Progress Report for 30 June 2004

A. Grant Number: 6-52618

B. Amount of Grant: $89,000

C. Project Title: Investigation of connectivity among coral communities using population genetic analysis

D. Grantee: Hawai‘i Coral Reef Initiative

E. Award Period: From 07/01/2003 – 12/31/2004

F. Period Covered by this Report: From 01/01/2003 – 06/30/2004

G. Summary of Progress and Expenditures to date:

1. Work Accomplishments
   a. Work Performed: The first three months of this grant (January to April) were spent running additional validation of the proposed AFLP methodology. Unfortunately, the method has not proved to be completely reproducible in multiple samples from the same individual. It is possible that the presence of zooxanthellae DNA in these extractions interferes with the consistent cutting of the DNA samples. In addition, new concerns regarding the use of AFLP techniques for assessing genetic connectivity have come to light, based on the lack of known structure underlying the bands produced in AFLP. Therefore, during the second three months of this grant (April to July), additional testing of an alternate protocol using microsatellites has been completed. Four microsatellite primers for *Pocillopora* species were obtained from a French colleague, developed with sperm from *P. meandrina* provided by Dr. Cox. These primers were ordered with appropriate dye labeling for the available Beckman sequencer at the Hawai‘i Institute of Marine Biology. Because the dyes are not identical to the dyes used in the original protocol developed by the French collaborators, it was necessary to optimize the protocol for work in our lab. We now have the first set of fragment analyses from 5 colonies and their larvae (testing the asexual or sexual nature of production of planulae as outlined in the original proposal) and will begin to collect the field samples for population genetics, focusing on *P. damicornis*, one of the three species originally outlined in the proposal. As no graduate students were available in March, we also hired a former student intending to apply to graduate school to assist with the DNA extraction and PCR amplifications. Pakki Reath began work in April and is currently being trained in the appropriate techniques.
   b. Work to be performed: The next six months will involve completing the field collections, DNA extraction and PCR amplification of the microsatellites, and analysis of the microsatellite fragment data.

2. Applications:
   a. Publications, presentations, workshops – apart from presentations for the HCRI committee, none have been completed at this date.
b. Applications to management or research – the lack of confirmation of the validity of
the use of AFLP for coral population genetics research is important in that no further
funds should be allocated to this specific line of research questions. AFLP may have
applications in coral species identification but does not appear to be acceptable for coral
population genetic studies.
c. Data – Branches from 10 colonies of *P. damicornis* and 5 to 10 larvae from each adult
were extracted and processed using the AFLP protocol. An additional set of branches
from 10 colonies of *M. capitata* were also extracted. Although these tests did not provide
reproducible data using AFLP, the DNA extractions from *P. damicornis* have been used
to optimize the microsatellite protocol. An additional set of 5 adult colonies and 10
larvae per colony were collected and processed for microsatellite analysis of larval origin.
We have results from the fragment analysis of these samples, although some of the
samples will need to be reamplified to optimize the fragment analysis procedure.
d. Partnerships – We are currently establishing the partnership with Leeward Community
College Marine Option Program for the collection of field samples this summer.

3. Expenditures:
a. Expenditures budgeted.
   Salary and Fringe Benefit: $55,105. Part time salary support was originally
provided for Dr. Cox, Dr. Lewis, and a graduate student.
   Materials and Supplies: $18,692.
b. Actual expenditures
   Salary and Fringe Benefits: $17,044 expended to date in this category.
   Materials and Supplies: $3,149 expended to date in this category.
c. Special problems, etc.
   As no graduate students were available for employment when this project
commenced in January 2004, we have hired a prospective graduate student to work on
this project. Pakki Reath began working part time for the project in April 2004, working
as a Casual Hire. She is currently being trained in DNA extraction and PCR
amplification techniques.
   We initially were using up supplies purchased for an earlier project to conduct the
validation of the AFLP methods. We have purchased two sets of pipetters, DNA
extraction kits, and PCR reagents using the funds from this project.

Prepared by:

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Co-Principal Investigator     Date: 3 July 2004