Essential Fish Habitat project status report

Reporting date: 9/28/2009

Project number: 2007-11C

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

PIs: Robert Stone (AFSC), Scott France (University of Louisiana)

Funding year: FY 2007

Funding amount: $14,050

Status: ☒ Complete ☐ Incomplete, on schedule ☐ Incomplete, behind schedule

Planned completion date if incomplete:

Reporting: Have the project results been reported? If yes, where were the results reported? No. We were not able to complete the project within the timeframe and with the funds received. The project will be completed with alternative funds being requested by Dr. France and will be reported at a later time.

Results: What is the most important result of the study? We developed microsatellite primers from *Primnoa pacifica* colonies collected in Tracy Arm, Holkham Bay. To our knowledge, this is the first attempt to develop microsatellite loci in *Primnoa pacifica* (or any other Alaskan octocoral). Initial tests of primers for four loci on 40 individuals from 4 separate collection sites reveal high levels of alleleic diversity within sites. More extensive optimization and testing will be required to further develop these loci (additional loci can likely be developed from the data we have already collected) for use in population studies of fjord corals. However, this preliminary study shows that this approach is likely to yield appropriate genetic markers to study fine-scale population processes of these cold-water corals.

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.

Objective 3: Examination of clone sequences revealed 6 repeat regions (4 tetra-, 1 tri- and 1 di-nucleotide repeat) that were suitable for PCR primer development (more such sequences are likely among the clones available and will be examined in our continuing research). We tested the 6 pairs of primers (= 6 presumptive microsatellite loci) initially on ≈10 colonies to determine whether the loci would amplify all individuals and to determine appropriate reaction conditions for optimal banding. All 6 loci produced reaction products in all individuals tested. We isolated and sequenced some of the reaction products to verify that we were amplifying the expected repeat regions (see locus and primer information below). Despite repeated manipulations of conditions, the dinucleotide repeat could not be effectively optimized, and was dropped from further analysis.

The remaining 5 loci were tested on 40 individuals from 4 collection sites within Tracy Arm, to look for variability (= multiple alleles) and if there were any preliminary evidence that this diversity might be structured within the fjord, i.e., differences in allele frequency among the collection sites. PCR reaction products were run out on 2.75% Metaphor agarose (Cambrex) gels to allow for discrimination of bands (alleles) between 160 and 300 nucleotides length. Four of five loci produced variable-size PCR products within an expected size range (based on the initial clone); the fifth locus included non-specific products and was not further analysed. For four loci we classified the products into allele classes, but acknowledge these could change somewhat when the products are amplified using a fluorescently-labeled primer and run on an automated sequencer for visualization; such methods provide higher spatial resolution among bands. All four loci showed high levels of band (presumptive allele) variation (Figure 1). Two of the loci (F10 and C01) produced sufficiently distinct bands to allow for further analysis of the

scored alleles (loci H10 and C09 produced banding patterns that did not conform to the expected diploidy, i.e., there were as many as 3 alleles per individual. Without further analysis, we could not determine which bands were real.).

At loci F10 and C01, many more homozygotes, and fewer heterozygotes, were observed than expected. This may be the result of one or a combination of the following:

- incomplete resolution of allelic bands. Although the Metaphor gel system has high resolution in the size range we targeted, it is possible that alleles of heterozygotes that differed by a single repeat unit were not recognized as distinct and scored as homozygotes.
- There is a high level of inbreeding or asexual reproduction at each site.
- Colonies collected from single sites actually come from separate interbreeding populations ("Wahlund effect").

At locus F10, site 4 (farthest up the Tracy Arm) did show frequency differences to sites 1 & 2 (individuals from site 3 did not produce strong enough bands to score for this locus), suggesting perhaps a different origin of individuals compared to other sites, but this will need confirmation with additional colonies and loci before any confidence can be put in this interpretation.

Microsatellite locus and primer information

F10 Tetranucleotide repeat (CTGT)
Primer 1: F10f: GCATTTCTCGACTTTCATGT
Primer 2: F10rev: gttTGACCAAGAAACAACAGACACA
Number of alleles scored: 14
Expected number of homozygotes: 3.7627
Observed number of homozygotes: 22
Expected number of heterozygotes: 26.2373
Observed number of heterozygotes: 8

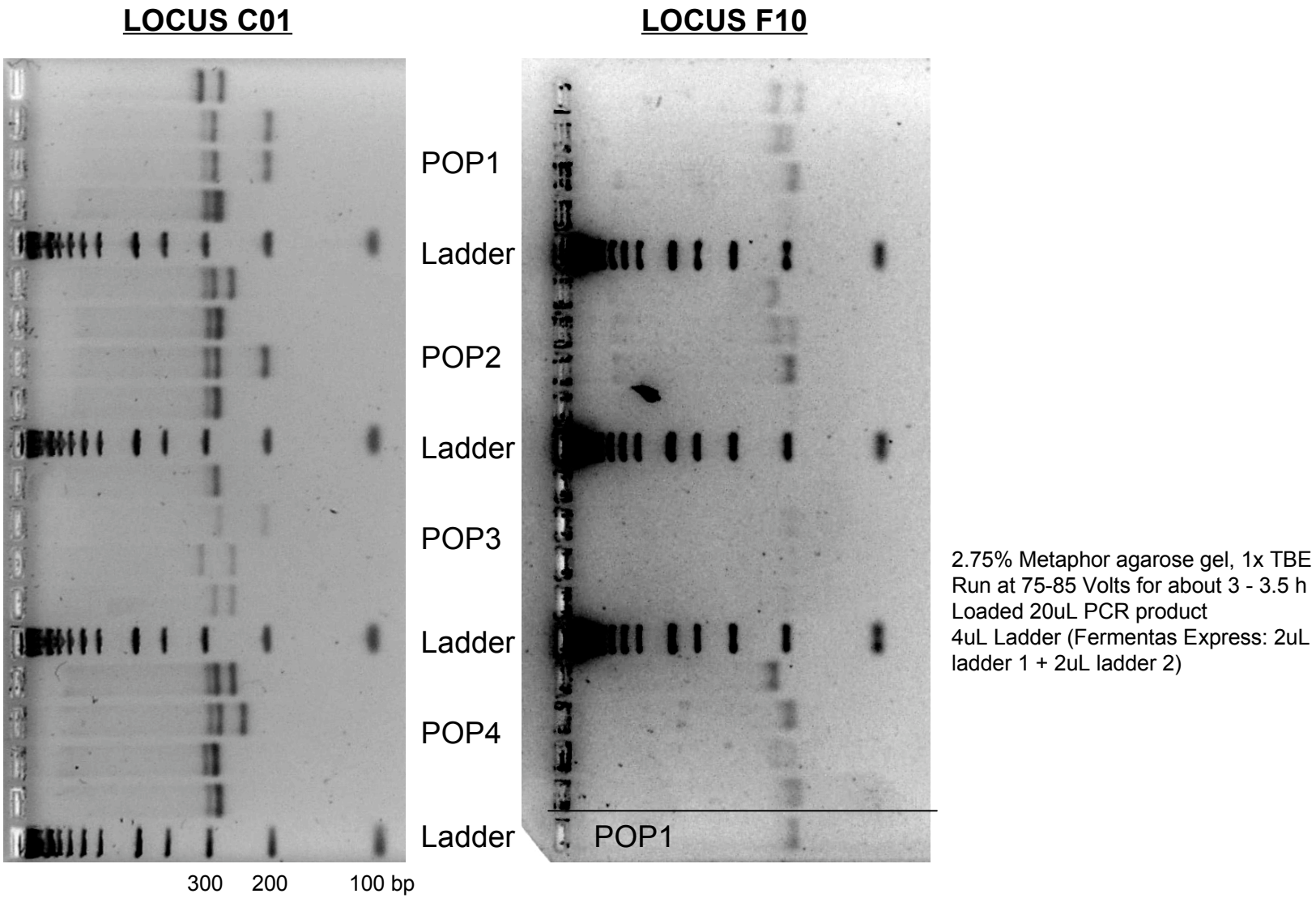
C01 Tetranucleotide repeat (GATT)
Primer 1: C01f: GCTGGCTACACAGGTCGTCT
Primer 2: C01rev: gTTTTTCCCGTGAACCCAAT
Number of alleles scored: 20
Expected number of homozygotes: 3.1688
Observed number of homozygotes: 21
Expected number of heterozygotes: 35.8312
Observed number of heterozygotes: 18

C09 Trinucleotide repeat (AGT)
Primer 1: C09f: GCGGCTTGGTATAGCTGATG
Primer 2: C09rev: gttTGATCCTGCTCCTATTCCTCT

H10 Tetranucleotide repeat (ACAG)
Primer 1: H10_P2f: CATTGTAGTGCCGGCCATC

Primer 2: H10_P2rev: gttTCTGTCTGTCTCTTGCTCTCT

B01 Tetranucleotide repeat (AACT)
Primer 1: B01f: aTGGCAGTGTAGCCACAGTA
Primer 2: B01rev: gtttGCAGAATCACAGACCACTCG



An example of size variability of PCR bands (presumptive alleles) for microsatellite loci C01 and F10. DNA extracted from individual colonies of *Primnoa pacifica* collected from four localities (POP1-4) in Tracy Arm, Holkham Bay were PCR amplified and run on the gels (migration from left to right); a different set of individuals is shown on each gel.