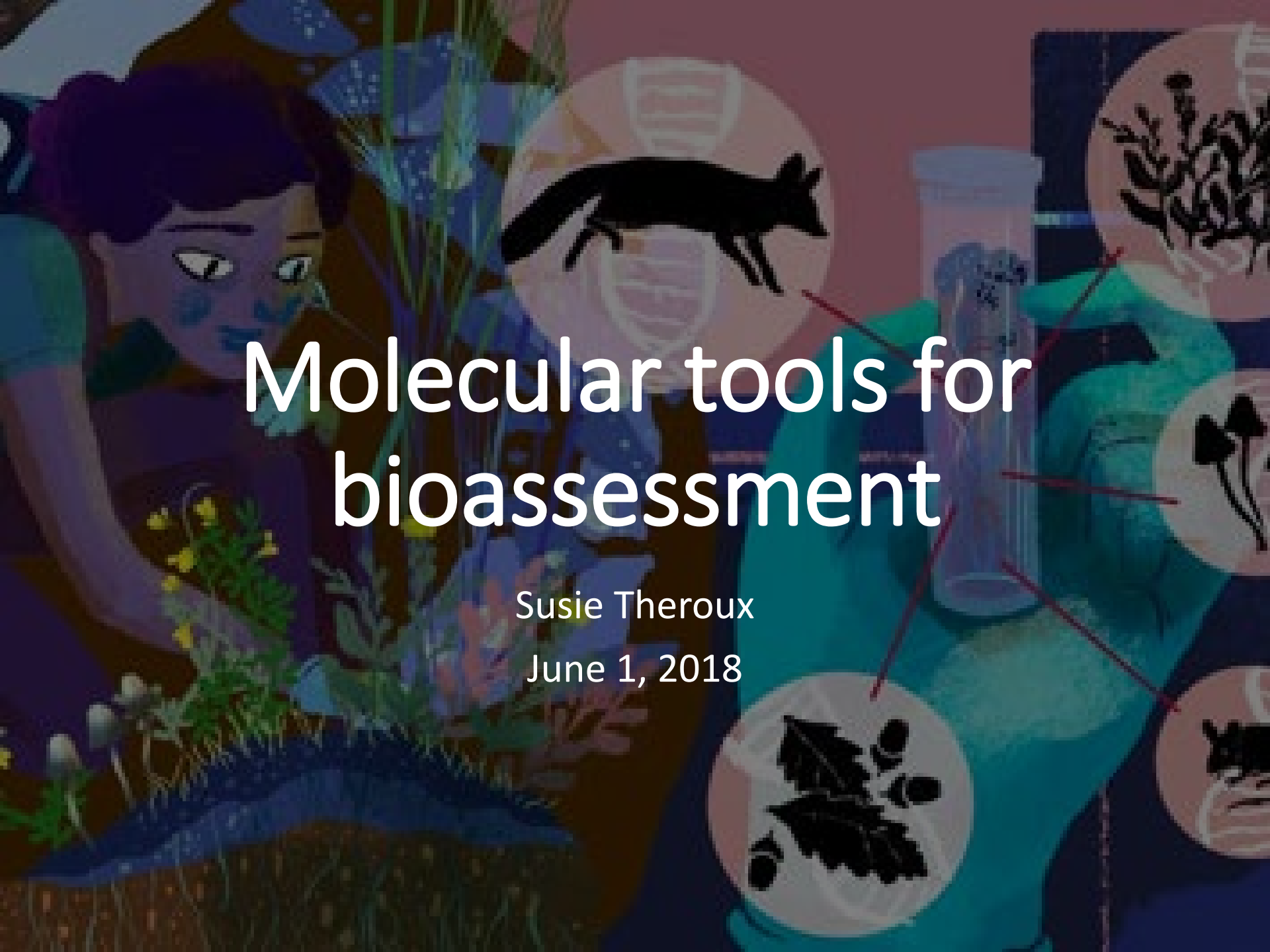


Molecular tools for bioassessment

Susie Theroux

June 1, 2018

Background

- Bioassessment is an integral part of regulatory programs
  - Invertebrates in wastewater outfall assessment
  - Invertebrates and algae for stream biointegrity
- Sensitive/endangered species monitoring critical for protecting beneficial uses
- Invasive species monitoring

Problems facing bioassessment

Spatial/temporal resolution

- Rare species are difficult to detect
- Need to be in the right place at the right time

Accuracy

- Certain species are difficult to identify using morphology
- Ambiguous/cryptic species assemblages in algae, invertebrates, fish

Capacity

- Generating taxonomy data takes TIME (~6 months/sample) and MONEY (~$1000/sample)

DNA-based solutions

Spatial/temporal resolution

- Able to detect trace levels of DNA
- DNA can persist after an organism is gone

Accuracy

- DNA sequencing can result in higher taxonomic resolution
- Can even detect sub-species populations

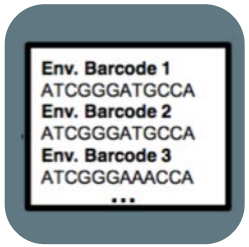
Capacity

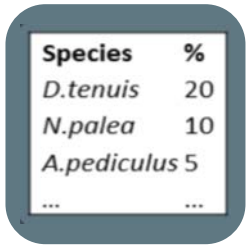
- DNA sequencing has the potential to generate data up to 10x faster and 10x cheaper than morphological approaches

Goals of this talk

- State of the science: DNA-based approaches
- SCCWRP's role in advancing DNA-based bioassessment
- How close are we to using these methods on a routine basis?

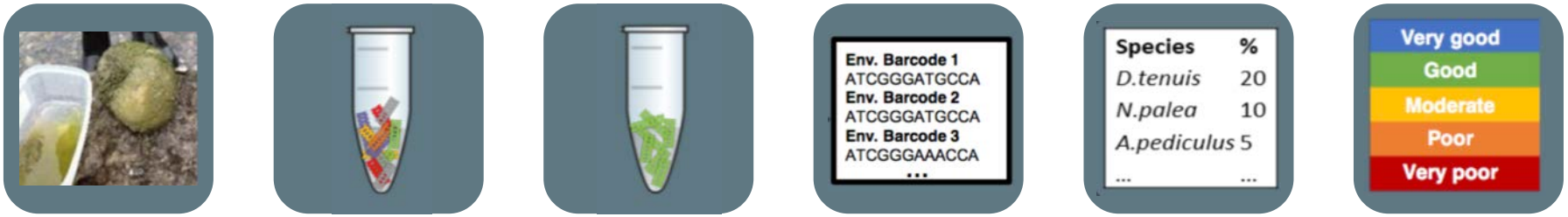
Six steps to generate taxonomy data for bioassessment





Sampling → DNA extraction → DNA sequencing → Bioinformatics → Taxonomy ID → Biological indices

Six steps to generate taxonomy data for bioassessment



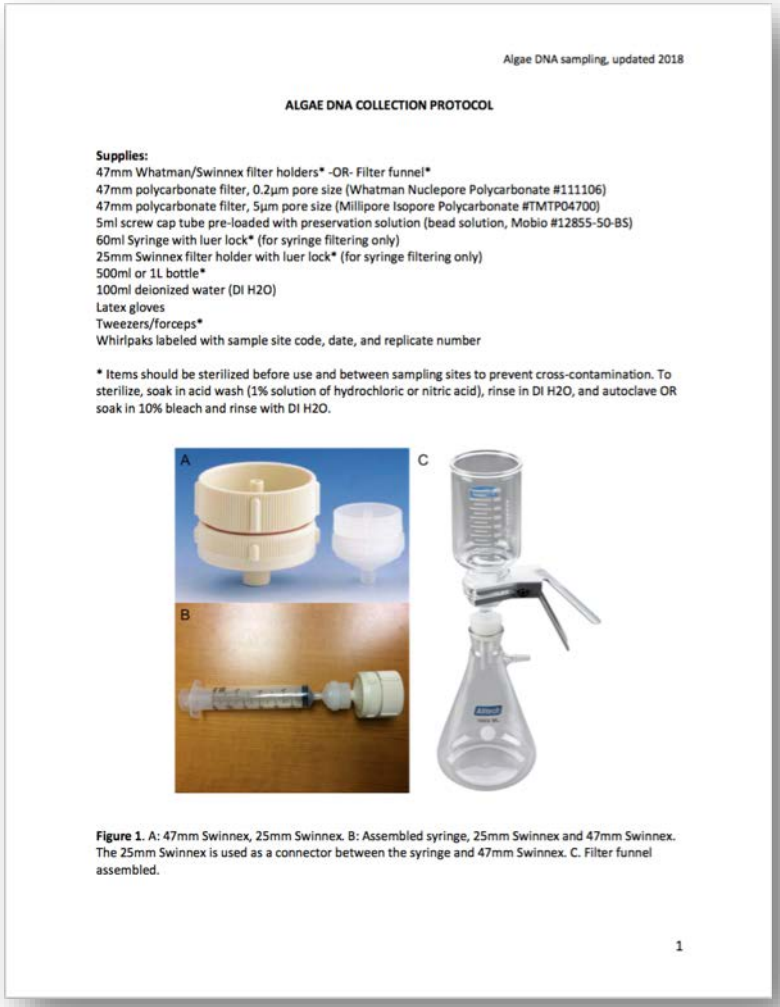
- Sampling and sequencing technologies more routine
- Efforts focused on adapting for regulatory programs

- Bioinformatics and sequence analyses evolving rapidly
- Focus of investigative studies

Step 1: Sampling

- SCCWRP is developing DNA sampling protocols for multiple species in multiple habitats:
  - Stream algae
  - Steam invertebrates
  - Marine invertebrates
  - Ichthyoplankton
  - Fish

Algal DNA sampling



Algae DNA sampling, updated 2018

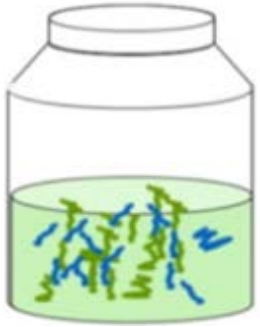
ALGAE DNA COLLECTION PROTOCOL

Supplies:
47mm Whatman/Swinnex filter holders* -OR- Filter funnel*
47mm polycarbonate filter, 0.2µm pore size (Whatman Nuclepore Polycarbonate #111106)
47mm polycarbonate filter, 5µm pore size (Millipore Isopore Polycarbonate #TMTP04700)
5ml screw cap tube pre-loaded with preservation solution (bead solution, Mobio #12855-50-BS)
60ml Syringe with luer lock* (for syringe filtering only)
25mm Swinnex filter holder with luer lock* (for syringe filtering only)
500ml or 1L bottle*
100ml deionized water (DI H2O)
Latex gloves
Tweezers/forceps*
Whirlpaks labeled with sample site code, date, and replicate number

* Items should be sterilized before use and between sampling sites to prevent cross-contamination. To sterilize, soak in acid wash (1% solution of hydrochloric or nitric acid), rinse in DI H2O, and autoclave OR soak in 10% bleach and rinse with DI H2O.

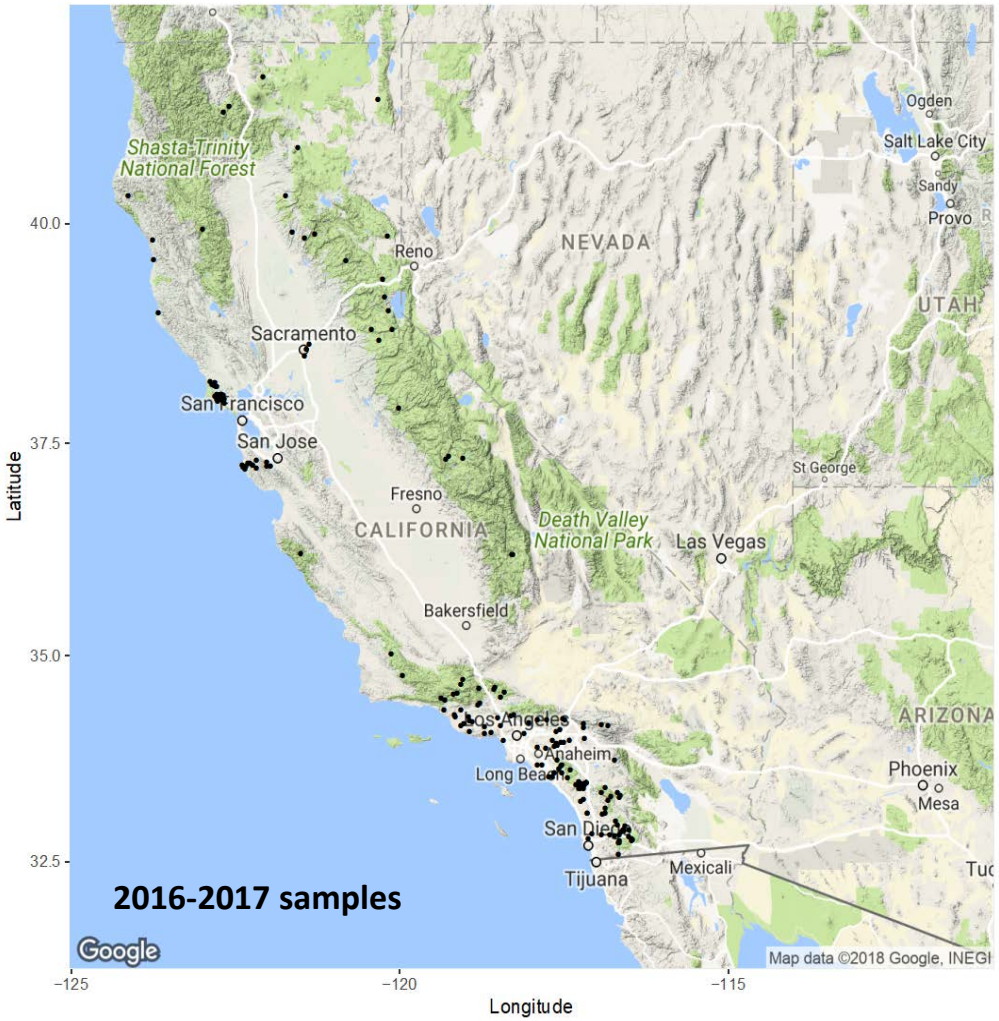
Figure 1. A: 47mm Swinnex, 25mm Swinnex. B: Assembled syringe, 25mm Swinnex and 47mm Swinnex. The 25mm Swinnex is used as a connector between the syringe and 47mm Swinnex. C. Filter funnel assembled.

1



Composite sample

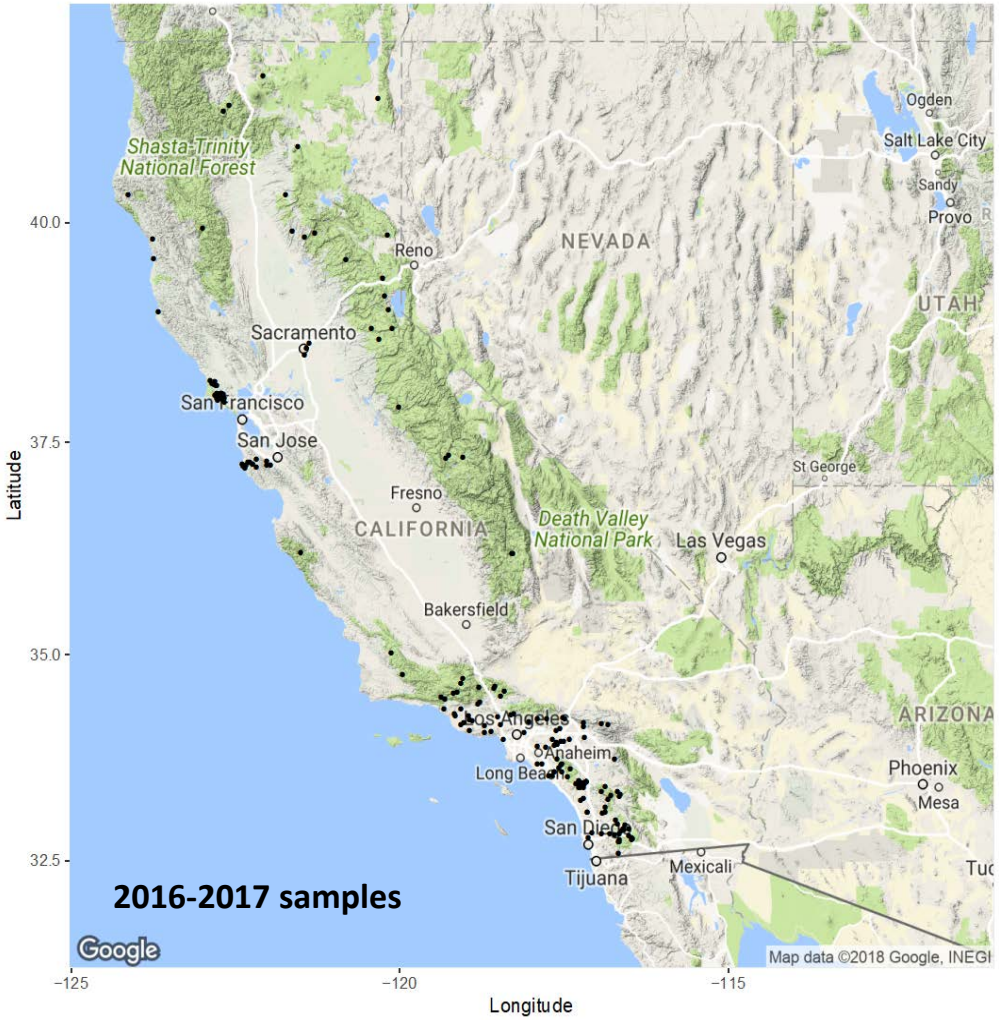
Algal DNA sampling



Partner sampling:

- Perennial Stream Assessment (PSA)
- Reference Condition Monitoring Program (RCMP)
- Stormwater Monitoring Coalition (SMC)
- Regional Water Boards 2, 4, 9

Algal DNA sampling



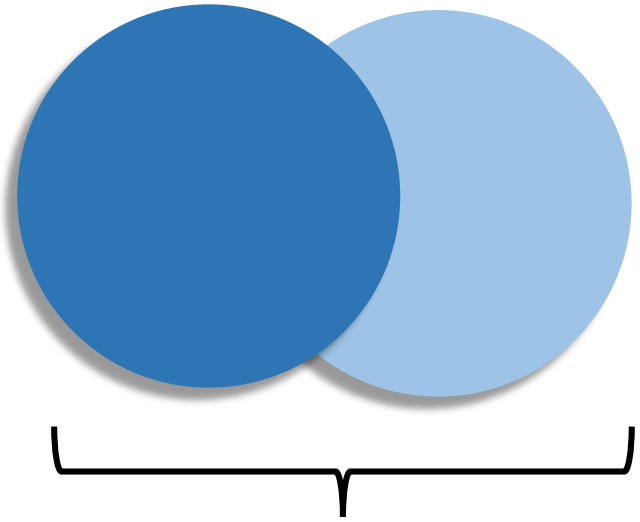
	Time	Cost/sample
Morphology	6 months	$1200
DNA	3 weeks	$300

Cheaper!
Faster!
Better?

Algal DNA: bias and repeatability

Morphology-based taxonomy

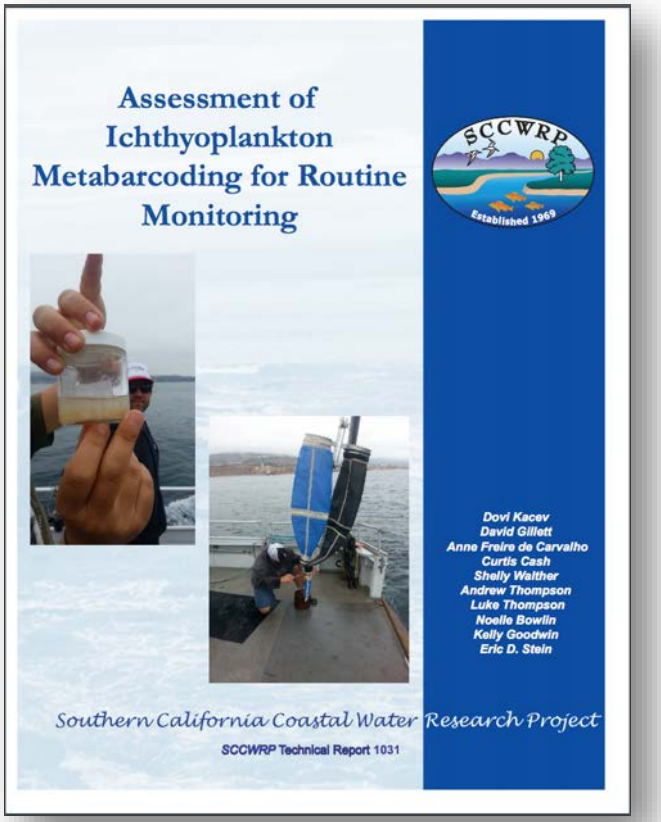
Taxonomist 1 Taxonomist 2



Algae DNA sampling: cost/time

Take-home:

- Algae DNA sampling is easily integrated into existing protocols
- DNA results delivered faster and lower cost/sample
- DNA sequencing results have better repeatability than morphology-results
- SCCWRP also has DNA sampling protocols for other organisms in other systems (ichthyoplankton, invertebrates)



Sampling | DNA extraction | DNA sequencing | Bioinformatics | Taxonomy ID | Biological indices

Step 2: DNA extraction

Application of high-throughput sequencing (HTS) metabarcoding to diatom biomonitoring: Do DNA extraction methods matter?

Valentin Vasselon[1,3], Isabelle Domaizon[1,4], Frédéric Rimet[1,5], Marla Kahlert[2,6], and Agnès Bouchez[1,7]

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[2]Department of Aquatic Sciences and Assessment, Swedish University of Agricultural Sciences, P.O. Box 7050, 75007, Uppsala, Sweden

Abstract: Current freshwater biomonit... of their silica skeleton. This standardis... pertise. Metabarcoding combined with ... biomonitoring applications but requires ... extraction method used, but the effect ... DNA extraction method for HTS metab... cell lysis and DNA purification to extra... with differing water quality. We compa... community inventories obtained from H... similarity between molecular and microsc... sensitivity Index (SPI). A method based o... but had the highest polymerase chain re... not affect operational taxonomic unit ... within Nitzschia, Amphora, Encyonema ... did not affect global diatom communit... inventories and molecular inventories h... purposes (high DNA quantity and low ... the SA-Gen method.

Key words: next-generation biomonitor... diatom communities

Diatoms are good bioindicators becau... sity, short life cycle, high sensitivity to ... tions, and widespread distribution in all ... (Stevenson and Pan 1999). Therefore, ... are used routinely for water-quality as ... ing programs and by environmental ag... tries. Well-established guidelines like ... in USA (US CWA) or the Water Frame... rope (EU WFD) help to standardize ... tries and laboratories. Classical diatom ... on the composition of environmental ... lies on morphological identification at ... the aid of microscopes and specialize... cies identification is challenging beca... sity of diatoms (Mann and Vanormelin...

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DOI: 10.1086/690649. Received 30 August 20... Freshwater Science. 2017. 36(1):162–177. © 2...

Biological Conservation 183 (2015) 53–63

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/biocon

Special Issue Article: Environmental DNA

Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA

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ARTICLE INFO

Article history:
Received 27 February 2014
Received in revised form 3 November 2014
Accepted 10 November 2014
Available online 3 December 2014

Keywords:
16S
Cytochrome c oxidase I
eDNA
Freshwater
Molecular: protocols
Targeted species detection

ABSTRACT

Environmental DNA (eDNA) is used to detect biodiversity by the capture, extraction, and identification of DNA shed to the environment. However, eDNA capture and extraction protocols vary widely across studies. This use of different protocols potentially biases detection results and could significantly hinder a reliable use of eDNA to detect biodiversity. We tested whether choice of eDNA capture and extraction protocols significantly influenced biodiversity detection in aquatic systems. We sampled lake and river water, captured and extracted eDNA using six combinations of different protocols with replication, and tested for the detection of four macroinvertebrate species. Additionally, using the same lake water technical replicates, we compared the effect of capture and extraction protocols on metabarcode detections of biodiversity using 16S for eubacteria and cytochrome c oxidase I (COI) for eukaryotes. Protocol combinations for capture and extraction of eDNA significantly influenced DNA yield and number of sequences obtained from next generation sequencing. We found significantly different detection rates of species ranging from zero percent to thirty-three percent. Differences in which protocol combinations produced the highest metabarcoded biodiversity were detected and demonstrate that different protocols are required for different biodiversity targets. Our results highlight that the choice of molecular protocols used for capture and extraction of eDNA from water can strongly affect biodiversity detection. Consideration of biases caused by choice of protocols should lead to a more consistent and reliable molecular workflow for repeatable and increased detection of biodiversity in aquatic communities.

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1. Introduction

Biodiversity assessment is a main goal as well as a tool used in ecology and conservation biology (Vermeulen and Kuzniell, 2002). Many different measuring approaches exist to assess biodiversity, and these various approaches are typically designed for specific groups of organisms. In recent years, the broadly applicable method of using environmental DNA (eDNA) as a tool to detect organisms in their environment has gained immense interest (Thomsen and Willerslev, 2015; Sutherland et al., 2012). Assessment of biodiversity using eDNA relies on a molecular workflow comprising several steps including the capture, extraction and identification of an organism's DNA from environmental samples such as soil or water. The use of eDNA to detect species and measure biodiversity is now at the forefront of approaches in the toolbox for ecologists and conservation scientists (Yoccoz, 2012). The rapid growth in its use, as well as an increased complexity and variation of molecular workflows used to detect eDNA (e.g., next generation sequencing technology (Shokralla et al., 2012)), make a consistent comparison of methodological procedures highly needed.

All molecular workflows currently used to analyze eDNA consist of capturing DNA from an environmental sample, followed by the extraction and purification of eDNA. Purified eDNA is then amplified for a specific gene target (e.g., metabarcode analysis) and categorized into biodiversity units. For each one of these steps there are a multitude of possible protocols that can be used (Table 1). This heterogeneity in laboratory protocols, however, is likely to challenge comparisons across eDNA studies and to create uncertainty in its application for detecting biodiversity (Wang et al., 2013). The inconsistent use of different molecular protocols across studies is likely due to the fact that research conducted thus far has focused on whether or not a particular species or

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http://dx.doi.org/10.1016/j.biocon.2014.11.018
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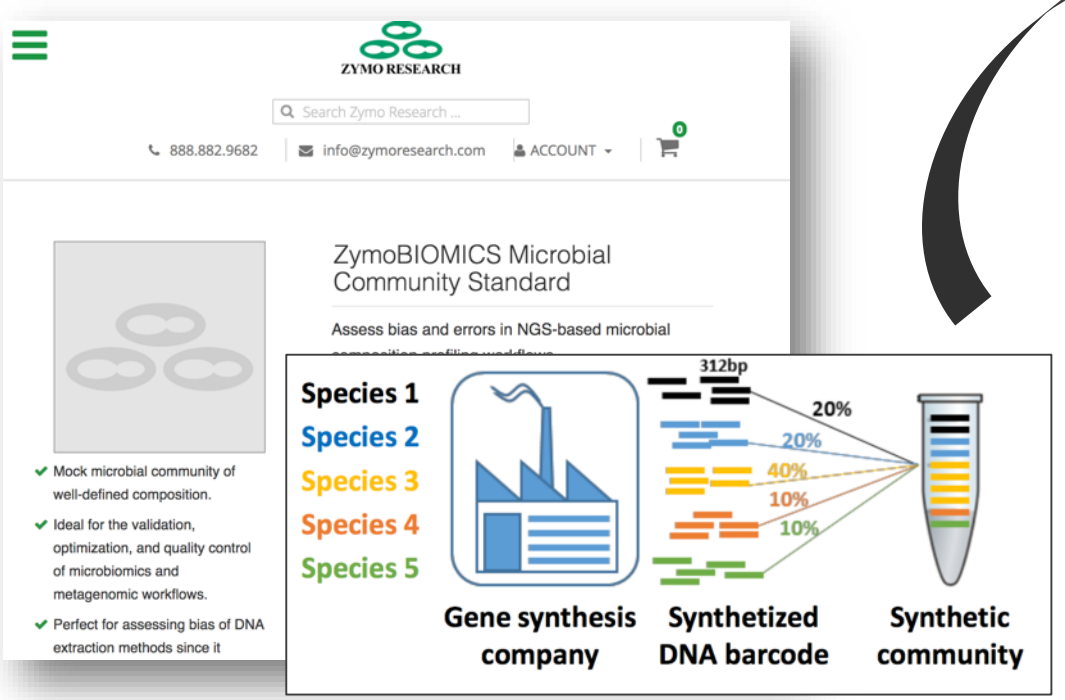
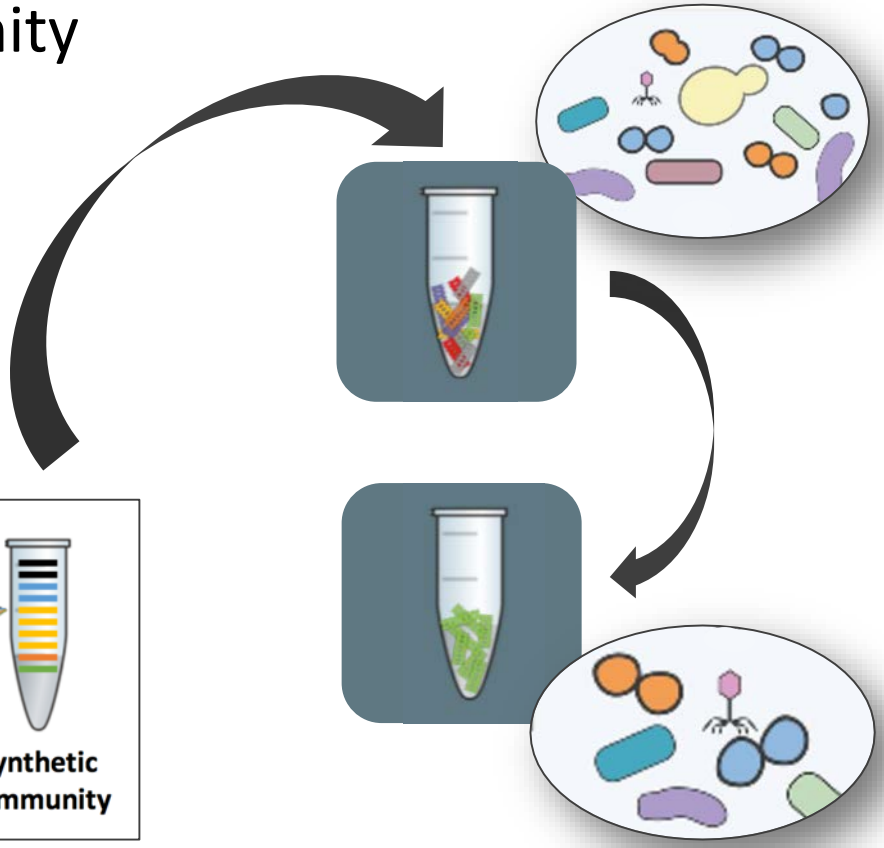
- Many commercial DNA extraction kits available
- Taxonomy results can vary depending on extraction method

Step 2: DNA extraction

- Use DNA standard to quantify DNA extraction efficiency
- Synthesized microbial community



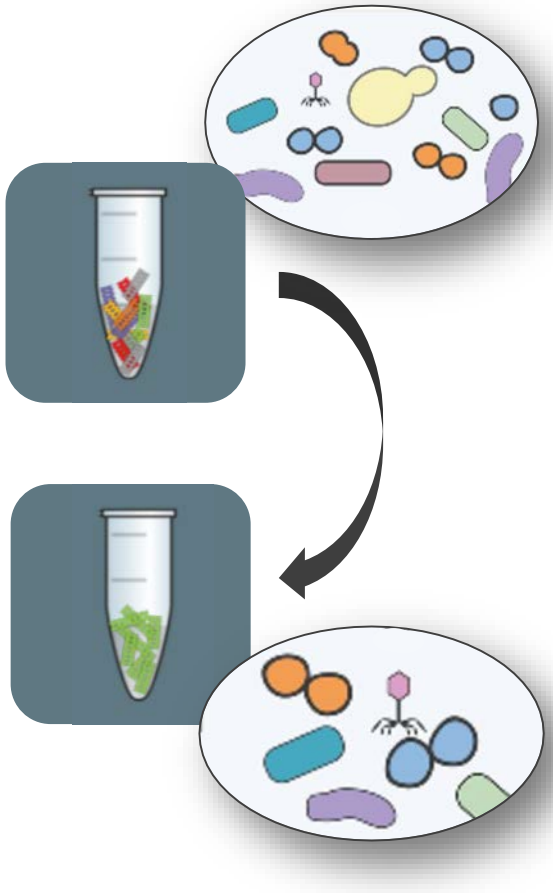


Valentin Vasselon

Step 2: DNA extraction

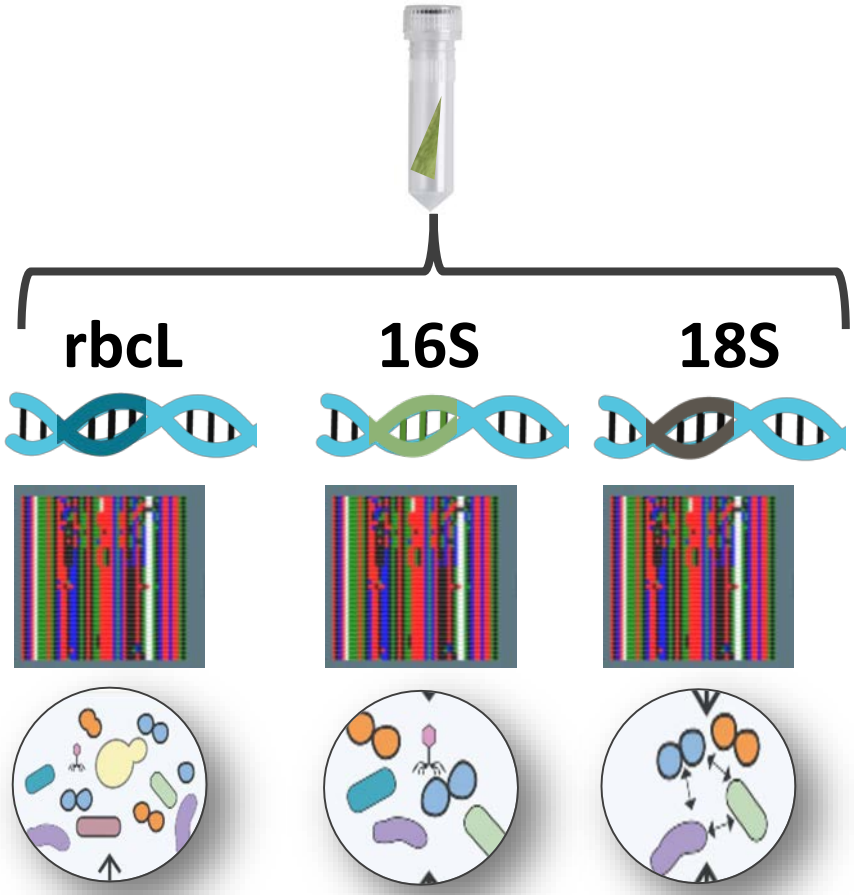
Take-home:

- DNA extractions with defined synthetic communities can be used to set quality control thresholds
- Will ensure that program-wide methods yield comparable data



Step 3: DNA sequencing

- There are many popular DNA (meta)barcode regions for sequencing environmental communities:
  - **16S**: bacteria
  - **18S**: eukaryotic organisms
  - **CO1**: eukaryotic organisms
  - **rbcL**: phototrophs
- Algae DNA pilot studies: compare taxonomy results using different barcode regions



Step 4: Bioinformatics

- Bioinformatics is a rapidly evolving field
- Many pipelines available to process raw DNA sequences and generate taxonomy data
- Every step in the bioinformatics pipeline can influence your end result
- SCCWRP is working to standardize these pipelines
- Create recommended pipelines that can be used by broader community

Example bioinformatics pipeline

@SRR038845.41 HWI-EAS038:6:1:0:1474
CCAATGATTTTTTTCCGTGT
GAATACGGTTAA
+SRR038845.41 HWI-EAS038:6:1:0:1474
BCCBA@BB@BBBBBAB@B9B@
=BABA@A:@693:@B
@SRR038845.53 HWI-EAS038:6:1:1:360
GTTCAAAAAGAACTAATTGTGTC
AATAGAAAACTC

QC

Sample1
Sample2
Sample3
> Seq1
AGTCTAGCTAGGCATC
ACGAGCTCGATGCATC
GGCTACGACTACTTAC
AGCATCAGCATTTCG
> Seq2
GTCGATGCTACGGGA

Cluster into OTUs

Seq1
Seq4
Seq3
Seq10
Seq2
Seq8
Seq9
Seq5

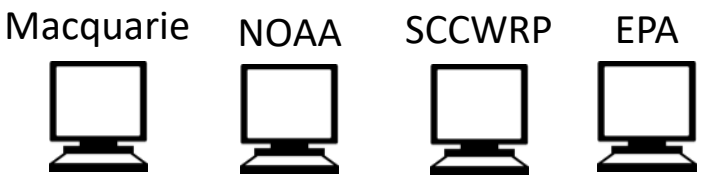
OTU1 AGTCTAGCTAGGCATTGA
OTU2 CGGCTAGTTAGGCACCGA
OTU3 GGTCTAGTTAGGCATCGA

Search against reference database

Assign taxonomy

Taxon1
Taxon2
Taxon3

Intercalibration study



- Setting standards for QA/QC helped resolve differences in pipeline output
  - Clustering method
  - DNA reference database
- **Take-home:** Bioinformatic QC guidelines will ensure results are comparable when generated by outside user community

Step 5: Taxonomy assignment

- **Your DNA taxonomy is only as good as your DNA library**
- The quality and completeness of your DNA reference database heavily influences the quality of resulting taxonomy data
- SCCWRP is spearheading the development of DNA libraries for:
  - Algae
  - Invertebrates

NCBI

BOLDDATA

Sampling | DNA extraction | DNA sequencing | Bioinformatics | Taxonomy ID | Biological indices

West Coast invertebrate DNA library

- Key partnerships to help create West Coast DNA library for invertebrates:
  - Bight program
  - WAML
  - Smithsonian Institution
- Coordinated sampling with member agencies and partner organizations to sample a broad geographic range

Western Association of Marine Laboratories (WAML)

Smithsonian

West Coast invertebrate DNA library

- Smithsonian will identify and sequence DNA barcode of organisms
- This effort will help fill in the critical gaps in the marine invertebrate DNA library
- Building capacity to use molecular approach for marine invertebrate bioassessment

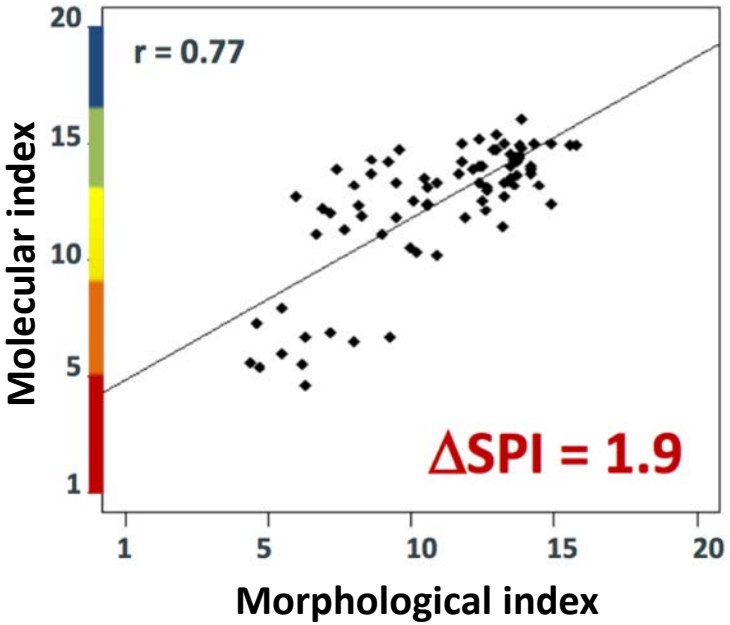
Western Association of Marine Laboratories (WAML)

Smithsonian

Step 6: Biological indices

- Adapting existing bioassessment indices to be compatible with molecular data
- Creating new bioassessment indices from DNA sequence data
- State Water Board prioritizing the development of DNA-compatible algal index

French diatom index (SPI)



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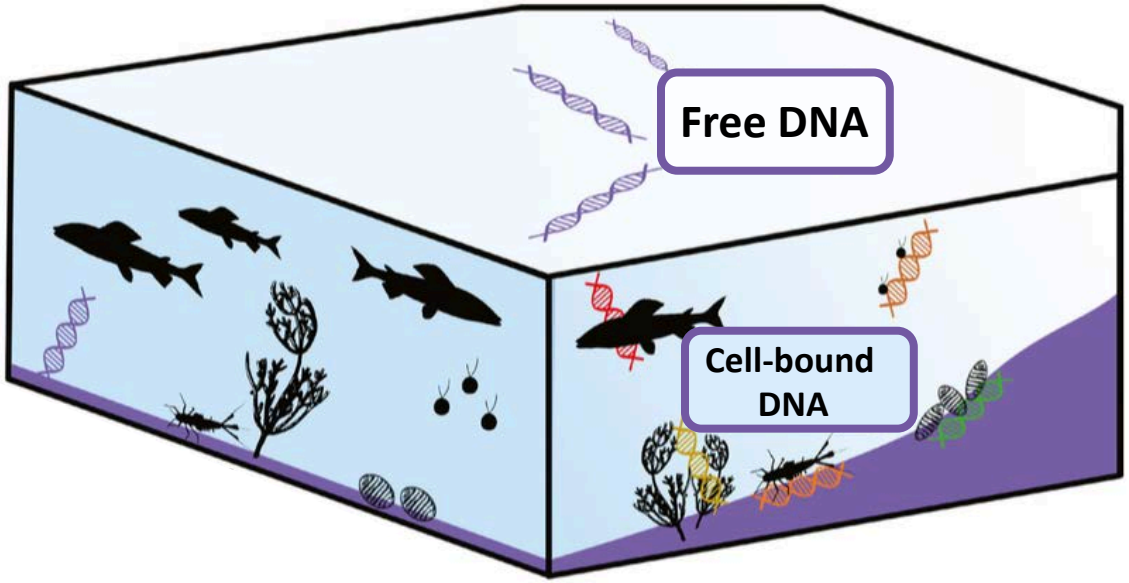
Sampling | DNA extraction | DNA sequencing | Bioinformatics | Taxonomy ID | Biological indices

eDNA sampling: the future of bioassessment



- eDNA = “environmental” DNA
- Excellent option for monitoring of sensitive, endangered, or invasive species
- Quantify DNA of interest using species-specific probes and qPCR

Understanding the fate of eDNA

eDNA "spiking" studies

- Use non-native DNA to track eDNA dispersal, degradation, and propagation
- Test under both "natural" and unnatural conditions

California mussel
(Mytilus californianus)

Coyote Creek

Upper San Juan Creek

Understanding the fate of eDNA

eDNA "spiking" studies

- Use non-native DNA to track eDNA dispersal, degradation, and propagation
- Test under both "natural" and unnatural conditions

California mussel
(Mytilus californianus)

Coyote Creek

Upper San Juan Creek

Implications of eDNA study

1. Standardized eDNA sampling protocols
   - Scalable
   - Consistent
   - Sterile
2. Guidance on predicting the fate of DNA
3. Recommendations regarding negative results
   - Setting confidence thresholds for non-detection

RB9 eDNA study

Status: DNA-based bioassessment

Algal bioassessment

- State Water Board is moving forward with developing algae DNA for bioassessment
- Field collection methods established
- Refining sequencing approach and bolstering DNA libraries

Invertebrate bioassessment

- Nationally, many efforts to test barcoding in invertebrates
- Sequencing approaches are standardized
- DNA library development still needed
- More CA-based studies needed

eDNA monitoring

- Sampling methods are standardized
- Sampling programs are scalable and adaptable to a variety of settings
- Pilot studies across California
- eDNA modeling on-going

How can SCCWRP support you?

- Joint studies
  - eDNA sampling for species of interest
  - eDNA spiking studies in variable systems
  - Paired morphology and DNA surveys for invertebrates, algae, ichthyoplankton
- Sampling for DNA library development
- Training in DNA sampling and computational analyses