DNA Extraction Protocol Optimization for Decades-Old Aquatic Macroinvertebrate Samples

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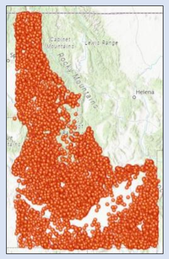
Abstract

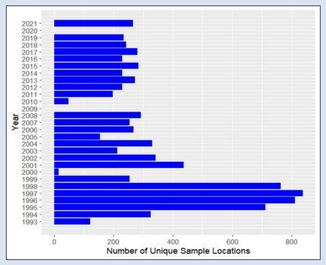
Stream benthic macroinvertebrate (BMI) communities are a valuable monitoring tool for assessing water quality. The Idaho Department of Environmental Quality's Beneficial Use Reconnaissance Program (BURP) has collected >8,600 samples at randomly selected sites throughout the state since 1994 and identified larval specimens to genus using morphology. Specimens were archived for many of the samples and the BURP samples could enhance detection of water quality and climate effects over the past three decades if species can be identified using DNA barcoding. DNA barcodes are a species-specific sequence of nucleotides found on the mitochondrial COI gene. Our objectives are to 1) develop an effective DNA extraction, amplification, and sequencing protocol and 2) to build a DNA barcode library for Idaho BURP BMI specimens. To date, we have modified DNA extraction protocols to optimize the concentration of extracted DNA. Experiments compared extraction yield to the number of elutions and incubation time. We determined two elutions with a twenty-four-hour incubation period provided the best yield. DNA from additional identified vouchers will be amplified using Polymerase Chain Reaction, sequenced, and submitted to the Barcode of Life (BOLD) database. DNA barcode libraries from will improve monitoring of species diversity in BMI samples and should increase the sensitivity of water quality and climate change studies in Idaho.

Background

- Stream benthic macroinvertebrates (BMI) transfer energy from primary to producers to consumers such as fish and are thus central to the complex trophic systems supported by streams and rivers (Baxter 2005).
- BMI communities are also a valuable tool in monitoring water quality and climate change.
- The IDEQ BURP program has collected and archived more than 8,600 BMI samples at randomly selected sites in Idaho since 1994.



- **Left**: BURP sample locations.
- **Right**: Number of BURP samples collected by year (8,643 samples total).



- Samples likely have unrecognized diversity (Jackson, et al. 2014) because most specimens could only be identified to genus or family using morphological characters of larval life stages.
- The samples could provide valuable insight into Idaho climate change as relates to water quality if the samples can be identified to species using DNA barcoding (Milner, et al. 2023).
- However, the DNA quality in these specimens declines over time, which could make extraction and barcoding difficult to accomplish.

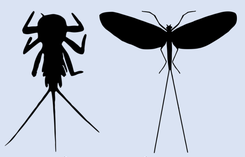
Goals

1. Optimize a DNA extraction, amplification, and sequencing protocol for BMI samples collected in the 1990s and early 2000s.
2. Begin to build a DNA barcode library using identified specimens from BURP and other museum voucher specimens.

Approach

The species *Baetis bicaudatus* and *Epeorus deceptivus* were selected because these mayflies are abundant in the samples and are reasonable representatives of the larger BURP database. We used 4 specimens of each species and a negative control for each test.

1. Select samples for DNA extraction



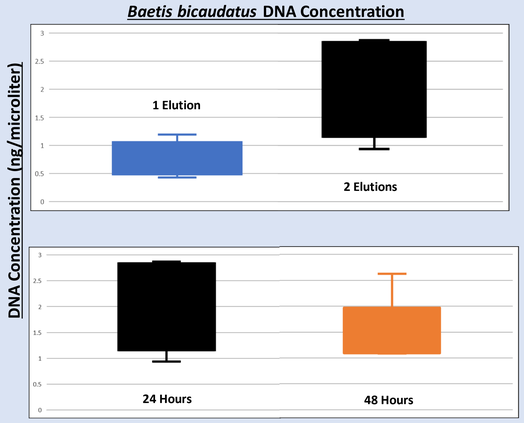
2. Test DNA extraction protocols:

The standard extraction followed Qiagen DNEasy Blood and Tissue protocol. This is a two-day extraction protocol where day one involves tissue lysis and day two purifies the DNA product. We experimentally altered the number of elutions and incubation time.

Vary the number of elutions

1 Elution | 2 Elutions

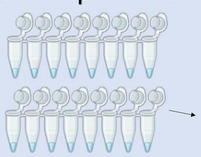
***Baetis bicaudatus* DNA Concentration**



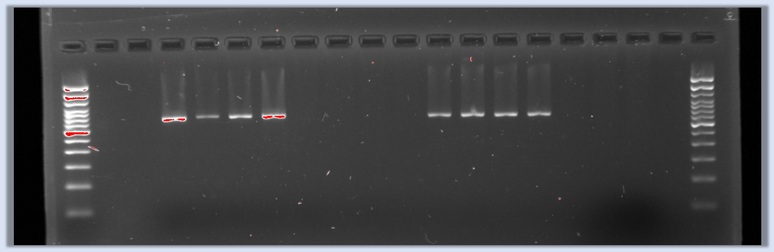
Vary the incubation time

24 hours | 48 hours

3. PCR Amplification



Gel Electrophoresis



Example gel from a PCR reaction performed to test extraction protocol. White bands below a well indicate the PCR for the sample was effective; left and right wells contained size standards.

4. DNA Sequencing

5. Submit sequences to the Barcode of Life Database (BOLD)

- BURP and museum specimens identified by expert taxonomists using morphology.
- DNA sequencing of specimen links morphological ID to a DNA barcode.
- Barcode of Life database will allow future researchers to identify new specimens using DNA.

Conclusions

- We found two 30 microliter elutions and a 24-hour incubation provided the best extraction yields out of those tested.
- Preliminary results suggest PCR success varies by species and future work to optimize PCR amplification may be needed.
- Our experiments have shown that, despite being nearly thirty years old, many museum specimens still have DNA that can be amplified with barcoding primers.
- DNA sequencing after additional extractions and amplifications will generate an initial DNA barcode library from the BURP samples.

Future Questions

- How many species are present in the BURP samples? Many species have been identified morphologically in the BURP sample set and past studies have revealed around twice as many species using DNA than morphological identifications in many groups (Jackson et al. 2014).
- How many midge species are revealed with barcodes? Midges (Chironomidae) compose a large proportion of individuals and species diversity in stream ecosystems but are only identified to family in most BMI samples.
- Does increased taxonomic resolution provided by DNA barcoding improve the ability of surveys such as BURP to detect environmental and climate change?

Literature Cited

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This research is made possible by Idaho NSF-EPSCoR Program (award OIA-1757324).