Howard 2002

Comments

I reviewed the Phase I proposal of this project, and provided an overall negative view of its likelihood of success, based on the lack of technical expertise by the PI (Greg Howard). After reviewing their putative preliminary success, my opinion has not changed. Here are my straightforward comments on this much larger request ($297K) for this risky venture.

1. Excuse my skepticism, but it appears to me that this husband and wife team set up Southern Aquatics L.L.C. as a woman-owned business solely to improve their chances to receive SBIR funds. Mrs. Howard provided no evidence of "extensive experience in business management and R+D", and Mr. Howard was a simple shell buyer with no experience in research or aquaculture. I suspect that neither has sufficient training in what they are attempting to do.
2. Phase II- Previous studies with the holding of riverine mussels in ponds has shown relatively high survival during the first year, but increasing mortality in years 2 and 3. Therefore, the high survival reported after year 1 in this study is expected but cannot be assumed in subsequent years. The PI (p. 13) begins by describing "flow-through trough culture tanks", then describes pond conditions, then describes growth in tanks (p. 14). What is his actual culture system?

Implantation- No actual data are presented on mabe production or quality. It is relatively easy to produce "domed pearls" by inserting plastic domes between the mantle tissue and shell. There is inadequate description of their "nuclei" or how they determined the rate of nacre deposition. Gonadal implants are much more difficult to achieve, and Tennessee Pearl Company was generally unsuccessful in producing such pearls, even with a Japanese implant-trained specialist. I don't see evidence of their success in this method, only that 58-71 % of the mussels survived the procedure. The bottom of pages 17-18 discuss topics beyond their capabilities and project objectives. There is no mention in this section as to whether the mussels are growing in their culture systems.

Phase II- Southern Aquatics has shown marginal success in my opinion because their report lacks the rigor for me to agree that, "the initial goals set forth in Phase I project have been reached".

"In order to make an impact on the existing pearl market, the volume of pearls produced will be an important factor". This was and is a major weakness that I described in my initial review of this project. China has produced ~500 tons of freshwater pearls in the late 1990's and is now pushing close to 1000 tons/year. They have out-competed the competitors and now rule the world market. They can sell baroque and round pearls cheaper than anyone else. So how do the Howards expect to break into the global freshwater pearl market without a market analysis, competitive costs, evaluation of Tennessee's Pearl Company's success, etc.? They are under the naïve assumption that if

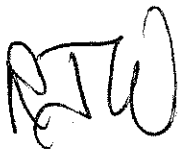
they produce pearls, people will buy them at their asking price to show a profit. They are way out of their league here and lack the business skills to succeed in this highly competitive trade.

The methods they propose are the same as those used by the Chinese over the last couple centuries, but their simplistic description (p. 22) of infesting host fish reflects their lack of biological training. The state of Alabama would prohibit the use of tilapia in ponds adjacent to the Tennessee River, and the notion that "Fresh-Water Prone" would do well in such ponds is wishful. Lacking sufficient biological knowledge to recognize the word prawn versus prone tells me that they are doomed to fail in their empirical trials of throwing various organisms into their mussel ponds.

They propose to construct more ponds of 4-6 ft. in depth. What will temperatures be in these ponds in the middle of the summer in Alabama? Mussels and most fish have an upper stress-level temperature of about 30°C. Certainly the upper level of the ponds will reach that, where the mussels are suspended below the surface. Again, the lack of training in biology or aquaculture is evident.

Bottom line is that this husband-wife team lacks the training and experience to succeed biologically and financially in this pearl making venture. Putting nearly $300,000 more into their highly experimental pearl farm is not a good investment. Even if they should happen to succeed biologically, the world's pearl market has no room for newcomers at this time nor is there any evidence that a "home-grown" niche exists at much higher prices. This is an aquaculture venture by 2 people who have no aquaculture training or experience. Therefore, the prospect for successfully producing and marketing freshwater pearls in a financially profitable venture is highly unlikely.

If SBIR ever does site visits as part of their review process, this is definitely a project that needs an on-site visit to see exactly what is being done and to assess the knowledge and ability of Mr. Howard.



Small Business Innovation Research
Cooperative State Research
Education and Extension Service
Stop 2243, Waterfront Centre
Washington, DC 20250-4690

UNITED STATES DEPARTMENT OF AGRICULTURE
SMALL BUSINESS INNOVATION RESEARCH
SOLICITATION NO. USDA / 02-1

OMB Approved 0524-0025

PHASE I AND PHASE II
PROPOSAL COVER SHEET

[redacted]

Proposal No. (for USDA use only)	
Date Received	

02-U3064

SUBMITTED	Firm: Southern Aquatics L.L.C.
	Mailing Address: 249 Bayless Avenue, Florence, AL 35630

Project Title: Fresh-Water Pearl Farming

Topic No. and Area (check appropriate box; see Section 8.0)

- ☐ 8.1 Forests and Related Resources
- ☐ 8.2 Plant Production and Protection
- ☐ 8.3 Animal Production and Protection
- ☐ 8.4 Air, Water, and Soils
- ☐ 8.5 Food Science and Nutrition
- ☐ 8.6 Rural and Community Development
- ☒ 8.7 Aquaculture
- ☐ 8.8 Industrial Applications
- ☐ 8.9 Marketing and Trade

Amount Requested: ($)	Proposed Duration (Mos.):	Congressional District No.: 5th

	YES	NO
1. The above concern certifies that it meets the first two criteria of a small business concern as stated in this solicitation (See subsection 2.2).	X	
2. The above concern certifies that it qualifies as a socially and economically disadvantaged small business as defined in this solicitation (See subsection 2.4). (For statistical purposes only).		X
3. The above concern certifies that it qualifies as a women-owned small business as defined in this solicitation (See subsection 2.5). (For statistical purposes only).	X	
4. The above concern certifies that the Principal Investigator's primary employment (at least 51%) will be with proposing firm at the time of any resulting award and during the conduct of the proposed research (See subsection 2.2(C)).	X	
5. The above concern certifies a minimum of two-thirds of the research (phase I) or one-half the research (phase II) will be performed by this firm (See subsection 2.2(D)).	X	
6. Will you permit the Government to disclose the title and technical abstract page of your proposed project, plus the name, address, and telephone number of the corporate official of your firm, if your proposal does not result in an award, to entities that may be interested in contacting you for future information?	X	
7. Do you plan to send, or have you sent, this proposal or a similar one to any other Federal agency? If yes, give acronym(s); e.g., DOE, NIH, NSF, etc.		X
8. Is the organization delinquent on any Federal Debt? (See subsection 5.11). (If yes, attach explanatory information).		X
9. Will the work in this proposal involve recombinant DNA, living vertebrate animals, or human subjects? (If yes, complete Form CSREES-2008).		X
10. Is this proposal a resubmission of a proposal submitted earlier to the USDA SBIR Program (See subsection 3.3(D)). If yes, list the proposal number _____		X

By signing and submitting this proposal, the prospective grantee is providing the required certifications set forth in 7 CFR Part 3017, as amended, regarding Debarment and Suspension and Drug-Free Workplace; and 7 CFR Part 3018 regarding Lobbying. (Please read the Certifications and Instructions included in this solicitation before signing this form.) In addition, the prospective grantee certifies that the information contained herein is true and complete to the best of its knowledge and accepts as to any grant award, the obligation to comply with the terms and conditions of the Cooperative State Research, Education, and Extension Service in effect at the time of the award. *Submission of the Social Security Number is voluntary and will not affect the organization's eligibility for an award. However, it is an integral part of the CSREES information system and will assist in the processing of the proposal.

PRINCIPAL INVESTIGATOR	AUTHORIZED ORGANIZATIONAL OFFICIAL
Name and Social Security Number*: D. Greg Howard 408-23-9581	Name: Amanda Howard
Title: CEO	Title: President
Address: 249 Bayless Avenue	Address: 249 Bayless Avenue
E-mail: southernintl@cs.com	
Telephone No.: 256-767-7506	Telephone No.: 256-331-5308
Fax No.: 256-767-7358	Fax No.: 256-331-5389
Signature: D Greg Howard	Signature: Amanda Howard
Date:	Date: 2/5/02

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0524-0025. The time required to complete this information collection is estimated to average 1.4 hours per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

PROPRIETARY NOTICE (IF APPLICABLE, SEE SUBSECTION 5.4)

The following pages (specify) contain proprietary information which (name of proposing organization) requests not be released to persons outside the Government, except for purposes of evaluation.

U.S. DEPARTMENT OF AGRICULTURE

SMALL BUSINESS INNOVATION RESEARCH
PHASE I AND PHASE II
PROJECT SUMMARY*

OMB Approved 0524-0025

FOR USDA USE ONLY			
Program Office:	Solicitation No.:	Proposal No.:	Topic No.:

TO BE COMPLETED BY PROPOSER

Name and Address of Firm:	Name and Title of Principal Investigator(s):
Southern Aquatics L.L.C. Liability Co. 249 Bayless Avenue Florence, AL 35630	D. Greg Howard, CEO

Title of Project (80-character maximum):

Fresh-Water Pearl Farming

Technical Abstract (200-word limit):

The goal of the Phase II project is to expand upon the research base from the Phase I project, developing the necessary knowledge of pearl culturing using American Fresh-water mussles in a controlled environment. Further research will define the requirement of finished pearl products and will determine the necessary steps to be taken to establish the culturing of mussle as a means of sustained aquaculture.

Anticipated Results/Potential Commercial Applications of Research (100-word limit):

The anticipated results is a set of procedures and methods which will inable the establishment of a new and valuable farm product for the United States. The techniques will be aplicial to most Southern states, allowing for an alternative to traditional forms of agriculture.

Keywords to Identify Technology/Research Thrust/Commercial Application (8-word maximum)

Pearl culturing, nuclei, pearl, mabé pearl

* The Project Summary must be suitable for publication by USDA in the event of an award. Do not include proprietary information on this page.

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E. Technical Content

(1). Identification and Significance of the Opportunity

Since the beginning of their history, beautiful pearls have attracted man and initiated many theories as to their origins. In the First Century A.D. men thought that when the weather was good the shellfish came to the surface and opened its shell, whereupon a drop of dew fell into the shell and soon grew to become a pearl. The scientists of ancient Rome believed the pearls to be the tear of the shell, or the crystallized tear of an angel. The ancient Greeks believed that lightning entering the sea caused the pearl. Columbus thought dew on the mangrove dropped into the sea and became a pearl. In 1825, Sir Edward Hume concluded that a pearl was formed when an egg of the mollusk died and was not discharged. (Cohen) Although many fantastic theories of pearl formation existed in early times, few experiments to produce pearls were ever attempted. The production of pearls apparently began in China during 13th Century A.D. Introducing a foreign substance between the mantle and the shell produced crude semispherical pearls. For several centuries the Chinese have been inserting tiny images of Buddha where the mussel would deposit a thin layer of nacre on the image.

It is accepted that Mikimoto of Japan developed the techniques that made it possible to commercially cultivate shellfish as a means of creating pearls. Before World War II, Japan was internationally famous for the production of such luxury items as silk and pearls. The name Mikimoto was known in every land as the inventor of a new commercial pearl. The gem was so lovely that it immediately captured the interest of the world. The appearance of this new commercially produced pearl inevitably set off arguments as to whether it was a "natural" or an "artificial" pearl. The argument resulted in the establishment of a new category, it became know as a cultured pearl. (Cohan)

Commercialization

By the Japanese being the first to develop the necessary culturing techniques, this gave them a tremendous head start on implementing the commercialization of pearl culturing. The methods that where found to be successful were held as a closely guarded secret until they became known after the defeat of the Japanese in World War II. Mikimoto first established his farm in the early 1900's. Additional farms where formed, most of which are located in four main areas of Japan: Mie Prefecture, Uwajima City, Toba City and Nagasaki.

Throughout most of the 20th century the Japanese have monopolized the pearl industry. They have, until recently held 80% of the total pearl market. In 1995 there were over two thousand farmers and over a hundred nuclei manufactures. (beads cut from American Mussel shells which are used as the nucleus for most all cultured pearls) In this same year, pearl sales were estimated to be $517.79 million. (Pearl Net World.com/Oct. 3, 2001)

In 1997, the Japanese control of the pearl industry came to a dramatic end. The Akoya oyster began to die in great numbers, up to 80% in most areas. (Pearl World, Feb., March 1999) The high mortality rate was attributed to several factors including red tide, pollution and mysterious viruses. This has become a persistent problem with no relief in sight. They have replenished their oyster farms with new animals each year only to see them perish. The Japanese have made a total effort to remedy the problem, but have had little success in defeating this illness that has caused such devastation.

Many pearl farmers and nuclei manufacturers (bead producers) have either gone bankrupt or have quit the business in the past four or five years. Just a few years

ago there were 300 nuclei manufacturers, now there is less than 30. The Japanese production of both pearls and nuclei has dramatically diminished compared to years earlier, pearl sales in 2000 were estimated to be $189 million, less than half that of 1995. (Pearl net world.com Oct. 3, 2001). There are almost no suppliers of some sizes of Akoya pearls available at the present time. Chinese Akoya pearls are being used in their place and are called Japanese Akoya Pearls. The pearls that they have been produced are of much poorer quality than their predecessor. (Torry, 2000)

China produces both fresh-water and salt-water pearls. In the past ten years they have greatly increased their production. The Chinese have the highest volume and the lowest quality than any other pearl producing area. In 2000, the fresh-water pearl harvest was estimated to be $180 million and the salt-water Akoya harvest was $20 million. The bulk of their output is in the form of non-nucleated irregular shaped fresh-water pearls. (Pearl Net World.com/Oct. 3, 2001)

Australia began its cultured pearl production in the 1950's. Most of the farms are located in Western Australia. Today there are sixteen licensed farms in operation, Paspaly being the largest, producing 50% of the areas over-all out put. Australia's annual sales are over $200 million with an average price of $180 to $200 per gram. Australia differs from the other pearl producing areas of the South Pacific due to the government's tight control over the wild oysters that are harvested each year. The licensed companies are given a quota of animals they will be allowed to take from the ocean each year. The number ranges from 15,000 to 100,000 animals depending on the size of the farm. The total yearly catch is around 570,000 oysters. (SPC Pearl Oyster Information Bulletin #14)

French Polynesia, famous for their black and colored pearls, began their cultivating operation in the 1970's. The industry has developed into a bi-level

configuration with a few large companies and many small or family operated farms. The industry employs, directly or indirectly, one out of four families. Their annual sales are around $160 million. French Polynesia holds 95% of the colored pearl market and 28% of the total pearl market. Pearls make up 95% of the countries total export. They differ greatly from the Australian and Japanese due to the large numbers of wild stock available to the farmers and the lack of control from the government. They have increased production in the past few years. The increase in supply has made the Black Pearls more readily available and has driven the price down. (SPC Pearls Oyster Information Bulletin #14)

The United States affiliated Pacific Islands have a small but rapidly expanding project in the Marshall Islands and the Federated States of Micronesia. The University of Hawaii Sea Grant and the College of Micronesia Land Grant programs have supported this expansion.

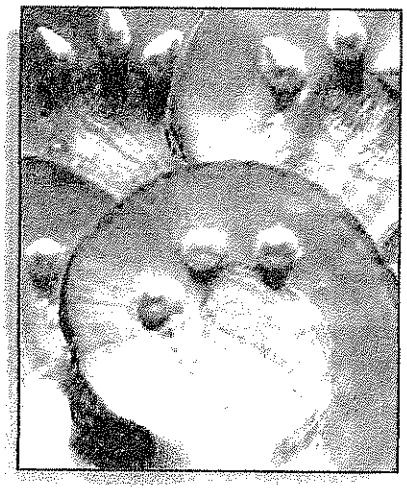
North American Pearls

Proof of pre-historic pearl gathering exists in North America in the pearls that have been found tucked away in caches as well as by the necklaces found inside the burial mounds of Indians. It was in the 19th Century that the exploiting of the North American riverbeds began to produce very high quality pearls. The pearls of Kentucky, Tennessee, Alabama and Arkansas came to be sought after for their beauty. (Tahiti Perles/Pearl Diving in North America) "Demand for U.S. freshwater pearls is a function of quality. The size, color, shape, degree of translucency, texture and luster determine quality. Usually, the quality of the U.S. pearls is as good or better than any other." (U.S. Geological Survey/Dept. of the Interior)

John Latandresse, the former owner of Tennessee Shell Company, established the first pearl farm in the U.S. in 1963. Their culturing techniques were self-developed

and have been kept as trade secret. They have been able to produce round, semi-round, and mabe' (half round) pearls. (Matlins, 1999) This operation is in the open waters of the Tennessee River, where permits for this type of operation are no longer available.

Mabe' or Half Round Pearls



Southern Aquatics is conducting experiments in a controlled environment. Where water from the Tennessee River is pumped into tanks and ponds that are used to house the native mussels. This type of configuration will allow for sustainable agriculture practices that could easily be expanded to allow for pearl farms to be established throughout the southeast.

(2). Background and Rationale

American Fresh-water Mussel Shells have been harvested for one reason or another for over a hundred years, beginning with a thriving button industry in the 1800's. It was discovered that the American Mussel Shell was by far the best material for nuclei manufacturing. The shells have been exported to Asia, primarily Japan, for decades. Nuclei made from U.S. mussel shell have long been a standard in the industry, any substitute is considered inferior.

The physical make up of all bivalves is very similar. The interior of the protective shell is made from nacre (calcium carbonate). The animal will secrete nacre as it matures causing the shell to enlarge with age. All bivalves produce nacre and have the ability to produce pearls. Natural pearls start with an irritant (sand or other types of debris) that, over several years, is covered with layers of nacre. The cultured pearl industry speeds up this process by inserting a nucleus, which will be coated with nacre as it irritates the animal. The faster the animal grows the greater its ability to produce nacre and the faster a pearl can be formed.

When compared to the Japanese oyster the American Mussel should have the ability to produce larger pearls at a much faster rate. The Akoya oysters that are used in Asia are small thin-shelled animals. Even at maturity the shell itself is no thicker than 2 mm. The American Mussel on the other hand can grow as thick as 30 mm with the average being around 10 mm at maturity. This would indicate that the American Mussel has a much greater production of nacre than that of the Akoya Oysters.

There is a wide variety of American Mussels species, but only about ten are accepted as commercial species for export from the Tennessee River. "The Southeast has the greatest diversity of fresh-water mussel species in the world, including an array of lotic species well suited for pearl production" (Neves, 1999). The nacre the shell excretes is what determines the color of the shell. Most of the animal's shells are white but some are colored. The Hill Splitter (Potamilus Inflatus) has a purple and lavender nacre, the Washboard (Megalonaias Nervosa) produces primarily white with a hint of blue and pink, the Three Ridge (Amblema Plicata), Maple Leaf (Quadrula Quadrula), and the Ebony (Fusconaia Ebena) produce white nacre. Southern Aquatics have also found that an animal may produce an entirely different color of nacre. They have seen gold, a very deep purple that is almost black, Champaign and silver. Due to the

individual characteristics of these animals a variety of pearls can be produced having a wide range of sizes and colors. All the American Mussels are much larger than those used in Japan and China and are equal in size if not larger than the animals used in the South Seas (Pinctada Maxima) oyster.

True colored pearls are produced primarily in French Polynesia by the (Pinctada Margeritifera) oyster. The Tahitians are known world wide for their famous black pearls. American Mussels will produce colored pearls in varieties that have never before been introduced to the pearl market!

Shell Structure

The shell is a complex structure containing 94% calcium carbonate in about 6% organic matrix. A cross section of the shell reveals an outer periostracum, middle prismatic calcite layer and inner nacreous or crystalline aragonite calcium carbonate layers. The outer periostracum is a thin layer of quinone-tanned concholin ($C_{32}H_{48}O_{11}$), a scleroprotein of keratin type. (Ram and Tripathi, 1992)

Anatomy

The soft body is compressed and covered with the mantle having two folds, one on either side of the shell valves. The mantle is primarily secretory in nature and also helps in repairing the damage to the shell. The shell is separated from the mantle by layer of extra pallial fluid. The secretory function of the mantle tissue through the extra pallial fluid in the formation of the three layers of the shell is a complex biomineralisation process (Simkiss and Wada, 1980). The mantle has an outer layer of secretory epithelial cells, middle connective tissue layer and an inner layer of ciliated epithelial cells. The mantle folds are free along the ventral and anterior margins. Posteriorly, the mantle folds are united to form two tube like openings called siphons. The lower inhalant siphon allows the water current into the body and

the upper exhalent siphon drains out the water along with excretory products. The water current helps in respiration and feeding (Ram and Tripathi, 1992).

Respiratory System

Water is inhaled through the siphon flows over the gills or ctenidia. Each gill has a series of septae separating the gills into a many compartments. These compartments serve as water tubes where the gaseous exchange takes place.

Digestive System

By nature, fresh-water mussels are filter feeders. The mouth is placed below the anterior adductor muscle. Leaf like palps are on both sides of the mouth. The oesophagus is located next to the mouth and leads to the stomach. The intestines are long and convoluted; it runs through the visceral mass. The rectum opens through the anus into the exhalent siphon.

Circulatory System

Fresh-water Mussels have a colorless blood, which contains leucocytes. Circulation is through contractile heart, arteries, veins and blood spaces or sinuses. The heart is comprised of a median ventricle and two lateral auricles. The anterior and posterior aortae join the ventricle while the auricles are connected to the gills and the mantle by branchial veins. The gills and mantle oxygenates the blood and it passes into the auricles and reaches the anterior and the posterior parts of the animal through the ventricle. The blood is carried by the vena cava from different regions, into the mantle, the gills and the kidney for purification.

Nervous system

Fresh-water mussels have a poorly developed nervous system. It consists of ganglia, commissures and connectives. There are pairs of cerebral, visceral and ganglia interconnected through cerebropleural ganglion. The organs of equilibration,

statocyst, is close to the pedal ganglion and the incoming water testing or sensory epithelial tissue called osphradium is in close proximity to the visceral ganglia.

Reproduction

The male releases sperm into the water, which enters into the branchial cavity of the female where fertilization is achieved. The fertilized ovum passes into the gill lamellae for development (Thomas, 1977). The embryo develops into a glochidium larva. The larva is passed through the exhalent siphon. The larva then finds a suitable host fish where it attaches itself to the gill or fins for further development. The larva will draw nourishment from the host for 60 to 70 days. At the end of this period a juvenile mussel will detach and fall to the bottom for further development.

Pearl Formation

Pearl nacre and the shell interior have more or less the same structural and chemical properties. When a foreign stimulus is trapped in the shell it leads to the depositions of micro-layers of pearl nacre around the stimulus. The epithelial cells of the mantle secrete a pearl sac surrounding the irritant nucleus forming the cellular basis for crystallization of calcium carbonate. The shape of the pearl is mostly determined by the nucleus. This is an abnormality in the normal biological processes. The abnormal response of the epithelium in pearl culturing is commercial biomineralisation process.

The hardness of a pearl is 3.5 to 4.5 in Moh's scale. The nacre that is deposited on the nucleus is arranged in concentric layers. The layers of nacre will continue to collect until the death of the mussel. It is the over lapping layers of nacre that gives a pearl a rough feeling. A pearl usually consist of 3.97% water, 3.83% organic matter and 91.53% calcium carbonate. (Bolman, 1941)

(3). Relationship with Research and Development

(a). Economic benefits of Fresh-water Pearl Culturing in the U.S.

"Today the cultured pearl industry provides 230,000 jobs and generates retail sales approaching $3 billion worldwide" (Neves, 1999)

It will be much more cost effective for the United States to produce high quality pearls than any other nation in the world. Unlike the Asian nations, all factors of production are contained in the Southeastern U.S. The Asian countries rely of the U.S. companies to supply them with American Mussel Shell for their nuclei. The Akoya oysters of Japan are reproduced in and raised to maturity in laboratories. This man-made reproduction system has caused the Akoya to become weaker than those found in the wild and less likely to survive the harsh conditions of the open water. It is very expensive for the Australians to collect wild oysters from the ocean, the total harvest cost is $20 per animal and they are allowed only a certain number. The U.S. is in a position where the cost of shell harvest will be much less costly than that of our foreign competitor. The existing shell industry can easily supply the demands for shell for nuclei and live animals to be used for implants. A program of this nature will be completely self-sufficient.

The potential economic benefits of pearl culturing for the Southeastern United States are tremendous. Almost every state east of the Rocky Mountains possesses the raw materials necessary to create and maintain a prosperous pearl farming industry. The basic requirements necessary to create a pearl farm are the following:

1. Fresh-water Mussel Shells (alive)
2. Nuclei (either purchased or produced in house)
3. Natural habitat (river or ponds re replicate living conditions)
4. Proper Implants and Grafting Techniques

(b). Costs Relative to Benefits

The United States could easily find its place in the Cultured Pearl Market and create an identity of its own. A study conducted by the Indian Council of Agricultural Research concluded that fresh-water pearl farming would reap a 45% return on capital. By means of the pond flow-through system in pearl culturing, American farmers will experience an even greater return than any other agriculture option available.

Phase One Research

Phase One Objectives

1. To determine if mussels can be held, with reasonable survivorship, in a controlled culturing environment.
2. Determine the best method of implanting nuclei in mussel to ensure the highest survival of implanted mussel under controlled culturing conditions.
3. Determine if implanted mussel held in culturing ponds will produce nacre in a timely manner

Pond/Holding Tank

Flow-through culturing tanks have been constructed along the banks of the Tennessee River. Each of the tanks is independently supplied with water pumped from the river. A portion of the tanks are placed in a terrace configuration where water is pumped into the highest tank and flows down into each of the lower one. Water flow controlled by valves and the water level is controlled by stand pipes. Adequate pond oxygenation is ensured by having: (1) sufficient turnover of water in each tank, which in this case is one hundred and sixty gallons per hour (2) by having diffused feeder lines with 1-2 foot drop from the point of discharge to the surface of the pond. By using this flow-though system with water from the Tennessee River, problems with

maintaining appropriate alkalinity, ph and hardness in the ponds are minimized. These parameters are however monitored on a regular basis. Furthermore, this design will also ensure adequate food supply. In essence, this system simulates the flow and living conditions as found in the river its self.

Growth And Survivorship

Mussels from five of the most common commercial species from the Tennessee River, the Washboard, Three Ridge, Maple Leaf, Hill Splitter and the Ebony have been used in the experiment. To date, there have been around five thousand animals, which have been implanted in one form or another. An additional four thousand animals have been used as donors for their mantle tissue or shells for nuclei production.

In order to monitor growth, mussels have been individually marked on the shell surface before being placed into the tanks. Each mussel has been measured to the nearest millimeter initially and again periodically throughout the course. Growth rates of ten randomly selected mussels from each of the tanks have been compared to the growth rates of the "control" mussels, which have no implants and are isolated from the implanted animals.

Survivorship of the mussels has been monitored by examining the proportion of mussels surviving in each tank and the method of implantation. There has been approximately a 97% survival rate among non-implanted animals. The mussels seem to adapt very well to their relocation. >2 yrs for mortality to occur

Nuclei implantation and post-implantation survival and growth

Procedures

Animals are harvested and placed into one of the tanks for a minimum of twenty-four hours. They are each given a number that is marked on the shell. Each species has its own set of numbers and this sequence of numbers continues through the phase one

project. A spreadsheet was established for each species to collect data on individual animals. The information on the spreadsheet includes: the date of harvest, the date of implant, the type of implant, size of the nucleus, sizes of the animal, the means in which the shell was opened, if mantle tissue was inserted and if it expires the date of death.

Implantation

The implants have been preformed in a variation of one of two ways, which has proved to be the two most successful in accomplishing the desired results of survivorship and nacre production.

1. **Mantle Cavity Insertion** - The Mantle tissue is a membrane that is between the inside of the shell and the body of the mussel. The shell is opened by force using forceps or by means of a drug (Propolin Phinixatal). A wedge is placed between the shell halves to keep it open. The mantle tissue is only attached to the shell around the outer edge. It is pulled away from the shell just large enough to accept the nuclei implant. The nuclei is passed through the opening and then placed in between the inside of the shell and the mantle tissue where the mantle holds it in place. The nuclei can be fixed to the shell by means of an adhesive, which cures in water. These implants can be preformed on both sides of the same animal. Survivorship has been very high among these animals averaging 79.8%. Most of the deaths were due to over exposure to Propolin Phinaxatal or by damage to the anterior adductor mussel, which can easily be torn when the shell is forced open.

This method will produce half round Mabe' pearls. The sample that Southern Aquatics has taken, show very clear nacre deposits. It is estimated that the mussels are coating the nuclei at a rate of .25mm every two to three months. A minimum of one

millimeter will be required before the pearl should be harvested. Nuclei as large as twenty millimeters have been successfully implanted.

Survival Rate Among Mantel Implants	
Washboard (Megalonaias Nervosa)	87%
Hill Splitter (Potamilus Inflatus)	77%
Three Ridge (Amblema Plicata)	77%
Maple Leaf (Quadrula Quadrula)	76%
Ebony (Fusconaia Edena)	82%

2. **Gonadal or internal insertion** - The shell is opened by the same means as the mantle implants. The gills are pushed up and away to expose the gonad. An incision is made in the epithelium covering the animal's gonadal area. Caution must be taken not to damage the intestines, the cut should not be deep. The nuclei along with a small piece of mantel tissue is placed in the opening and gently pushed into the body of the animal. The mantel tissue contains nacre-producing cells. Survivorship has been good averaging around 65%. The samples collected show nacre deposits collecting on the nuclei. It is not yet determined the amount of nacre to be deposited. The Gonadal implants will produce round, semi round and Baroque (odd shaped) pearls.

Survival Rate Among Gonadal Implants	
Washboard (Megalonaias Nervosa)	68%
Hill Splitter (Potamilus Inflatus)	71%
Three Ridge (Amblema Plicata)	69%
Maple Leaf (Quadrula Quadrula)	59%
Ebony (Fusconaia Ebena)	58%

Post Implantation

After the animals have been implanted they are placed in a tanks where they are monitored for five days. The mussels that are unable to survive the procedures will normally expire within the five-day period. The animals will then be relocated to a community tank along with animals that have been implanted in the same manner.

Southern Aquatics has isolated the animals by the type of implantation procedures employed and a control group of non-implanted animals. This is being done to examine the efficacy of the two methods. The dependant variables that have been monitored are (1) survival, (2) growth and (3) quantity of nacre.

The results of the phase one research have demonstrated that it is feasible to rear pearl mussels and culture American Mussels in a controlled pond environment. Previous research has provided the basis for future examination of the production of quality fresh-water pearls on a large commercial scale. From the information derived from continued research we will be able to refine estimates of nacre production rates in order to better predict and then verify the time necessary for going from implantation to marketable product. In addition, knowing the efficacy of pond rearing will allow us to examine several pearly mussel species for use in production of a variety of pearl sizes and colors. Continued research will build on survivorship results of wild animals held in ponds. The intent is to examine the flow-through rates, optimal nutritional regime, and pond stock levels of mussels appropriate for maximizing mussel's survival and thus the rate of pearl production both in terms of finished product and in nacre production. Additionally, mussel reproduction in an effort to produce juvenile mussel from wild stock will be studied. Young mussels may subsequently be grown for on-site culturing or for re-seeding natural populations in the Tennessee River.

It is our intention to examine linking pearl culturing to other forms of aquaculture. Host fish for each of the mussels employed in the project will be introduced in the pond in an effort to reproduce each species. Additionally, other commercial fish species will be stocked in the ponds. The maximum levels of production with the ponds being inhabited by both the fish and the mussels will be studied.

These results will substantially enhance the knowledge of base of pond-rearing fresh-water mussels and culturing American Mussels. The technical information will benefit farmers in the southeast by giving them the protocol for another option in product production.

(4). Phase II Technical Objectives

Southern Aquatics has proven from the Phase one research that river mussels can be successfully held in culturing tanks and that the animals can live through the nucleated implantation procedures. The animals have shown their ability to deposit nacre coatings on the implanted nuclei. Now that the initial goals set forth in Phase one project have been reached, Southern Aquatics will investigate the long-term commercial objectives in the pearl culturing of American Fresh-Water Mussels. The following technical objectives need to be addressed:

1. Determine the best long-term method of implanting nuclei in river mussels to ensure the highest survival in culturing pond conditions.
2. Refine the procedures and methods of round and half round pearls
3. Determine the time duration to produce a quality pearl.
4. Explore the types of pearls the animals can produce.
5. Determine the colors of pearls that can be produced.
6. Determine the type of implant best suited for commercial culturing

7. Assess whether other forms of aquaculture can be introduced into the same living space without being harmful to the mussels. And if so, which fish type will be the most commercially viable to inhabitation common living space with the mussels?
8. Study the feasibility of mussel reproduction in a controlled pond environment.
9. Establish the growth rate of mussels hatched in the ponds.
10. Expand the original research project into a commercial operation.
11. Develop the facility requirement for a commercial operation.
12. Determine the value of the produced pearls.
13. Develop a marketing and sales program for the product.

The technical objective will be addressed by the following means:

1. Growth and survivorship will continue to be monitored among the implanted animals.
2. Random samples will be taken quarterly; this will provide information as to the production of nacre, color of nacre and the success of the types of implants.
3. Fish species will be stocked in the ponds; they will include both commercial species and those species that act as a host for each type of mussel.
4. Each fish species will be isolated in an individual pond along with the implanted mussels.
5. The fish and the mussels will be stocked in different levels of density to determine the full capacity of the ponds.

6. Juveniles born and reared in the ponds will be monitored regularly to establish growth rates.

(5). Phase II Work Plan

In order to make an impact on the existing pearl market the volume of pearls produced will be an important factor. It is essential that American pearls create an identity all of it's own. A volume of pearl production can only accomplish this, where the pearls will be promoted as an "American Pearl" in multiple markets.

Research Plan

The number of implantations preformed on a daily bases will be dramatically increased. The implanted animals will be treated in much the same way as they were in the Phase one research. Each mussel will be numbered and cataloged into the database. It will be recorded as to:

1) The date of implant, thus allowing for an estimated harvest date for the pearl.
2) The size of the implanted nuclei, this allows for an estimate of the size of the finished product.
3) The type of nuclei: round, half-round, teardrop, oval, etc.
4) The method of implant, which will determine the shape of the pearl.
5) The animals will be placed in four foot by four-foot baskets, which will be suspended below the pond surface and tied to a cable that is stretched across the pond. These baskets will be dispersed in intervals throughout the pond.
6) Which pond it will be located in.

7) The specie of the animal. The animals will be segregated by species in different ponds. This will allow host fish for each species to be stocked and concentrated in the ponds providing a much greater chance of inoculation and reproduction.

The date of the animal's implant is very important. This information will allow for an estimated harvest date for the pearl. Animals that are implanted in the relative same time frame will be grouped together and upon the harvest date will be collected simultaneously. This will prevent disturbing the other mussels.

The data collected on the size of the implanted nuclei will provide information on the size of the pearls at the time of harvest. This will forecast the product availability in the future harvest. The information of the nuclei size and shapes will provide a basic knowledge of the finished produced. Effort can be made for the advanced market and sale of the individual pearls.

The methods of implant will determine the type of pearl.

- Mantle Cavity Implants will be employed to produce Mabe' pearls. These will include round, teardrop, oval and heart shaped half-round pearls in various colors depending on the animal specie.
- Gonadal Implants will be used to create round, semi-round and baroque pearls. As with the Mantel Implants the animal will determine color.

The animals will be placed approximately five inches apart from one another in a four foot by four foot baskets. These will be suspended below the ponds surface and tied to a cable, which will be stretched across the pond. The baskets will be distributed evenly throughout the pond. By placing the animals in the basket it will

prevent over crowding and provide for better monitoring and management. All the mussels in a single pond will be harvested at the same time. The basket can be moved along the cable to either end of the pond to allow fish to be collected in the same pond.

The mussels will be segregated by their species in different ponds. This will allow for the host fish of each species to be stocked and concentrated in a single area. This will greatly encourage inoculation and reproduction. The spats and juveniles will be reared in isolation in individual ponds.

Commercial Fish species will be stocked in with the mussel. It is our intent to experiment with several different species, Catfish, Tilapia and Fresh-Water Prone to determine which is best suited for polyculture with the mussel. Each of these experiments will be conducted in separate ponds and the pond's yield at the end of the growing season will be compared for cost and commercial value.

Production

An average size pearl farm in Asia will have at least one hundred thousand animals implanted at any given time. This is an example of just one of a great many farms throughout the Pacific Ocean. In order to create an identity for the American pearl the product must be available. By the time it is available on the market it will become known not as a pearl but as an American Pearl as in the case with the Japanese Akoya, South Seas and Tahiti Black Pearls.

A graft technician can implant one hundred animals in a single day. It is the plan of Southern Aquatics to employ and train five technicians. The mussels will be implanted equally in both the Mantle Cavity and the Gonadal manner. These implants will be performed on a daily bases. This will calculate into a total of one hundred

thirty thousand implanted animals in a one-year period of time. These regimens will continue into the second year. Samples will be taken to determine when the pearls will be ready for harvest. When the pearls have reached a finished quality they will be harvested in groups, which were all implanted within a thirty-day time period of each other. By this means of continued implanting, it will reach a point were as pearls can be harvested monthly.

Commercial Pond Construction

water temp ?

Ponds will be constructed at various sizes depending on the terrain at a depth between four to six foot. The ponds will be in a terrace configuration. Fresh river water will be pumped into these ponds where standpipes will feed the water onto the next pond. Valves will be installed in order to lower water levels or drain the ponds for the pearl and fish harvest. Steel cables will be stretched tight running the length of the ponds, which will be used to suspend the mussel. Waterfall will be enhanced as much as possible to increase oxygenation. Sufficient water turn over will be necessary to ensure an adequate food supply.

Marketing and Sales Plan

American consumers make up 70% of the total amount of pearls sold in the world. Therefore, the bulk of our marketing efforts will be done in the U.S. The market for cultured pearls is far from saturated but yet is fully established. Marketing of the American Cultured Pearls will primarily utilize the marketing routes established by our foreign counterparts. The route chosen to introduce this product to the market will be extremely important. It will take careful marketing, advertisement, and public relations to ensure the public understands the quality of the American Cultured Pearl.

Every effort will be made to publicize the operation and it's future product. Pearl World Magazine has shown a desire to publish a number of articles starting with the beginning of the culturing process and finishing with the finished products. **Southern Aquatics was first published in Pearl World Magazine in 2000**, "Business Plan for Proposed Pearl Farm in the Southeastern U.S.", Pearl World, The International Pearling Journal, May/June 2000. By promoting the new "American Cultured Pearl" in trade publications, name recognition and quality assurances will be made before the product is introduced through pearl auctions. The primary pearl auction is the yearly pearl auction that is held in Hong Kong. This is where most of the Asian farmers sell their products to jewelry manufacturers. It would be feasible to finish the jewelry items in house or subcontract with jewelry manufacturers and offer them on the wholesale and the retail level at the auction.

The very expensive finished items will be marketed on the wholesale level. We plan for these items to be sold in the most exclusive jewelry stores in the world. These pearls will be the most rare produced while maintaining the same quality as Asian pearls, which will make them very sought after and expensive.

The pearls of lower quality will be marketed on a less expensive level. The lower quality pearls would be a perfect item for Television Marketing shows like QVC where they could be sold in very large quantities. Large department stores will be a conducive environment to market a large portion of these pearls.

Recent Auction Results

*The following information was taken from Pearl World Magazine, The Millennium Edition

- Paspaley Pearl Auction, Hong Kong in September of 1999 reported total revenue of $12,002,371.00 for 48,134 total pearls. The

average price was $345.00 per pearl. One lot of three round 15mm pearls (white) was sold for $33,689.00

- Robert Wan's Tahiti Perles Auction in Hong Kong in September of 1999 brought a record $9.3 million dollars, 47% above the reserve price. 90,237 pearls in 242 lots were sold. The average price was $168.00 per pearl.
- Pearlautore Auction in Sydney, Australia during September of 1999 achieved sales of $11.72 million dollars with an average price of $245.00 per pearl.
- Shima Shokai Auction in Japan sold 206 lots or 54,185 pieces for a total of $9.19 million for an average of $227.00 per pearl.
- Gee Auction in Hong Kong sold 93,143 pearls for $199.00 each.

(6). Related Research and Development

In 1992, the Indian Council of Agricultural Research completed "A Manual on Freshwater Pearl Culture". This study fully documents the pearl culturing potential of fresh water mussels in India. Although it is proposed to use similar implantation techniques it is anticipated that a more natural environment would cause these mollusks to flourish and therefore secrete nacre at a much faster rate.

The University of Honolulu Sea Grant Extension Service has published numerous articles and videos completely documenting proper grafting and implantation techniques of saltwater bivalves. Southern Aquatics plans to expand on these studies to determine the most productive implantation techniques for freshwater mussels.

The Marine Environmental Sciences Consortium of Alabama published "New Opportunities for Economic Benefits for the American Southeast in the International Pearl Industry" by Maria C. Haws and Leonard DiMichele in 1999. This study assessed

options for the United States to reap economic benefits from pearl culturing. It was concluded that in the Southeastern United States all factors are present to create a freshwater pearl industry and that by doing so the United States could dramatically change the economic situation of these states. It is stated that "...Stabilization of the shell industry can be enhanced through establishment of domestic pearl farms, thus providing a domestic market for nuclei and creating an economic incentive to preserve mussel stocks. Once these cornerstones have been laid, the even more potentially lucrative role of technology development and delivery can be assumed by the southeastern private sector and research institutions."

Richard Neves published the "Biological Feasibility of Freshwater Mussel and Pearl Culture in Gulf Coast States" in 1999. He concluded that "economic success is possible for an effectively run pearl farm in the U.S. Southeast" (Neves, pp. 107). It is also stated that "Once the implantation technique becomes more widely known, quality pearls of various shapes and colors could become economic by-products of our mussel fauna in southeastern rivers, further justifying the protection of water quality and biological resources of long-term sustainability" (Neves, pp. 107).

Southern Aquatics conducted a SBIR Phase One Research Project on the feasibility of pearl culturing. For details concerning this project see page 13 – 17 of this paper.

(7). References

Cahn, A.R. 1949. "Pearl Culture in Japan." Fishery Leaflet 357. Fish and Wildlife Service, Department of the Interior, Washington, DC.

Bolman, J., 1941. The Mystery of the Pearl, E.J. Brill, Leiden, 170 pp.

Fassler, R.C. 1996a. "The American Mussel Crisis: Effects on the World Pearl Industry. Part 1. Aquacult. Mag. July/Aug.: 42-54.

Fassler, R.C. 1996b. "The American Mussel Crisis: Effects on the World Pearl Industry. Part 2. Aquacult. Mag. Sep./Oct.: 59-70

GIE-Perles De Tahiti. 2000. webpage: http://www.tahiti.blackpearls.com.

Haws, M.C. and DiMichele, L. "New Opportunities for Economic Benefits for the American Southeast in the International Pearl Industry." Gulf of Mexico Science, 1999 (2), pp. 109-122.

Matlins, A.L. "The Pearl Book: The Definitive Buying Guide," 2nd ed., Gemstone Press, Woodstock, 1999.

Neves, R.J. "Biological Feasibility of Freshwater Mussel and Pearl Culture in Gulf Coast States"Gulf of Mexico Science, 1999 (2), pp. 103-108.

Neves, R.J. "Conservation and Commerce: Management of Freshwater Mussel (Bivalvia: Unionoidea) Resources in the United States" Malacologia, 1999, 41 (2): 461-474.

"Pearl Statistics: Good, Bad or Indifferent," Pearl World, Feb./March 1999, pp. 10.

Pearl Net World. Com., Oct. 2001, How large is the pearl industry?

Ram, K.J., and S.D. Tripathi. 1992. "A Manual on Freshwater Pearl Culture. Manual Series 1. Central Institute of Freshwater Aquaculture, Bhubaneswar, India

Thomas, E.I., 1977. The bionomics and development of the freshwater mussel, Lamellidens marginalis (Lamarck. Ann. Zool. (Agra), 10 (4): 71-169.

Tisdell, C.A., Bernard Poirine, 2001. Socio-economics of pearl culture, SPC Pearl Oyster Information Bulletin #14

Torrey, Richard, Editor, Pearl World, The International Pearling Journal. Personal Interview – July 2000

Tahiti_Perles.com., 2001, Pearl Diving in North America

Dept. of the Interior, 2001, U.S. Geological Survey, Pearls

Ward, Fred, "Pearl Fundamentals," Pearl World, The International Pearling Journal, July/August/September 2000, pp. 13.

(F). Key Personnel and Bibliography

Greg Howard will serve as principal investigator, with key contributions being made by Amanda Howard and Kenley Austin. Mr. Howard has over 16 years experience working with the mussel industry in the

United States and was President of the world's second largest mussel exporting firm. He gave up his position to devote has time to develop a pearl industry in the U.S. Mr. Howard completed extensive on-site training by leading experts in the field at Awaji Island, Osaka and Kobe, Japan. Mrs. Howard has worked with the mussel industry for over 5 years and has extensive experience in business management and R&D in areas directly related to the proposed work. Mr. Austin is currently employed with T.V.A. and has worked directly with fisheries for over 10 years.

(G). Facilities and Equipment

Property will be needed for the construction of permanent ponds. The project sight must be in close proximity of the Tennessee River. Piping will be installed where the river water can be circulated with in the ponds. A large water pump will be needed to supply a sufficient turn-over of water. Southern Aquatics will provide equipment for the production of nuclei and surgical equipment for the implantations.

(H). Outside Services

In an effort to reduce cost, it is the intent of Southern Aquatics that this project will be as self sufficient as possible. Requiring no outside service.

(I). Satisfaction of Public Interest

Successful development of nuclei implantation procedures and grafting techniques as well as the documentation of fresh water mussels creating cultured pearls will lead to an aquaculture industry in the Southeastern United States. Furthermore, successful development of a viable economic venue for the 600 species of mussel shell will force the Southeastern United States to increase the value that is

placed on these species. There will be great benefits with the development of the new alternative agriculture industry. This industry will create many new jobs in the rural areas as it develops. Spin off businesses will be created in the form of nuclei manufacturers, equipment producers and distributors, excavation contracting firms, biology and fisheries specialists and marketing and sales, to name a few. The farming techniques can be applied in all the areas where there is a supply of mussel shells and offer traditional farmers other options in potential crops. This would allow for greater economic opportunities in rural areas.

The United States consumers have been completely dependent on the import of pearls. With this industry consumers will have greater options in the market. Due to the universal appeal of quality pearls and the low production of Japanese pearls, this will be an ideal export product.

(J). Potential Post Applications

Southern Aquatics was founded in 1999, with research and initial preparations beginning in 1995. The company has four direct employees and subcontracts with over 5 individual mussel divers. Southern Aquatics has been involved in the mussel export business for over 6 years. In 1999 it began to focus all efforts on research and development towards creating a sustainable cultured pearl industry for the Southeastern United States.

Potential Commercial Applications

This new industry will offer a number of opportunities for farmers and businesses. The following exert was taken from an article published in "Gulf of Mexico Science" by Maria C. Haws and Leonard DiMichele in 1999.

The five principal means by which economic benefit can be derived from global pearl culturing activities are 1) fresh-water mussel shell fishery, 2)

manufacture and marketing of fresh-water shell nuclei, 3) pearl production, 4) commercialization of pearls and 5) provision of services and materials to the global industry.

1. Shell harvesters from the existing shell industry will supply wild stock of mussels, while a fisheries program can be established. When completed, this will supply a large portion of the shells for both implantations and nuclei production
2. The manufacturing of nuclei will have economic potential in the United States. The nuclei can be sold to U.S. farmers as well as sold internationally as an export item to all countries producing pearls. In the past the Japanese have monopolized this market.
3. The production of pearls and pearl farms will be used as a means of alternative agriculture providing greater profit potential than any other product currently available to small farmers.
4. Economic opportunities will become available for the finishing of raw pearls.

Marketing, Sales and Distribution as well as consulting services will be needed by beginning pearl farmers in the form of grafting technician and biologist. Facilities construction will also be needed as individual pearl farms develop.

(K). Current and Pending Support

Southern Aquatics has no current support or has no pending support.

(L). Budget

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0039
Expires 03/31/2004

BUDGET

ORGANIZATION AND ADDRESS	USDA AWARD NO.			
Southern Aquatics L.L.C. 249 Bayless Avenue, Florence AL 35630	DURATION PROPOSED MONTHS: 24 Funds Requested by Proposer	DURATION PROPOSED MONTHS: ____ Funds Approved by CSREES (If different)	Non-Federal Proposed Cost-Sharing/Matching Funds (If required)	Non-federal Cost-Sharing/Matching Funds Approved by CSREES (If Different)
PROJECT DIRECTOR(S) Greg Howard, Amanda Howard				

A. Salaries and Wages	CSREES-FUNDED WORK MONTHS						
1. No. Of Senior Personnel	Calendar	Academic	Summer				
a. ____ (Co)-PD(s)				$ 114,400	$	$	$
b. ____ Senior Associates							
2. No. of Other Personnel (Non-Faculty) a. ____ Research Associates/Postdoctorates							
b. ____ Other Professionals							
c. ____ Paraprofessionals							
d. ____ Graduate Students							
e. ____ Prebaccalaureate Students							
f. ____ Secretarial-Clerical							
g. 2 Technical, Shop and Other				58,240			
Total Salaries and Wages →				172,640			
B. Fringe Benefits (If charged as Direct Costs)							
C. Total Salaries, Wages, and Fringe Benefits (A plus B) →				172,640			
D. Nonexpendable Equipment (Attach supporting data. List items and dollar amounts for each item.)				5,000			
E. Materials and Supplies				81,500			
F. Travel				5,000			
G. Publication Costs/Page Charges				5,000			
H. Computer (ADPE) Costs							
I. All Other Direct Costs (In budget narrative, list items and dollar amounts, and provide supporting data for each item.)				28,200			
J. Total Direct Costs (C through I) →				124,700			
K. F&A/Indirect Costs (If applicable, specify rate(s) and base(s) for on/off campus activity. Where both are involved, identify itemized costs included in on/off campus bases.)							
L. Total Direct and F&A/Indirect Costs (J plus K) →				297,340			
M. Other →							
N. Total Amount of This Request →				$	$	$	$

O. Carryover – (If Applicable) ..... Federal Funds: $	Non-Federal funds: $	Total $

P. Cost-Sharing/Matching (Breakdown of total amounts shown on line N)		
Cash (both Applicant and Third Party) →	$	$
Non-Cash Contributions (both Applicant and Third Party) →	$	$

NAME AND TITLE (Type or print)	SIGNATURE (required for revised budget only)	DATE
Project Director Greg Howard	Greg Howard	2/5/02
Authorized Organizational Representative Amanda Howard	Amanda Howard	2/5/02
Signature (for optional use)		

BUDGET

Salaries & Wages		
	Senior Associates:	
	Principal Investagater (Greg Howard) $31,200 per year/2year period	$62,400
	Co-Princibal Invertagater (Amanda Howard) $26,000 per year/2year period	$52,000
	2 Employees @ $7.00 per hour/40 hrs per week/2years	$58,240
	TOTAL	**$172,640**
Non-expendable Equipment		
	Water pump	$5,000
	TOTAL	**$5,000**
Materials & Supplies		
	Fish Stock	$25,000
	Chemicals for maintaining pond water	$1,500
	Basket Material	$2,000
	Water Piping	$3,000
	Mussel Shell	$50,000
	TOTAL	**$81,500**
Other Direct Costs		
	Equipment Rental	$6,000
	Rent	$15,000
	Utilities & Telephone	$7,200
	TOTAL	**$28,200**
Travel		$5,000
Publication/Promotion		$5,000
TOTAL BUDGET		**$297,340**

The following amounts will be needed to supplement or fund salaries:

- Greg Howard, Principal Investigator @ $31,200 per year for two years, Total $62,000.00
  ** Mr. Howard has agreed to participate in this study for the above stated amount and any additional salaries sought will be supplemented by Southern Aquatics.*
- Amanda Howard, Organizational Representative and Co-PI @ $26,000.00per year for a two year period Total $52,000.00
  **Mrs. Howard has agreed to participate in this study for the above stated amount and any additional salaries sought will be supplemented by Southern Aquatics.*
- Two General Laborers will be employed @ $7.00 per hour based on a 40 hour workweek for two years, $14,560.00 each Total %8,240.00

The following non-expendable equipment items must be purchased for this research:

One Commercial Water Pump $5,000.00

The following materials and supplies will be necessary:

- Chemicals for maintaining pond water and anesthesia for mussels $1,500.00
- Water Piping for piping water from river to flow through system for all ponds @ $3,000.00
- Mussel Shell for nuclei production and implantations @ $1.00 per animal for 50,000 animals. Forty thousand will be used for implantations and approximately ten thousand will be used for nuclei production.
- Material to construct the hanging baskets. $2,000.00
- Fish Stock, estimated @ $25,000.00

dead mussels

- Note: Southern Aquatics will fund any additional animals, materials, equipment or

Direct Cost

Equipment Rental For the construction of the ponds$6,000.00

Property Rent for two years, $15,000.00

Utilities and Telephone for two years, $7,200.00

Travel to Trade Shows $5,000.00

Publication and Promotion, Catalog cost and booth rental at trade shows, $5,000.00

(M). Documentation of Multiple Phase II Awards

No prior Phase II Awards have been awarded to Southern Aquatics or any principals thereof.

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approval 0524-0039
Expires 03/31/2004

ASSURANCE STATEMENT(S) - FOR RESEARCH PROJECTS

STATEMENT OF POLICY - Institutions receiving CSREES funding for research are responsible for protecting human subjects, providing humane treatment of animals, and monitoring use of recombinant DNA. To provide for the adequate discharge of this responsibility, CSREES policy requires an assurance by the institution's Authorized Organizational Representative (AOR) that appropriate committees in each institution have carried out the initial reviews of protocol and will conduct continuing reviews of supported projects. CSREES also requires AOR certification by citing a timely date that an appropriate committee issued an approval or exemption.

NOTE: Check appropriate statements, supplying additional information when necessary.

1. INSTITUTION

Southern Aquatics L.L.C.
249 Bayless Avenue
Florence, AL 35630

2. CSREES PROJECT NUMBER OR AWARD NUMBER (if known)

3. PROJECT DIRECTOR(S)

Amanda Howard

4. TITLE OF PROJECT

Fresh-Water Pearl

A. BIOSAFETY OF RECOMBINANT DNA

- [X] Project does not involve recombinant DNA.
- [ ] Project involves recombinant DNA and was either approved ( ) or determined to be exempt ( ) from the NIH Guidelines by an Institutional Biosafety Committee (IBC) on _____ (Date).

This performing organization agrees to assume primary responsibility for complying with both the intent and procedures of the National Institutes of Health (NIH), DHHS Guidelines for Research Involving Recombinant DNA Molecules, as revised.

B. CARE AND USE OF ANIMALS

- [X] Project does not involve vertebrate animals. AH
- [ ] Project involves vertebrate animals and was approved by the Institutional Animal Care and Use Committee (IACUC) on _____ (Date).

This performing organization agrees to assume primary responsibility for complying with the Animal Welfare Act (7 USC, 2131-2156), Public Law 89-544, 1996, as amended, and the regulations promulgated thereunder by the Secretary of Agriculture in 9 CFR Parts 1, 2, 3, and 4. In the case of domesticated farm animals housed under farm conditions, the institution shall adhere to the principles stated in the Guide for the Care and Use of Agricultural Animals in Agricultural Research and Teaching, Federation of Animal Science Societies, 1999.

C. PROTECTION OF HUMAN SUBJECTS

- [X] Project does not involve human subjects.
- [ ] Project involves human subjects and
  - [ ] Was approved by the Institutional Review Board (IRB) on _____ (Date). Performing Institution holds a Federalwide assurance number _____; if not, a Single Project Assurance is required.
  - [ ] Is exempt based on exemption number _____.
  - [ ] Specific plans involving human subjects depend upon completion of survey instruments, prior animal studies, or development of material or procedures. No human subjects will be involved in research until approved by the IRB and a revised Form CSREES-2008 is submitted.

This performing organization agrees to assume primary responsibility for complying with the Federal Policy for Protection of Human Subjects as set forth in 45 CFR Part 46, 1991, as amended, and USDA regulations set forth in 7 CFR 1c, 1992. All nonexempt research involving human subjects must be approved and under continuing review by an IRB. If the performing organization submits a Single Project Assurance, supplemental information describing procedures to protect subjects from risks is required.

SIGNATURE OF AUTHORIZED ORGANIZATIONAL REPRESENTATIVE	TITLE	DATE
Amanda Howard	President	2/5/02

According to the Paperwork Reduction Act of 1995, an agency may not conduct or sponsor, and a person is not required to respond to a collection of information unless it displays a valid OMB control number. The valid OMB control number for this information collection is 0524-0039. The time required to complete this information collection is estimated to average .50 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information.

CSREES-2008 (12/02/00)

UNITED STATES DEPARTMENT OF AGRICULTURE
COOPERATIVE STATE RESEARCH, EDUCATION, AND EXTENSION SERVICE

OMB Approved 0524-0039
Expires 03/31/2004

National Environmental Policy Act Exclusions Form

Project Director Name	Institution
Amanda Howard	Southern Aquatics L.L.C.
Address	
249 Bayless Avenue, Florence Alabama 35630	

Under 7 CFR Part 3407 (CSREES's implementing regulations of the National Environmental Policy Act of 1969 (NEPA)), environmental data or documentation is required in order to assist CSREES in carrying out its responsibilities under NEPA, which includes determining whether the proposed activity requires the preparation of an environmental assessment or an environmental impact statement, or whether such activity can be excluded from this requirement on the basis of several categories. Therefore, it is necessary for the applicant to advise CSREES whether the proposed activity falls into one of the following Department of Agriculture or CSREES categorical exclusions, or whether the activity does not fall into one of these exclusions (in which case the preparation of an environmental assessment or an environmental impact statement may be required). Even though the applicant considers that a proposed project may or may not fall within a categorical exclusion, CSREES may determine that an environmental assessment or an environmental impact statement is necessary for a proposed project should substantial controversy on environmental grounds exist or if other extraordinary conditions or circumstances are present that may cause such activity to have a significant environmental effect.

Please Read All of the Following and Check All Which Apply

[ ] **The proposed activity falls under the categorical exclusion(s) indicated below:**

Department of Agriculture Categorical Exclusions
(found at 7 CFR 1b.3 and restated at 7 CFR 3407.6 (a)(1)(i) through (vii))

[ ] (i) Policy development, planning and implementation which are related to routine activities such as personnel, organizational changes, or similar administrative functions
[ ] (ii) Activities that deal solely with the functions of programs, such as program budget proposals, disbursements, and transfer or reprogramming of funds
[ ] (iii) Inventories, research activities, and studies such as resource inventories and routine data collection when such actions are clearly limited in context and intensity
[ ] (iv) Educational and informational programs and activities
[ ] (v) Civil and criminal law enforcement and investigative activities
[ ] (vi) Activities that are advisory and consultative to other agencies and public and private entities, such as legal counseling and representation
[ ] (vii) Activities related to trade representation and market development activities abroad

CSREES Categorical Exclusions
(found at 7 CFR 3407.6(a)(2)(i) through (ii))

The following categories of CSREES actions are excluded because they have been found to have limited scope and intensity and to have no significant individual or cumulative impacts on the quality of the human environment:

(i) The following categories of research programs or projects of limited size and magnitude or with only short-term effects on the environment:
[ ] (A) Research conducted within any laboratory, greenhouse, or other contained facility where research practices and safeguards prevent environmental impacts
[ ] (B) Surveys, inventories, and similar studies that have limited context and minimal intensity in terms of changes in the environment
XX (C) Testing outside of the laboratory, such as in small isolated field plots, which involves the routine use of familiar chemicals or biological materials
[ ] (iii) Routine renovation, rehabilitation, or revitalization of physical facilities, including the acquisition and installation of equipment, where such activity is limited in scope and intensity

OR

[ ] **Proposed activity does *not* fall into one of the above categorical exclusions**
(**NOTE**: If checked, please attach an explanation of the potential environmental impacts of the proposed activity. May require completion of an environmental assessment or an environmental impact statement.)