CULTURE OF FRESHWATER MUSSEL GLOCHIDIA IN AN ARTIFICIAL HABITAT UTILIZING COMPLEX LIQUID GROWTH MEDIA

S P E C I F I C A T I O N

TO ALL WHOM IT MAY CONCERN:

Be it known that we, Billy G. Isom and Robert G. Hudson, citizens of the United States, and residents of Killen, County of Lauderdale, and State of Alabama, and Clinton, County of Laurens , and State of South Carolina, respectively, while employed by the Tennessee Valley Authority, a corporation existing under and by virtue of an Act of Congress, as a full time employee and under the provisions of a personal services contract, respectively, by virtue of and incidental to such employment, have invented a new and useful improvement in CULTURE OF FRESHWATER MUSSEL GLOCHIDIA IN AN ARTIFICIAL HABITAT UTILIZING COMPLEX LIQUID GROWTH MEDIA, of which the following is a specification.

Mailed to P.O. 12-3-81

FIELD OF INVENTION.

The present invention relates to a method and composition of matter essential to the transformation of the glochidia of freshwater mussels to the juvenile stage. More particularly, the present invention relates to a process whereby the normal period of host specific parasitism on fish is reproduced in an artificial habitat, which is essential to commercial or conservation management of some species, especially where the specific fish host requirement is unknown.

BACKGROUND OF THE INVENTION

Due in part to the complex life-cycle of freshwater mussels usually involving a parasitic stage on fish host and to unknown environmental or habitat requirements, many freshwater mussel species have been recognized as declining in numbers and/or extirpated from historical habitats. Therefore, approximately 15 percent of all animal species recognized by law as endangered include various species of freshwater mussels.

Initiation of research leading to the present invention was for the specific purpose of developing a culture medium for use in conservation of endangered freshwater mussels. However, the instant invention is highly relevant to management and conservation of commercial freshwater mussel species shells of which were used historically for making buttons and presently for making nuclei for cultured pearls.

Historically, Ellis et al (1926, Science, Vol. 54, No. 1667: pp 579-580; and 1929 Trans. Amer. Fish Soc., Vol. 59: pp 217-223) claimed to have obtained transformation of freshwater mussel glochidia to young adult mussels; however, if they did, neither the process nor the composition of matter was ever published to the extent their work could be reproduced. The research described herein utilized processes and composition of matter unavailable during Ellis's research and certainly during the period of their purported successes.

Since each gravid mussel may have tens of thousands of glochidia, it is obvious that if they could be cultured artifically there would be

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Abstract of the Disclosure

A process, and a composition of matter utilized therein, to obtain transformation of the glochidia of freshwater mussels to the juvenile stage. In nature, glochidia normally transform while as parasites on fish. The instant artificial process involves the use of cell culture and bacteriological techniques to best assure environmental integrity during the protracted culture period. The composition of the media includes a combination of the blood serum of fish and commonly available tissue culture fluids and inorganic salts. A nonspecific component of fish serum in combination with the media supra was determined to be essential for glochidial transformation to juveniles.

ADVANTAGES OF THE INVENTION

A primary advantage of the present invention over use of natural means of mussel propagation is that a mussel species host determination is unnecessary.. Identification of natural mussel fish host is made difficult, not only by the diversity of fish fauna that have to be tested, but also the host identification is complicated by the fact that a true host may be rejected due to natural immune responses. The present invention obviates this problem since there can be no immune response development in the artificial cultures.

Another advantage of the present invention is that a colony of very rare or endangered mussels could be developed as a base for use in ultimate discovery of a host fish for natural propagation. The invention has potential application to management of commercial mussel species used in pearl culture.

Still another potential advantage of the present invention is that unlike in the vissitudes of nature, where most glochidia are thought to die, thousands of glochidia released by a single female mussel could be grown for ultimate release or management.

DESCRIPTION OF THE INVENTION

The present invention will be better understood from a consideration of the following description taken in connection with the accompanying tabulated data of the essential compositions of matter and the various steps of the process used in carrying out the invention.

Composition of Matter Used to Transform Freshwater Mussel Glochidia to Juveniles

Complex Artificial Growth Medium (CAGM)

The complex artificial growth media, or CAGM as it will hereafter be referred to for the sake of convenience, comprises (1) inorganic salts; (2) amino acids grouped as essential amino acids and nonessential amino acids; (3) vitamins; and (4) glucose.

The composition of inorganic salts used in the CAGM is a modification of the "unionid ringers fluid" introduced by Ellis, Merrick, and Ellis (1930,

significant commercial and conservation potential. There appear to be no historical data base other than the claims of Ellis et al, supra, relevant to the present invention.

Since glochidia parasitizing fish are known to obtain some essential stimulus and nourishment from the fish blood serum in order to transform from the larval or glochidial stage to the young adult state, it was thought that the best chance of artificial habitat culture was to simulate this complex chemical environment in the artificial habitat. Thus, a complex medium was developed which, when combined with blood serum of fish in a process, was found to be essential for glochidial transformation to the juvenile stage.

PRIOR ART

Biologists have long recognized the parasitic relationship between freshwater mussels and their specific fish host. However, researchers in this area apparently are not aware of any prior art specifically related to transformation of glochidia of freshwater mussels by artificial means.

OBJECTS OF THE INVENTION

It is therefore an object of the present invention to develop a method or process whereby glochidia of freshwater mussels can be handled during an extended period necessary for their transformation to juvenile mussels.

Another object of the present invention is to report development of a composition of matter essential to the transformation of freshwater mussel glochidia under artificial conditions.

Still further and more general objects of the present invention will appear from the more detailed description set forth in the following description and examples.

Table II (continued)

Nonessential Amino Acids

Compound	Concentration (mg/l) Limits	Preferred
L - alanine	1-12	8.9
L - asparagine	1-15	13.2
L - aspartic acid	1-15	13.3
glycine	1-10	7.5
L - glutamic acid	1-20	14.7
L - proline	1-15	11.5
L - serine	1-15	10.5
taurine	3-35	31.0
L - ornithine	1-12	10.0

The vitamins present in the CAGM, the same as those used by Eagle, supra, for cell and tissue cultures, are shown in Table III below.

Table III

Vitamins

Compound	Concentration (mg/l) Limits	Preferred
Choline chlorine	0.1-2	1.0
Folic acid	0.1-2	1.0
Inositol	0.2-4	2.0
Nicotinamide	0.1-2	1.0
Calcium pantohthenate	0.1-2	1.0
Pyridoxal	0.1-2	1.0
Riboflavin	0.1-2	0.1
Thiamine	0.1-2	1.0

The remaining components in the artificial portion of the medium are shown in Table IV below.

Table IV

Other Compounds

Compound	Concentration (mg/l) Limits	Preferred
Glucose	100-1500	1000.0
Phenol red (optional)	1-15	10.0

The antibiotics and antimycotics used in the CAGM are listed in Table V below.

Bulletin Bur. Fish, Vol. 56, pages 509-542) and is found in Table I below. Essential modification of the fluid included deletion of dibasic sodium phosphate, and addition of 2.2 gm $NaHCO_3$ per 1000 milliliters of unionid ringers fluid.

Table I

Inorganic Salts

Compound	Concentration (mg/l) Limits	Preferred
$CaCl_2$	600-1400	1200
$MgCl_2 \cdot 6H_2O$	500-1200	1000
NaCl	700-1700	1530
KCl	50-125	99
$NaHCO_3$	1100-2400	2200

The amino acids in the CAGM are the same as those used by Eagle (1959, Science, Vol. 130, pages 432-437) for cell and tissue cultures with the exception of the addition of taurine and ornithine which are constituents of fish blood. The amino acids are shown in Table II below.

Table II

Essential Amino Acids

Compound	Concentration (mg/l) Limits	Preferred
L - arginine	10-120	105
L - cystine	2-26	24
L - histidine	3-35	31
L - isoleucine	5-55	52
L - leucine	5-55	52
L - lysine	6-60	58
L - methionine	1-20	15
L - phenylalanine	3-35	32
L - threonine	5-50	48
L - tryptophane	1-12	10
L - tyrosine	3-40	36
L - valine	5-50	46

force) for 10 minutes followed at 3000 rpm (approximately 1000 RCF) for 10 minutes, in a refrigerated centrifuge, and the supernatant blood serum and a few formed cellular elements, such as for example blood cells are removed. The antibiotics and an antimycotic are added to the serum in the same concentration as shown in Table V, supra. The serum should be pressure filtered through a 0.45 μm or smaller pore size filter as described in the process below. This natural portion of the medium is used as 20-80 percent of the total glochidial medium.

The Invention Process

Following selection of gravid mussels from their habitat, they should be transported to the laboratory site in water obtained from the immediate habitat area.

Upon receipt of the gravid mussels, a specimen is selected, the valves are pried open gently, and both the anterior and posterior adductor muscles are severed utilizing a sterilized knife blade or scapel. Gills containing glochidia are severed with sterile surgical type scissors and removed to a small beaker (250-500 ml) containing sterile deionized water. Use of deionized water is essential to prevent premature closure of the glochidia, i.e., if they sense the presence of certain ions in the water, for instance Cl^-, they close up prematurely. At this point, all washing and transfer work is conducted in a laminar airflow cabinet of the high-efficiency particulate air (HEPA) filter type. Standard bacteriological and/or tissue culture sterile techniques are adhered to in all processing and transfer of media and glochidia.

Glochidia are separated from the gill tissue to the extent practical. In some species the glochidia are in a mucoid or conglutinate mass. When fully mature the glochidia should separate from this mass by agitation or gentle movement in the deionized water utilizing tweezers.

The glochidia are washed several times with sterile deionized water, utilizing the following simple procedure. Set up a standard glass filtering

Table V

Antibiotics and Antimycotic

Compound	Concentration
Antibiotics	
Streptomycin sulfate	800 µg/ml
Penicillin G. sodium salt	800 U/ml
Oxytetracycline	50 µg/ml
Antimycotic	
Amphotericin B	5 µg/ml

The antibiotics and antimycotic concentrations noted in Table V, supra, are the basic concentrations used in cell culture experiments. In our experiments, it was determined that even four times or greater a concentration can be used to control infection. All the agents listed in Table V are used in the concentration noted in the basic medium except oxytetracycline which is only used to control bacterial infections when penicillin and streptomycin prove ineffective.

The above components (Tables I-V) are combined and 2.85 ml of 1.25 M NaOH added per 100 ml of CAGM solution to bring the pH up to the range of about 7.8 to about 8.0. All of the above solutions are pressure filtered through a standard 0.45 µm or less pore size membrane for final sterilization. A larger pore size will not retain all of the bacteria and a smaller pore size makes filtration difficult; it clogs up prematurely. This complete artificial portion, or pH adjusted CAGM with antibiotics and antimycotic, which is hereinafter referred to for the sake of convenience and conciseness as pH-CAGM-AA, is used as 20-80 percent of the total glochidial medium.

Whole fish serum serves as a natural protein source in the glochidial medium. Fish blood is obtained by cardiac puncture of any number of fresh-water fish including bass, catfish, carp, buffalo, and suckers, with a sterile syringe coated with sterile sodium heparin, in concentration of 1000 U/ml, and a 18 gauge needle. We have found that a larger size needle will pick up tissues and a smaller size needle tends to rupture red blood vessels. The blood is centrifuged at 1000 rpm (approximately 100 relative centrifugal

the length of which, of course, varies with certain conditions such as temperature as well as species of glochidia being transformed, the glochidia mature to the juvenile stage, after which some may be transferred back to natural habitat wherein same are allowed to further mature to adult mussels. Usually the protracted period for freshwater mussels ranges from about 14 to about 21 days.

INVENTION PARAMETERS

After sifting and winnowing through the data herein presented, as well as other results and operations of our novel process including the use of our new and novel compositions of matter utilized therein for ensuring the transformation of glochidia of freshwater mussels to the juvenile stage in artificial habitat, which processes and compositions of matter are eminently suited to ensure environmental integrity during the artificial and protracted cultural period and thereby effect significant commercial and conservation management relations, the operating variables and preferred conditions for carrying out our process wherein our new compositions of matter are utilized therein are summarized below.

flask with a tube to fit a pasteur pipette. Attach a sterile pipette and vacuum the water from the glochidia. Add more water and swirl the glochidia gently; vacuum water off again. Repeat this procedure several times to restore tissue debris, dead glochidia, bacteria, and protozoa. At this point, the glochidia are ready to be transferred to the complex media, described supra.

Although it is not absolutely necessary, a quick check of glochidial condition and maturity can be accomplished as follows: Transfer a few glochidia (up to 100) to 2-3 ml of artificial medium. The glochidia will exhibit almost immediate closure. Sixty to ninety percent or greater closure indicates that the glochidia are in good condition and/or mature.

All media should be tested bacteriologically the day prior to use by culture on nutrient-Agar. Check the bacteriological samples the day of use for positives. Filtration should be used to sterilize contaminated media, or make new media.

The growth medium is measured into tissue culture dishes of the following types: either the 60 mm wide by 15 mm deep, which are preferred, or 100 mm wide by 20 mm deep tissue culture dishes with surface treated for cell attachment and that are optically clear. Pipette 3 mls of media into the 60 mm wide dishes or 10 mls into the 100 mm wide dishes. Use a pasteur pipette to transfer from 50-200 glochidia into each dish. Glochidia are then transferred to an airflow CO_2 incubator. The CO_2 incubator is essential to control the pH between about 7.2 to about 8.3, preferably between about 7.2 and 7.4 since a carbonate buffer system is present in the media. Twenty-three degrees Celsius should be used for incubation of the glochidia.

In the initial cultures, centrifuged, unfiltered blood serum can be used in the medium. At least the first change of media can be with unfiltered serum. In order to keep down infections, pressure filtered (0.45 μm) serum is preferred initially and is used thereafter. The culture media should be changed at least every three days, preferably by the use of a pasteur pipette attached to a vacuum source. After a protracted period,

80:20 experimental replicates with a decreasing rate of development down to 20:80. Development was infrequent in the 100:0, 10:90, and 0:100 dishes, with no live glochidia by the fourth day. In terms of yield, a test involving the same concentrations of serum with six replicate cultures each, produced transformation only in the dishes with high serum concentration. See Table VI below.

Table VI

Number of Developing Glochidia in Six Repetitions of Cultures at Different Concentrations of Serum

Serum concentration (in % of total medium)	Percent of glochidia closed at one day range and mean	($S\bar{x}$)	Percent of closed showing advanced transformation, range and mean	($S\bar{x}$)
0	51.0-68.3, 50.1	(2.3)	0, 0	(0)
10	66.3-87.6, 77.7	(3.6)	0, 0	(0)
20	77.8-90.5, 84.7	(2.0)	0, 0	(0)
40	73.9-90.2, 85.2	(2.5)	0-7.1, 2.1	(1.3)
60	80.3-93.1, 87.2	(1.8)	19.4-62.1, 33.8	(7.8)
80 (only 3 reps)	80.2-86.9, 83.3	(2.0)	44.6-57.8, 51.2	(3.8)

The 20-80 percent serum allowed initial closure and development significantly higher than the 0 to 10 percent serum concentrations. Additionally, the advanced transformation was best on 80 percent, decreasing in 60 and 40 percent and absent in 20 percent.

While we have shown and described particular embodiments of our invention, modifications and variations thereof will occur to those skilled in the art. We wish it to be understood, therefore, that the appended claims are intended to cover such modifications and variations which are within the true scope and spirit of our invention.

Variables	Range	Preferred
CAGM		
Inorganic salts	Modified unionid ringers fluid Table I, supra	Modified unionid ringers fluid Table I, supra
Amino acids	Modified eagles Table II, supra	Modified eagles Table II, supra
Vitamins	Eagles Table III, supra	Eagles Table III, supra
Other compounds		
Glucose, mg/l	100-1500	1000
Phenol red, mg/l	1-15	10
NaOH (sufficient to adjust pH)	7.2-8.3	7.2-7.4[1]
Antibiotics/Antimycotics (final solution)[2]		
Streptomycin sulfate, µg/ml	400-2000	800
Penicillin G, sodium salt U/ml	400-2000	800
Amphotericin B, µg/ml	1-25	5
Oxytetracycline, µg/ml	50-300	50
Total blood serum, percent		
Initial culture	20-80	80
Final culture	0-80	20
Total artificial medium, percent (pE-CAGM-AA)		
Initial culture	20-80	20
Final culture	20-100	80
Incubator		
pH	7.2-8.3	7.2-7.4[1]
Temperature, ^{o}C	18-28	23
Rate filtered air to incubator ℓ/hr	Sufficient to maintain pH range, supra	
Rate CO_2 to incubator, ℓ/hr	Sufficient to maintain pH range, supra	

[1] 7.3 most preferred.
[2] In CAGM, supra, or blood serum, infra, or both.

Fish blood serum was necessary to stimulate development in all species tested. The concentration of this serum determined the rate of development as well as the number of glochidia developing. In the initial medium, 80 percent serum was used with 20 percent artificial medium, i.e., pE-CAGM-AA. Test cultures were made varying the present serum and artificial medium respectively as follows: 0:100; 10:90; 20:80; 40:60; 60:40; 80:20; 100:0. In the above cases, early development occurred most rapidly in the

Inorganic salts

Compound	Concentration (mg/l)
$CaCl_2$	600-1400
$MgCl_2 \cdot 6H_2O$	500-1200
NaCl	700-1700
KCl	50-125
$NaHCO_3$	1100-2400

Essential Amino Acids

Compound	Concentration (mg/l)
L - arginine	10-120
L - cystine	2-26
L - histidine	3-35
L - isoleucine	5-55
L - leucine	5-55
L - lysine	6-60
L - methionine	1-20
L - phenylalanine	3-35
L - threonine	5-50
L - tryptophane	1-12
L - tyrosine	3-40
L - valine	5-50

Nonessential Amino Acids

Compound	Concentration (mg/l)
L - alanine	1-12
L - asparagine	1-15
L - aspartic acid	1-15
glycine	1-10
L - glutamic acid	1-20
L - proline	1-15
L - serine	1-15
taurine	3-35
L - ornithine	1-12

Vitamins

Compound	Concentration (mg/l)
choline chlorine	0.1-2
folic acid	0.1-2
inositol	0.2-4
nicotinamide	0.1-2
calcium pantohthenate	0.1-2
pyridoxal	0.1-2
riboflavin	0.1-2
thiamine	0.1-2

Other compounds

glocose	100-1500
phenol red (optional)	1-15

What we claim as new and desire to secure by Letters Patent of the United States is:

1. A process for the transformation of glochidia of freshwater mussels to the juvenile stage wherein the normal period of host specific parasitism on fish is reproduced in an artificial habitat, which process comprises the steps of:

(1) removing from gravid mussels the glochidia therein and transferring same into sterile deionized water wherefrom are removed the undesirable portions thereof including tissue debris, dead glochidia, bacteria, and protozoa;

(2) removing at least a portion of the resulting decontaminated glochidia from said sterile de-ionized water and transferring same into a mixture of complex artificial growth medium and fish blood serum, said complex artificial growth medium having an adjusted pH of about 7.8 to about 8.0 and said complex artificial growth medium and said blood serum containing predetermined quantities of antibiotics and antimycotics, said mixture of artificial growth medium and blood serum ranging from about 20 to about 80 percent and about 80 percent to about 20 percent, respectively; said predetermined quantities of antibiotics antimycotics in said artificial growth medium and said blood serum ranging in concentrations therein as follows:

Compound	Concentration,	
Antibiotics		
Streptomycin sulfate	800-3200	µg/ml
Penicillin G. sodium salt	800-3200	U/ml
Oxytetracycline	50-200	µg/ml
Antimycotic		
Amphotericin B	5-20	µg/ml

and said complex artificial growth medium comprising predetermined quantities of inorganic salts, amino acids, including essential amino acids and nonessential amino acids, vitamins and other compounds including glucose as follows:

2. The process of claim 1 wherein the initial culture medium subjected to said incubation means contains about 40 to 20 percent by weight complex artificial growth medium and about 60 percent to about 80 percent by weight blood serum, wherein said culture medium proportions are adjusted during subsequent substitutions thereof in said incubation means such that the final concentration of said mixture ranges from about 80 to about 60 percent artificial complex growth medium and from about 20 to about 40 percent blood serum.

3. The process of claim 1 wherein the initial culture medium subjected to said incubation means contains about 20 percent by weight complex artificial growth medium and about 80 percent by weight blood serum, wherein said culture medium proportions are adjusted during subsequent substitutions thereof in said incubation means such that the final concentration of said mixture contains about 80 percent artificial complex growth medium and about 20 percent blood serum.

4. The process of claim 1 wherein the inorganic salts, amino acids, vitamins, and other compounds in said artificial growth medium are as follows:

(3) subjecting said glochidia in said mixture of artificial complex growth medium and said blood serum to incubation means wherein the temperature is maintained from about $18^{\circ}C$ to about $28^{\circ}C$ and wherein carbon dioxide enriched air is introduced and withdrawn in sufficient quantities to effectively control the pH in said mixture in the range from about 7.2 to about 8.3;

(4) subsequently and periodically removing from contact with said glochidia the resulting culture medium comprising said mixture of complex artificial growth medium and blood serum and substituting therefor a quantity of fresh culture medium, the length of time between said periodic substitution of fresh culture medium ranging from about 1 to about 3 days and the number of substitutions being sufficient to ensure the maturing of said glochidia to the juvenile stage; and

(5) subsequently removing from said incubation means the resulting juvenile mussels and returning same to natural habitat.

5. The process of claim 4 wherein the initial culture medium subjected to said incubation means contains about 40 to about 20 percent by weight complex artificial growth medium and about 60 to about 80 percent by weight blood serum, wherein said culture medium proportions are adjusted during subsequent substitutions thereof in said incubation means such that the final concentration of said mixture ranges from about 80 to about 60 percent artificial complex growth medium and from about 20 to about 40 percent blood serum.

6. The process of claim 4 wherein the initial culture medium subjected to said incubation means contains about 20 percent by weight complex artificial growth medium and about 80 percent by weight blood serum, wherein said culture medium proportions are adjusted during subsequent substitutions thereon in said incubation means such that the final concentration of said mixture contains about 80 percent artificial complex growth medium and about 20 percent blood serum.

7. The process of claim 1, or 2, or 3, or 4, or 5, or 6 wherein the temperature in said incubation means is maintained at about 23°C and wherein the pH of the culture medium therein is maintained in the range of about 7.2 to about 7.4.

Inorganic Salts

Compound	Concentration (mg/l)
$CaCl_2$	1200
$MgCl_2 \cdot 6H_2O$	1000
NaCl	1530
KCl	99
$NaHCO_3$	2200

Essential Amino Acids

Compound	Concentration (mg/l)
L - arginine	105
L - cystine	24
L - histidine	31
L - isoleucine	52
L - leucine	52
L - lysine	58
L - methionine	15
L - phenylalanine	32
L - threonine	48
L - tryptophane	10
L - tyrosine	36
L - valine	46

Nonessential Amino Acids

L - alanine	8.9
L - asparagine	13.2
L - aspartic acid	13.3
glycine	7.5
L - glutamic acid	14.7
L - proline	11.5
L - serine	10.5
taurine	31.0
L - ornithine	10.0

Vitamins

Compound	Concentration, (mg/l)
choline chlorine	1.0
folic acid	1.0
inositol	2.0
nicotinamide	1.0
calcium pantohthenate	1.0
pyridoxal	1.0
riboflavin -	0.1
thiamine	1.0

Other Compounds

Compound	Concentration, (mg/l)
glucose	1000.0
phenol red (optional)	10.0

Inorganic Salts

Compound	Concentration (mg/l)
$CaCl_2$	600-1400
$MgCl_2 \cdot 6H_2O$	500-1200
NaCl	700-1700
KCl	50-125
$NaHCO_3$	1100-2400

Essential Amino Acids

Compound	Concentration (mg/l
L - arginine	10-120
L - cystine	2-26
L - histidine	3-35
L - isoleucine	5-55
L - leucine	5-55
L - lysine	6-60
L - methionine	1-20
L - phenylalanine	3-35
L - threonine	5-50
L - tryptophane	1-12
L - tyrosine	3-40
L - valine	5-50

Nonessential Amino Acids

Compound	Concentration (mg/l)
L - alanine	1-12
L - asparagine	1-15
L - aspartic acid	1-15
glycine	1-10
L - glutamic acid	1-20
L - proline	1-15
L - serine	1-15
taurine	3-35
L - ornithine	1-12

Vitamins

Compound	Concentration (mg/l)
choline chlorine	0.1-2
folic acid	0.1-2
inositol	0.2-4
nicotinamide	0.1-2
calcium pantohthenate	0.1-2
pyridoxal	0.1-2
tiboflavin	0.1-2
thiamine	0.1-2

Other Compounds

Compound	Concentration (mg/l)
glucose	100-1500
phenol red (optional)	1-15

8. A new composition of matter eminently suitable for effecting transformation of glochidia of freshwater mussels to the juvenile stage wherein the normal period of host specific parasitism on fish is reproduced in an artificial habitat, said composition of matter comprising a mixture of complex artificial growth medium and fish blood serum, said complex artificial growth medium having an adjusted pH of about 7.8 to about 8.0 and said complex artificial growth medium and said blood serum containing predetermined quantities of antibiotics and antimycotics, said mixture of artificial growth medium and blood serum ranging from about 20 to about 80 percent and about 80 percent to about 20 percent, respectively; said predetermined quantities of antibiotics and antimycotics in said artificial growth medium and said blood serum ranging in concentrations therein as follows:

Compound	Concentration
Antibiotics	
Streptomycin sulfate	800-3200 µg/ml
Penicillin G. sodium salt	800-3200 U /ml
Oxytetracycline	50-200 µg/ml
Antimycotic	
Amphotericin B	5-20 µg/ml

and said complex artificial growth medium comprising predetermined quantities of inorganic salts, amino acids, including essential amino acids and non-essential amino acids, vitamins, and other compounds including glucose as follows:

Inorganic Salts

Compound	Concentration (mg/l)
$CaCl_2$	1200
$MgCl_2 \cdot 6H_2O$	1000
NaCl	1530
KCl	99
$NaHCO_3$	2200

Essential Amino Acids

Compound	Concentration (mg/l)
L - arginine	105
L - cystine	24
L - histidine	31
L - isoleucine	52
L - leucine	52
L - lysine	58
L - methionine	15
L - phenylalanine	32
L - threonine	48
L - tryptophane	10
L - tyrosine	36
L - valine	46

Nonessential Amino Acids

L - alanine	8.9
L - asparagine	13.2
L - aspartic acid	13.3
glycine	7.5
L - glutamic acid	14.7
L - proline	11.5
L - serine	10.5
taurine	31.0
L - ornithine	10.0

Vitamins

Compound	Concentration, (mg/l)
choline chlorine	1.0
folic acid	1.0
inositol	2.0
nicotinamide	1.0
calcium pantohthenate	1.0
pyridoxal	1.0
riboflavin	0.1
thiamine	1.0

Other Compounds

Compound	Concentration, (mg/l)
glocose	1000.0
phenol red (optional)	10.0

9. A new composition of matter eminently suitable for effecting transformation of glochidia of freshwater mussels to the juvenile stage wherein the normal period of host specific parasitism on fish is reproduced in an artificial habitat, said composition of matter comprising a mixture of complex artificial growth medium and fish blood serum, said complex artificial growth medium having an adjusted pH of about 7.8 to about 8.0 and said complex artificial growth medium and said blood serum containing predetermined quantities of antibiotics and antimycotics, said mixture of artificial growth medium and blood serum ranging from about 20 to about 80 percent and about 80 percent to about 20 percent, respectively; said predetermined quantities of antibiotics and antimycotics in said artificial growth medium and said blood serum ranging in concentrations therein as follows:

Compound	Concentration
Antibiotics	
Streptomycin sulfate	800-3200 µg/ml
Penicillin G. sodium salt	800-3200 U/ml
Oxytetracycline	50-200 µg/ml
Antimycotic	
Amphotericin B	5-20 µg/ml

and said complex artificial growth medium comprising predetermined quantities of inorganic salts, amino acids, including essential amino acids and nonessential amino acids, vitamins, and other compounds including glucose as follows: