

Essential Fish Habitat project status report

Reporting date: 10/31/2008

Project number: 2007-11C

Title: Biological parameters to estimate the recovery of disturbed benthic habitat in Alaska, study C: Coral genetics

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Funding year: FY 2007

Funding amount: \$14,050

Status: Complete Incomplete, on schedule Incomplete, behind schedule

Planned completion date if incomplete: September 15, 2009. The project is behind schedule because we had to revisit the sites a second time (in May 2008) to recollect samples. The original samples collected in April 2007 were not of sufficient quality to produce viable DNA for our analyses. We tried 3 different preservative techniques for the coral tissue samples collected in 2008 and one of them produced excellent high-molecular weight DNA for analysis.

Reporting: Have the project results been reported? If yes, where were the results reported? No.

Results: What is the most important result of the study? A summary of the project history and findings to date follows:

Tissue samples for genetic analysis from 150 *Primnoa pacifica* colonies were collected in 2007 & 2008 from Tracy Arm and Endicott Arm, Holkham Bay. Our initial objective was to extract high molecular weight (HMW) DNA to be used to construct enrichment libraries for detecting and developing taxon-specific microsatellite markers. These markers typically show sufficient variation that they can detect fine-scale population structure and, if variation is present, would allow for an assessment of the relative contribution of asexual reproduction in patches of coral. Subsequent to the library construction, our objective is to screen sequenced clones for microsatellite loci, and test them for variability among the *P. pacifica* samples collected.

Objective 1: In March, 2007, 80 samples were collected from four sites. Although we were able to extract DNA from these samples, we did not have success obtaining the unsheared, HMW DNA required for the library construction. We concluded the problem may have been in the initial preservation of samples, i.e., DNA was degrading before tissues were preserved. The best solution was to resample colonies, paying particular care to rapid preservation, and this effort took place in May 2008 (70 samples from 5 sites).

DNA extractions on a subset of these specimens did produce HMW DNA from multiple individuals.

Objective 2: HWM DNA from three extractions was sent to the SREL (Savannah River Ecology Lab) DNA Lab in September 2008 for construction of an enriched genomic library for microsatellite development. The following steps have been successfully completed by the SREL DNA Lab:

- 1) DNA digestion and linker ligation, cloning, double enrichment with biotinylated probes, and PCR amplification of inserts.
- 2) DNA sequencing of inserts from > 100 clones

DNA sequences of clones (>100) containing microsatellite motifs were delivered by SREL to UL Lafayette on 10/27/2008.

The next phase of the project will involve analysis of the DNA sequences for microsatellite motifs that are of appropriate size and structure to be used in a population study. Primers will be designed to flank these regions and then tested on the set of samples collected in 2007 & 2008. This will involve completing DNA extractions of all collected tissue samples, PCR amplification, optimization of reaction parameters, and analysis of output for variability. This phase will require significant lab effort, and funding was budgeted for one graduate student assistant to complete the work during the summer of 2009.