ECALS MILESTONE REPORT

ACRCC Framework Item: 2.6.3

Milestone: High Throughput Technique complete

Milestone Date: May 2012

Agencies Involved: USACE, USGS, USFWS

Brief Description of Milestone: Develop a higher-throughput DNA extraction capability. Combine commercial extraction protocols with available large format high-throughput (HT) DNA extraction instruments such that average per sample extraction time is reduced, without statistically significant reductions in eDNA extract results relative to standard protocol.

How does the accomplishment of the milestone contribute to ECALS and eDNA overall?
Reduces time required to process eDNA samples, reducing costs and critical reporting time.

Was the milestone accomplished?
75% complete. Significant progress has been made to develop data or processes that will reduce the overall eDNA sample processing time. Those processes and studies are described in the following text.

- We reduced the time required to break down samples for DNA extraction by about 80% by incorporating a Mini-Beadbeater 96 (Bio Spec Products, Inc., Bartlesville, OK) into a revised protocol. This change, however, reduces the overall time to completely process a typical 120-sample batch by only one hour.

- We evaluated the BuccalAmp™ DNA QuickExtract™ Solution (Epicentre Biotechnologies, Madison, WI) as an alternative DNA extraction kit. The QuickExtract™ solution provides one of the most rapid DNA extraction protocols currently available. Unfortunately, while this kit worked well on lab samples, it generally failed to remove enough environmental inhibitors to allow for successful downstream processing of samples. No extraction or PCR amendment was found that could reverse that result.

- We observed that the effectiveness of the PowerWater® DNA Isolation Kit (Mo Bio Laboratories, Inc., Carlsbad, CA) kit that is currently used for eDNA sample extraction seemed to be inhibited when more than 2 filter papers were processed in cell lysing

tubes. We surmise that the kits ability to remove PCR inhibiting compounds may become saturated when 3 or more filter papers are processed in a single lysing tube. A small-scale test in the lab corroborated this observation. Subsequently, eDNA samples from the Asian carp monitoring efforts and other eCALS tasks were extracted with a limit of 2 filter papers per lysing tube. Unfortunately this discovery increases the time required to process a sample (filtering some samples may require 3 or more filters and thus may require multiple extractions per sample) but improves accuracy of the assay.

- We assessed the relative efficacy of another alternative DNA extraction protocol. The Qiagen DNeasy Blood & Tissue Kit (Qiagen Inc., Valencia, CA) is a kit commonly used for DNA extraction from many types of samples. This kit and the PowerWater (QAPP) kit were compared by determining the lowest amount of DNA detected following extraction of laboratory-prepared samples. DNA from deionized water samples (2L) that were spiked with 5 different amounts (4,000 ng to 250 ng) of silver carp DNA source material was extracted using both kits. Deionized water was used in this initial study to provide a consistent, standard matrix for testing all samples without the presence of interfering substances that could affect the absolute DNA extraction efficiency of the kit. One subset of samples were filtered and extracted according to the current Asian carp eDNA QAPP. The other subset of samples were extracted using the Qiagen kit according to manufacturer protocols, with a minor modification required for extracting DNA from the glass fiber filter. Silver carp DNA was detected in only 3 samples (3/5 or 60%) in trials with the lowest amount of DNA starting material (250 ng) and the PowerWater kit. The PowerWater kit required a silver carp DNA loading of >2000 ng/sample to achieve a 100% positive detection rate. A 100% positive detection rate was achieved using the Qiagen kit in samples starting with as little as 250 ng silver carp DNA material. Samples with 250 to 1,000 ng silver carp DNA material showed positive results in only 9 of 15 trials with the PowerWater kit, while all samples (15/15) in this same DNA range showed positive results with the Qiagen kit.

  Based on these comparisons, inter-laboratory round robin testing of the two extraction kits is planned to begin in September 2012. The USGS UMESC will coordinate inter-laboratory comparisons of the PowerWater and Qiagen kits with participation by the USACE ERDC and USFWS NEFC; additional laboratories (e.g. USGS CERC, USFWS La Crosse Fish Health Center) will be included if needed. The USGS UMESC will prepare a study protocol for this testing based on standard inter-laboratory method validation protocols. Once the inter-laboratory testing has been completed, the group will determine whether to revise the QAPP to use the Qiagen kit as the standard DNA extraction kit. If this revision to the QAPP is incorporated, the DNA extraction time would be reduced by about 50% and the per sample extraction costs would decrease

from about $7.50 using the PowerWater kit to close to $2.50 per sample using the Qiagen kit (a savings of roughly 67%)

- We assessed the degree to which eDNA samples degrade when held at room temperature for several hours instead of being placed immediately in cold storage. The current requirement to move samples quickly from the field to cold storage impacts the efficiency of field sampling and results in several logistic difficulties for field and filtering teams. We found that samples lose a significant amount of DNA when held at temperature above 0°C for 24 h, which supported the requirement to move samples quickly to cold storage.