Carlon, Year 8: Annual Progress Report

A. Grant Number: 655568

B. Amount of Grant: $77,125

C. Project Title: Sources and sinks of a keystone herbivore on Hawaiian coral reefs

D. Grantee: David B. Carlon

E. Award Period: From: 10-01-05 To: 12-31-06

F. Period Covered by this Report: From 10-01-05 To: 6-30-06

G. Summary of Progress and Expenditures to Date:

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

a. Provide a brief summary of progress, including results obtained to date, and their relationship to the general goals of the grant;

Three objectives and results so far:

Objective 1: To determine source and sink populations by assigning individuals to natal source populations.

We have successfully obtained samples from 30-60 individual Tripneustes gratilla (collector urchins) from 3-5 populations on each of four Main Hawaiian Islands (Table 1). Tissue samples (several spines and tube-feet) were collected non-destructively, and all urchins were released to the original site of collection unharmed. We extracted genomic DNA from a total of 703 samples.

Table 1. Location of sampling sites on four Hawaiian Islands

<table>
<thead>
<tr>
<th>Island</th>
<th>Site (= population)</th>
<th>Aspect</th>
<th>Sample size</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kauai</td>
<td>Hanalei Bay</td>
<td>Windward</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Kahala Pt.</td>
<td>Leeward</td>
<td>41</td>
</tr>
<tr>
<td></td>
<td>Koloa Landing</td>
<td>Leeward</td>
<td>50</td>
</tr>
<tr>
<td>O'ahu</td>
<td>Barking Sands Beach</td>
<td>Leeward</td>
<td>49</td>
</tr>
<tr>
<td></td>
<td>Power Plant</td>
<td>Leeward</td>
<td>47</td>
</tr>
<tr>
<td></td>
<td>Honolulu Sewer Pipe</td>
<td>Leeward</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Turtle Bay</td>
<td>Windward</td>
<td>49</td>
</tr>
<tr>
<td>Mau'i</td>
<td>Honolulu Bay</td>
<td>Leeward</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>Coral Garden</td>
<td>Leeward</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>White Rocks</td>
<td>Leeward</td>
<td>49</td>
</tr>
<tr>
<td>Hawai'i</td>
<td>Richardson Beach Park</td>
<td></td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Poho'iiki Bay</td>
<td></td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Punalu'u</td>
<td></td>
<td>43</td>
</tr>
<tr>
<td></td>
<td>Two-Steps</td>
<td></td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Puako Bay</td>
<td></td>
<td>30</td>
</tr>
</tbody>
</table>
Our efforts have focused on two types of genetic markers. The first marker is the *cytochrome oxidase I* gene (COI) of the mitochrondion genome. We used PCR and direct sequencing on ABI capillary sequencers to sequence a 431 bp portion of this gene, and so for a total of 425 individuals from the four main islands. We found a total of 42 polymorphic (variable) sites in this gene resulting in 47 haplotypes (unique sequences). Our populations were dominated by 4 common haplotypes with the remainder being rare (~2%), or found in only one single individual.

We have also successfully developed and applied microsatellite loci for *Tripneustes gratilla*. These nuclear markers have co-dominant inheritance and evolve at high rates providing the potential to detect more recent (10s to 100s of thousands of years). We subcontracted Genetic Identification Services, Chatsworth, CA (GIS) to develop genomic libraries, and they successfully developed three libraries enriched for three different repeat motifs for *T. gratilla*. We assayed 39 loci for evidence of polymorphism and selected 8 putative polymorphic loci to optimize for automated genotyping. We have now optimized multiplex PCR for automated genotyping on ABI 3730 capillary sequencers. We have scored three of eight loci, and analyzed genetic structure within and among islands with these markers.

The general result from analyzing both COI and our three microsatellite loci is that there is no evidence for genetic structure within, or between islands. A common metric of population structure: $F_{ST}$ was not significant in a hierarchical AMOVA model (Analysis of MOlecular VAriance) at either: 1) populations within islands or 2) between the four main islands. This means that there is an equal probability of sampling the same genetic variants on Kauai as on the Big Island. It also implies that larvae are regularly dispersing this distance and exchange is high.

**Objective 2.** To determine source and sink populations by assigning individuals to natal source populations.

**Objective 3.** Construct a map of the genetic landscape of sampling sites within and among Hawaiian Islands. This map will indicate larval corridors, sources, and sinks of successful larval recruitment that can be used by managers to identify critical habitats that act as nursery areas for keystone species.

Accomplishments related to these two objectives will depend on the final analyses from the complete set of microsatellite loci. Also see section b below.

*b. Provide a brief summary of work to be performed during the next year of support, if changed from the original proposal; and indication of any current problems or favorable or unusual developments; and any other significant information pertinent to the type of project support by COP, or as specified by the terms and conditions of the grant.***

We see no changes to the original proposal, or problems in meeting all three objectives. In the next year, we will be genotyping and scoring the remaining five microsatellite loci to increase our power to detect genetic structure. Objectives 2 and 3 will depend on the degree of genetic structure we detect in the total sample. If we find no structure, then by implication, gene flow and larval dispersal are high among all islands.

We are also planning on using the genetic data obtained to infer the demographic history and to estimate the effective population size of *Tripneustes gratilla*, information that could be used to set a baseline for future management strategy. For example we will be able to detect expansions or contractions in the Hawaiian population over a time frame of thousands of years.
2. Applications:
   a. Publications, presentations, workshops;
      1. Oral presentation on Kauai and Hawaii for the HCRI “Road Show.”
      2. Oral presentation at the Benthic Ecology Meeting 2006 (Québec, Canada)
      3. Oral presentation at the HCRI committee on 16 May 2006
      4. Workshop: “Molecular Tools for Reef Managers” to be held in October 2006
   b. Applications to management or research;
      1. Data from genetic structure indicates that transplantation of urchins among sites around islands
         or even between islands to control invasive macroalgae will not impact the evolutionary potential
         of this species.
      2. It is genetically feasible to stock problem areas where Tripneustes are abundance
   c. Data and/or information products;
      1. We have established a genetic baseline of population structure of Tripneustes gratilla around the
         four main Hawaiian Islands.
      2. We have 8 new microsatellite loci that will be added to the NCBI genbank database, and will
         submit a “Primer Note” to the journal Molecular Ecology to communicate these markers to the
         scientific and conservation community.
   d. Partnerships established with other federal, state, or local agencies, or other research institutions (other
      than those already described in the original proposal).
      We have established contacts with the following DAR staff: Ivor Williams, Bill Walsh, Skippy Hau,
      and Wade Ishikawa. These people have been generally supportive of our research and very helpful in
      locating sampling sites and putting us in touch with people with local knowledge of fishing practices.

3. Expenditures:
   a. Describe expenditures scheduled for this period.
      Salary $44,031.00
      Materials and Supplies $9,920.00
      Travel $1,600.00
   b. Describe actual expenditures this period.
      Salary $33,023.00
      Materials and Supplies $7,678.91
      Travel $2,386.98 (completed)
   c. Explain special problems, differences between scheduled and actual expenditures, etc.
      I have requested reallocation of funds among categories that will reduce the total Salary and
      increase funds in Materials and Supplies. We will not need as much technical time to complete the project
      as originally planned, but we do need additional molecular supplies to complete genotyping with
      microsatellite loci. Travel exceeded original budget because of higher than anticipated costs of sampling
      on Kauai.
Signature of Principal Investigator                                           Date: June 29, 2006

NOAA COP Annual Progress Report Form
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.

Public reporting burden for this collection of information is estimated to average 300 minutes per response, including time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed and completing and reviewing the collection of information.

Send comments regarding this burden estimate or any other aspects of this collection of information, including suggestions for reducing this burden, to the National Ocean Service, CSCOR/COP Office, 1315 East-West Highway, Silver Spring, MD 20910. Grant files are subject to the Freedom of Information Act (FOIA). Confidentiality will not be maintained—the information will be made available to the public. However, unpublished research results shall not be published without prior permission from the recipient.

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