxa of living freshwater bivalves (Burky 1983; Russell-Hunter 1983). Of the North American freshwater bivalve fauna, most belong to two families of Unionacea: Margaritiferidae and Unionidae (Fuller 1974) and one family of Corbiculacea: Sphaeriidae. Unionidae is the largest family of freshwater mussels, with greatest diversity in eastern North America and in southeastern Asia (Banarescu 1990). There are 297 species and subspecies of unionoid bivalves known in North America (Williams et al. 1993). Generally, unionacean mussels associate with large, permanent drainage systems, whereas sphaeriid clams often live in small transient bodies of freshwater (Burky 1983). The region of the Mississippi River drainage basin is of special importance because of the great diversity of species (Pennak 1978). Richness and diversity are the key features of the North American freshwater mussel fauna.

DECLINE IN NATIVE MUSSEL POPULATIONS

Historical Overview

The history of the freshwater mussel fishery in North America can be divided into two phases: pearling and shelling. The pearling phase dates back to at least 1857, when the first American pearl was discovered in Notch Brook River near Paterson, New Jersey (Fassler 1991b). Modest numbers of freshwater mussels were taken as sources of food

and pearls, which were assumed to have no major impact on native mussel populations. The thick shells, composed of pearly nacre, of a variety of unionids gradually lead to their use as the primary raw material for the button industry. The shelling industry developed after 1891, initially exploiting the yellow sandshell (*Lampsilis teres*). Harvesting of shells gradually became intense, and the pearl button industry was finally established. By 1912, there were 196 factories in 20 states along the Mississippi system and a few rivers entering the Gulf of Mexico east of the Mississippi River. About 41 species, most belonging to *Quadrula* and *Lampsilis*, were used; and annual production of shells at that time was from 40, 000 to 60, 000 tons, mainly from the Mississippi River system (Coker 1921; Pennak 1878; Landau 1990).

The replacement of pearl shell by plastics as the raw material for buttons marked the demise of the pearl button industry. The reduced harvest of freshwater mussels allowed mussel populations to recover. During the period from the end of the pearl button industry to 1965, virtually no interest existed in harvesting shell in the Mississippi River (Grantham 1969). However, another shell fishery emerged after discovery of the utilization of thick shells of freshwater mussels as raw material for nuclei in the cultured pearl industry. An increase in harvesting of native freshwater mussels then resumed. The extensive harvesting beginning in the 1960s resulted in the reduction of mussel resources and consequent decrease in shell production.

The current status of the North America freshwater mussel fauna is in a critical condition. Among the 297 taxa of freshwater mussels, 21 (7.1 %) are listed as endangered

but possibly extinct, 77 (25.9 %) as endangered but extant, 43 (14.5 %) as threatened, 72 (24.2 %) as of special concern, 14 (4.7 %) as undetermined, and only 70 (23.6 %) as currently stable (Williams et al. 1993). Aquatic biodiversity in the U. S. will be greatly impoverished if these species are lost. The stability of aquatic ecosystems also are in jeopardy since freshwater mussels play an important role in organic matter utilization and energy flow in rivers.

Causes of Decline

Habitat destruction, commercial exploitation and invasion of exotic species are the major factors resulting in the depletion of freshwater mussel resources in North America.

Habitat Destruction

Adverse anthropogenic activities such as channelization, dredging and damming of rivers for navigation, flood control or improving drainage, siltation and pollution have destroyed natural mussel habitats and led to the depletion of mussel resources. These activities changed water flows, disrupted reproductive processes, eliminated glochidial hosts, caused siltation, or altered substrate composition (Fuller 1974). Water quality degradation is another major problem. As filter feeders, freshwater bivalves could not tolerate heavy loads of silt resulting from headcutting, gravel washing, coal washing, poor agricultural practices, cutting of riparian forests, and clear cutting of major portions of the watershed (Bogan 1993). Chemical pollutants which included wood product wastes, acid mine wastes, pesticides, other industrial pollutants and eutrophication by

effluents from municipal sources were the major factors contributing to the depletion of mussels in many areas (Fuller 1974). The most detrimental effect of dams was likely the disruption of the reproductive cycle by eliminating host species (Williams et al. 1993).

One characteristic of the North American freshwater mussel fauna is that many are endemic, highly differentiated species, which implies that these species have limited adaptability to environmental alteration of their habitats. Generally, habitat preference of adult unionaceans and their host fish coincide closely because of their dependence on host fish for dispersal (Kat 1984). This dependence greatly limited their distribution, resulting in highly endemic species. On the other hand, their extended life spans, delayed maturity, low effective fecundity, reduced power of dispersal, high habitat selectivity, poor juvenile survival and extraordinarily long turnover time make unionaceans highly vulnerable to adverse anthropogenic perturbations (McMahon 1991). The adverse alteration of their habitat is fatal, in many instances, to those mussel populations.

Commercial Exploitation

Overharvest as a result of demand for shells, first by the pearl button industry and later by the cultured pearl industry, contributed to the depletion of native freshwater mussel resources. In the era of the pearl button industry, the overharvest of freshwater mussels by the industry resulted in a decrease of native mussel resources in the Mississippi River basin. This was mirrored by a reduced activity and irregularity of shelling, and also by a gradual decrease in the proportion of larger shells (Coker 1921). The average size of mussels in most overfished populations rapidly declined, and areas

that once supported large mussel populations were often near depletion (Landau 1990). The possible extirpation of commercial mussel species in the Mississippi River and its large tributaries from overharvest by the pearl button industry was of concern (Lefevre and Curtis 1910b).

The demand for shell nacre as the nuclei for the marine cultured pearl industry in Japan revived shell harvesting in the 1960s. In some areas, native freshwater mussels were extensively harvested and their populations declined. As a result, the annual tonnage of shells in the Tennessee River dropped from 10,000 tons in the 1940s and 1950s to 2,000 tons in 1964 to 1967; and the present mussel fauna in this river now consists of only 44 species, whereas there were 100 species before the first Tennessee Valley Authority impoundment in 1936 (Isom 1969, in Pennak 1978). In 1991, 9,000 short tons of raw shell were exported to Japan; while in 1992 and 1993, about 4,500 short tons were shipped (Baker 1993, in Williams et al. 1993). The shells of mussel species were also used for the culture of freshwater pearls in the United States (Isom and Hudson 1982; Fassler 1991a, 1991b).

Introduced Species

The introduction and spread of nonindigenous mollusks has also contributed to the depletion of native freshwater mussels (Williams et al. 1993). The large populations of asian clam (*Corbicula fluminea*), established permanently in North American waters, competed with native unionoids and caused a decline or possible extirpation of native freshwater bivalves (Clarke 1988, In: Bogan 1993). Zebra mussels (*Dreissena*

polymorpha), native to Eurasia, were first found in Lake St. Clair in 1988. Now this exotic species inhabits 20 U.S. states and 2 Canadian provinces. Their negative influence on unionid populations in some water bodies is through attachment to the larger native mussels which reduces host feeding efficiency. Another important impact is the competition for food with native species since both are filter feeders. Large zebra mussel populations also affect the ecology of aquatic ecosystems by altering the energy flow through the food web via their feeding activity, have an oligotrophic effect on water quality and change the structure of the benthic community (Griffiths 1993).

CULTURE OF FRESHWATER MUSSELS

North America

At the turn of the century, as a result of concern for the decline of important commercial species in the Mississippi River, the U.S. Bureau of Fisheries initiated research on artificial propagation and culture of freshwater mussels. The research focused on the artificial infestation of fish with glochidia of mussels, natural history and habitat requirements, and experiments on growout (Landau 1990). Lefevre and Curtis (1910b) produced metamorphosed juveniles by experimental infestation but only retained post-metamorphosed juveniles for 4 weeks. Howard (1914b) cultured the Lake Pepin mucket, *Lampsilis luteola*, in a floating crate in the Mississippi River. From June 10 to November 24, the largest young mussel reached 32 mm. Meanwhile, his effort on the yellow sandshell, *Lampsilis teres*, was not as successful as that of the Lake Pepin mucket. In that

period, different culture methods using 1) floating cages with fine or coarse meshed net; 2) pens and fixed cages with wooden bottoms, 3) earthen ponds, concrete ponds, and troughs, 4) crates, tanks, aquaria, etc.; and different mussel species -- *Lampsilis luteola, L. ventricosa, L. anodontoides, L. ligamentina* and *Quadrula pustulosa*, were used in experiments (Corwin 1920, 1921; Coker et al. 1921; Howard 1922). The results of many experiments in which *Lampsilis luteola* was reared under diverse conditions indicated that this valuable species could be reared in quantities under controlled conditions. However, experiments with other species generated less consistent results (Coker et al. 1921). Researchers found that host fishes could be infested in seine nets and transferred to ponds or other natural water bodies (Landau 1990). However, little work was undertaken after the demise of the pearl button industry because of less concern for overharvest of freshwater mussels.

The culture of freshwater mussels attracted the attention of researchers again when resurgence of harvesting of freshwater mussels was initiated for the cultured pearl industry in 1960s and much later for the protection of rare species. Isom and Hudson (1982) reported a method for transformation of glochidia to juveniles in vitro rather than by their natural host. Subsequently, they used a modified mariculture procedure to raise juveniles transformed by in vitro methods and obtained from host fishes (Hudson and Isom 1984). Keller and Zam (1990) simplified the in vitro method by the substitution of commercial culture media and horse serum to culture glochidia of three freshwater mussels -- *Utterbackia imbecillis, Lampsilis teres* and *Villosa lienosa*. Johnson et al.

(1993) reported that juvenile *Utterbackia imbecillis* could be maintained and tested within a few days of transformation without food and silt, although no successful juvenile culture methods were developed. Gatenby (1994) tested different combinations of live algae, bacteria, silt and commercial mixtures to culture juvenile mussels indoors. She reported that the tri-algal diet with silt generated the maximum growth and survival for rearing newly metamorphosed juvenile *Villosa iris* and *Pyganodon grandis*. Burress (1995) used suspended buckets to attempt to rear newly metamorphosed *Villosa iris* in two static ponds, but no survival occurred. However, by 1991, three U. S. firms were holding adult freshwater mussels for pearls, using a variety of mussel species (Fassler 1991a, 1991b).

Overseas Propagation

China is one of the leading countries in global freshwater pearl production, culturing the species *Hyriopsis cumingii* and *Cristaria plicata*. Natural lakes, reservoirs, rivers and ponds are used to produce cultured pearls. Culture techniques for those species were developed in 1970s. Now the freshwater cultured pearl industry relies mainly on artificially produced populations. In the last two decades, this industry fluctuated due to the market value of freshwater cultured pearls, deterioration of the culture environment, and infectious diseases.

In Japan, freshwater cultured pearls are limited to lakes Biwa and Kasumingaura. Commercial production began in 1935, and used the species *Hyriopsis schlegelii*. For the most part, no artificial nucleus is employed. In 1989, the total area of 70 culture farms

was 100 ha in Lake Biwa and 6 farms totaled 4 ha in Lake Kasumingaura. The maximum annual pearl production for freshwater species in Japan was estimated at 10 tons (Mizumoto 1976; Ikenous and Kafuku 1992).

In Europe, research has been completed on the biology and conservation of the freshwater pearl mussel, *Margaritifera margaritifera*. Buddensiek (1995) reported a method of raising juvenile *M. margaritifera* in cages in rivers of northern Germany. Survival rates were equal to those of free-living juveniles, and growth was equal or slower than under natural conditions.

LIFE HISTORY OF FRESHWATER UNIONIDS

Special parasitic stage

The most unusual aspects of the reproductive biology of unionids have been 1) embryonic development in the female's marsupium, and 2) a parasitic stage on host fish (Lefevre and Curits 1910a, 1910b; Coker et al. 1921; Howard 1922; Fuller 1974). With respect to breeding seasons, North American unionids are classified into two groups: summer brooders (tachytictic) and winter brooders (bradytictic). There are also two types of glochidia; hooked and hookless. In late summer, ripe eggs are released from ovaries into the suprabranchial chambers of females. Sperm are shed into the water by males and carried into the female with her inhalant water to fertilize the eggs. Fertilized eggs are brooded inside the female's marsupium and develop into glochidia. From there, glochidia are liberated and released into the water, where they attach themselves to host fish,

usually on gills and fins. They then become encysted by host cells; the cyst results from migration of gill epithelial cells, but not hyperplasia (Nezlin et al. 1994). Glochidia finally metamorphose into juveniles, are shed from the fish body to the water bottom, and live a free benthic existence. Duration of parasitism is variable and temperature dependent (Lefevre and Curtis 1910b).

Studies of the relationship between host fish and unionids showed that the blood of fish could stimulate glochidia to clap their valves rapidly and violently, and that different species of fishes were not equally susceptible to glochidial infection (Lefevre and Curtis 1910b). Surber (1912) described two classes of natural host fishes with respect to freshwater mussels; specific distributors of particular species, and accidental or occasional hosts. Repeated heavy infections on fish will result in fish immunity to the attachment of glochidia, even for host fish (Reuling 1919). The glochidial hosts play an important role in dispersion of freshwater mussels, since the mussels have no free-swimming larval stage (Fuller 1974).

Longevity

Unlike freshwater clams, freshwater mussels usually have long life spans. For example, *Unio pictorum, U. tumidus* and *Anodonta antina* are three large, slow-growing, riverine mussels with reported maximum life spans of 9 - 15 years (Russell-Hunter and Buckley 1983). It takes roughly 12 years for the freshwater pearl mussel (*Margaritifera margaritifera*) to become mature, and most mussels then survive to reproduce for another 30 - 50 years (Young and Williams 1983). Unionids have extremely long life spans

ranging from < 6 to > 100 years and age at maturity ranging from 6 to 12 years, compared with sphaeriids (< 1 to > 5 year life spans; 0.17 to 1.0 year age at maturity), *Corbicula fluminea* (1 to 5 year life span; 0.25 to 0.5 year age at maturity) and *Dreissena polymorpha* (4 to 7 year life span; 1 to 2 year age at maturity) (McMahon 1991).

Fecundity

The fecundity of freshwater mussels varies from 200,000 to 17,000,000 glochidia per mature female each year (Parker et al. 1984; Paterson 1985). The recruitment to populations, however, is extremely low due to their specialized life cycle. It was estimated that only 1 of 100,000,000 shed glochidia become a successfully settled young mussel (McMahon 1991). The parasitic link of the glochidial stage of unionacean clams (selection ratio > 5,000,000 : 1) is tied to host fish movements, which limits both larval survival and dispersal within each system (Burky 1983). However, their low recruitment is counter-balanced by a high life expectancy and the vast numbers of glochidia released by females (Young and Williams 1984). These characteristics infer that these animals are competent in a stable habitat and are not readily adaptable to environmental alterations.

Unknowns

Although much valuable research has revealed the different aspects of life histories of freshwater mussels, there remain many unknowns, especially for the early life stages. Some of these factors are believed to be crucial to the success of culturing efforts, such as foods, water currents, optimal ranges of water quality parameters for growth and survival, and predators. All these factors may make their artificial culture difficult.

MATERIALS AND METHODS

OBJECTIVES

The primary goal of this project was to develop a culture technique for rearing juvenile freshwater mussels, to restore native populations and to prevent the extinction of rare species. There were two specific objectives: 1) to rear juvenile mussels in indoor facilities, using sediment bed culture and floating container culture; and 2) to rear juvenile mussels in outdoor facilities, using tank culture and floating container culture.

INDOOR SEDIMENT BED CULTURE

Culture System

This experiment was designed to develop a suitable indoor culture technique to rear juvenile mussels, using *Villosa iris* as the cultured species. Newly metamorphosed juveniles were the experimental animals. Because this was a preliminary study in culture, its purpose was to test the feasibility of the culture technique used. Usually the entire experiment was cultured under the same conditions, with three replicates per experiment.

The sediment bed was designed to provide juveniles with gently flowing water, adequate food and sediments. The sediment bed culture system was composed of a sediment bed (made from PVC sheet), a reservoir water tank (elliptical, 378 L) or living stream (rectangular, 569 L), lights (four 40 W cool white fluorescent lights) and a pump (1/6 or 1/12 horsepower). The sediment bed consisted of six culture compartments

(length x width x height: 30 x 20 x 10 cm), an inlet channel, an outlet channel and a buffering channel (Figure 1). Water had to pass through small holes on the partition between the input and buffering channels to enter the buffering channel, and then enter the culture compartments through small multiple notches on the partition. A gentle water flow was then created. Culture compartments were shallow (actual water depth was 9 cm and sediments 1 cm) so that adequate light could support algae growth on sediments and to allow water flow across the sediment surface where juveniles live. Water was recirculated within the system. This culture technique combined water flow with inoculation of algae, lighting of a 12:12 h dark:light cycle, fertilization and a pre-operation phase to enhance a presumed suitable growth environment for juvenile mussels.

All experiments were conducted indoors at ambient room temperature in the Aquaculture Center, Virginia Polytechnic Institute and State University, Blacksburg, Virginia.

Experimental Conditions

Gravid *Villosa iris* were collected from the North Fork Holston River, Smyth County, Virginia. Mussels were held in a living stream indoors where water was chilled to maintain a low temperature to prevent release of glochidia. Host fish were rock bass (*Ambloplites rupestris*) caught by electrofishing in the New River, near McCoy, or in Tom's Creek near its confluence with the New River, Montgomery County, Virginia. The

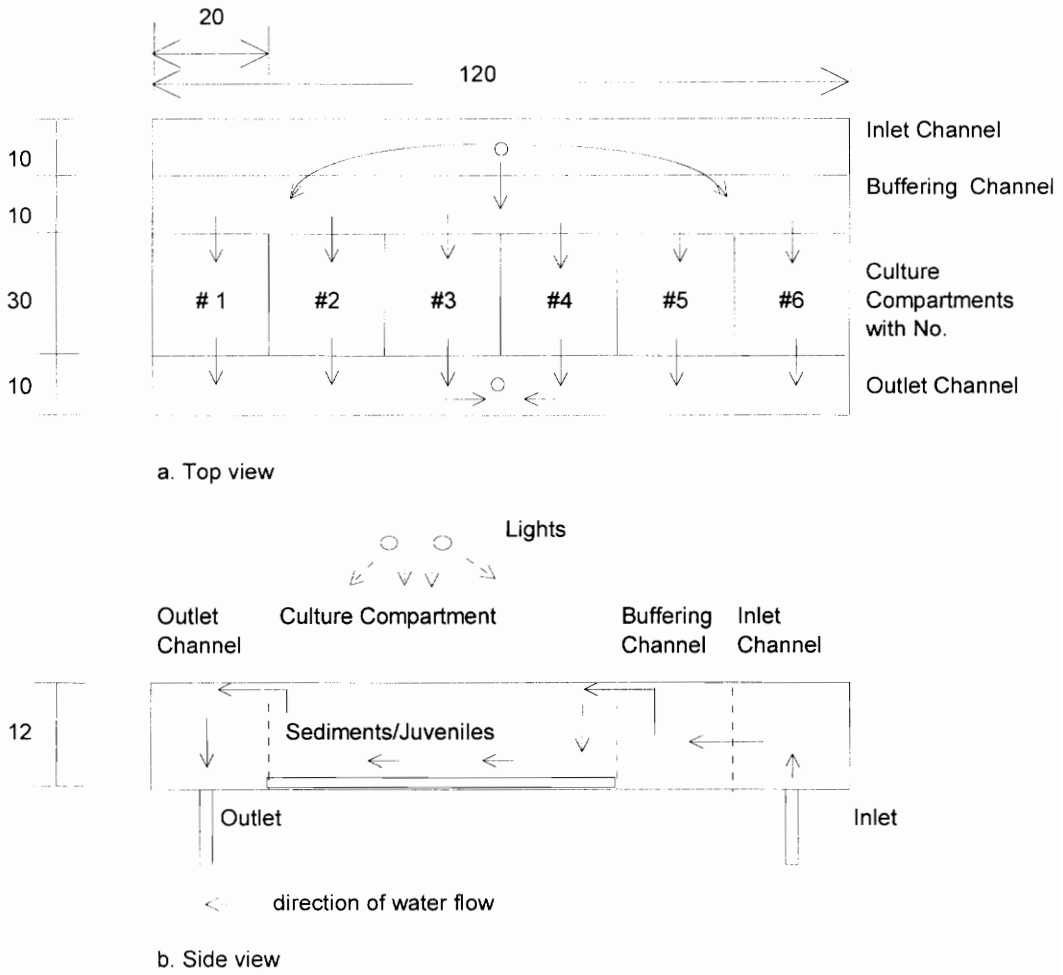

Figure 1.—Schematic of sediment bed for culturing juvenile mussels indoors (units in cm).

newly metamorphosed juveniles of *V. iris* were produced by induced infestations of host fish (Appendix A).

Bottoms of culture compartments were covered with a 1 cm thick layer of sediments. Sediments were collected from Craig Creek in Jefferson National Forest, Montgomery County, Virginia, and passed through a 130 μm sieve to obtain a uniform silt with particle sizes smaller than 130 μm. The main reason for using sediments of such small and uniform particle size was to make the retrieval of juvenile mussels reliable by sieving sediments siphoned through a sieve of 130 μm to retain the juveniles (>130 μm) on the sieve screen. The sediments were sterilized by being boiled for 10 min, twice in 3 d (a technique similar to pasteurization) or with one application of bleach (chlorine). One experiment used sediments of a variety of particle sizes (< 2 mm).

Dechlorinated Blacksburg town water or town water mixed with well water was the initial water source. Water was recirculated through PVC pipes by the pump between the sediment bed and reservoir tank or living stream. During the culture experiments, in order to compensate for water loss by evaporation, well water was added whenever the water level became low. Frequency of refill depended on the evaporation rate and ranged from 1 to 3 times a week.

Prior to the experiment, ammonium nitrate (NH_4NO_3) and monobasic sodium phosphate (NaH_2PO_4) were added to the water to provide additional nitrogen and phosphorus sources for algal growth. Fertilization rates were 1 g NH_4NO_3 and 60 mg NaH_2PO_4 for 378 L of water. During the culture experiment, these chemicals were added

when the water chemical analyses showed the need, usually about once every 1 to 1.5 months.

Three species of algae; *Neochloris*, *Bracteacoccus* and *Phaeodactylum* (Appendix B), were inoculated into culture compartments before the pre-operation phase and stocking of juveniles, and during the culture experiment. Dense suspensions of uni-algal cultures were obtained from the Biology Department, Virginia Tech (Appendix C).

A pre-operation phase was established between the inoculation of algae and application of N and P salts, and the stocking of juveniles, when the system was operating with continuous light. This period usually lasted a week or more and was intended to allow time for algae to grow on sediments as a food source for the incoming juvenile mussels. This time also allowed the establishment of microbial communities that are crucial to maintaining good water quality. Before the pre-operation phase, the entire system was disinfected with bleach; washed three times and then flooded with water for one day. All water was changed, and the system was ready for use one day later.

Newly metamorphosed juveniles of *Villosa iris* were collected daily from host fish artificially infested with glochidia (Appendix A). When juveniles met the number required for stocking, all juveniles collected in that period were placed together as one group. They were then counted and stocked into the culture compartments. Their mean age in days was used as the age of juveniles stocked, usually about 7 d old. Water flow was halted temporarily when stocking to avoid washing away the young juveniles, but water was aerated to provide sufficient oxygen for the newly stocked juveniles. Water

began recirculation 12 h later. No juveniles were found in the filtered contents of the sieve kept at the water outlet; mesh size (75 μm) was small enough to retain all newly metamorphosed juveniles.

The culture system was checked daily to ensure that it was operating properly. Water inlet notches of culture compartments were cleaned mechanically with a brush about twice per week to ensure the constancy of water flow. A 75 μm sieve was placed under the water outlet to collect large zooplankton in order to control their quantity in the system. Large zooplankton may compete with juveniles for planktonic algae. The entire culture period was scheduled to last 3 months.

Water was sampled once every week to measure temperature, pH, dissolved oxygen, hardness, NO_3^--N, NO_2^--N, NH_3-N and PO_4^--P. These indices were measured using a spectrophotometer (DR/2000, Hach Co.) by the following methods (Hach DR/2000 Spectrophotometer Handbook, Hach Co.):

NH_3-N: Nessler method;

NO_2^--N: Diazotization method;

NO_3^--N: Cadmium reduction method;

PO_4^--P: Amino acid method;

Other measurements:

Hardness: EDTA titration;

Dissolved oxygen: Dissolved oxygen meter (YSI, Model 58);

Water temperature: Temperature tester (Pocket Pal™, Hach Co.);

pH: pH tester (Pocket Pal™, Hach Co.);

Lighting was set in a 12:12 h light:dark cycle except for the experiment I when light was continuous. Four 40 W cool white fluorescent lights provided the lighting for one sediment bed. The light intensity on the water surface of culture compartments was measured with a photometer (Model LI-185B, Li-Cor Inc.).

Water exchange rate (L / h) were calculated from the water volume flowing through the sediment bed in a certain period of time. Algal composition was evaluated by examining a pooled sample of sediment from culture compartments, and counting the frequency (%) of each algal genus. Ten fields (400x) were examined for each sample.

Before stocking, 30 juveniles were randomly sampled, and their shell lengths were measured using a dissecting microscope (Figure 2). Sampling of juveniles took place monthly to monitor juvenile growth. During the sampling, sediments of about one-sixth area of the compartment bottom were siphoned into a 300 μm sieve. In the first sampling, an additional sieve of 200 μm also was used to prevent smaller juveniles from escaping. The sieved contents were examined using a dissecting microscope. All live juveniles were collected and counted. Twenty individuals were randomly selected, and their shell lengths measured. Sediments of the same type and quantity were added to replace those being siphoned. All collected juveniles were returned to their original compartments. In some experiments, the lengths of dead valves also were measured to provide additional information on juvenile growth conditions. Total five sediment bed

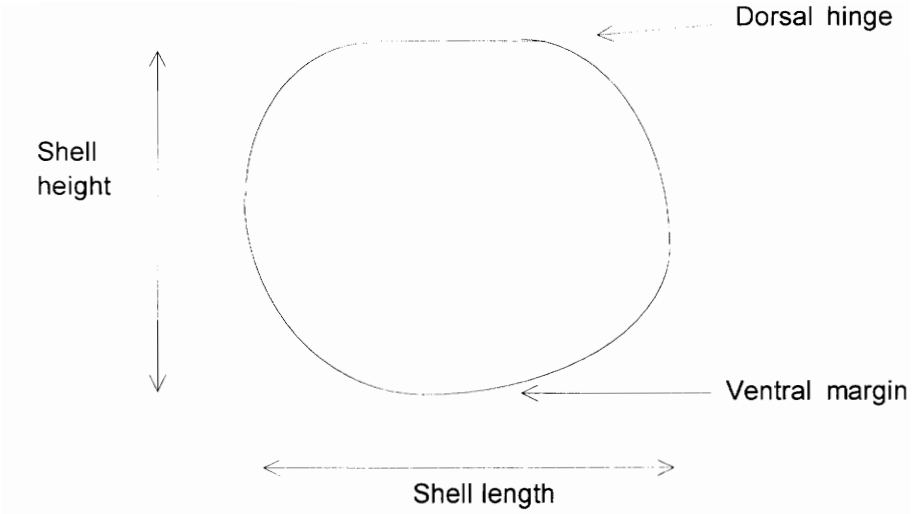

Figure 2.—Juvenile mussel measurements.

culture experiments have been undertaken, and some variations of culture measures were applied in those experiments (Table 1).

Experiment I

The experiment was conducted from March 22 to April 26, 1995, and used a culture system with a 378 L elliptical reservoir water tank. A total of 280 juveniles of *Villosa iris* were stocked in each of the three culture compartments (three replicates), with a stocking density of 47 individuals / 100 cm^2.

Three species of algae (3 ml / compartment); *Neochloris* (1.76 x 10^7 cells / ml), *Bracteacoccus* (5.25 x 10^6 cells / ml) and *Phaeodactylum* (6.24 x 10^7 cells / ml) were inoculated into the sediments on March 15, a week before the stocking of juveniles. N and P salts also were added and the pre-operation phase began. A week later when juveniles were stocked, many green patches of algae were observed on the sediment surface. When sampled with a pipette and observed using a microscope, *Neochloris* and *Bracteacoccus* were found. Then, 5 ml of additional algal suspension per species were applied to each compartment.

Water chemistry indices were monitored weekly (Table 2). The first monthly sample of juveniles was taken on April 25 - 26, 1995. In the beginning, only the sediment of one-sixth area of each compartment was sampled. Because few live juveniles were found, all sediments in the three culture compartments were siphoned and examined. Live juveniles were counted and their shell lengths were measured. The lengths of 20 dead valves from each culture compartment also were measured. Algal composition of

Table 1.— Variations in culture conditions within sediment bed culture (SBC) experiments [a].

Experiment No.	SBC I	SBC II	SBC III	SBC IV	SBC V
Light (dark:light cycle)	constant	12:12 h	12:12 h	12:12 h	12:12 h
Water flow (L / h)	50	113 - 120	118 - 122	118 - 132	128 - 135
Sediments (μm)	< 130	< 130	< 130	< 2,000	< 130
Stocking density (juveniles/100 cm^2)	47	83	83	83	83 or 166
Sampling within culture period	Yes	Yes	Yes	No	Yes
Reservoir tank volume (L)	378	569	569	569	569
Duration	1 month	80 d	3 months	4 months	3 months

a) other measures such as inoculation of algae, algae species, fertilization, a pre-operation phase, etc. were same in all experiments

Table 2.—Mean (± SD) of weekly water chemistry characteristics in sediment bed culture (SBC) experiments.

Characteristic	SBC I	SBC II	SBC III	SBC IV	SBC V
Water temperature (°C)	24.1 ± 3.8	28 ± 2.2	23.7 ± 2.2	23.7 ± 1.5	23.8 ± 1.6
pH	8.8 ± 0.2	9.1 ± 0.3	8.4 ± 0.6	8.2 ± 0.6	7.9 ± 0.4
Dissolved oxygen (mg/L)	8.3 ± 0.9	8.5 ± 0.6	7.6 ± 1.1	7.6 ± 0.8	7.6 ± 0.4
Hardness ($CaCO_3$ mg/L)	99 ± 24	253 ± 129	351 ± 47	396 ± 71	422 ± 61
NH_3-N (mg/L)	0.13 ± 0.15	0.16 ± 0.23	0.09 ± 0.07	0.08 ± 0.06	0.06 ± 0.02
NO_2^--N(mg/L)	0.26 ± 0.22	0.08 ± 0.21	0.04 ± 0.08	0.03 ± 0.06	0.01 ± 0.003
NO_3^--N (mg/L)	2.1 ± 1.1	2.7 ± 2.2	1.5 ± 0.3	1.7 ± 0.4	1.8 ± 0.5
PO_4^- (mg/L)	2.74 ± N/A[a)]	1.75 ± 0.63	1.81 ± 1.83	1.52 ± 1.49	1.06 ± 0.51
Light intensity (micro-einsteins . m^2. s^{-1})	100	57	63 ± 3	62 ± 3	62 ± 3
Water exchange rate (L / h)	50	113 - 120	118 - 122	118 - 132	128 - 135

a) not applicable

sediments was examined (Table 3). The experiment was terminated at that time, only 1 month after initiation.

Experiment II

This experiment began on April 21 and ended on July 10, 1995, for a time period of 80 d. Some modifications to the techniques of sediment bed culture were made because experiment I yielded unsuitable results (Table 1):

. (1) The entire system was moved into a smaller room of the laboratory.

(2) A rectangular living stream of 569 L served as a larger reservoir water tank.

(3) Five hundred juveniles were stocked in each culture compartment.

(4) A 12:12 h light:dark cycle was set instead of constant light.

5) An increase in the water exchange rate was introduced.

NH_4NO_3 (1.47 g) and NaH_2PO_4 (0.88 g) were added on April 10, 1995, and the same algae suspensions used in experiment I were inoculated (10 ml / species / compartment). The system began its pre-operation phase at that time.

Host fish (rock bass) were infested with glochidia of *Villosa iris* on March 21, and juveniles began to drop off on April 4. Five hundred post-metamorphosed juveniles, 8 d old, were stocked into each of three culture compartments on April 21, for a stocking density of 83 individuals / 100 cm^2.

Water chemistry measurements were taken weekly, and other culture conditions also were measured (Table 2). Water was changed by about one third on May 1 and May

Table 3.—Algae composition (%) in sediments of the sediment bed culture (SBC) and indoor floating container culture (IFCC) experiments after 1 month.

	SBC I	SBC II	SBC III	SBC IV	SBC V	IFCC I	IFCC II
Neochloris	11	0.26	0.2				
Bracteacoccus	1					0.83	
Phaeodactylum	12						
Scenedesmus	75	95.5	76.17	46.25	41.44	66.12	27.9
Navicula		3.71	18.55	37.79	45.53	20.66	58.77
Cyclotella			1.37	6.51	5.64		10.43
Fragilaria		0.09		3.26		8.26	1.95
Closterium					1.17	0.83	
Chlamydomonas	1	0.44	3.71	6.19	6.23	3.31	0.95
Total	100	100	100	100	100	100	100

2 due to the high concentration of nitrite. Live algae suspensions and N and P salts were then applied to provide additional cells and nutrients for the algae community.

The first monthly sample of juveniles was taken on May 18. A sufficient number of juveniles was collected from each compartment to make up the required sample size of 20 individuals. A second sample was taken on June 20. More juveniles than the required sample size were collected from each compartment. Sample 3 was scheduled for July 21. However, because of an accidental oil-leak of the pump on July 10, the experiment was terminated at that time (11 d early).

Experiment III

This experiment repeated the second sediment bed culture experiment and used the same culture techniques with three replications (Table 1). The experiment was conducted from August 25 to November 27, 1995.

Three species of algae (20 ml / compartment); *Neochloris* (4.75×10^6 cells / ml), *Bracteacoccus* (3.58×10^6 cells / ml) and *Phaeodactylum* (4.48×10^7 cells / ml) were inoculated. After inoculation of algae and application of N and P salts, the system started its pre-operation phase on August 12. On August 21, because filamentous algae had colonized the sediment surface, a secondary application of bleach was used to eliminate them. The entire system was washed three times and refilled with well water. Re-inoculation of algae and additional of nutrients were applied on August 22. Both were added into the culture compartments, and the water was not circulated in order to accelerate algal growth.

Post-metamorphosed juveniles of *Villosa iris* were stocked on August 25, 1995. This group of juveniles was shed from host fish between August 12 and August 21, and were 10 d old at the time of stocking. Five hundred individuals were stocked in each of the three culture compartments, for a stocking density of 83 juveniles / 100 cm^2. Water chemistry indices were measured weekly (Table 2). Since water temperature decreased seasonally, two 60 W electro-heaters were added in the living stream.

Despite the additional disinfection with bleach before stocking of juveniles, filamentous algae recolonized the sediment surface of culture compartments during the last 2 months of the experiment. Though these algae were removed mechanically during this period, they still grew extensively. Two dominant filamentous algae were identified: *Oscillatoria sp.*, a blue-green alga, and *Spirogyra sp.*, a green alga (Appendix B).

Monthly samples of juveniles were taken. Based on the desire to reduce the extent of disturbance on juveniles, if a sample with juveniles was less than the required sample size (20 individuals), no additional sampling was attempted. Sampling was mainly intended to monitor juvenile growth, and the shell length data obtained from more than 10 individuals were relatively reliable. The first sample was taken on September 28, the second on October 26 and the final on November 27. In the second sampling, less than 20 juveniles were collected from the one-sixth area of all three culture compartments.

Experiment IV

This experiment also was another trial of the sediment bed culture of juvenile *Villosa iris*, with some minor variations. It used the same culture technique and

procedures as the previously described experiments, except for the particle size of sediments (< 2 mm), a 4 month culture period and no sampling of juveniles during this period (Table 1). There were also three replications, which lasted from August 25 to December 31, 1995.

The system started operating on August 12. As with experiment III, the occurrence of filamentous algae in culture compartments required application of bleach on August 21 to eliminate the filamentous algae. Re-inoculation of algae and addition of N and P salts occurred on August 22.

Newly metamorphosed juveniles of 10 d old were stocked on August 25. Five hundred individuals were stocked in each of the three culture compartments, for a stocking density of 83 juveniles / 100 cm^2.

Water chemistry indices were measured weekly (Table 2). Two 60 W electrical heaters were used to raise water temperature as the season progressed. No samples of juveniles were taken during the culture process. At the end of the experiment, on December 31, all the sediments were siphoned and sorted through a 600 μm sieve. Those filtered contents were partitioned into smaller components. They were placed in a circular dish together with some water, and shaken so that live juveniles would come to the surface. Sediments were examined then with a dissecting microscope. Live juveniles were collected with a pipette. This procedure was repeated until no live juveniles were found in three consecutive attempts. To confirm the suitability of this method, 20 juveniles were mixed with the same type of sediments and re-collected using the same

method. In three attempts, 17, 18 and 16 juveniles were recaptured, indicating that the method was relatively reliable.

The second application of bleach did not eliminate filamentous algae from the system. These algae grew extensively on the surface of sediment in the culture compartments throughout the latter 3 months of the experiment, in spite of continuous mechanical removal. The dominant filamentous algae were *Oscillatoria sp.* and *Spirogyra sp.* (Appendix C).

Experiment V

There were originally two purposes for this experiment: 1) to replicate the sediment bed culture of juvenile mussels, and 2) to examine the effect of stocking density on growth and survival of juvenile mussels. This experiment was divided into two components; a low stocking density (500 juveniles / compartment; 83 juveniles / 100 cm^2) in compartments #1, #3 and #5; and a high stocking density (1,000 juveniles / compartment; 166 juveniles / 100 cm^2) in compartments #2, #4 and #6. All other procedures followed those of the previous experiments (Table 1). *Villosa iris* was the cultured species, and the experiment was conducted from September 26 to December 26, 1995.

On September 18, three species of algae (20 ml / compartment) were inoculated: *Neochloris* (4.75 x 10^6 cells / ml), *Bracteacoccus* (3.58 x 10^6 cells / ml) and *Phaeodactylum* (4.48 x 10^7 cells / ml). Because this experiment used the same reservoir living stream as described previously, no initial application of N and P salts occurred.

The system started its pre-operation phase on September 18. Five hundred or a thousand juveniles of *V. iris* were stocked in each of the six culture compartments on September 26.

Water chemistry parameters were measured weekly (Table 2). The first sample of juveniles was taken on October 27. A sufficient number of juveniles was sampled from one-sixth area to make up the required sample size. The second sample was taken on November 28. Less than 20 juveniles (the pre-determined sample size) were collected from the one-sixth area of most (5 out of 6) culture compartments. No further samples were taken to eliminate the disturbance to juveniles. All juveniles were collected on December 26. A sample of dead valves also was taken from each culture compartment.

Because this experiment used the same reservoir living stream as experiments III and IV, the filamentous algae problem also occurred. Filamentous algae colonized on the sediment surface of culture compartments during the latter 2 months of this experiment. Continuous mechanical removal did not prevent them from growing extensively. The species were *Oscillatoria sp.* and *Spirogyra sp.* (Appendix B).

INDOOR FLOATING CONTAINER CULTURE

Culture System

These experiments were intended to evaluate the use of floating containers for culturing juvenile mussels indoors. Based on the same considerations as sediment bed culture experiments, the experiments had one treatment with three replicates.

The indoor floating container culture system consisted of floating containers, buoys, a reservoir water tank and lights. Floating containers were made from cylindrical plastic containers (17 cm diam; 2.8 L volume), with two windows on sides and the container bottom (Figure 3). The windows were covered with 0.8 mm mesh. The reservoir water tank that held the floating containers was a rectangular living stream (569 L) or an elliptical plastic tank (378 L). The living stream was the same one used in the second sediment bed culture experiment; thus, the same water conditions and manipulations were applied. Floating containers floated on the water surface by buoys made from PVC pipes and plastic bottles. Culture conditions consisted of a pre-operation phase, water flow, lighting, inoculation of algae and N and P salts.

Experimental Conditions

This experiment used the same mussel species (*Villosa iris*), host fish (*Ambloplites rupestris*), sediments, water sources and enrichment salts (N and P salts) as those of the sediment bed culture experiments. The floating container bottom was covered with a layer of sediments of 1 cm thickness. The water in the living stream or tank was aerated with compressed air to produce water currents and to increase dissolved oxygen. Dense suspensions of three species of algae; *Neochloris, Bracteacoccus* and *Phaeodactylum* were added to floating containers. Aeration was turned off for about 2 h to let algae settle on the sediments. The system began to operate days before stocking of juveniles to provide time for algae to colonize sediments and provide food source.

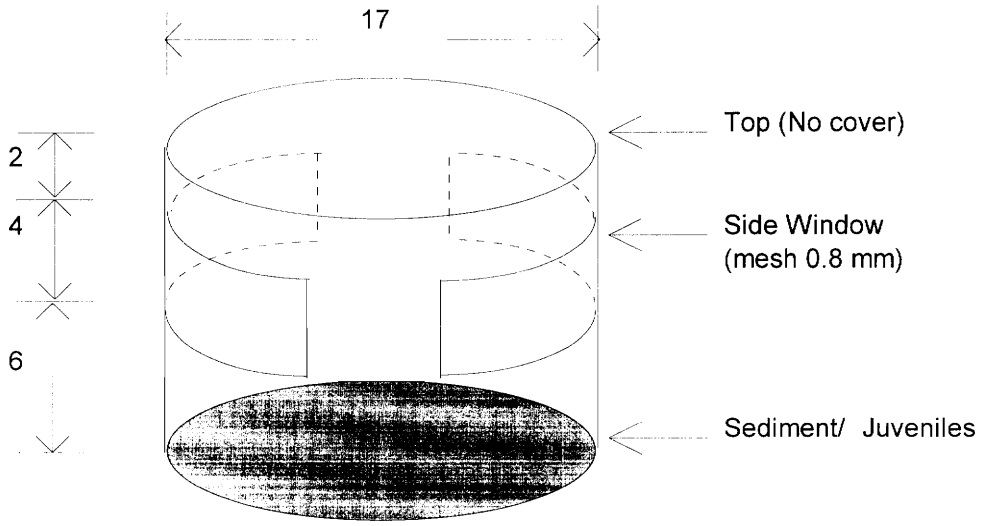

Figure 3.—Diagram of indoor floating container used to culture juvenile mussels (side view, units in cm).

Newly metamorphosed juvenile *V. iris* were produced by induced infestation of host fish with glochidia (Appendix A). Five hundred individuals were stocked in each floating container, for a stocking density of 220 juveniles / 100 cm^2.

The culture system was checked daily. Well water was added frequently to compensate for water losses due to evaporation or spilling. The screens on the floating containers were cleaned 2 - 3 times per week to remove fouling algae and improve water exchange. Weekly water chemistry measurements of temperature, pH, dissolved oxygen, hardness, NO_3^--N, NO_2^--N, NH_3-N and PO_4^--P were taken, using the same methods as those described for the sediment bed culture experiments. Light was provided by four 40 W cool white fluorescent lights, operated in a 12:12 h light:dark cycle. Algal composition of sediments also was evaluated using the same method described for the sediment bed culture.

The sampling method was the same as that used in the sediment bed culture. Thirty juveniles were randomly sampled at stocking and their shell lengths were measured. Sampling of juveniles (N = 20) was conducted monthly. After the sampling, new sediments were added to replace those siphoned. Sampled juveniles were returned to their original floating containers. In experiment II, the lengths of randomly selected dead valves were measured to provide additional information on juvenile growth conditions.

Experiment I

Newly metamorphosed juveniles of *V. iris* were reared from April 21 to July 10, 1995, for a period of 80 d, with one treatment and three replicates.

Three species of algae were introduced (10 ml / container): *Neochloris* (1.76 x 10^7 cells / ml), *Bracteacoccus* (5.25 x 10^6 cells / ml) and *Phaeodactylum* (6.24 x 10^7 cells / ml). They were inoculated on April 10, and N and P salts were applied. The system then began its pre-operation phase for 11 d before stocking of juveniles. Five hundred juveniles of 8 d old were stocked into each of three culture floating containers on April 21, with a stocking density of 220 juveniles / 100 cm^2. The juvenile *V.* iris stocked in this experiment were from the same group as those used for sediment bed culture II.

Weekly water chemistry measurements was taken during the culture experiment (Table 4). The first monthly sampling was taken on May 18, and the second was on June 20. A sufficient number of live juveniles was sampled from a one-sixth area of each floating container in both samplings. Those floating containers were placed in the same living stream used in sediment bed culture II. This experiment was concluded when the accidental leak of oil from the pump occurred 11 d before the scheduled termination. No filamentous algae were found during the entire culture period.

Experiment II

This was a replication of the indoor floating container culture I. The only difference was that floating containers were placed in an elliptical tank (378 L) instead of a living stream because of space availability. This experiment lasted for 90 d, from September 26 to December 26, 1995.

On September 18, three species of algae were inoculated (20 ml / container): *Neochloris* (4.75 x 10^6 cells / ml), *Bracteacoccus* (3.58 x 10^6 cells / ml) and

Table 4.—Mean (± SD) of weekly water chemistry characteristics in indoor floating container culture (IFCC) experiments.

Characteristic	IFCC I	IFCC II	Level of significance [a)]
Water temperature (°C)	29.1 ± 2.0	20.8 ± 1.2	$P < 0.001$
pH	9.1 ± 0.3	7.9 ± 0.5	$P < 0.001$
Dissolved oxygen (mg/L)	8.5 ± 0.6	8.3 ± 0.5	NS
Hardness ($CaCO_3$ mg/L)	253 ± 129	345 ± 29	$P < 0.05$
NH_3-N (mg/L)	0.16 ± 0.23	0.11 ± 0.09	NS
NO_2^--N(mg/L)	0.08 ± 0.21	0.04 ± 0.06	NS
NO_3^--N (mg/L)	2.7 ± 2.2	2.0 ± 0.4	NS
PO_4^- (mg/L)	1.75 ± 0.63	1.37 ± 0.97	NS
Light intensity (micro-einsteins . m^2. s^{-1})	48	43	

a) result of t-test of two samples with homogenous variance

Phaeodactylum (4.48 x 10^7 cells / ml). N and P salts also were added and the system started its pre-operation phase. The juvenile *V.* iris stocked in this experiment were from the same group as those used for sediment bed culture V. Five hundred juveniles were stocked in each floating container on September 26, with a stocking density of 220 juveniles / 100 cm^2.

Well water was added frequently to maintain the water level in the reservoir tank. During the later period, a 60 W electrical heater was used because of decreasing water temperatures. Routine water chemistry measurements were taken weekly (Table 4). Juveniles were sampled monthly, the first on October 27, the second on November 28, and the final on December 26. A sample of dead valves also was taken for measurement from each floating container during the final collection. Filamentous algae were absent in this floating container culture system during the entire culture period.

OUTDOOR TANK CULTURE

Culture System

The purpose of this experiment was to develop a suitable outdoor culture technique to rear juvenile mussels. Each experiment had one treatment with three replicates. The outdoor tank system was composed of nine plastic rectangular culture tanks and a large circular reservoir tank (Figure 4). Culture tanks were 92 x 52 x 48 cm, with a volume of 230 L. The reservoir tank was 152 cm (diam) x 61 cm (height), with a volume of 1,106 L. The culture tanks and reservoir tank were connected by PVC pipes.

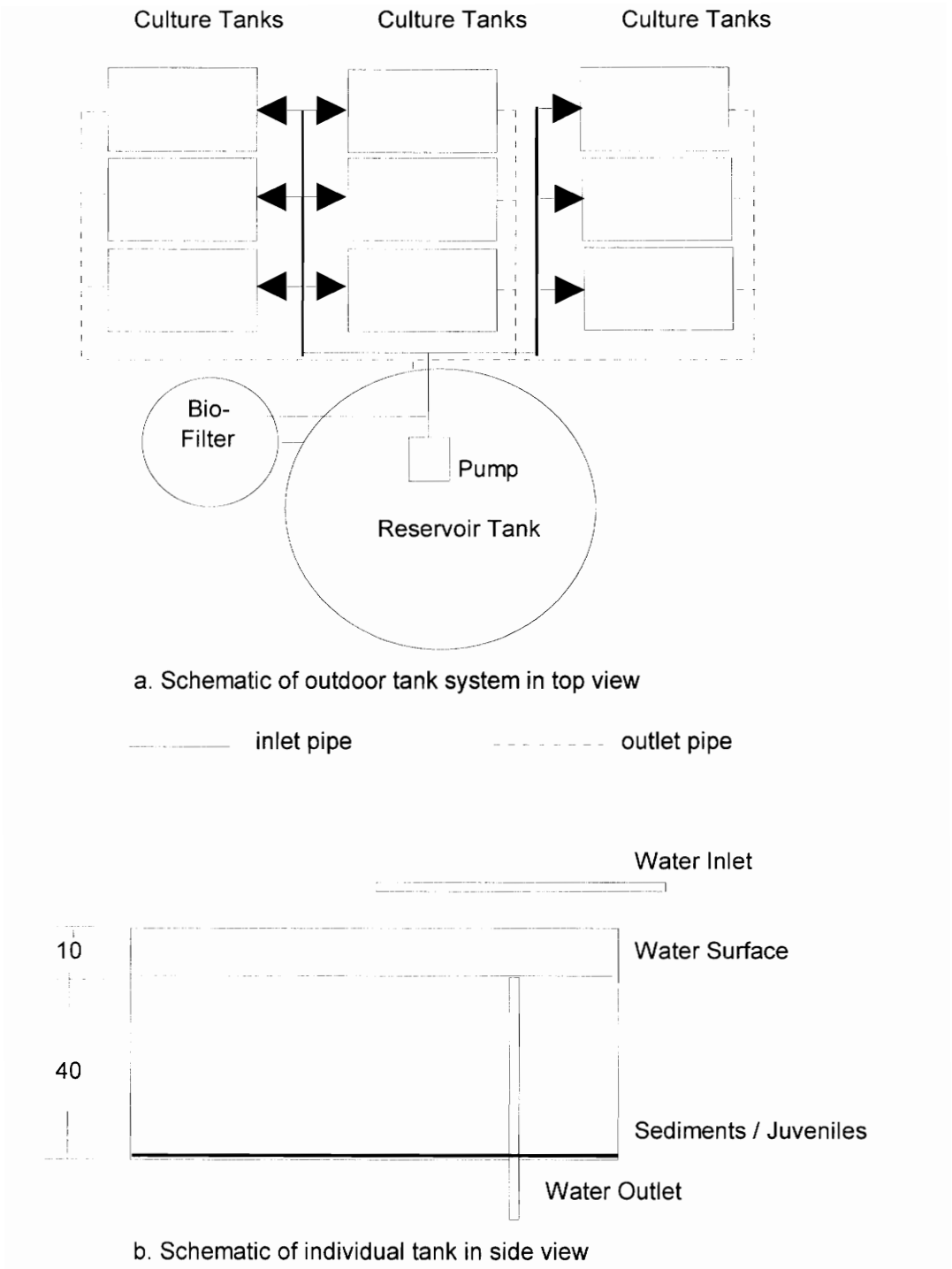

Figure 4.— Outdoor tank used to culture juvenile mussels (side view, units in cm).

Water was recirculated within the system and kept at 40 cm deep. No biofilter was included in the 1994 experiment, whereas a biofilter was connected to the system in 1995. In 1995, due to the suspected interference or predation by insect larvae, all culture tanks were covered by a screen (mesh 1.0 mm), and the reservoir tank was covered by a thick plastic membrane.

Experimental Conditions

One species, *Villosa iris*, was used in this tank culture experiment. Gravid mussels were collected from the Clinch River or North Fork Holston River in southwestern Virginia. Its host fish, rock bass (*Ambloplites rupestris*), was collected as previously described. Metamorphosed juveniles were produced by induced infestation of rock bass with glochidia of *V. iris* (Appendix A).

Sediments were collected from Claytor Lake, Pulaski County, or from Craig Creek, Montgomery County, Virginia, and passed through a 300 μm sieve before placement into the tanks. The sediment layer depth was 5 mm or 1 cm. Dechlorinated Blacksburg town water was the initial water source. The water depth in the culture tanks was 40 cm. The same 3 algal species described previously were inoculated into the tank system. This tank system was set up at least a week before stocking of juveniles, to provide time for algae to colonize the sediments as food supply for introduced juveniles.

Newly metamorphosed juvenile *V. iris* were stocked into the culture tanks; 1,000 (stocking density, 21 individuals / 100 cm^2) or 2,000 juveniles (stocking density, 42 individuals / 100 cm^2) per tank.

The culture system was checked frequently. Well water was added to compensate for evaporation, spillage, or overflow, whenever water level was low. No additional lighting or temperature control facility were provided. In 1995, weekly water chemistry measurements of temperature, pH, dissolved oxygen, hardness, NO_3^--N, NO_2^--N, NH_3-N and PO_4^--P were taken, using the same methods as those described for the sediment bed culture experiment.

Thirty juveniles from each container were randomly selected and measured using a dissecting microscope when stocking of juveniles. Monthly sampling of juveniles was conducted by siphoning all the sediments in one-sixth of the total tank bottom area, and sieving the sediments through a 300 μm mesh. When collecting juveniles, all the sediments were passed through a 600 μm sieve. The sieved contents were examined using a dissecting microscope. All live juveniles were collected, counted and measured.

Experiment I

This experiment occurred from August 12 to November 14, 1994, with three replicates in tanks #1, #5 and #9. Each tank was stocked with 1,000 juveniles. Sediments were obtained from Claytor Lake. The depth of sediments was 5 mm, and sediments were not disinfected.

The tank system began operating on July 30, 13 d before the stocking of juveniles. Twenty ml of *Neochloris* (2.30×10^7 cells / ml), *Bracteacoccus* (4.15×10^6 cells / ml) and *Phaeodactylum* (4.54×10^7 cells / ml) were inoculated into each of the 9 culture tanks. One day post-metamorphosed juveniles were stocked on August 12. The first sample of

juveniles was taken on September 14. This experiment ended on November 14, about 3 months after stocking.

Experiment II

Three tanks of the outdoor tank system, tank #3, #6 and #9, were used in this experiment. Each tank was stocked with 2,000 juveniles and covered with a screen. The increase in stocking density was based on the concern that natural survival rate of age-0 mussels is low, and more juveniles needed to be stocked to increase the probability that some juveniles would survive. Sediments were obtained from Craig Creek and disinfected with bleach before use. Sediment in culture tanks was 1 cm deep.

This experiment began on September 1 and ended on November 20, 1995. Because the tank system was already in use before the stocking of juvenile mussels, only a 3 d pre-operation period was applied. Juveniles of *V. iris* were stocked on September 1, when 30 juveniles from each tank were randomly sampled and measured. No samples were taken during the culture period. The experiment was concluded on November 20, about 80 d after the stocking of juveniles. Water chemistry indices were monitored weekly (Table 5).

OUTDOOR FLOATING CONTAINER CULTURE

Culture System

The main purpose of this experiment was to find a method to rear juvenile mussels outdoors. Floating containers were made from cylindrical plastic buckets, with a

Table 5.—Mean (± SD) of weekly water chemistry characteristics in outdoor tank culture and outdoor floating container culture (OFCC) experiments in 1995.

Characteristic	Tank culture	OFCC
Water temperature (°C)	10.9 ± 7.8	17.4 ± 7.8
pH	7.8 ± 0.6	8.4 ± 0.8
Dissolved oxygen (mg/L)	11.2 ± 2.0	10.8 ± 1.8
Hardness ($CaCO_3$ mg/L)	360 ± 51	283 ± 111
NH_3-N (mg/L)	0.08 ± 0.10	0.12 ± 0.16
NO_2^--N(mg/L)	0.005 ± 0.003	0.008 ± 0.006
NO_3^--N (mg/L)	0.7 ± 0.3	1.3 ± 0.7
PO_4^- (mg/L)	1.49 ± 0.73	1.77 ± 0.75

circular window on the top and two rectangular windows on sides (Figure 5). All three windows were covered with 0.8 mm mesh. These floating containers were suspended in a pond at a depth of 50 cm or placed in the outdoor tank system. The pond was located at the Prices Fork Research Station, Virginia Tech, Blacksburg, Virginia. Pond dimensions were 36 x 36 x 2 m, and water chemistry was as follows: dissolved oxygen, 10.0 mg / L; pH, 8.0; alkalinity, 205 mg / L; hardness, 257 mg / L in August, 1993 (Burress 1995). The outdoor tank system was the same as previously described.

Experimental Conditions

Gravid *Villosa iris* and its host fish, rock bass (*Ambloplites rupestris*), were used to produce metamorphosed juveniles, and had the same sources as those of the above outdoor tank culture experiment. The same sediments as those of outdoor tank culture experiments were used. Floating containers were placed in the pond a week before the stocking of juveniles. Newly metamorphosed juvenile *V. iris* or infested fish were stocked into floating containers.

Floating containers were checked frequently. Container screens were cleaned mechanically with a brush at least twice a month to improve water exchange. In 1995, the following water chemistry indices were measured weekly: temperature, pH, hardness, dissolved oxygen, NO_3^--N, NO_2^--N, NH_3-N and PO_4^--P. Analytical methods were those previously described.

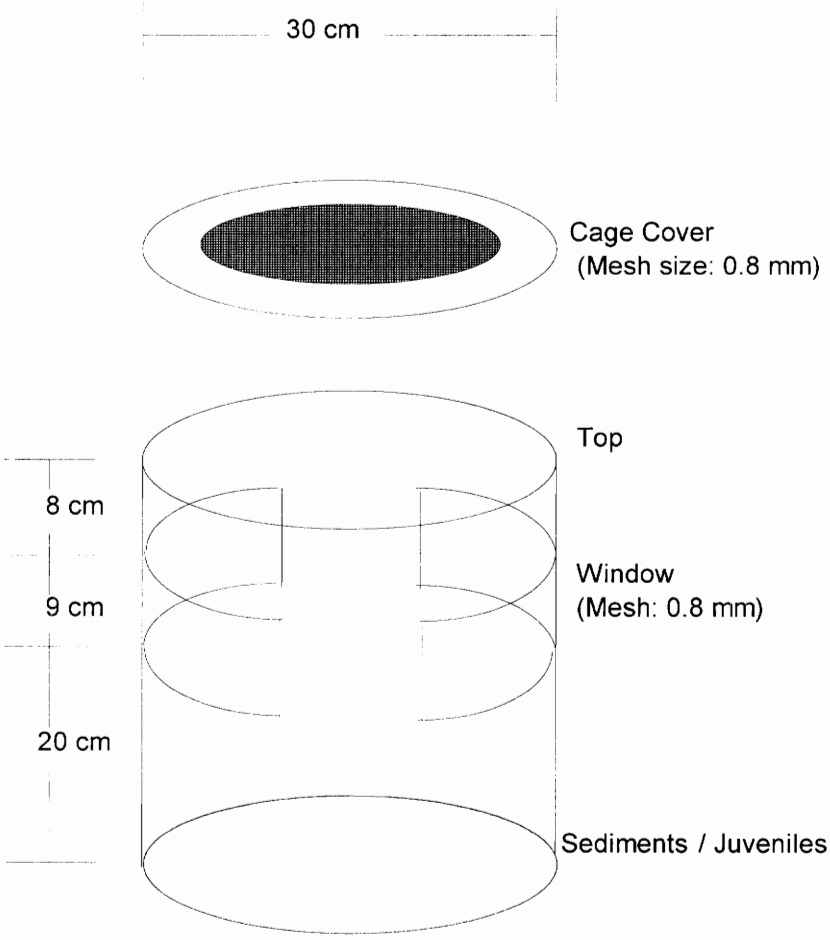

Figure 5.—Diagram of outdoor floating container to culture juvenile mussels (side view, units in cm).

At stocking, 30 juveniles were randomly sampled, and their shell lengths were measured using a dissecting microscope. Monthly samples of juveniles were taken by the same sampling method as described for the outdoor tank culture experiment.

Experiment I

Experiment I was designed to test both the feasibility of using floating containers to culture juvenile mussels and the hypothesis that there was a difference in growth rate and survival between quick-transformed juveniles and slow-transformed ones, i.e., the duration of metamorphosis (the time glochidia remaining on fish) effected later growth. Two groups of floating containers were used in this experiment. One group was composed of juveniles collected on July 3 - 5 (Group 2), the other were obtained on July 13 (Group 1). The two groups of juveniles were collected 9 d apart from the same group of rock bass infested with glochidia from the same group of female *V. iris*. Two hundred juveniles were stocked in each container. This experiment was conducted from August 18 to November 25, 1994.

Before stocking of juveniles, floating containers were placed for one week in the pond. The first monthly sample was taken on September 28. About one quarter of the sediments of the bottom of each floating container were examined. Collected sediments were returned to the original floating containers. When the experiment ended, all sediments of each floating container were collected and examined. Because many hydra were found in each floating container, they were counted from all sieved contents of three randomly selected floating containers.

Experiment II

This experiment evaluated an alternative stocking method by using infested fish instead of post-metamorphosed juveniles. The main goal of this attempt was to reduce the handling stress on newly metamorphosed juveniles due to routine handling.

Each of three floating containers was stocked with one rock bass infested with glochidia of *V. iris* and placed in a culture tank of the outdoor tank system on May 19. These fish were infested with glochidia of *V. iris* on the same day. Since infested fish were stocked instead of juvenile mussels, no pre-operation time occurred. Fish were held there for 24 d before they died. Slides of the gill squashes of the those fish were examined using a microscope. Fewer glochidia attached to the gills, compared those from the fish of a concurrent experiment. Those fish were infested with glochidia at same time and died earlier. Water chemistry indices also were measured (Table 5). This experiment was terminated on October 20.

RESULTS

INDOOR SEDIMENT BED CULTURE

Experiment I

The results of the one month juvenile culture experiment were a mean percental increase of shell length of 53 %, and an average survival rate of only 0.95 % (Table 6). No juveniles survived in compartment #2, whereas a few juveniles survived in compartment #3 and #4. The algal community in the sediments consisted of more genera than were intentionally introduced (Table 3). The dominant taxon was *Scenedesmus,* a genus that was not purposely introduced. The genera inoculated were present at lower densities. Water temperature during the experiment was 24.1 ± 3.8 °C, with the highest standard deviation of all sediment bed culture experiments (Table 2).

Experiment II

Culture conditions were modified for this experiment (Table 1). Results from this experiment suggested that the sediment bed culture technique was potentially useful for raising juvenile mussels, as measured by survival rate and growth rate. The mean survival rate for 80 d was 51.0 %, and juveniles reached a mean shell length of 3.22 mm (Table 7, Figure 6). This corresponded to a 705 % increase in shell lengths (Figure 7). During the course of the experiment, many young mussels were observed burying themselves in sediments.

Table 6.—Growth and survival of juvenile *Villosa iris* in indoor sediment bed culture I, as determined by live juveniles and dead valves.

Time period		Culture compartment			
		#2	#3	#4	Mean
Initial	Number	280	280	280	
	Shell length (mm)				
	Mean ± SD		0.45 ± 0.03		
1 month	Shell length (mm)				
	Mean ± SD	N/A [a)]	0.69 ± 0.12	0.68 ± N/A	0.69 ± 0.12
Survival rate (%)		0	2.5	0.36	0.95
Dead valves	N	20	20	20	
	Shell length (mm)				
	Mean ± SD	0.53 ± 0.05	0.54 ± 0.08	0.51± 0.05	0.53 ± 0.06

a) not applicable

Table 7.—Growth and survival of juvenile *V. iris* in indoor sediment bed culture II, as determined by live juveniles.

Time period		Culture compartment			
		#1	#2	#3	Mean
Initial	Number	500	500	500	
	Shell length (mm)				
	Mean ± SD		0.40 ± 0.08		
1 month	Shell length (mm)				
	Mean ± SD	0.79 ±0.15	0.95 ± 0.25	0.88 ± 0.20	0.88 ± 0.21
2 months	Shell length (mm)				
	Mean ± SD	1.37 ± 0.39	2.02 ± 0.51	2.03 ± 0.68	1.81 ± 0.61
3 months	Shell length (mm)				
	Mean ± SD	2.56 ± 0.72	3.67 ± 0.77	3.42 ± 0.97	3.22 ± 0.95
Survival rate (%) 80 d		33.8	61.0	58.2	51.0

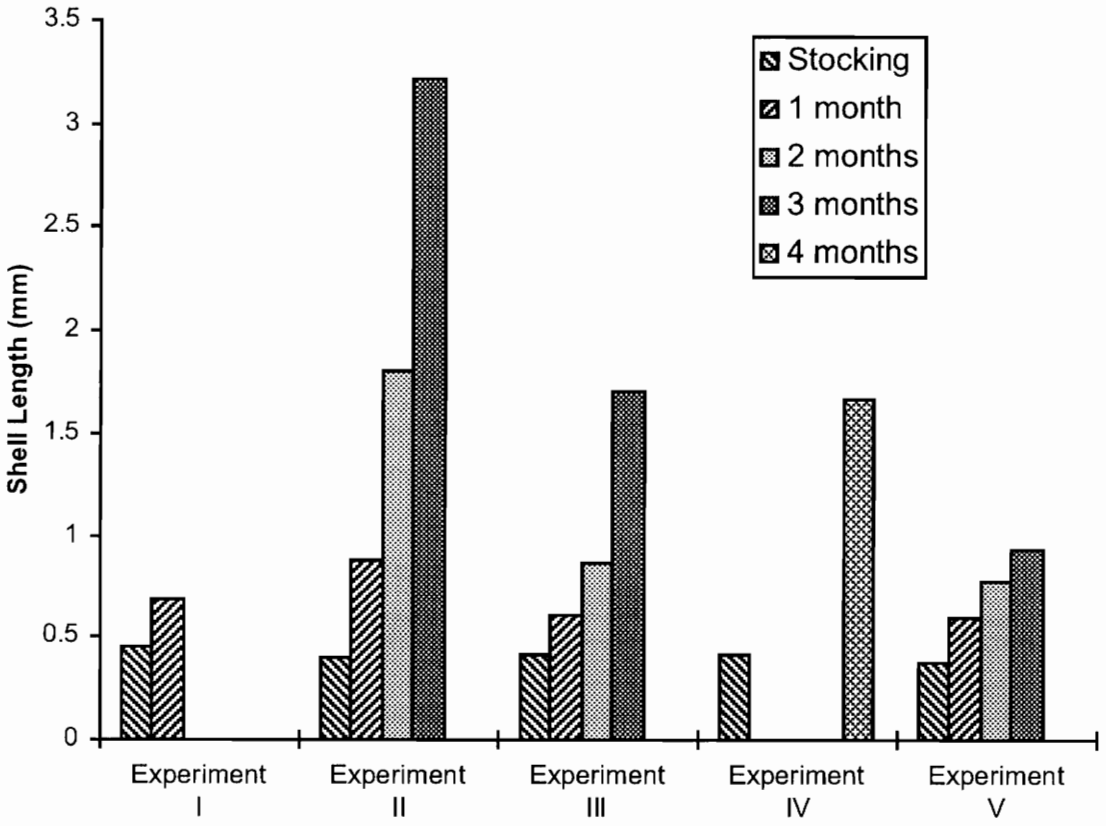

Figure 6.—Comparison of juvenile mussel growth (mm) at monthly intervals in sediment bed culture experiments.

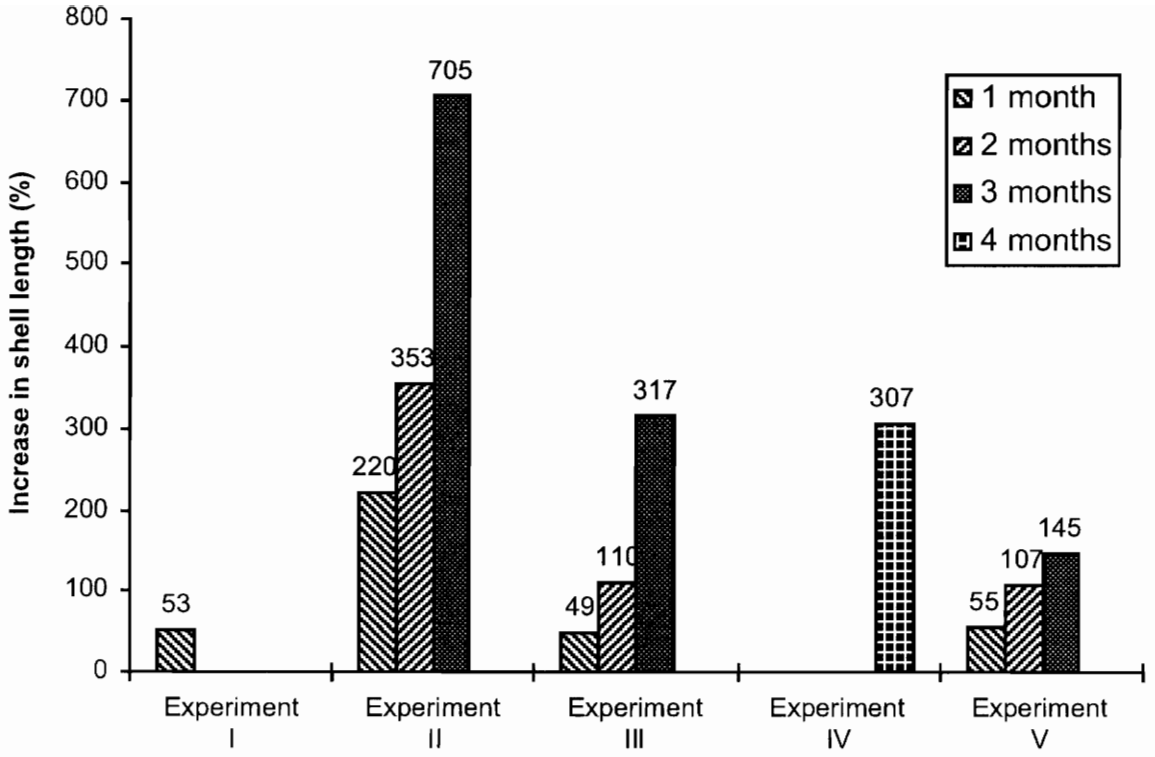

Figure 7.—Percent increase in shell lengths of juvenile *Villosa iris* in sediment bed culture experiments.

Experiment III

This experiment resulted in poor survival, with an average of 1.27 % over the three month culture period (Table 8). Surviving juveniles increased in size by 317 % (Figure 7). During the experiment, filamentous algae grew extensively on the sediment surface of all culture compartments in the second month and thereafter.

Experiment IV

This experiment used the same culture conditions as the former two with exceptions (Table 1). Juveniles increased their shell lengths by 307 % (Figure 7), and achieved a survival rate of 4.9 % (Table 9). The continuous growth of filamentous algae on the sediment surface occurred in the second month and thereafter.

Experiment V

Although the two groups had different stocking densities, mean shell lengths of juveniles of both groups were similar at each sampling (Table 10). Analysis by t-test showed no significant difference in shell lengths of juveniles of all monthly samples between these two groups ($P > 0.05$), which indicated that there was no significant difference in the growth rates of juveniles at the high stocking density vs low stocking density. Then the shell length data of two stocking density groups were pooled (Table 10, Figure 6, 7). The survival rates were 1.40 % for the low density group and 5.37 % for the high density group, respectively; weighted average survival rate was 4.04 % (Table 10). Unfortunately, filamentous algae grew extensively on the sediment surface in the second month and thereafter.

Table 8.—Growth and survival of juvenile *V. iris* in indoor sediment bed culture III, as determined by live juveniles.

Time period		Culture compartment			
		#1	#2	#3	Mean
Initial	Number	500	500	500	
	Shell length (mm)				
	Mean ± SD		0.41 ± 0.04		
1 month	Shell length (mm)				
	Mean ± SD	0.61± 0.13	0.56 ± 0.08	0.66 ± 0.10	0.61± 0.11
2 months	Shell length (mm)				
	Mean ± SD	0.91± 0.25	0.88 ± 0.10	0.79 ± 0.16	0.86 ± 0.20
3 months	Shell length (mm)				
	Mean ± SD	1.73 ± 0.77	1.63 ± 0.26	1.72 ± 0.69	1.71 ± 0.70
Survival rate (%) (3 months)		2.0	0.6	1.2	1.27

Table 9.—Growth and survival of juvenile *V. iris* in indoor sediment bed culture IV, as determined by live juveniles.

Time period		Culture compartment			
		#4	#5	#6	Mean
Initial	Number	500	500	500	
	Shell length (mm)				
	Mean ± SD		0.41± 0.04		
4 months	Shell length (mm)				
	Mean ± SD	1.63 ± 0.37	1.37± 0.27	1.81± 0.39	1.67± 0.39
Survival rate (%) (4 months)		4.8	1.8	8.0	4.9

Table 10.—Growth and survival of juvenile *V. iris* in indoor sediment bed culture V, as determined by live juveniles and dead valves.

Time period		Group		
		Low density	High density	Mean
Initial	Stocking density	83	166	
	(juveniles / 100 cm^2)			
	Shell length (mm)			
	Mean ± SD		0.38 ± 0.03	
1 month	Shell length (mm)			
	Mean ± SD	0.61± 0.09	0.59 ± 0.09	0.60 ± 0.09
2 months	Shell length (mm)			
	Mean ± SD	0.78 ± 0.13	0.79 ± 0.13	0.79 ± 0.13
3 months	Shell length (mm)			
	Mean ± SD	0.92 ± 0.18	0.93 ± 0.13	0.93 ± 0.14
Survival rate (%) (3 months)		1.40 ± 1.56	5.37 ± 3.08	4.04 ± 3.08
Dead valves				
	N	30	30	
	Shell length (mm)			
	Mean ± SD	0.61± 0.06	0.56 ± 0.09	0.59± 0.09

COMPARISON OF SEDIMENT BED CULTURE EXPERIMENTS

Comparison of Growth

Experiment I was quite different from other trials since it only lasted one month rather than 3 or 4 months. Therefore, the data of this experiment were not used in the growth comparison.

Compared to the other experiments, experiment II produced good growth and survival rates. Juveniles in this experiment increased their shell lengths by 705 % in 3 months, compared to 317 % in experiment III in 3 months, 307 % in experiment IV in 4 months, and 145 % in experiment V in 3 months (Figure 7). They had shell lengths significantly greater than those of other experiments after 1 month. This trend continued through the entire course of the experiment (Table 11, Figure 6). These results showed that juveniles of experiment II grew much faster than those of other experiments in the same length of time. Experiment II was conducted at a water temperature ($P < 0.001$), pH ($P < 0.01$) and dissolved oxygen ($P < 0.02$) significantly higher, and harness ($P < 0.05$) significantly lower than those of sediment bed culture III, IV and V, and no occurrence of filamentous algae, which likely contributed to the faster growth rates.

Juveniles of experiment III showed a shell length less than experiment II, not significantly different from experiment IV, and greater than experiment V (Table 11 and 12). Of importance is that experiment IV had a 4 month culture period and sediments of variable particle sizes, while other experiments lasted 3 months and used uniform sediments (Table 1). Juveniles of experiment V had a significantly slower growth rate

Table 11.—Comparisons of mean shell lengths (mm) of sampled juvenile *V. iris* in sediment bed culture II with those of other sediment bed culture experiments (t-test).

Time period	Experiment			
	II	III	IV	V
	Shell lengths (mm)			
Initial	0.40	0.41	0.41	0.38
1 month	0.88	0.61 [a)]	—	0.60
2 months	1.81	0.86	—	0.79
3 months	3.22	1.71	1.67 [b)]	0.93

a) underlined values are significantly different from experiment II ($P < 0.001$)
b) shell lengths of the result of 4 month culture

Table 12.— Comparisons of mean shell lengths of sampled juvenile *V. iris* in sediment bed culture III with those of other sediment bed culture experiments (t-test).

Time period	Experiment		
	III	IV	V
Initial	0.41	0.41	0.38 [a)]
1 month	0.61	—	0.60
2 months	0.86		0.79
3 months	1.71	1.67 [b)]	0.93

a) underlined values are significantly different from experiment III ($P < 0.02$)
b) shell lengths of the result of 4 month culture

than those of other experiments (Figure 6, 7, Table 11, 12). Water quality parameter comparisons (t-test) showed that, as for the eight water quality parameters measured, there were no significant differences between experiments III and IV, between experiments IV and V. The only significant difference between experiments III and V was that experiment V had a higher hardness ($P < 0.01$).

Comparison of Survival Rates

Experiment I had the lowest survival rate of juveniles among all five sediment bed culture experiments, compared to the minimum survival rates of the others (Table 13). The minimum survival rates of experiment II through V were calculated from the number of live juveniles collected during the first sampling process one month after stocking .

Experiment II generated high survival rates. Juveniles in this experiment had a 3 month survival rate of 51.0 %, compared to 1.27 % for experiment III, 4.9 % for experiment IV, and 4.04 % for experiment V. Yate's corrected Chi-square test (Norman and Streiner 1994) was used to compare survival rates between those experiments (Table 14). Experiment II had a significantly higher survival rate than the others ($P < 0.001$).

Experiment IV yielded a survival rate higher than that of experiment III ($P < 0.001$), but not significantly different from that of experiment V ($P > 0.05$) (Table 14). The survival rate of juveniles in experiment V also was higher than that of experiment III ($P < 0.001$) (Table 14).

Table 13.—Comparison of survival rate of juvenile *V. iris* of sediment bed culture I and the minimum survival rates of other sediment bed culture experiments after 1 month.

Experiment No.	Juveniles stocked	Juveniles collected	Minimum survival rate [a] (%)
I	840	8	0.95
II	1,500	127	8.47
III	1,500	125	8.33
IV	1,500	74	4.9 [b]
V	4,500	762	16.93

a) (live juveniles collected / juveniles stocked) x 100 %

b) derived from final survival rate at 4 months.

Table 14.—Comparison of survival rates of juvenile *V. iris* between sediment bed culture (SBC) II and other sediment bed culture experiments, using Yate's corrected chi-square test.

Culture test survival rate	SBC II 51.0 %	SBC III 1.27 %	SBC IV 4.9 %	SBC V 4.04 %
SBC II 51.0 %		$P < 0.001$	$P < 0.001$	$P < 0.001$
SBC III 1.27 %			$P < 0.001$	$P < 0.001$
SBC IV 4.9 %				NS
SBC V 4.04 %				

INDOOR FLOATING CONTAINER CULTURE

Experiment I

In 80 d, juveniles increased their shell lengths by an average of 418 %, from 0.40 to 2.07 mm (Figure 8). Their mean survival rate during the same period was 27.0 %, ranging from 11.4 to 38.8 % (Table 15). There were no filamentous algae growing in the floating containers during the entire culture period.

Experiment II

This experiment generated good survival rates of juveniles over 3 months, ranged from 11.4 to 35.6 %, with an average of 23.3 %. However, the juveniles grew slowly during this time, with a total increase of 126 % in shell lengths, from 0.38 to 0.86 mm (Table 16, Figure 8). No filamentous algae were found during the entire culture period.

Comparison between indoor floating container experiments

Although both indoor floating container culture I and II used the same techniques and procedures, there were significant differences between growth rates of juveniles (Table 17). Juveniles of comparable size at stocking became significantly different in shell lengths after they had been reared in indoor floating containers for a month ($P < 0.001$), and the difference ($P < 0.001$) remained throughout the experiments. Juveniles in experiment I grew much faster than those in experiment II. On the other hand, the survival rates in these two experiments also showed a significant difference (Table 17). Apparently the difference in culture conditions also affected the growth and

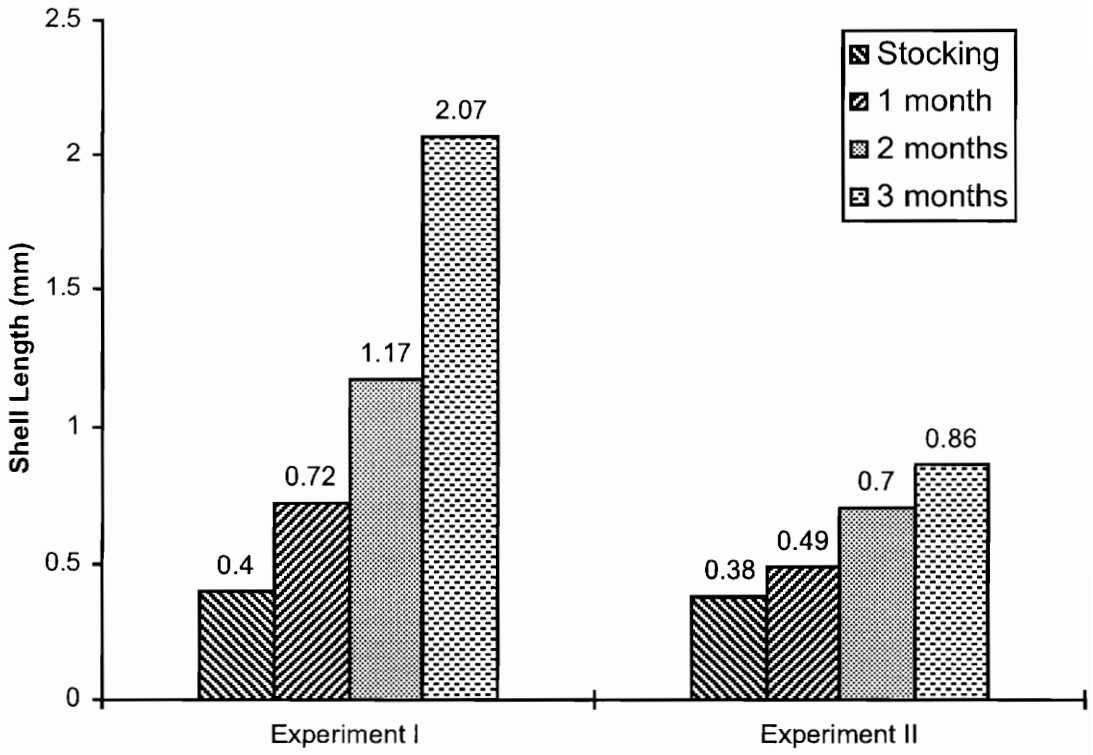

Figure 8.—Comparison of juvenile mussel growth (mm) at monthly intervals in indoor floating container culture experiments.

Table 15.—Growth and survival of juvenile *V. iris* in indoor floating container culture I, as determined by live juveniles.

Time period		Floating container			
		#1	#2	#3	Mean
Initial	Number	500	500	500	
	Shell length (mm)				
	Mean ± SD		0.40 ± 0.08		
1 month	Shell length (mm)				
	Mean ± SD	0.73± 0.12	0.76± 0.12	0.67± 0.12	0.72± 0.12
2 months	Shell length (mm)				
	Mean ± SD	1.22± 0.31	1.33± 0.40	0.97± 0.24	1.17± 0.35
3 months	Shell length (mm)				
	Mean ± SD	2.18± 0.54	2.09± 0.61	1.94± 0.56	2.07± 0.57
Survival rate (%)	3 months	38.8	31.4	11.4	27.0

Table 16.—Growth and survival of juvenile *V. iris* in indoor floating container culture II, as determined by live juveniles and dead valves.

Time period		Floating container			
		#1	#2	#3	Mean
Initial	Number	500	500	500	
	Shell length (mm)				
	Mean ± SD		0.38± 0.03		
1 month	Shell length (mm)				
	Mean ± SD	0.47± 0.07	0.50 ± 0.05	0.49 ± 0.04	0.49± 0.06
2 months	Shell length (mm)				
	Mean ± SD	0.71± 0.12	0.72 ± 0.17	0.68 ± 0.13	0.70 ± 0.14
3 months	Shell length (mm)				
	Mean ± SD	0.85 ± 0.16	0.87 ± 0.18	0.85 ± 0.17	0.86 ± 0.17
Survival rate (%)	(3 months)	22.8	35.6	11.4	23.3
Dead valves					
	N	30	30	30	
	Shell length (mm)				
	Mean ± SD	0.64 ± 0.11	0.65 ± 0.13	0.64 ± 0.08	0.64 ± 0.11

Table 17.—Comparison of shell lengths and survival rate of sampled juveniles in indoor floating container culture I with those in indoor floating container culture II.

Time period	Mean shell length (mm) in IFCC I	Mean shell length (mm) in IFCC II	Significance
Initial	0.40	0.38	NS
1 month	0.72	0.49	$P < 0.001$
2 months	1.17	0.70	$P < 0.001$
3 months	2.07	0.86	$P < 0..001$
Survival rates (Yate's corrected chi-square test)			
	27.0 % (IFCC I)	23.3 % (IFCC II)	$P < 0.025$

a) indoor floating container culture

juveniles, and the comparison of water quality parameters showed that temperature, pH and hardness were significantly different (Table 4).

COMPARISON OF SEDIMENT BED CULTURE AND INDOOR FLOATING CONTAINER CULTURE

The major differences between the these two experiment types are: stocking density of 220 juveniles / 100 cm^2 in the indoor floating container vs 83 juveniles / 100 cm^2 in the sediment bed; water flow across the sediment surface in the sediment bed culture vs water flow generated by compressed air in the indoor floating container culture experiments. Because both sediment bed culture II and indoor floating container culture I, and sediment bed culture V and indoor floating container culture II, were stocked with the same group of juveniles and were conducted concurrently, their growth and survival rates were compared.

Juveniles in indoor floating container culture I had significantly lower growth rate ($P < 0.001$) and survival rate ($P < 0.001$) when compared to those in sediment bed culture II (Table 18). Since both experiments located in the same living stream, water quality parameters were similar (Table 2, 4).

Indoor floating container culture II had a survival rate (23.3 %) significantly higher than that of sediment bed culture V (4.04 %) (Table 18). However, in the shell length comparison, the shell lengths of juveniles in the sediment bed culture V were all significantly greater than those of the indoor floating container culture at all three stages.

Table 18.—Comparison of shell lengths and survival rate of sampled juveniles in sediment bed culture experiments with those in indoor floating container culture experiments. Shell lengths were compared with t-test, and survival rates were compared with Yate's corrected chi-square test.

Sediment bed culture II (SBC II) vs indoor floating container culture I (IFCC I)

	Mean shell lengths (mm)		
Time period	SBC II	IFCC I	Significance
Initial	0.40	0.40	NS
1 month	0.88	0.72	$P < 0.001$
2 months	1.81	1.17	$P < 0.001$
80 d	3.22	2.07	$P < 0..001$
	Survival rate (%)		
80 d	51.0	27.0	$P < 0.001$

Sediment bed culture V (SBC V) vs indoor floating container culture II (IFCC II)

	Mean shell lengths (mm)		
Time period	SBC II	IFCC I	Significance
Initial	0.38	0.38	NS
1 month	0.60	0.49	$P < 0.001$
2 months	0.79	0.70	$P < 0.001$
3 months	0.93	0.86	$P < 0.01$
	Survival rate (%)		
3 months	4.04	23.3	$P < 0.001$

(Table 18). Water chemistry parameters of these two experiments were compared by t-test. Among the 8 parameters, three were significantly different: temperature, pH and hardness (t-value and significance were; 5.048 and $P < 0.001$, -3.723 and $P < 0.01$, and 3.778 and $P < 0.01$, respectively).

OUTDOOR TANK CULTURE

Experiment I

The first sample of juveniles was taken on September 14, 1994. Two tanks (#1 and #5) failed to collect live juveniles, while 20 juveniles were sampled from tank #9. One concern at that time was that the juveniles may be too small to be retained by the 300 μm sieve; i.e., they could not be separated from sediments of particle sizes less than 300 μm. No further sampling was conducted until the end of experiment. In the 3 months, juveniles grew 296 % in shell length, but only 0.7 % of individuals survived (Table 19). During the first and final samplings, many insect larvae were found in sediments.

Experiment II

Although two modifications were made, using screen lids and increasing stocking density, this experiment still generated poor results for both growth rate and survival (Table 19). The application of screen lids was not a practical way to get rid of insect

Table 19.—Growth and survival of juvenile *V. iris* in the outdoor tank culture experiments, as determined by live juveniles.

Time period		Tank			
		#1	#5	#9	Mean
		Outdoor tank culture I			
Initial	Shell length (mm)				
	Mean ± SD	0.27 ± 0.05	0.25 ± 0.004	0.24 ± 0.003	0.25 ± 0.02
1 month	Shell length (mm)				
	Mean ± SD	N/A[a)]	N/A	0.61 ± 0.24	0.61 ± 0.24
3 months	Shell length (mm)				
	Mean ± SD	1.01 ± 0.35	0.96 ± N/A	0.99 ± 0.12	0.99 ± 0.10
Survival rate (%) (3 months)		0.7	0.1	1.3	0.7
		Outdoor tank culture II			
Initial	Number	2,000	2,000	2,000	
	Shell length (mm)				
	Mean ± SD		0.43 ± 0.04		
Survival rate (%)		0	0.5	0	N/A

a) not applicable

larvae because they could not cover the culture system tightly. There were still many insect larvae found in the tanks.

OUTDOOR FLOATING CONTAINER CULTURE

Experiment I

No live juveniles were found during the first and final samplings of the experiment (Table 20). Many hydra were found, and their numbers (65 ± 38.4 hydra / container) were counted for all sediments of three randomly selected containers (Table 20).

Experiment II

No live juveniles were found during the final sampling. Since the outdoor floating containers were placed in the outdoor tank system and no hydra were found during final sampling, the potential hydra predation problem was eliminated.

Table 20.—Growth and survival of juvenile *V. iris* in outdoor floating container culture I, as determined by live juveniles. Samples of hydra are also included.

	Group 1			Group 2		
	#1	#2	#3	#4	#5	#6
Initial Shell length (mm)						
Mean ± SD		0.39 ± 0.01			0.38 ± 0.01	
Survival rate (%) 3 months		0			0	
Hydra count (number of hydra / container)						
Mean ± SD			65 ± 38.4			
Density[a)]			9 hydra / 100 cm^2			

a) The bottom area of cage was 706.5 cm^2.

DISCUSSION

INDOOR SEDIMENT BED CULTURE

The main design features of the sediment bed system to culture juvenile mussels were derived from characteristics of the natural habitat of *Villosa iris* and the protocols for rearing juvenile mussels in Asia. Since little information was available on the growth requirements of this species, culture experiments were conducted under conditions that were assumed to be suitable. The major considerations were:

1) *V. iris* occurs in a flowing water environment;

2) Nano- and micro- algae (2 - 200 μm) are important food sources for juvenile mussels;

3) The presence of silt enhances juvenile mussel growth;

4) Growth of algae requires light and nutrients.

In this study, sediment bed culture I resulted in a poor survival. A comparison of its culture conditions with those of the other experiments indicated the following possible causes for poor success: constant light, low water exchange rate, and temperature fluctuations. The constant light may have interfered with the regular circadian rhythm of juveniles. Some freshwater bivalves, such as *Corbicula fluminea*, *Toxolasma parva* and *Ligumia subrostrata,* display diurnal cycles of metabolic activities (McMahon 1991). Bright light stimulates mussel movement, probably to start the mussel on its quest for deeper water (i.e., less light) and away from the shallows with the hazards of desiccation and predation (Fuller 1974). Other possible factors were low water exchange rate and

temperature fluctuation, which effects on juvenile growth and survival will be discussed later.

The likely factor contributing to the faster growth of juveniles in experiment II was water quality, which included significantly higher water temperature (28.0 ± 2.2 °C), higher pH (9.1 ± 0.3), higher dissolved oxygen (8.5 ± 0.6 mg / L) and lower hardness (253 ± 128.6 mg $CaCO_3$ / L) than experiment III, IV and V. The low survival rates of juveniles in experiments III, IV and V may be the result of interference of filamentous algae, which grew extensively on the sediment surface. An observation was that there was no difficulty to sample the required sample size (20 individuals) in the first monthly sampling in all sediment bed culture experiments and the second monthly sampling in experiment II (Table 13). However, the second monthly sampling of most culture compartments (89 %) in experiments III and V failed to sample 20 or more juveniles. This coincided with the occurrence of filamentous algae on the sediment surface, which were present in the second month and thereafter, in these experiments. The effects of water quality parameters and filamentous algae on juvenile growth will be discussed later.

Juveniles in experiments III and IV had similar percent increases in shell lengths. Both experiments used juveniles produced from the same female mussel, and were located in the same culture system and at the same time. No significant difference of water quality parameters was found. The differences between these two experiments were as follows: for experiment IV, 1) no monthly sampling during the culture period except

for the initial and final, i.e., no disturbance during rearing; 2) variable particle size (< 2 mm) of sediments; and 3) a longer culture time (Table 1). One possible explanation for the two experiments having similar shell length increases was that the influence of filamentous algae. This factor was dominant over other factors and likely altered the results of both experiments in a similar manners. Two filamentous algae were dominant species; namely, *Oscillatoria sp.* and *Spirogyra sp* (Appendix B), a blue-green and a green alga, respectively. Mechanical removal was the only method used to crop filamentous algae because of the possible sensitivity of juvenile mussels to algicides. Despite the continuous removal efforts, algae still grew profusely on the sediment surface. As for the comparison of experiments III and V, juveniles in experiment III had a greater initial shell lengths ($P < 0.01$) and lived in water with less hardness ($P < 0.01$), which likely contributed to the faster growth rate of juveniles in experiment III than in experiment V.

To summarize, the sediment bed culture technique showed great potential in rearing juvenile mussels, with respect to growth and survival rates. Although current experimental results varied, which may have been caused by the interference of filamentous algae, most experiments generated acceptable survival rates (4.04 - 51.0 % for 3 months) and modest growth rates (145 - 705 % increase in shell length in 3 months). This culture technique needs improvement, especially in the methods to prevent the interference of filamentous algae. My suggestion is to use an additional algae incubation tank, which is connected to the current system, to produce dense planktonic algae. This

tank can act as a constant supply of algae for the micro- and nano- algal community in sediments to suppress filamentous algae, and a supplemental food source over time. If filamentous algae are found on the sediment bed then it is necessary to shade the sediment beds or turn off lights to reduce light, and use the algae incubation tank as the main food sources for 1 - 2 weeks.

INDOOR FLOATING CONTAINER CULTURE

These experiments applied similar culture measures as the sediment bed culture. Both indoor floating container culture experiments generated good survival rates (23.3 - 27.0 % for about 3 months) and modest growth rates (86 - 207 % increase in shell length in about 3 months). This culture technique was a suitable method to raise juvenile mussels.

Comparison of sediment bed culture and indoor floating container culture experiments (Table 18) showed that the sediment bed culture technique provided better growth conditions for juvenile *V. iris* and the seemingly inconsistent survival results were due to the interference of filamentous algae in sediment bed culture V.

OUTDOOR TANK CULTURE

Repeated attempts to use an outdoor tank system to culture juvenile *Villosa iris* met with poor survival (< 1 %), suggesting that my outdoor tank culture system was not suitable for this species. One possible reason for this was the lack of water flow at the

bottom of the tanks. Water in the culture tanks was maintained at a depth of 40 cm, and both the water inlet and outlet were at the surface. The rate of water entering the culture tank was not high since it was common for the outlet pipe to get blocked by air bubbles which resulted in an overflow. Though water was exchanged rapidly in terms of tank turnover, there was possibly little or no water current near the tank bottom where the juveniles lived. *Villosa iris* is a stream species that occurs in streams and creeks with clean, flowing water. Therefore, water currents may be crucial for their growth and survival. Another problem was the water temperature of 10.9 ± 7.8 °C, too low for juveniles to exhibit considerable growth. My suggestion to improve the tank system is to modify the culture tank: 1) to use a shallow tank (height about 20 cm), 2) to keep a water depth of 10 cm, and 3) to place the water inlet and outlet on the opposite short sides of the tank to provide water flow across sediments.

OUTDOOR FLOATING CONTAINER CULTURE

No juveniles survived in either experiment of this study. Similar outdoor culture experiments in suspended bucket in two static ponds using juvenile *Villosa iris* produced no survival either (Burress 1995). Possible causes for failure of these experiments were the following reason.

1) Predation by hydra. Large numbers of hydra (9 hydra / 100 cm^2) inhabited the sediments in experiment I. Hydra have been a problem in marine mussel culture (Quayle and Newkirk 1989), and they can prey on crustaceans, worms, insect

larvae and others they encounter (Slobodkin and Bossert 1991) and young mussels (Li-fan Jiang 1996, pers. comm.).

2) Unsuitable environment. Because *V. iris* lives in flowing water and has a high requirement for dissolved oxygen (Li-yen Chen 1995, pers. comm.), floating container culture in static water may not be suitable.

My suggestions to improve the outdoor floating container culture are: 1) decrease the distance between side windows and container bottom from 20 cm to 5 -10 cm; 2) place the containers in a gently flowing water body or use aerators to generate water flow.

COMPARISON WITH OTHER CULTURE EXPERIMENTS

Some experiments in culturing juvenile mussels have been reported, but it is difficult to compare growth rates among species due to inherent growth rate differences. After being in floating cages for 67 d, *Lampsilis luteola* exhibited a 52-fold increase in shell length (Howard 1922). Hudson and Isom (1984) reported an 18-fold increase in shell length of *Utterbackia imbecillis* juveniles in 74 d, with a survival rate of less than 1.0 %. In a creekside trial, newly metamorphosed juvenile *Velesunio angasi* almost doubled in size, and 93 % individuals survived after 7 d (Humphrey 1990). Gatenby (1994) reported a 250 % increase in shell length and 51.6 % survival rate for juvenile *Villosa iris* in 60 d. Buddensiek (1995) cultured juvenile *Margaritifera margaritifera* in cages for up to 52 months, and recorded a maximum shell length of 6.40 cm. On the other hand, juvenile mussels suffer high mortality during early age. Most mortality of *M.*

margaritifera occurs at the young mussel stage, just after leaving the fish host, and it is density independent. Only 5% of young mussels survived between leaving the fish host and establishing in the substrate (Young and Williams 1983, 1984). Buddensiek (1995) recorded most mortality during the first months in his culture experiment of *M. margaritifera.* No data on the natural mortality of juvenile *V. iris* are available. Furthermore, different culture techniques usually involved different culture conditions and affected juvenile growth and survival, which was confirmed by the comparisons of juvenile growth and survival in sediment bed culture experiments with those in indoor floating container culture experiments (Table 18).

RELATED ENVIRONMENTAL FACTORS

Water Temperature

Although freshwater bivalve Q_{10} values (the acute, temperature-induced changes in the metabolic rate with a 10 °C increase in temperature) vary between and within species, ranging seasonally from < 1.0 to 9.76 (Burky 1983), an immediate temperature increase causes a corresponding increase in the metabolic rate of ectothermic bivalves (McMahon 1991). Water temperature affects the growth rate of juveniles since growth is the direct result of organism metabolic processes. An experiment with *Utterbackia imbecillis* revealed a barely significant growth increase at 30 °C over 23 °C (Hudson and Isom 1984). Water temperature affected not only the success of reproduction of *Margaritifera margaritifera*, but also the growth of adult mussels (Hruska 1992). Water

temperature showed a highly significant positive correlation with the growth and mortality of juvenile *M. margaritifera*, which illustrated the delicate balance necessary for a species adapted to cold water (Buddensiek 1995). In my sediment bed culture II, higher water temperature was the likely reason that juveniles grew faster than those in other sediment bed experiments. A higher water temperature ($P < 0.001$) was the most likely reason that juveniles in indoor floating container culture I grew faster than in indoor floating container culture II.

Freshwater mussels are ectothermic animals. Species have specific upper and lower limits for survival and reproduction (Burky 1983), with a limited capacity for the regulation of metabolic rate. Three types of acclimation occur in freshwater bivalves: 1) no capacity for acclimation; 2) partial acclimation; 3) reverse acclimation (McMahon 1991). Juvenile *Utterbackia imbecillis* exhibited inverse acclimation which was different from that of adult mussels (Polhill and Dimock 1996). In my study, the fluctuations of water temperature in sediment bed culture I may also have affected juvenile growth and survival. Juvenile *V. iris* can survive water temperatures up to 31 °C.

Algae

Filamentous Algae Filamentous algae seemed to have negative effects on juvenile mussels by depleting nutrients required for the growth of smaller planktonic and benthic algae. The latter are the important food sources for juvenile mussels. Filamentous algae also colonized the sediment surface and then likely interfered with the feeding and locomotion activities of juvenile mussels. In the pond culture of hard clam (*Meretrix*

lusoria), filamentous algae, such as *Enteromorpha* and *Chaetomorpha*, often develop and compete with phytoplankton for nutrients (Chen 1990). Large green algae, such as *Ulva* and *Entermorpha,* may cause fouling problem in marine bivalve culture (Quayle and Newkirk 1989). Filamentous algae, such as *Spirogyra*, *Zygnema* and *Mougeotia,* could not only attach to mussel shells and interfere with mussel's respiration and feeding, but consume inorganic nutrients needed for plankton and influence mussel growth (Cai and Huang 1989). Filamentous algae can be removed manually, by stocking fish that consume filamentous algae (Higginbotham 1983; Chen 1990; Lee 1993), or using a shallow seine (Heinen et al. 1988).

Algal Composition Samples of sediments showed a complex algal community in the sediments of the sediment beds and indoor floating containers. Although the inoculated algae could be found in the samples, the dominant algae were other genera. Inoculating algae to build an algal community on sediments to supply food for juvenile mussels was not as effective as presumed. Algal counts also showed that species composition was favorable, composed mainly of green algae and diatoms.

Water Currents

Flow, or water exchange, is important for determining the growth potential of mussels since mussels are passive filter feeders and rely on water currents to bring in food and remove metabolic wastes. Water exchange rates will affect mussel growth. Four marine bivalves, *Choromytilius meridionalis, Aulacomya ater, Mytilus galloprovincialis* and *Perna perna*, grew more rapidly at a high water circulation site than in one with

restrict circulation (Van Erkon Schurink and Griffiths 1993). Water current also can be critical for juvenile mussel survival. A larger flow coming in contact with the mussels provided enough total plankton for growth of juvenile *Utterbackia imbecillis*, while other designs with less flow failed (Hudson and Isom 1984). Humphrey et al. (1990) indicated that the only requirement for successful field rearing of one-day-old *Velesunio angasi* in both a creekside system and an *in situ* enclosure was a constant turnover of creek water. Growth rate differences were presumed to be caused by differences in water turnover and availability of potential food items. Decreasing water exchange rate reduced the survival rate of juvenile *Hyriopsis cumingii*; juvenile survival rate was 31 % and 4.4 % when water exchange rate was 0.5 - 2 tons / h and 0.25 tons / h, respectively, and all juveniles eventually died when water exchange rate was only 0.025 tons / h (Ye et al. 1990).

In my study, although no specific experiment on the effect of water flow was undertaken, some results suggested its effects. Water currents may be critical for growth and survival of this riverine species, *Villosa iris*. Lack of water currents in the tank bottom zone may be the main cause for the failure of the outdoor tank culture experiments. In the first outdoor floating container culture, except for the hydra predation problem, less water flow may be another important factor causing the death of all juveniles since outdoor floating containers were placed in a small static pond. Low water exchange rate may be one cause of low survival rate in sediment bed culture I as well.

Other Factors

Sediments The importance of silt in the practice of culturing juvenile mussels has been confirmed (Hudson and Isom 1984; Gatenby 1994). Sediment types also affected shell growth of *Lampsilis radiata* (Hinch et al. 1986), and mussels have showed an ability to select habitat (Bailey 1989). Fine, uniform sediments were used in my experiments. The main purpose of using this type of sediment was to ensure the reliability of sampling juveniles, because of the practical difficulty of finding mussels less than 1 cm long, even in extensively sieved substrate samples (Young and Williams 1984). This type of substrate is different from that in natural habitat, composed of particles of variable sizes. Uniform, fine sediments may have influenced juvenile growth and survival. However, it is not a crucial factor since the majority of experiments in my study used this type of substrate and some of them had very high growth and survival rates.

Water Chemistry Indices Although calcium ions are most important for freshwater mussels (Burkey 1983), no published values of requirements for mussel species are available (Fuller 1974). In my sediment bed culture V, the high hardness (422 $\pm$ 61 mg $CaCO_3$ / L, $P < 0.01$), together with smaller initial juvenile size (0.38 mm, $P < 0.01$), likely contributed to slower growth of juveniles than in sediment bed culture III ($P < 0.001$). Mussels can tolerate a relatively wide range of pH values, and pH has less of an impact on mussels than is suspected (Fuller 1974). Although the majority of species prefer alkaline waters with pH above 7.0, they can grow and reproduce over a pH range of 5.6 - 8.3 (McMahon 1991). Some "riffle species", including juvenile and adult

Amblema plicata, require 2.5 mg / L dissolved oxygen for survival at laboratory temperatures (Imlay 1971). Among water chemistry indices, phosphate, calcium and pH has shown a relationship to mortality rate; and eutrophication not only inhibited the growth of young mussels in the critical stage, but it also caused a steep rise in mortality of older mussels (Bauer 1983). In my study, pH, hardness and dissolved oxygen, together with water temperature, may have contributed to the differences in juvenile growth and survival.

RECOMMENDATIONS FOR JUVENILE MUSSEL CULTURE

Juvenile mussels should be reared in sediments in a flowing water system (water exchange rate > 120 L / h). Initial inoculation of algae (green algae and diatoms, multiple species), fertilization with N and P salts (N:P = 15:1), and provision of time (> 7 d) could be used to improve the algal community in the system and provide food for juveniles. Light with a light:dark cycle is provided for the algal growth, with a light intensity of 50 - 60 micro-einsteins.m^2.s^{-1} . Filamentous algae, hydra and other fouling organisms and predators should be controlled.

The recommended water parameter ranges for culturing juvenile *V. iris* are as follows:

water temperature	28 °C,
pH	7.5 - 8.5,
dissolved oxygen	> 8.0 mg / L,

hardness	200 - 300 mg $CaCO_3$ mg / L,
PO_4-P	1.5 - 2.0 mg PO_4 / L,
NH_3-N	< 0.15 mg / L,
NO_2^--N	< 0.08 mg / L,
NO_3^--N	2.0 - 3.0 mg / L.

SUMMARY

Sediment Bed Culture

The combination of water flow, inoculation of algae, lighting in a 12:12 h dark:light cycle, fertilization, a pre-operation phase, and the sediment bed culture technique produced modest growth rates (145 - 705 %, with a mean of 369 %, increase in shell lengths in 3 or 4 months) and variable survival (1.27 - 51.0 %, with a mean of 15.3 %, in 3 or 4 months) of juvenile *Villosa iris*. This indoor technique was a potentially suitable culture technique for rearing newly metamorphosed juveniles, but needs further improvement, especially the prevention of filamentous algae from colonizing sediments.

Indoor Floating Container Culture

The experimental results showed that using indoor floating containers, with the application of water flow, inoculation of algae, lighting in a 12:12 h dark:light cycle, nutrient enrichment, and a pre-operation phase, provided a suitable method to rear newly metamorphosed juvenile mussels. Juvenile *V. iris* increased their shell lengths by 86 - 207 % (mean 147 %); and had survival rates of 23.3 - 27.0 % (mean 25.2 %), in about 3 months.

Outdoor Tank Culture

The technique tested was not suitable for rearing juvenile mussels. Unsuitable culture conditions were problematic, and lack of water flow near the tank bottom where juveniles lived was presumably detrimental to juvenile survival.

Outdoor Floating Container Culture

These experiments yielded negative results, at best partially due to unsuitable culture conditions and predation by hydra.

Related Factors

Water temperature affected growth and survival of juvenile mussels in both the sediment bed culture and the indoor floating container culture systems. Higher temperatures accelerated the growth of juveniles. In sediment bed culture experiments, filamentous algae likely influenced juvenile growth and survival. Water flow may have had an important effect on the growth and survival of juveniles of *Villosa iris* in both outdoor and indoor culture systems. Other water quality parameters such as pH, dissolved oxygen and hardness may also contribute to juvenile growth and survival.

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APPENDIX A PRODUCTION OF JUVENILE MUSSELS

Mussel -- Collected gravid *Villosa iris* were kept indoors in a living stream with chilled water to prevent them from releasing their glochidia. Some gravid mussels collected in late October of 1994 overwintered in the outdoor tank system without losing their glochidia.

Fish -- Rock bass (*Ambloplites rupestris*) were caught by electrofishing.

Before Infestation -- Before being put into the holding tank in the laboratory, fish were treated with 1% salt solution for 10 - 20 min to get rid of possible external parasites. Fish were then kept for at least 3 d to adapt to the new environment before the glochidial infestation.

Collecting Glochidia -- Some glochidia were flushed into a small dish with a clinical syringe with water. Their maturity was tested with a small amount of salt added to the water. Glochidial movement was observed through a dissecting microscope. If glochidia clapped their valves rapidly, they were mature and ready for infesting fish. Then, all glochidia were flushed out from the marsupial gills of gravid mussels.

Infesting Fish -- Usually, two fish were infested with glochidia from one gill of the mussel. Fish were kept in a 5 gallon bucket with 1/5 -1/4 water. Glochidia were dumped into the water. After being in contact with glochidia for about 20 -- 30 min at room temperature, fish were taken out and moved into aquaria. Each fish was kept in a separate aquarium.

Maintenance -- A third of the water was changed every other day to avoid water quality degradation in aquaria. This was crucial in the first several days because fish slough mucus after being infested with glochidia. Fish were not fed throughout the infestation.

Collecting Juveniles -- The aquarium bottom was siphoned on alternate days and filtered through a 150 or 200 μm sieve. The filtered contents were examined through a dissecting microscope. Metamorphosed juveniles were collected with a pipette.

Holding Juveniles -- Juveniles were held in small containers with silt (particle size < 130 μm). Water was aerated and changed every day. Juveniles were fed with algae cultures of the same three species used in culture experiments. What should be pointed out was that this holding was good only for a short period. Juveniles should be stocked into permanent culture containers as soon as possible. My experience showed that most would not survive for 30 d.

APPENDIX B DESCRIPTION OF RELATED ALGAE GENERA

Bracteacoccus Green alga. Cells spherical with many discoid chloroplasts and without pyrenoids. 5 - 20 μm. Reproduction by zoospores.

Chlamydomonas Green alga. 10 - 20 μm, unicellular species. Cell wall may be smooth at the anterior end, or may be one or two apical papillae. The inner wall is thin, smooth, and very finely striated with parallel fibrils. The chloroplast has one or more pyrenoids with starch sheaths. Asexual reproduction by zoospores. Most species reproduce sexually through isogametes (Prescott 1968).

Chlorella Green alga. Cells spherical or spheroidal, 3 - 8 μm. There is one thin, parietal chloroplast either with or without pyrenoids. Species are both marine and freshwater. Autospores are the only known reproductive elements (Prescott 1968).

Closterium Green alga. Cell sickle-shaped, bow-shaped or fusiform with finely attenuated apices. Two clearly ridged chloroplasts contain several pyrenoids (Pentecost 1984).

Cyclotella Diatom. 5 - 10 μm. Cells solitary or in short chains, pill-box shaped, often seen in girdle view. These small diatoms are widely distributed, particularly in the plankton (Pentecost 1984). Reproduction is by vegetative cell division (Prescott 1968).

Fragilaria Diatom. Frustules joined into ribbons by the valve faces. Valves fusiform, sometimes centrally gibbons or with capitate ends. Striae are fine and usually parallel (Pentecost 1984).

Navicula Diatom. 5 - 10 μm. It includes many common benthic diatoms. They are pennate diatoms with a true raphe on both valves. They occur in all kinds of aquatic habitats (Pentecost 1984). Reproduction is by vegetative cell division (Prescott 1968).

Neochloris Green alga. 5 - 12 μm. Round cells having cup-shaped chloroplasts and pyenoids. Reproduction usually by zoospores (Prescott 1968).

Oscillatoria Filamentous blue-green alga. Trichomes straight or bent, often of great length and either isolated or in dense bundles. Sheaths are absent and gliding motility is common in the benthic forms. Trichomes olive, blue-green or brownish (Pentecost 1984). It was not considered as a good mussel food.

Phaeodactylum Diatom. 5 - 10 μm. There is polymorphism of cell form, usually fusiform or oval.

Scenedesmus Green alga. Cells in small colonies with or without regularly arranged spines on the two end cells. Each colony consists f a single, or occasionally double row of (2)4-16 cells, the walls of which are often minutely granular or ridged. The species are abundant in all kinds of water but mainly in eutrophic ponds, often among vegetation (Pentecost 1984).

Spirogyra Filamentous green alga. The chloroplasts shich are spiral ribbons vary in number from one to as many as 15 and may be loosely or tightly coiled. The three-layered cross wall shows a middle lamella of pectose bounded on either side by layers of pectin. Reproduction is either scalariform or lateral, and occurs at different time of the year according to species. Zygospores form in the female gametangium (Prescott 1968).

APPENDIX C CULTURE OF ALGAE IN LABORATORY

All uni-algal cultures of the three species used in this study were cultured in the laboratory using different liquid culture media.

Neochloris: Neochloris medium or Bristol's plus soil extract

Bracteacoccus: OCM or Bristol's plus soil extract

Phaeodactylum: Bristol's plus soil extract

Culture media composition followed Gatenby (1994). Culture media were sterilized through autoclaving. Uni-algal cultures were inoculated into media and placed inside an incubator with cool white fluorescent lights at 20 °C. Media were shaken frequently to accelerate algal growth.

A hemacytometer was used to count algae cells, and then the number was converted to algal cells per milliliter. For final sampling of those algal cultures, they were concentrated by being centrifuged at 5,000 rpm for 20 min. They were then suspended in city water in a high concentration.

Concentrated algal cell suspensions were kept dark in refrigeration at 4 °C.

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Engineer, Laboratory of Fish Disease, Zhejiang Institute of Freshwater Fisheries, Huzhou, Zhejiang, China.

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Assistant Engineer, Laboratory of Fish Disease, Zhejiang Institute of Freshwater Fisheries, Huzhou, Zhejiang, China.

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Assistant Engineer, Laboratory of Aquaculture, Zhejiang Institute of Freshwater Fisheries, Huzhou, Zhejiang, China.

PUBLICATIONS

Yang, C., J. Dong, and G. Yang. 1991. Epidemiological investigation of the outbreak disease of freshwater fishes in Zhejiang province. Freshwater Fisheries (Chinese) 1991(6):1-3.

Chen, Y., C. Yang,, and L. Yao. 1988. The relationship between hemorrhage disease of grass carp (*Ctenopharygodon idellus*) and ecological environment. Acta. Ecologica Sinica (Chinese) 8(3): 242-249.

RESEARCH EXPERIENCE

Culture and artificial production of juvenile freshwater mussels in laboratory.

Research on prevention and treatment of viral hemorrhage disease of grass carp through immunization and culture environment improvement.

Research on prevention, treatment and diagnosis of bacterial diseases of cultured fishes.

Pond culture of fishes.

Diagnosis and treatments of fish and other aquatic cultured animal diseases.