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Expiration Date: 9/30/2006

COP Format for Annual Progress Report

A. Grant Number: 102263

B. Amount of Grant: $42,981

C. Project Title: Assessment of Genetic Diversity and Connectivity in Fish Replenishment Areas in the Hawaiian Yellow Tang (*Zebrasoma flavescens*).

D. Guarantee:

E. Award Period: From 2/23/04 To 12/31/04

F. Period Covered by this Report: From 2/23/04 To 6/1/04

G. Summary of Progress and Expenditures to Date:

l. Work Accomplishments: (as related to project objectives and schedule for completion)

a. In accordance with the goals and objectives outlined in the revised proposal, we have completed;

1. Construction of a microsatellite DNA library for the Yellow tang. This library consists of four bacterial clone libraries, each enriched for a different tetranucleotide microsatellite motif. The library is housed at Washington State University in a -80^{0}C freezer. Construction of the library also entailed the design and optimisation of polymerase chain (PCR) primers for approximately 35 polymorphic microsatellite sequences, which were identified during the library construction process.

2. Sample collection and DNA extraction from approximately 400 Yellow tangs collected from Fish Replenishment Areas along the west coast of Hawai`i. In addition, several samples were collected from the Maui Ocean Center, and DNA extracted from these also.

3. Purification and quantitation of all DNA samples. The DNA has been transferred into 96 well microplate format and is stored at -20^{0}C until required for analysis.

4. Fluorescent PCR primers have been developed and are presently being optimised for analysis of microsatellite loci in the extracted Yellow tang DNA. Additionally to our proposal, we successfully tested the Yellow tang PCR primers in other coral reef species. This finding is of great interest to the Hawaiian scientific community and should form the basis of a subsequent funding proposal.

b. This project is presently on track. However, we have been able to collect DNA from approximately twice as many samples as originally proposed. Continuing work includes the following;

1. Optimisation of fluorescent microsatellite markers, and generation of raw microsatellite genotypic data from the Yellow tang DNA samples is expected to be completed by the end of October 2004.

2. Computer-aided population statistical analysis is scheduled to occupy the period between mid-October and the end of November.

3. Final report writing and manuscript preparation is scheduled in December.

2. Applications:

a. Presentations have been prepared and submitted to each of the quarterly HCRI meetings to date and the upcoming presentation is in preparation. Noakes travelled from Washington State University Vancouver to Honolulu in order to present the previous presentation. A short information session was also presented to a college class by Noakes during this period. A manuscript is in preparation for publication submission, explaining the characterisation of the microsatellite library and detailing variability at microsatellite loci in the Yellow tang. A short communication is also in preparation, detailing the usefulness of Yellow Tang microsatellite PCR primers in other coral reef fish species.

b. Interest generated during Noakes' visit to Hawai`i is expected to foster future collaborative links between WSU Vancouver and UH, and also between James Cook University, Australia. The additional research findings in other coral reef species will benefit the Hawaiian research community and some preliminary research is presently being undertaken in Hawai`i that is based on the research findings of our project thus far. We anticipate a subsequent funding proposal to continue this aspect of the present project and also, to develop collaborative links in this area.

c. The following data has been generated but not yet published;

1. DNA sequence of approximately 100 microsatellite clones from the library.

2. Approximately 270 oligonucleotide PCR primer sequences have been developed for Yellow tang microsatellites. Of these, 34 have been synthesised for use in fluorescent PCR analysis.

3. Eight Yellow tang individuals have been tested for each of the 34 synthesised primers and examined for polymorphism (see attached example agarose gel electrophoresis).

4. Nine of the above PCR primers have been selected and are presently being optimised for fluorescent PCR analysis in 400 Yellow tang samples.

5. Two PowerPoint presentations have been prepared, presented, and copies submitted to HCRI.

3. Expenditures:

a. Expenditures scheduled for this period include;

1. Salaries, wages and employee benefits.
2. Construction of microsatellite library.
3. Travel to Hawai`i
4. Consumables and reagents for Yellow tang tissue collection and DNA extraction.
5. Consumables, reagents and fluorescent primers for PCR.
6. Consumables and reagents for ABI3100 automated genetic analyser.
7. Desktop computer for statistical population analysis and literature preparation.

b. Actual expenditures this period include all of the above. Project budget is on schedule.

Prepared By: _____ - 6/10/2004
Signature of Principal Investigator Date

NOTICE

Subsequently, all NOAA COP recipients with approved grants will be asked to file a COP Annual Progress Report in the specified format. The first section of the proposed format is taken from the COP implementation plan and has some advantages in that previously-funded investigators will be familiar with the format. Consistency in reporting requirements for competitive research grant programs is desirable and this is behind COP's efforts in proposing a standardized format. This annual report format will enable COP program staff to monitor each project supported by an award.

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