

# Addressing Some Critical Bottlenecks to Commercially Viable Hatchery and Nursery Techniques for Black-Lip Pearl Oyster Farming in Micronesia, and Population Genetics of the Black-Lip Pearl Oyster (*Pinctada margaritifera*)

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## General Information

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*Participants*                         **Maria Haws**, Ph.D., Director, Pearl Research and Training Program  
Pacific Aquaculture and Coastal Resources Center, University of Hawaii at Hilo

Simon Ellis  
Mid-Pacific Marine Consultants

Eileen Ellis, Biologist  
Ponape Agriculture and Trade School (PATS)

Teresa Lewis, Ph.D., Assistant Researcher  
Hawaii Institute of Marine Biology (HIMB), University of Hawaii at Manoa

Matang Ueanimatang, Aquaculture Extension Agent  
College of the Marshall Islands

Greg Muckenhaupt, Director  
PATS

Deirdre Hon, Fred Martin, John Carran, Mikelson Hemil, Cristin Gallagher, and  
Jayson Silbanuz  
PATS

Quentin Fong, Ph.D.  
University of Alaska Fairbanks at Kodiak

Manoj Nair R., Ph.D., Aquaculture Research Scientist  
USDA Land Grant Program, College of the Marshall Islands

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## Objectives

### ***Hatchery and Nursery Methods Component***

The overall goal of this component is to conduct applied research to address some previously identified, critical bottlenecks to black-lip pearl oyster hatchery and nursery techniques in Micronesia.

1. Develop simple, cost-effective, land-based early nursery techniques for black-lip pearl oysters, and perform a cost-benefit analysis of these techniques compared with established ocean-based culture techniques.
2. Determine natural spawning seasons for black-lip pearl oysters in Pohnpei, FSM and share data with a similar effort to be conducted in Majuro, RMI so that hatchery operators may better time their activities thus increasing the cost-effectiveness of these operations. The same information will also assist in scheduling and preparing for grafting operations.

### ***Pearl Oyster Population Genetics Component***

The overall objective of this component is to quantify genetic differences between regional pearl oyster stocks. This objective is extremely important if hatcheries are

to address the genetic aspects and issues of broodstock management and the advisability of transferring localized stocks of pearl oysters within the region.

1. Collect samples from hatcheries in Hawaii, FSM, and RMI as well as from natural stocks to provide genomic DNA for population genetics analysis.
2. Perform amplified fragment length polymorphism (AFLP) analysis for genetic fingerprint.
3. Screen microsatellite library to identify diagnostic loci for genetic fingerprint.

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## Anticipated Benefits

### **Hatchery and Nursery Methods Component**

*The industry in CTSA's region relies on hatchery-propagated spat, and improved hatchery and nursery methods would be highly beneficial.*

In most of the region, the pearl industry is dependent on hatchery production for farm stock, and this has represented a major impediment to development of a strong industry. Now that pearl oyster hatcheries are present or being developed in Hawaii, RMI, FSM, and Palau, it is crucial that hatcheries be made more efficient by lowering mortality, increasing growth rates, improving the dependability of production, and lowering costs. The frequently high predation rates associated with ocean-based spat culture are problematic, but can be partially overcome if means can be found to promote spat growth rates so that spat more quickly reach sizes at which they are less susceptible to predation. If successful, this component of the work will yield information leading to improved hatchery and nursery methods that will assist local facilities in producing more spat with more regularity and at lower costs. It will also assist farmers and hatchery managers in determining the best methods for nursery rearing. All information generated by this study will be shared with regional stakeholders and incorporated into the next edition of the pearl oyster hatchery manual published by CTSA.

### **Pearl Oyster Population Genetics Component**

*Understanding pearl oyster population genetics will promote better stock management decisions.*

An improved understanding of basic pearl oyster genetics is key to conservation of the biodiversity of wild stocks, management of farmed stocks, and broodstock management in hatchery situations. Pearl oysters are rare, endangered, or protected in many areas of our region, and there is evidence from previous research in the South Pacific and Australia that genetically distinct populations may exist. If this is the case, then this has important implications for managing stocks, particularly in regards to the ability to transfer hatchery-produced spat or wild adults from one area to another. As there is also evidence that genetics may have a strong role in

determining pearl attributes that influence price (e.g., growth rates, color), more information is needed so that the industry can make good decisions about stock management without risking losing unique genetic characteristics that have economic implications. All information generated by this study will be shared with regional stakeholders and incorporated into regional discussions and decision-making relevant to managing pearl oyster stocks.

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## Work Progress and Principal Accomplishments

### ***Hatchery and Nursery Methods Component***

***Objective 1: Develop simple, cost-effective, land-based early nursery techniques for black-lip pearl oysters, and perform a cost-benefit analysis of these techniques compared with established ocean-based culture techniques.***

#### **Evaluating and optimizing land-based nursery techniques**

***Milestone 1: Supplies and equipment for the larval rearing run and research trial will be ordered. (S. Ellis, E. Ellis, and Haws).***

Most of the supplies needed for this portion of the work were purchased by the end of June 2004.

***Milestone 2: Algal room operation will begin two months prior to the larval rearing run to ensure trouble-free production. (S. Ellis and E. Ellis).***

The existing algae room was cleaned, and necessary minor repairs were completed by March 31, 2004. New axenic cultures of *Pavlova salina*, *Chaetocerus muelleri*, *Isochrysis galbana*, and *Tetraselmis suecica* were received on April 2, 2004. Staff members (Deirdre Hon, John Carran, Mikelson Hemil, and Fred Martin) were trained in all aspects of algae culture techniques from April to June. A PATS student intern (Jayson Silbanuz) was trained for six weeks during the larval rearing and spat settlement period.

***Milestone 3: Work will begin on routinely establishing a reliable “green water” bloom of phytoplankton for cost-effective, land-based nursery of spat. A 27,000-L tank will be filled with 1-micron filtered seawater (FSW), commercially available algae fertilizer will be added, and aeration provided. Water will be monitored for phytoplankton content and density. This process will be repeated several times throughout the year until researchers are***

*satisfied that a reliable method has been determined for “green water” culture. (S. Ellis and E. Ellis).*

This work is planned to commence in December 2004.

***Milestone 4:*** *Larval rearing run will be conducted to obtain spat for experiments. (S. Ellis, E. Ellis, Muckenhaupt, and Haws, in conjunction with other PATS staff).*

Oysters were spawned on June 16, resulting in the production of 18.67 million fertile eggs. The hatch rate was 77.25%, and a total of 3.6 million larvae were stocked into six 300-L hatchery tanks at the Marine Environmental Research Institute of Pohnpei (MERIP) laboratory. Spat were raised in the hatchery until August 2, when a sufficient number of spat exceeded 1.3 mm (dorso-ventral height, DVH) in order to start the experiment.

***Milestone 5:*** *At day 60 (or at an approximate size of 2 mm), juvenile pearl oysters will be removed from the larval rearing tanks and placed into 1-mm spat bags at treatment densities of 100, 200, and 300 spat per bag. Each stocking density will be replicated three times per tank. Bags will be randomly hung in one of four land-based 300-L treatment tanks, which will be supplied with TMD at densities of 150,000, 300,000, 450,000, or 600,000 cells/larva via a drip-feeding method. Water will be exchanged in all tanks twice per day and moderate aeration provided to ensure adequate mixing. Bags will also be rotated regularly to remove any “tank” effect in the treatment tanks. A triplicate set of spat at each treatment density will also be placed into 1-mm and 1.5-mm spat bags on an established farm in Pohnpei lagoon as a control. A comparison of growth and survival of spat in the differently sized bags will also be made. Bags hung in the ocean nursery will be checked once a week and cleaned when necessary. Predatory snails will be removed and enumerated when encountered on the lagoon farm, and water will be filtered to 25 microns in the land-based treatment tanks to prevent snail larvae from entering the tanks. Temperature will be recorded daily in each tank. (S. Ellis and E. Ellis).*

At the end of the spat rearing period, in preparation for this study, spat were selected for the study by grading them using sieves. Some spat that should have fallen through the sieve did not because they clung to larger spat. The spat used to start this study were a mean DVH of 1.7 mm (sd = 0.35 mm), but 10% were 1.3 mm or smaller and could fit through the mesh diagonally. We did not want to introduce bias by attempting to hand select spat by size, so we randomly assigned spat to treatment bags regardless of size and intentionally overstocked the bags by 10%. Bags were stocked with 110, 220, and 330 spat for the stocking density treatments. This was done in the expectation that 10% of spat were small enough to fall through the mesh bags.

Spat were stocked into 1-mm mesh bags only. Following a recommendation by Mr. Masahiro Ito of the College of Micronesia Land Grant pearl project, we used a polyvinyl chloride (PVC) frame (30 cm x 45 cm) made of ½-inch (1.3-cm) pipe as a substrate inside the spat bags. Mr. Ito's design includes a sheet of shade cloth attached to the frame for spat settlement, but in our experience we found the spat did not settle on the cloth, but on the PVC pipe only, so we used a pipe frame with no cloth on it.

The microalgal diet used in this study consisted of equal numbers of *Isochrysis galbana* (Tahitian strain), *Chaetocerus muelleri*, and *Tetraselmis suecica* cells. Algae rations were added to experimental tanks in two batches, morning and evening, rather than by drip-feeding. During the larval rearing run prior to the start of the experiment, we determined that the drip-feeding method did not always deliver the entire quantity of algae and thus was not accurate enough to use for a feeding study. Water was exchanged 100% every two days during the study.

**Milestone 6:** *Twenty days after stocking, twenty randomly selected spat will be measured from each bag. Spat in each bag will be counted and feeding rates adjusted accordingly. (S. Ellis and E. Ellis).*

Spat were enumerated and samples were measured on August 23<sup>rd</sup>. Spat in bags kept on a farm in Pohnpei lagoon were brought to the laboratory for this day and returned to the farm the following day. To avoid disturbing the spat unnecessarily, we did not remove them from the PVC pipe substrate while counting and measuring. The sample of 20 spat was randomly selected by starting from one corner of the frame and measuring the first 20 spat encountered along the frame.

**Milestone 7:** *Forty days after stocking, 20 randomly selected spat will be measured from each bag. Spat in each bag will be counted and feeding rates adjusted accordingly. (S. Ellis and E. Ellis).*

Spat were enumerated and samples were measured on September 13<sup>th</sup> and 14<sup>th</sup>.

**Milestone 8:** *Sixty days after stocking, 40 randomly selected spat (all the total remaining spat if less than 40) will be measured from each bag and the number of spat remaining in each bag counted. (S. Ellis and E. Ellis)*

For the final measurement, all spat were removed from the PVC frames. A sample of no less than 40 spat was randomly selected for measurement (DVH). To avoid introducing bias, all spat taken for this random sample were measured (40 to 60 spat).

**Milestone 9:** *Data will be analyzed to determine optimum stocking and feed density. (S. Ellis, E. Ellis, and Haws).*

The data are currently being analyzed.

**Objective 2: Determine natural spawning seasons for black-lip pearl oysters in Pohnpei, FSM and share data with a similar effort to be conducted in Majuro, RMI so that hatchery operators may better time their activities thus increasing the cost-effectiveness of these operations. The same information will also assist in scheduling and preparing for grafting operations.**

*Milestone 1: Necessary supplies will be ordered. (S. Ellis, E. Ellis, and Haws).*

The majority of the supplies for this study were purchased by June 30<sup>th</sup>.

*Milestone 2: A temperature recorder will be installed in the Pohnpei lagoon. Temperature data from the recorders will be downloaded every three months. Rainfall data will be obtained from the NOAA weather stations at each site. (S. Ellis and E. Ellis).*

Temperature recorders in underwater housings were placed in the three sites in Pohnpei lagoon selected for this study (on May 5<sup>th</sup> for Awak Pah and Aru sites and on May 10<sup>th</sup> for the Napoli site). The sites are located as follows: Napoli 06° 51.68' N 158° 21.21' E, Aru 06° 56.33' N 158° 19.34' E, and Awak Pah 06° 57.75' N 158° 12.82' E. The shuttle device for downloading temperature data in the field has proven unreliable and thus the data will not be downloaded until the study is ended after one year of sampling and the recorders are returned to the laboratory.

*Milestone 3: A total of 5–10 wild pearl oysters will be collected from three different sites in each lagoon within a 2–3-day period every six weeks throughout the year. (S. Ellis and E. Ellis).*

Rather than searching for oysters for each sample date, all oysters for the study were collected and deployed on the reef at each site at a depth of 15 meters by January 26<sup>th</sup>, over three months prior to the first sampling. Oysters were put in wire cages for protection, and cages were tagged with an identification number to aid in data collection. Six oysters from each site were collected and brought to the MERIP laboratory for each of the sampling dates so far: May 5<sup>th</sup>, June 16<sup>th</sup>, July 28<sup>th</sup>, and September 7<sup>th</sup>.

*Milestone 4: Oysters will be opened, and a qualitative estimate of gonad condition will be made with reference to presence or absence of gametes and degree of gonad fullness. Digital images of each gonad will be taken using a digital camera for comparative documentation and later use in training*

*materials to demonstrate the various stages of development. Gonads will also be measured. Gonad tissue will be examined microscopically to assess the stage of the development of the gametic material. DVH and weight will be recorded for each oyster. (S. Ellis and E. Ellis)*

Oysters were measured and then opened. Gonads were assessed for potential spawning condition and photographed with a digital camera. In order to avoid having to sacrifice oysters, gonads were not measured. A biopsy was performed to determine condition of gonad tissue. Oysters were then placed in cold water (22°C) overnight. On the following day, they were placed in warm water (32°C) for spawning induction. Spawning activity for each oyster was recorded.

### **Pearl Oyster Population Genetics Component**

#### **Objective 1: Collect samples from hatcheries in Hawaii, FSM, and RMI as well as from natural stocks to provide genomic DNA for population genetics analysis.**

*Genomic DNA has been extracted from 130 tissue samples from nine different sites in the region.*

Collection efforts have yielded 130 total tissue samples to date. Mantle tissue samples have been collected from a number of locations in the Federated States of Micronesia (Ahnt, Pohnpei, Nukuoro, and Chuuk) and the Republic of the Marshall Islands (Arno, Majuro, Jaluit, and Namdrik). Additionally, samples from Kaneohe Bay, Oahu were collected. Additional sites throughout Hawaii need to be surveyed and samples collected for analysis, and increased sample numbers need to be obtained from all sites excluding Arno in order to have enough tissue replicates for a statistically sound analysis (30 samples from each site).

Genomic DNA has been extracted and archived for all samples collected to date. Tissue samples are also archived.

#### **Objective 2: Perform amplified fragment length polymorphism (AFLP) analysis for genetic fingerprint.**

*Most of the samples have been processed to perform AFLP analysis, and more samples will also be collected.*

Approximately 100 total samples from FSM, RMI, and Hawaii have been processed to perform AFLP analysis at HIMB. Preliminary results of these experiments indicate there are significant genetic differences between farmed and wild populations of oysters and among oysters from various regions included in the study. However, in most instances the number of oysters included in this analysis (i.e., only three from Kaneohe Bay) is much too low to provide statistical confidence in these results. Additional samples are being collected to correct this.

#### **Objective 3: Screen microsatellite library to identify diagnostic loci for genetic fingerprint.**

Samples of genomic DNA have been extracted and pooled for production of a microsatellite library in order to develop diagnostic microsatellite loci to identify a genetic fingerprint for *P. margaritifera*. This material has been submitted to Genetic Identification Services (GIS), a commercial enterprise based in Chatsworth, CA, USA, that will produce the library and provide primers to screen samples at HIMB to identify diagnostic loci. This work is currently in progress at GIS.

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## Work Planned

### ***Hatchery and Nursery Methods Component***

Data from the spat rearing experiment will be analyzed, and recommendations for hatchery production of spat will be formulated. Trials to determine feasibility of the production of “green water” to rear pearl oyster spat will begin in December.

Gonad assessment of oysters from each of the three lagoon sites will continue to be conducted every six weeks until the end of the one-year time frame. Data will be collated, and any seasonal or site-specific trends will be noted. This work has also been an excellent learning and training experience for MERIP and PATS staff and students.

### ***Pearl Oyster Population Genetics Component***

Sample collection is still ongoing. S. Ellis, E. Ellis, Muckenhaupt, Haws, and others are currently working to obtain enough samples for processing and analysis by the end of 2004. Hawaiian *P. margaritifera* samples will be collected in early 2005 for processing and analysis. While most of the samples collected and returned to the University of Hawaii at Hilo and HIMB for processing have yielded genomic DNA of relatively high molecular weight and integrity for analysis, some sets of samples have not been successfully preserved for transport, and this problem is currently under investigation. New sets of samples, storage buffer, and collection tubes were provided to overcome this problem.

Additional AFLP primers are going to be included in future analysis to identify how many AFLP markers are required to produce a true genetic fingerprint. More importantly, inclusion of more samples for AFLP will be performed once Objective 1 has been completed.

Once GIS has provided primers for screening of samples, microsatellite analysis of samples will commence at HIMB. It is anticipated that this work will begin

some time in December as the samples collected by S. Ellis, E. Ellis, Muckenhaupt, Haws, and others in the fall of 2004 are provided to Lewis at HIMB.

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## Impacts

### ***Hatchery and Nursery Methods Component***

Since the experiments are still in progress and some of the data remain to be analyzed, the full impact of the research element of the work cannot yet be reported. This work has proved to be an excellent training exercise for the staff at MERIP/PATS, which includes Micronesian staff and marine science students.

### ***Pearl Oyster Population Genetics Component***

Once the genetic fingerprint of *P. margaritifera* has been determined and comparisons of oyster stocks from various farmed and wild populations accomplished, the results will be published in the CTSA newsletter and disseminated to stakeholders. Knowledge of the genetic relatedness of various populations of pearl oysters throughout the region will allow managers to make informed decisions regarding transfers of localized stocks between locations within the region. Future development of a selective breeding program for oysters also hinges on the results of the project's genetics component.

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## Publications in Print, Manuscripts, and Papers Presented

Lewis, T.D., C. Martin, C. Muir, M. Haws, S. Ellis, M. Ueanimatang, D. David, and M. Nair R. 2004. Population genetics of the black-lip pearl oyster *Pinctada margaritifera*. Page 350 in *Aquaculture 2004: Book of Abstracts*. March 2–5, 2004, Honolulu, Hawaii. World Aquaculture Society, Baton Rouge, Louisiana, USA.