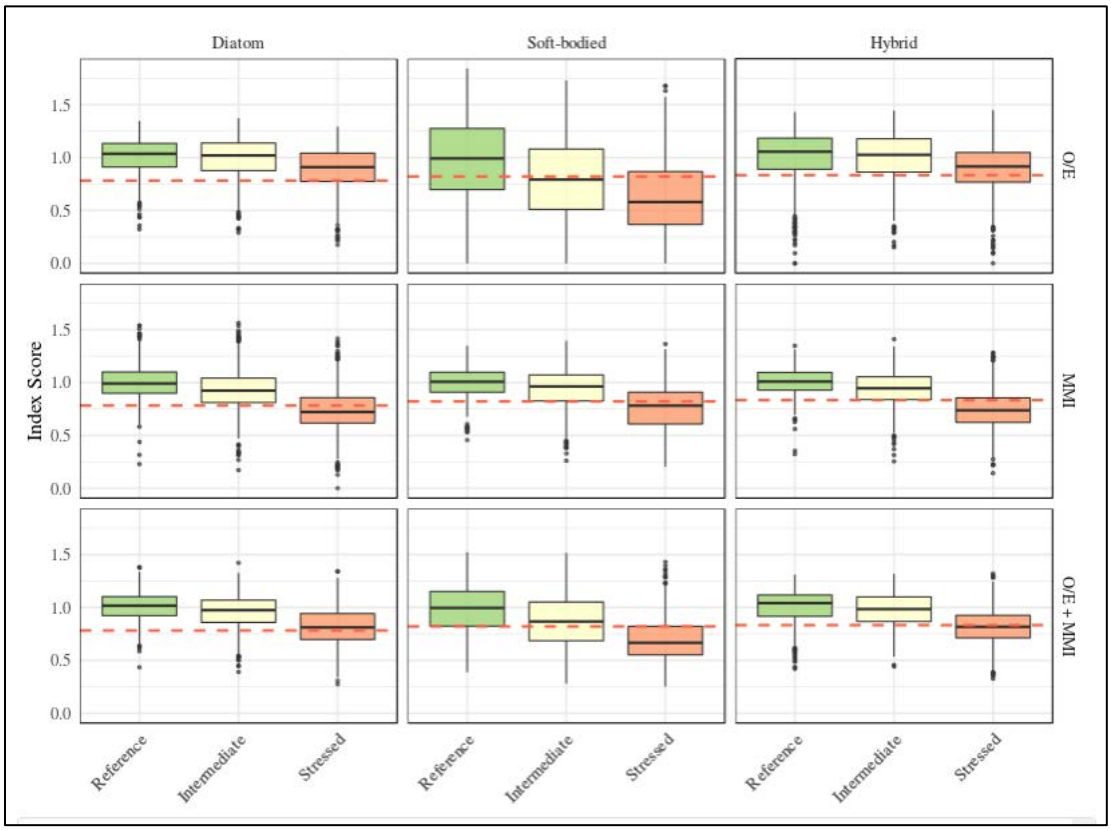
Is a water quality index an appropriate substitute for a biotic index?

While the draft Algal Stream Condition Index (ASCI) was developed to respond to a general stressor gradient, the component metrics appear to be in-line with a water quality index. In addition, each of the "target" water quality constituents is routinely monitored. While algal assemblages have been used for decades as a water quality indicator, this index is intended to address aquatic life beneficial uses (i.e., does the algal community look similar to reference). Water quality specific metrics seem to be an indirect means to answer questions about water quality which seem unnecessary given the abundance of direct measurement data available. Such water quality monitoring provides high quality data, reduces the inherent spatial and temporal of biomonitoring, is far less expensive to conduct, and has a much faster turnaround time.

Do the proposed indices offer sufficient resolution along a disturbance gradient?

Very few of the proposed indices appear to clearly differentiate between reference sites and those with intermediate levels of anthropogenic stress (Figure 1) and the ability to discriminate between such a stressor gradient is at the very core of biotic indices. While better discrimination is observed between reference and stressed sites, it is likely that far less expensive and more consistent observations could provide the same information.

Figure 1. Various ASCIs and Response to a Stressor Gradient



Is soft-bodied algal (SBA) taxonomy robust enough to include in a regulatory program?

Morphological SBA taxonomy is currently problematic due to high cost[1], a lack of taxonomic capacity[1], and documented inconsistency among taxonomists[2]. SCCWRP researchers are currently working to circumvent these problems with the development of molecular algal taxonomy methods. With the doubts surrounding the inclusion of SBA in any ASCI, does it make scientific sense to take pause and increase certainty?

Has the reference condition been sufficiently defined for a statewide algal assessment?

Application of a predecessor to the CSCI, the Southern Coastal California Index of Biotic Integrity (IBI), was hampered by a lack of relevant reference sites leading to an incomplete understanding of the reference condition for certain geographical regions (e.g., low-gradient coastal streams)[3]. The modeled reference approach, at least, partially addressed the concerns surrounding underrepresented environmental variables in the reference condition. Does the ASCI reference pool sufficiently characterize low gradient, low elevation, large watershed systems?

Were redundant metrics sufficiently screened?

Multi-metric index development commonly includes analyses for exclusion of redundant metrics[4,5,6]. The SBA metrics appear to be highly redundant. BCG 3 taxa richness, proportion non-reference taxa, and proportion tolerant taxa seem to all tell the same story. Should redundant metrics be included and were such metrics sufficiently addressed?

Should "BCG Taxa" be used as metrics?

The BCG process was a subjective (expert opinion based) and not entirely successful effort to bin sites based on ecological function. Whether or not taxa are often observed in samples falling into a specific bin seems overly subjective, inconsistent, and open to human bias. Further, if the State opts to use a reference based approach and not the BCG, is reliance upon products coming from the BCG work technically defensible?

---

[1] Molecular Tools for Bioassessment (2018). Presented to SCCWRP Commission, June 1, 2018. Attachment 1.

[2] Weech, S., Orr, P., White, M., and C. Fraser. 2014. Inter-laboratory Comparison Reveals Critical Issues with Periphyton Community Assessment. Presented at the SETAC North America annual meeting, Vancouver, British Columbia. Attachment 2.

[3] Diamond, Jerry. Reference Conditions and Bioassessments in Southern California Streams. July 31, 2009. Memorandum to Phil Markle of the Sanitation Districts. Attachment 3.

[4] Ode, P. R., Rehn, A. C., & May, J. T. (2005). A quantitative tool for assessing the integrity of southern coastal California streams. *Environmental management*, *35*(4), 493-504.

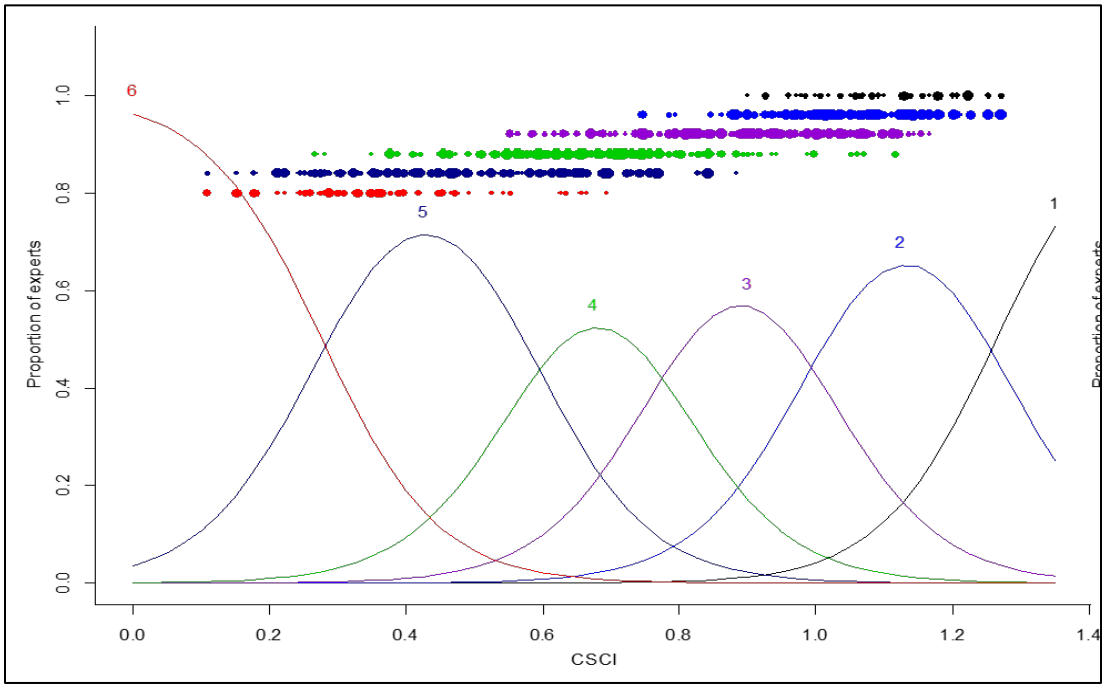
[5] Rehn, A. C., P. R. Ode, and J. T. May. 2005. Development of a Benthic Index of Biotic Integrity (B-IBI) for Wadeable Streams in Northern Coastal California and its Application to Regional 305(b) Assessment. Final Technical Report, State Water Resources Control Board, Sacramento, CA.

[6] Rehn, A. C. (2009). Benthic macroinvertebrates as indicators of biological condition below hydropower dams on west slope Sierra Nevada streams, California, USA. *River Research and Applications*, *25*(2), 208-228.

Was the BCG process successful at communicating ecological structure, function, and beneficial use attainment?

The BCG output has created additional confusion among entities quite familiar with the reference condition. The CSCI is based on a well vetted, objective, index which will give you the same score every time with the same taxa list (excluding insignificant changes across iterations). However, when looking at the BCG to CSCI crosswalk (Figure 2), one can see that a CSCI score of 1.0 (the mean of reference) is most likely in BCG bin 3. Bin 3 is described as a group "in which some changes in structure due to loss of some rare native taxa; shifts in relative abundance of taxa but sensitive–ubiquitous taxa are common and abundant; ecosystem functions are fully maintained through redundant attributes of the system." There appears to be a disconnect between the expert opinion-based and modeled approaches. Can they both be correct? In addition, the BCG practitioner's guide recognizes the challenges and shortcomings of most monitoring programs to assess ecosystem function[7] and notes that the BCG conceptual model "includes ecosystem function for future application." Has the BCG either addressed or communicated ecosystem function any better than the reference condition approach?

Figure 2. Relative Distribution of BCG Bins vs. CSCI Scores



Has a mechanistic linkage been sufficiently demonstrated between the biotic indices and eutrophication?

The technical team describes use of these organisms for diagnostic indicators as "caveated" because organism and population measures of health are impacted by a variety of different stressors in a complex environment which is not easy to model. Sites with elevated nutrients are likely to have elevated conductivity and any other ubiquitous water quality sign of development. Further, the models'

[7] USEPA. 2016. *A Practitioner's Guide to the Biological Condition Gradient: A Framework to Describe Incremental Change in Aquatic Ecosystems*. EPA-842-R-16-001. U.S. Environmental Protection Agency, Washington, DC.

underlying data tends to have impacted sites and non-impacted sites. The impacted sites are typically impacted by nutrients, habitat alteration, urban/agricultural runoff, etc. The unimpacted sites tend to be unimpacted by nearly anything. Does this inability to isolate variables coupled with the two-step translation (index scores to eutrophication impacts to biostimulatory substance thresholds) limit certainty and applicability of these tools? Does the associative stressor modelling with the CSCI and the ASCI sufficiently diagnose eutrophication as expected by organizing assumption #1?

Can eutrophication be prevented at biostimulatory substance levels above those correlated with high biotic index scores?

The nutrient concentrations correlated with "protecting aquatic life beneficial uses" are unattainable. Would decoupling the eutrophication from the aquatic life beneficial uses provide a technically defensible, and potentially attainable, "first step"? While the technical team's initial investigation (Figure 3) suggests that it will not, are there any recommendations of ways to further explore this potential decoupling?

Figure 2. Initial Investigation of Biomass Thresholds to Support Recreational Use

Comparison of Cover Targets: Aquatic Life Versus Recreational Use (Literature-Based)

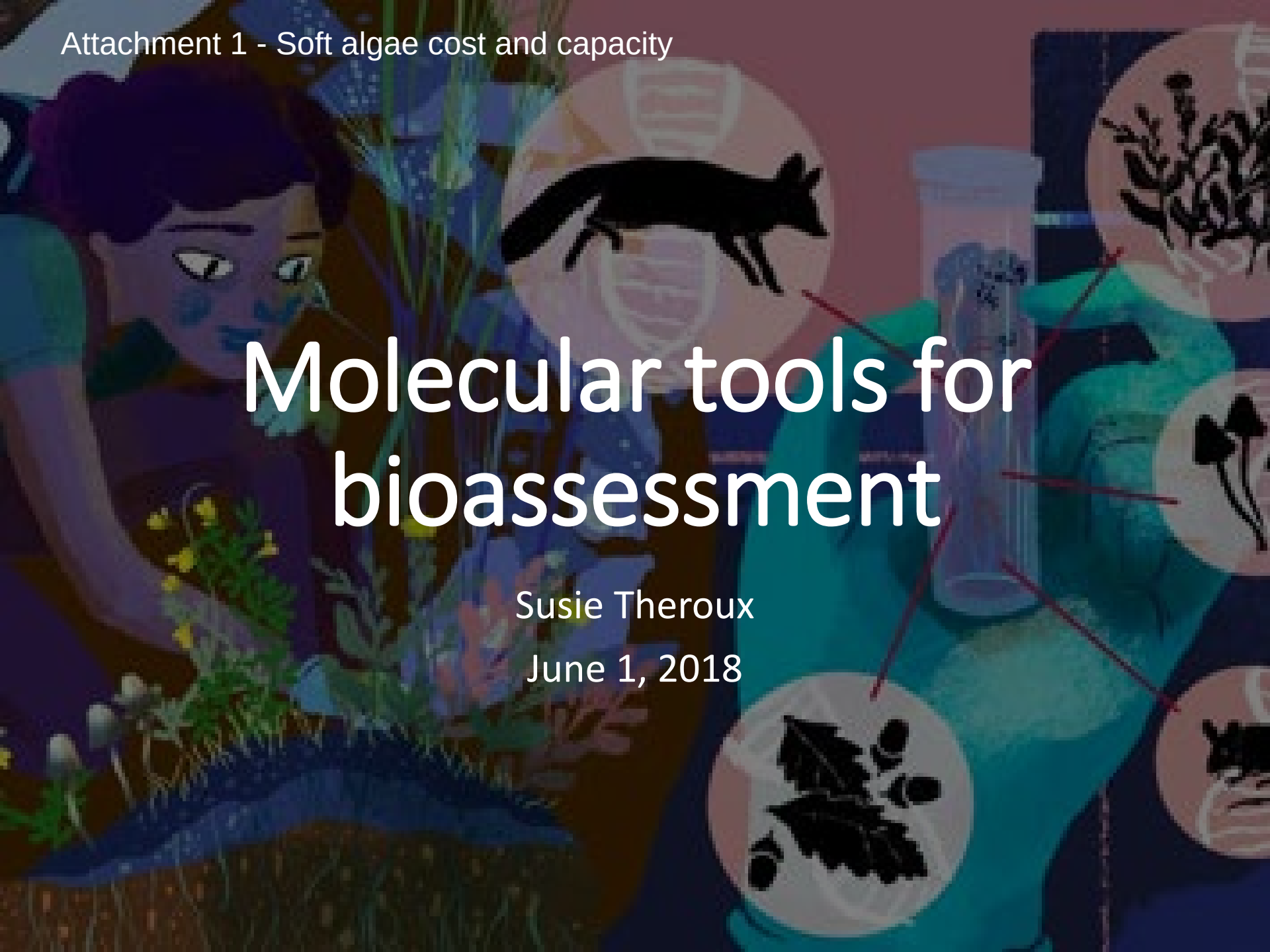
CSCI- 90% Prob REf10	ASCI- 90% Prob REf10	Literature Recreational Use
13%	21 %	> 20 to 25% Cover

Comparison of Biomass Thresholds (mg/m-2) with 90% Prob. Of Meeting Aquatic Life Versus Range of % Cover Goals*

CSCI-REf10	ASCI-REf10	13% Cover	30% Cover	50% Cover
28	58	19	41	123

* Numbers are provisional, pending model validation

Guiding Principle #1 states that "the amendment should address both nutrient pollution and biostimulatory conditions." Have biostimulatory conditions been sufficiently addressed?



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

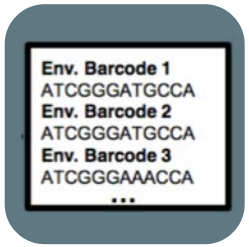
Capacity

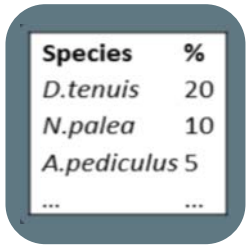
- DNA sequencing has the potential to generate data up to 10x faster and 10x cheaper than morphological approaches

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?

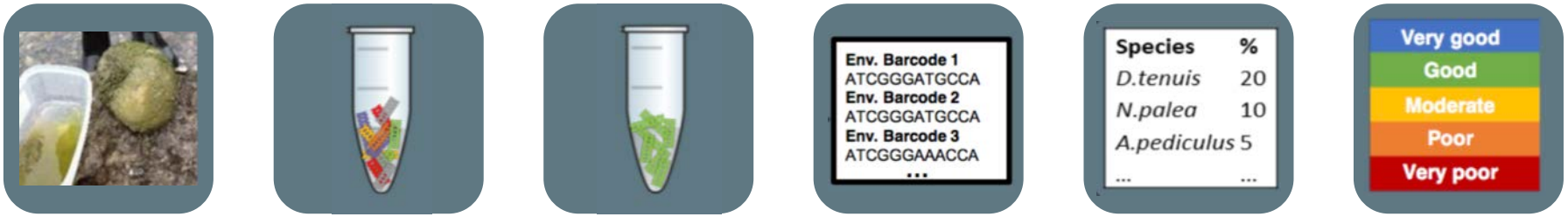
Six steps to generate taxonomy data for bioassessment





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

Six steps to generate taxonomy data for bioassessment



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

- 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

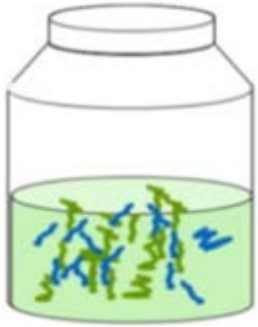
Algal DNA sampling

ALGAE DNA 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

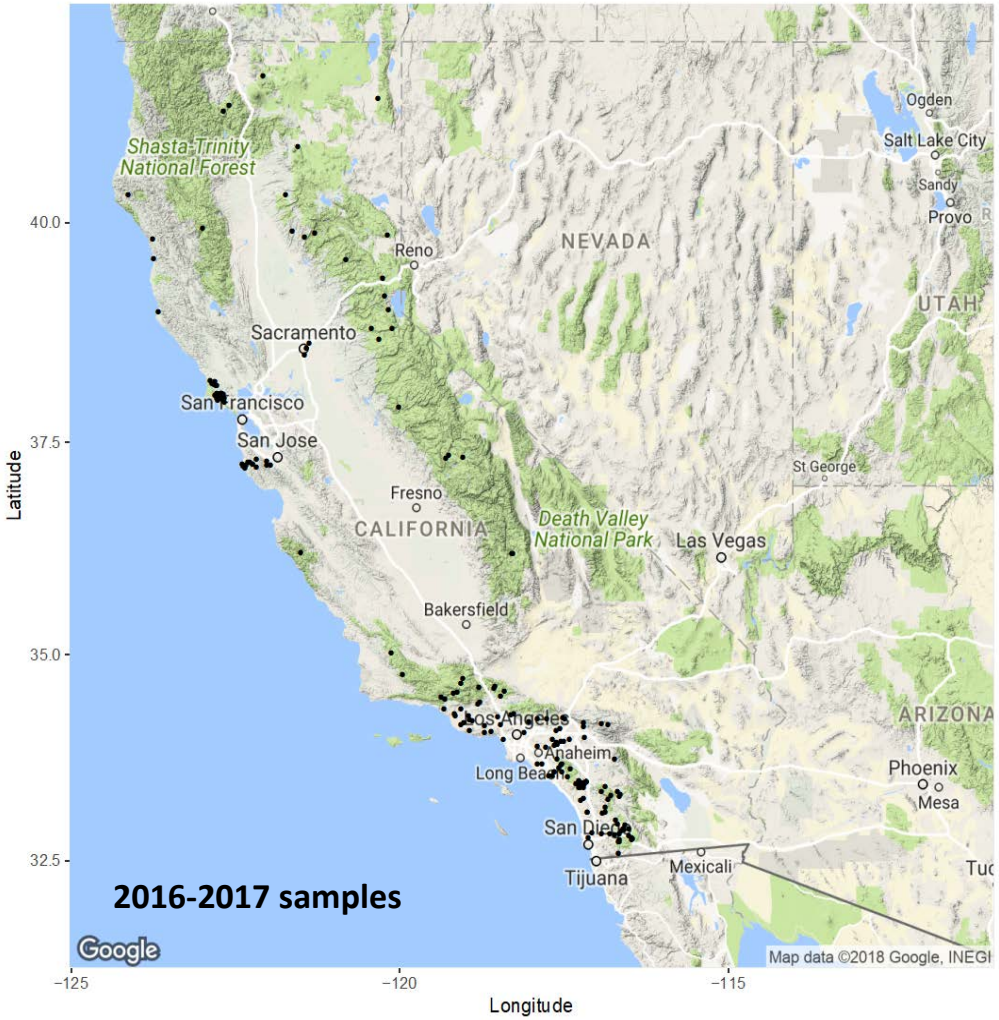
* Items should be sterilized before use and between sampling sites to prevent cross-contamination. To sterilize, soak in acid wash (1% solution of hydrochloric or nitric acid), rinse in DI H2O, and autoclave OR soak in 10% bleach and rinse with DI H2O.

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.



Composite sample

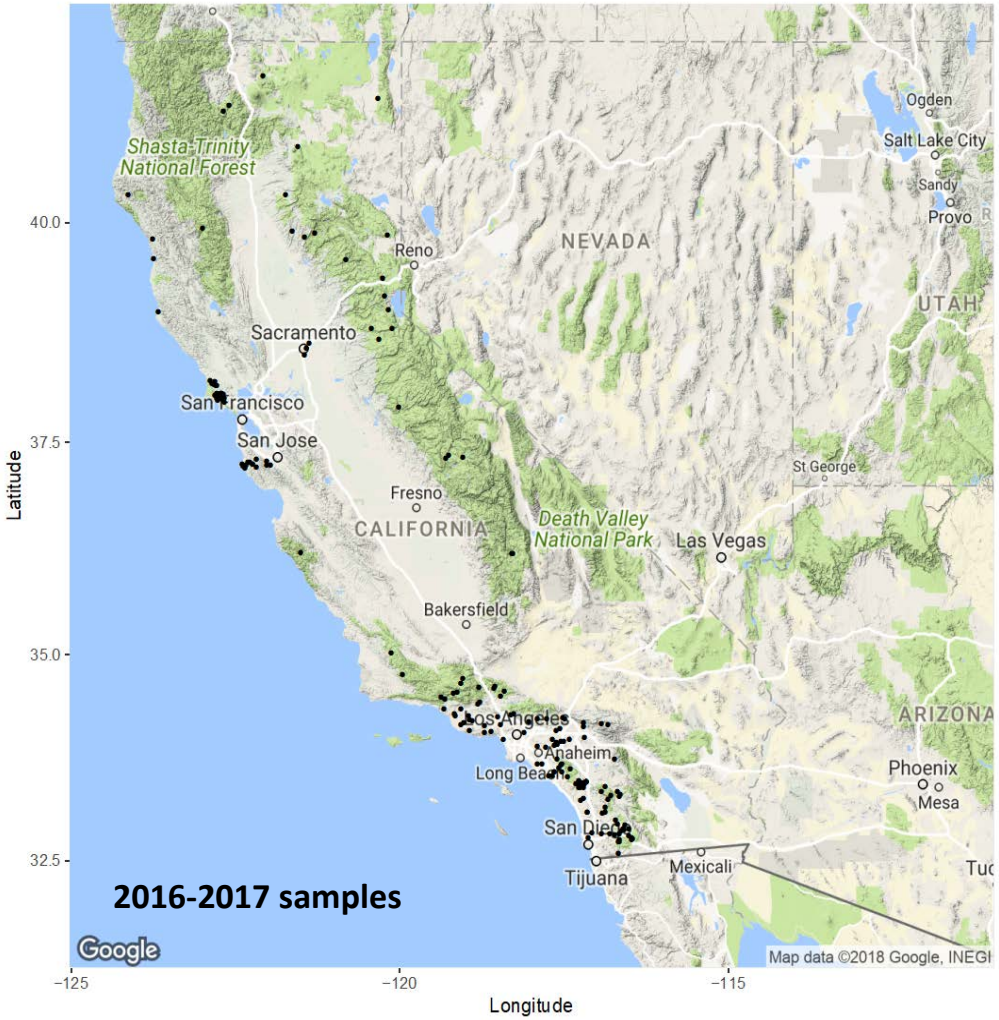
Algal DNA sampling



Partner sampling:

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

Algal DNA sampling



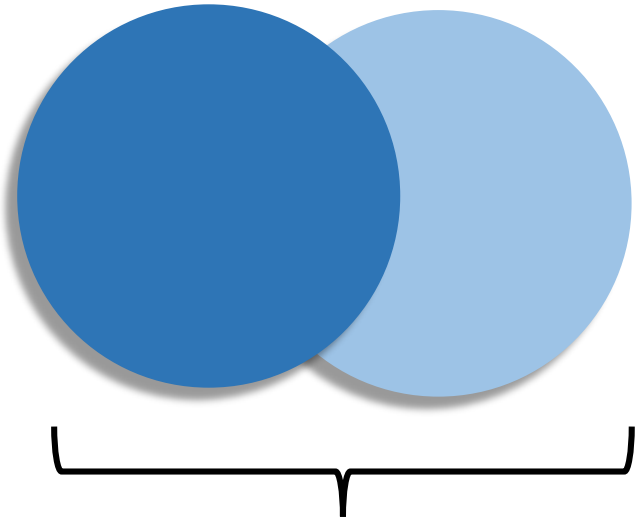
	Time	Cost/sample
Morphology	6 months	$1200
DNA	3 weeks	$300

Cheaper!
Faster!
Better?

Algal DNA: bias and repeatability

Morphology-based taxonomy

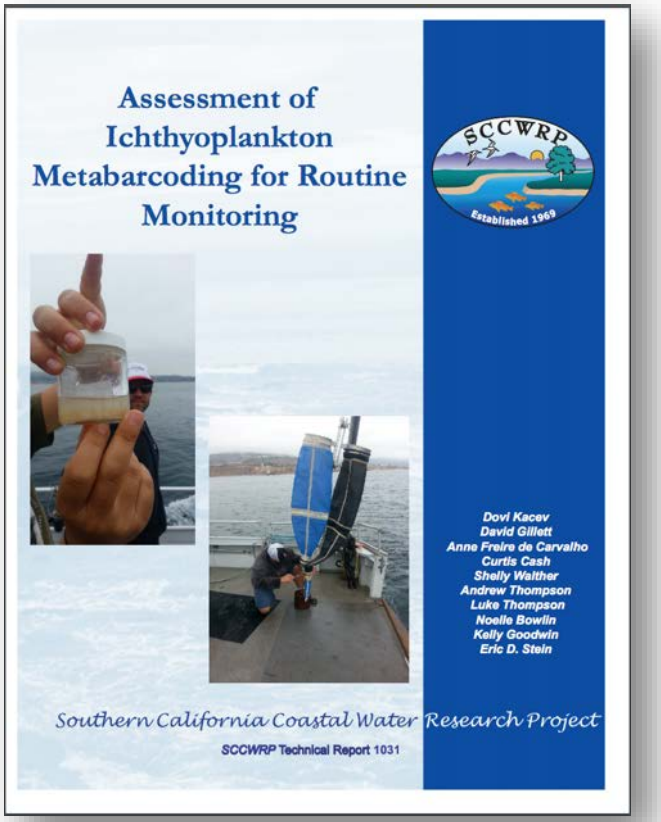
Taxonomist 1 Taxonomist 2



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 Vansomeliq...

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Special Issue Article: Environmental DNA

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

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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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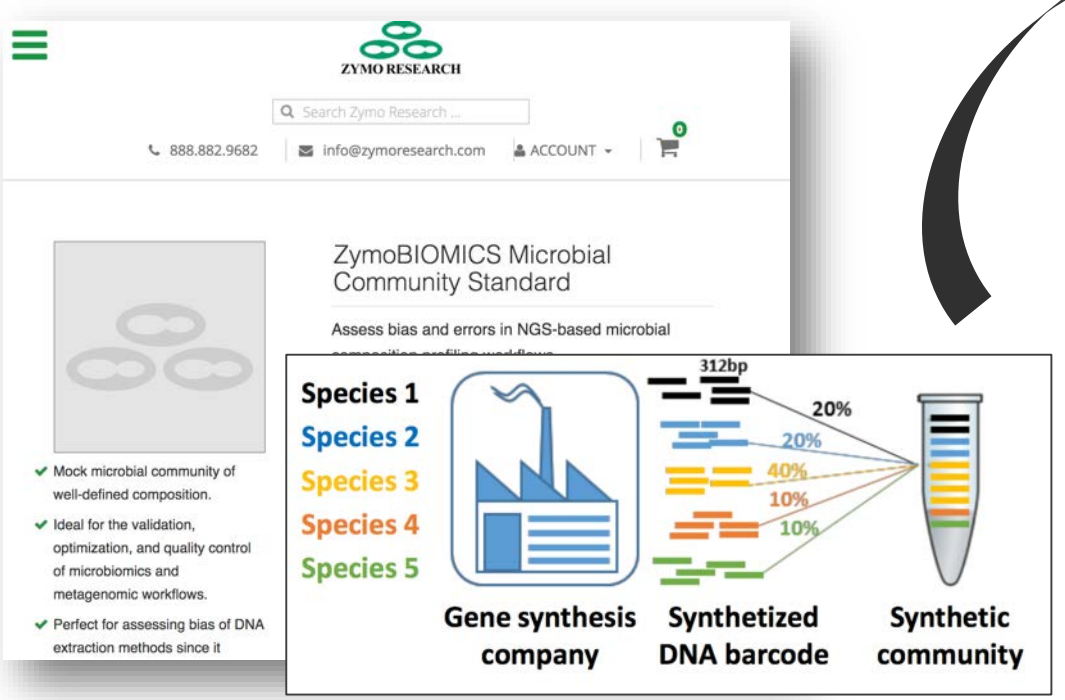
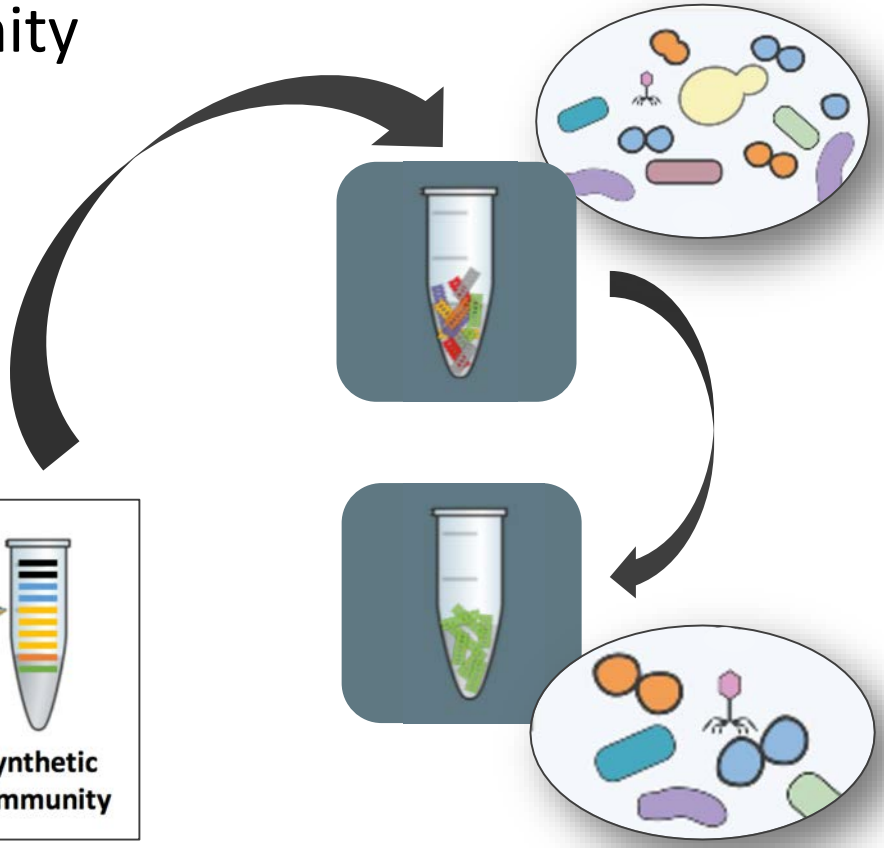
- Many commercial DNA extraction kits available
- Taxonomy results can vary depending on extraction method

Step 2: DNA extraction

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



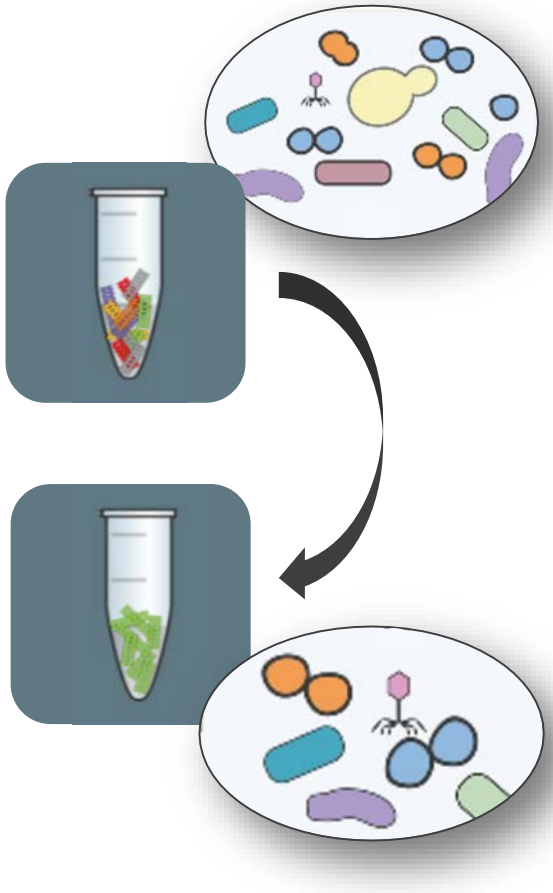


Valentin Vasselon

Step 2: DNA extraction

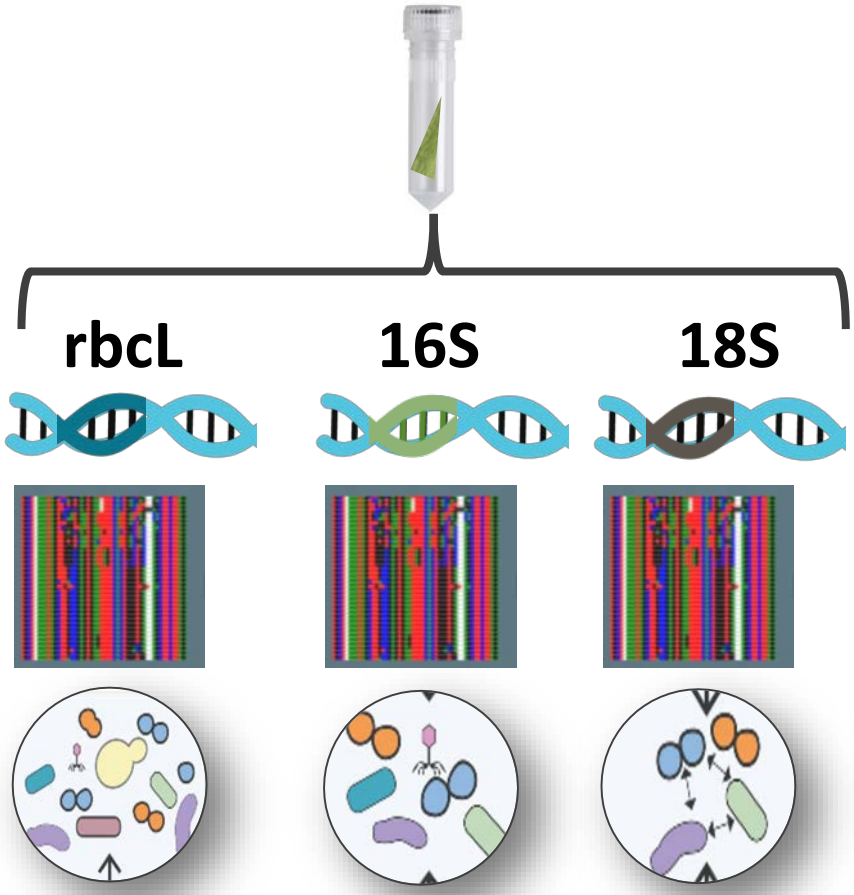
Take-home:

- 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

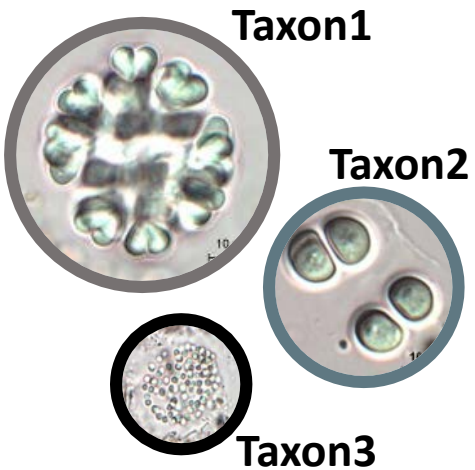
OTU1 AGTCTAGCTAGGCATTGA

OTU2 CGGCTAGTTAGGCACCGA

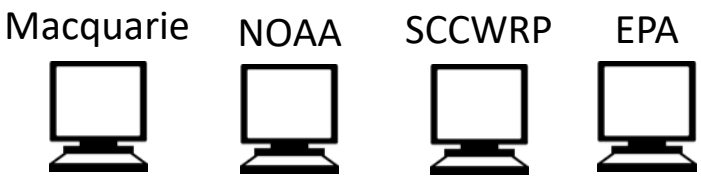
OTU3 GGTCTAGTTAGGCATCGA

Search against reference database

Assign taxonomy



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

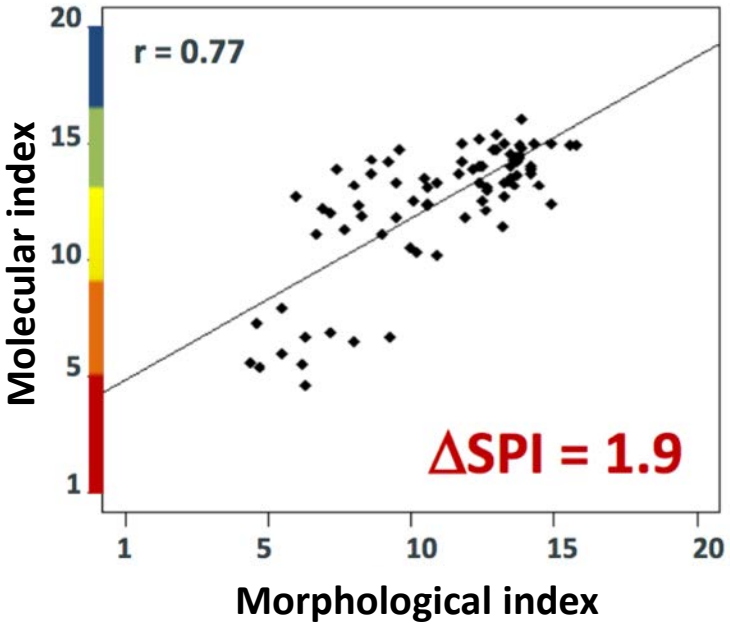
Western Association of Marine Laboratories (WAML)

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

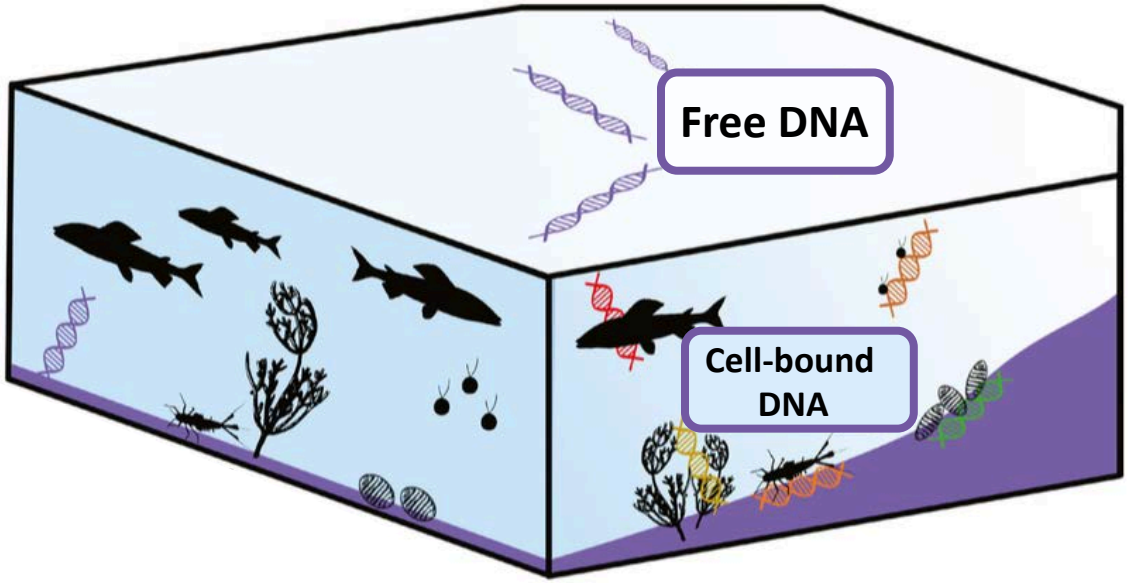
French diatom index (SPI)



Valentine Vasselon

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

Inter-laboratory Comparison Reveals Critical Issues with Periphyton Community Assessment

minnow environmental inc.

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Carla Fraser – Teck Coal Ltd.

Why should you care?

- Analysis of periphyton community structure is routinely requested by some Canadian regulators as part of aquatic baseline and operational monitoring programs for mines
- Few commercial laboratories provide this type of analysis (Canada and US included)
- Differences between community endpoints in mine-exposed compared to reference areas may be taken as evidence of mine-related effect, but…
  - What if this is simply due to methodological issues encountered during sample analysis?

Study Overview

- Study implemented in September 2013, one component being to identify if different laboratories give comparable results
- Four different commercial laboratories were sent split samples from seven different field locations, representing both reference and mine-exposed conditions (one lab initially turned down work)
- Duplicate analysis of at least one sample requested (as a measure of QA/QC) and copies of SOPs
- Results compared to determine (in)consistencies in taxonomic identification and enumeration among laboratories

Periphyton – ID Variability: Nomenclature

Group	Name used	Interlab synonyms
Diatoms	*Achnanthes ventralis*	*Navicula ventralis*
	Achnanthidium alpestre	*Achnanthes deflexa var. alpestris*
	Achnanthidium gracillimum	*Achnanthes minutissima var. gracillima, Achnanthes gracillima*
	Achnanthidium minutissimum	*Achnanthes minutissima*
	Achnanthidium minutissimum var. scoticum	*Achnanthes microcephala f. scotica*
	Achnanthidium pyrenaicum	*Achnanthes pyrenaica*
	Achnanthidium rosenstockii	*Achnanthes rosenstockii*
	Didymosphenia geminata	*Echinella geminata, Gomphonema geminatum*
	Encyonema minutum	*Cymbella minuta*
	Encyonema silesiacum	*Cymbella silesiaca*
	Encyonopsis microcephala	*Cymbella microcephala*
	Eucocconeis flexella	*Achnanthes flexella*
	Eucocconeis laevis	*Achnanthes laevis*
	Fragilaria capucina var. vaucheriae	*Fragilaria vaucheriae*
	Fragilaria recapitellata	*Fragilaria vaucheriae var. capitellata*
	Gomphoneis olivaceum	*Gomphoneis olivacea*
	Gomphonema parvulum var. micropus	*Gomphonema micropus*
	Hannaea arcus	*Ceratoneis arcus*
	Planothidium lanceolatum	*Achnanthes lanceolata*
	Reimeria sinuata	*Cymbella sinuata*
	Rhoicosphenia abbreviata	*Rhoicosphenia curvata*
	Staurosirella leptostauron	*Fragilaria leptostauron*
	Staurosirella pinnata	*Fragilaria pinnata*
Cyanophyte	*Heteroleibleinia sp.*	*Lyngbya sp.*

*** List only includes synonyms used in this study. Many more exist. ***

Periphyton – Inter-lab ID Variability: Species Level

Station	Criteria	Lab A	Lab B	Lab C	Lab D	Combined lab species Richness	Instances where all 4 labs identified same species
BUUQ	Total # of species identified	13	22	31	30	68	1
	At least one match with another lab	3	11	10	9		
	% of spp. identified that were also counted by at least one other lab	23%	50%	32%	30%		
WIHR	Total # of species identified	16	18	21	26	53	2
	At least one match with another lab	9	5	10	10		
	% of spp. identified that were also counted by at least one other lab	56%	28%	48%	38%		
LIDSL-SHR2	Total # of species identified	13	19	25	21	49	3
	At least one match with another lab	7	11	14	8		
	% of spp. identified that were also counted by at least one other lab	54%	58%	56%	38%		
Combined Stations (7)	Total number of unique species identified by each lab	33	46	67	41		

Periphyton – Inter-lab ID Variability: Genus Level

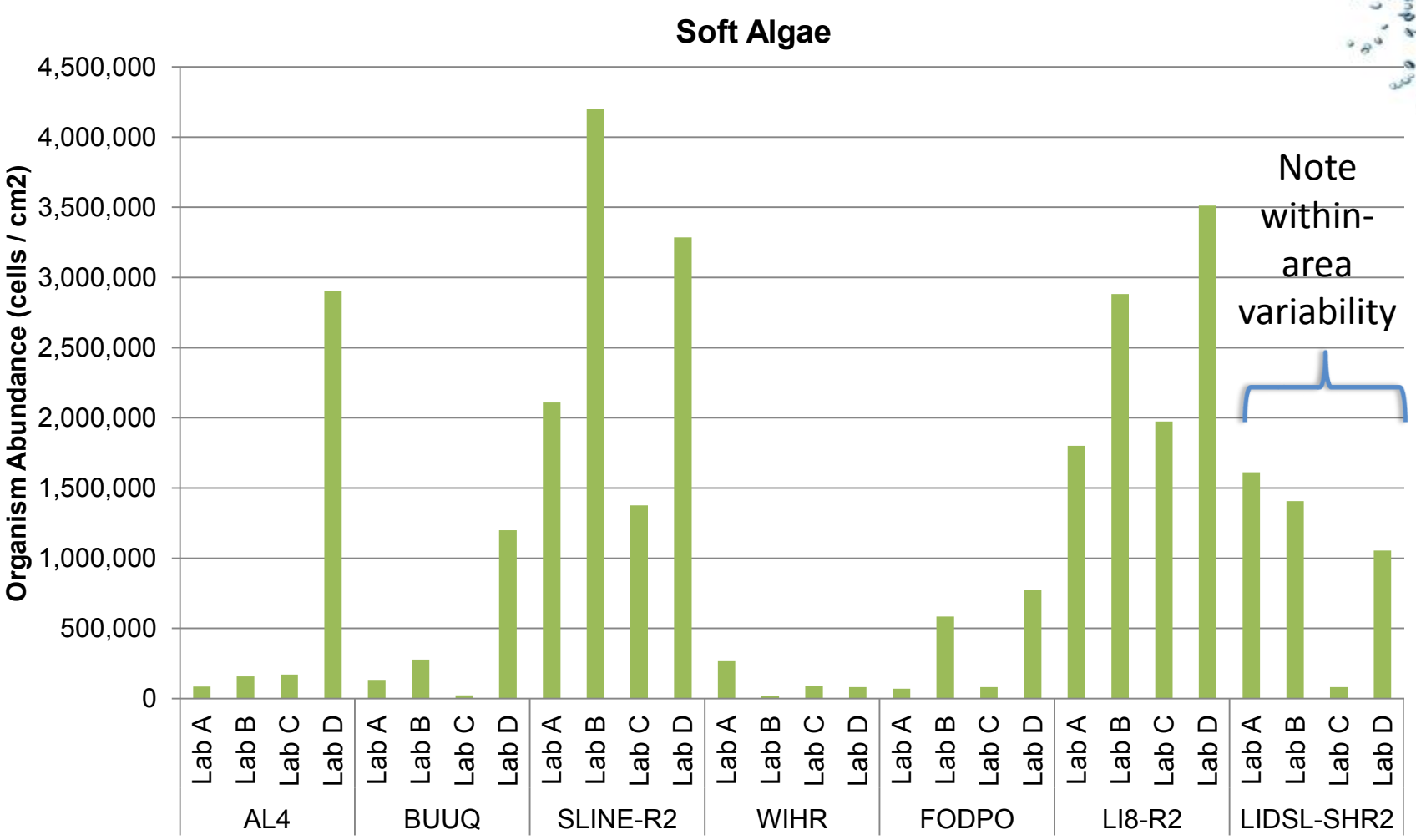
Station	Criteria	Lab A	Lab B	Lab C	Lab D	Combined lab genera Richness	Instances where all 4 labs identified same genera
BUUQ	Total # of genera identified	13	17	19	23	38	4
	At least one match with another lab	9	13	14	15		
	% of genus identified that were also counted by at least one other lab	69%	76%	74%	65%		
WIHR	Total # of genera identified	14	10	12	21	27	6
	At least one match with another lab	12	8	11	13		
	% of genus identified that were also counted by at least one other lab	86%	80%	92%	62%		
LIDSL-SHR2	Total # of genera identified	12	13	15	19	27	7
	At least one match with another lab	10	11	13	13		
	% of genus identified that were also counted by at least one other lab	83%	85%	87%	68%		
Combined Stations (7)	Total number of unique genera identified	28	26	33	31		

Laboratory Duplicate Results

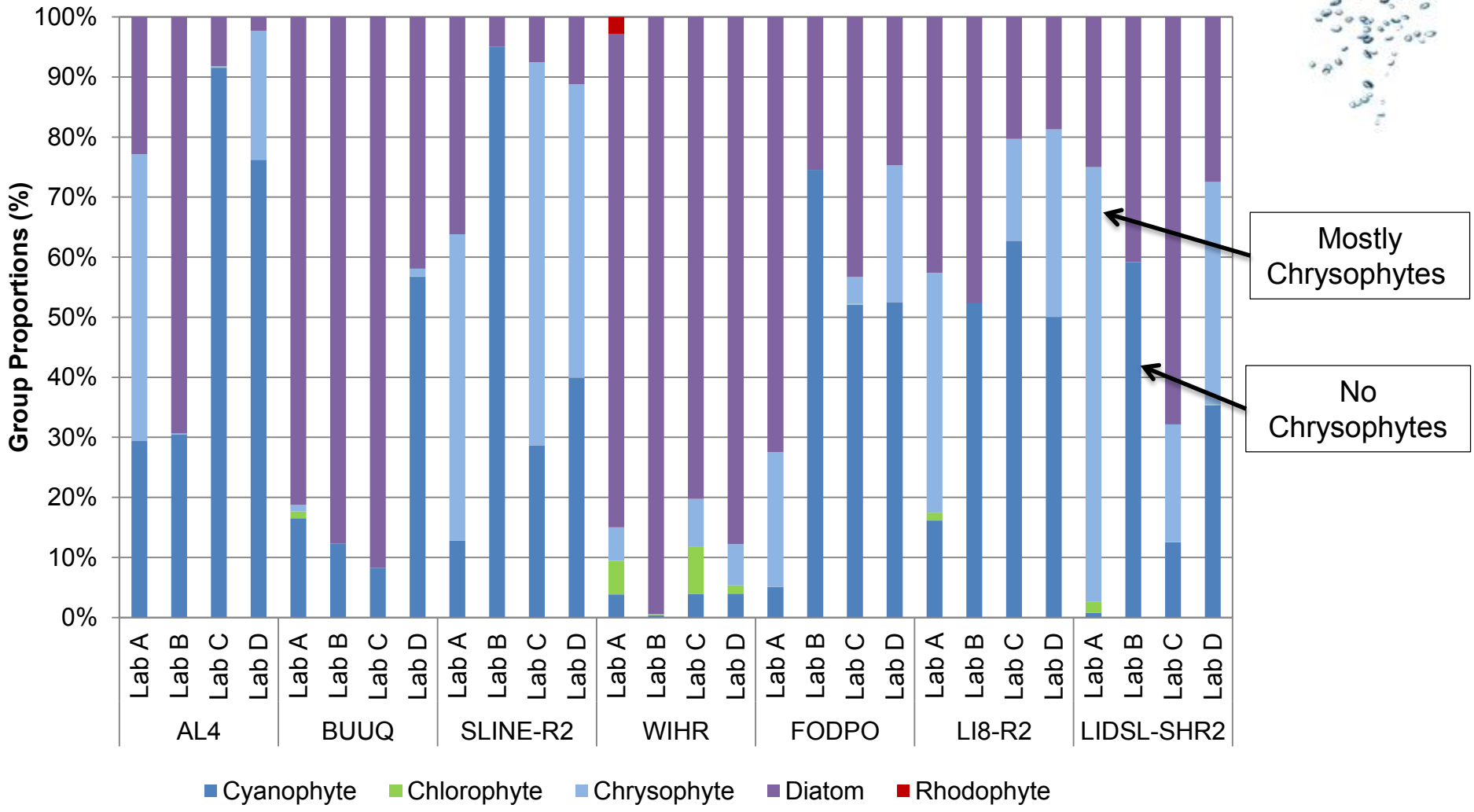
Criteria	Laboratory A						Laboratory B		
	AL4	AL4Q	RPD	LIDSL-SHR2	LIDSL-SHR2Q	RPD	WIHR	WIHR-QAQC	RPD
Total counted taxa	10	11	10%	13	14	7%	19	13	38%
Total cell density	109,950	108,645	1%	527,236	198,366	91%	3,118,286	3,283,308	5%
Number of unique taxa	1	2	-	2	3	-	8	2	-
Number of unique taxa identified by at least one other lab at same station	0	1	-	0	0	-	2	0	-

Criteria	Laboratory C						Laboratory D		
	BUUQ (non-diatom algae only)	BUUQ-dup (non-diatom algae only)	RPD	LI8-R2 (diatoms only)	LI8-R2-dup (diatoms only)	RPD	L18-R2	L18-R2-QAQC	RPD
Total counted taxa	5	6	18%	17	16	6%	30	29	3%
Total cell density	23,157	20,141	14%	502,501	502,501	0%	4,319,692	3,190,720	30%
Number of unique taxa	0	1	-	5	4	-	3	2	-
Number of unique taxa identified by at least one other lab at same station	0	1	-	2	2	-	0	0	-

Periphyton Inter-lab study
Soft algae density variability



Periphyton Inter-lab Study
Proportional variability



Standard Operating Procedure Variability

- Sample preparation: ranged from nothing to high-pressure filtration for soft algae and nitric acid digestion for diatoms
- Magnification levels – 200 to 1000X, with or without contrast/oil immersion
- Minimum # of cells counted: 100 of dominant species vs. 300-400 natural units of soft algae or 400-600 diatom valves
- Counting techniques: random fields vs. transects

Data Qualifiers

- Not consistently used. One lab used only one qualifier (i.e., sp.) while one used all of the following:
  - sp. – unknown single species of known genus
  - spp. – multiple unknown species belonging to same genus
  - cf. – looks like a particular organism, but not confirmed
  - < – organism identified in overall chamber scan, but not found during counts
  - ? – possibly unknown genus
  - UID - unidentified
  - '/' between two species – 1 set of counts for both species combined (species could not be separated)

Summary

- Total lack of agreement in algal taxonomy and densities among 4 labs sent split periphyton samples
  - possibly 7 species of *Achnanthidium* present, but one lab reported only *Achnanthidium minutissimum*)
  - Large differences even at major group level of identification (cyanophyte, chrysophyte, chlorophyte, etc.)
- Nomenclature not standardized
- No standard QA/QC requirements for laboratory methods or reporting

Conclusions

- Periphyton taxonomic identification and sample handling procedures are not sufficiently standardized at the present time to use data in regulatory assessment programs:
  - How do we know if reported data are an accurate reflection of relative taxon abundances?
  - What are the implications of methodological variations on the outcome of an impact assessment?
- Need to ensure that all laboratories being used by government, industry, and consultants provide accurate, reproducible data so results are useable

Recommendations

- Evaluate effect of method variations on results to determine “best” standard method for laboratory sample processing
- Agree upon standard nomenclature
- Develop program for taxonomic certification, such as exists for benthic invertebrate taxonomists
- Determine standard QA/QC reporting to verify sample sub-sorting accuracy and precision

Question: Who should be responsible for leading/funding this?

MEMORANDUM

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DATE: 31 July 2009
TO: Phil Markle
FROM: Jerry Diamond, Ph.D.

SUBJECT: Reference conditions and bioassessments in southern California streams

All bioassessment methods depend on having appropriate reference conditions with which to base an assessment; i.e., bioassessment data for a given site cannot be accurately interpreted by themselves—interpretation or assessment of the site data is done within the context of the biology that can be expected to occur naturally, given the type of habitat present, the type of aquatic system, and the physiographic region (i.e., ecoregion) of the country (Stoddard et al., 2006). Identifying appropriate reference conditions for certain types of aquatic systems, habitats, and ecoregions can be problematic because of wide-scale human land use changes such as hydrological modification (e.g., dams, levees, concrete channelization), urbanization (e.g., increased runoff, removal of riparian vegetation, bank protection structures), and agricultural/ livestock effects (e.g., water removal for irrigation, removal of riparian vegetation).

Southern California (Los Angeles, San Diego and surrounding counties) is an area that has experienced intense land use changes over the past 50 years, particularly in terms of urbanization and its many environmental consequences (e.g., changes in the natural hydrology, changes in stream geomorphology, etc.). In particular, low gradient as well as low elevation streams in this region have been especially prone to land use effects. This situation has resulted in high uncertainty regarding appropriate reference conditions for low gradient and low elevation streams in this region.

This observation was identified in a Technical Report I and others at Tetra Tech prepared for the Los Angeles Regional Water Quality Control Board (Tetra Tech, 2005; 2006). In that report we evaluated stream biological condition with respect to a generalized human disturbance gradient in the region, as part of an EPA-funded project to evaluate the possibility of developing tiered aquatic life uses (TALU) for southern California coastal streams. Relying on SWAMP and other data for the region, we attempted to use the recently developed southern California IBI (SoCal IBI, Ode et al., 2005) to define certain attributes of the Biological Condition Gradient for the region, which could then be used to develop TALU (Davies and Jackson, 2006). We observed that the BCG should be different (i.e., expectations lower) for low versus high elevation streams

in that project and that low elevation streams lacked a clear reference condition in this region. Working with a Technical Advisory Committee (TAC) on this project (consisting of regional experts from California Fish & Game, State Water Resources Control Board, other Regional Boards, EPA Region 9, and universities), we identified a lack of appropriate reference sites for low elevation/low gradient streams as a critical data gap in moving forward with TALU. A fairly extensive search of existing biological data in the region by Tetra Tech and the TAC indicated that suitable reference sites at lower elevations and/or for lower stream gradients were not available with which to benchmark a biological condition gradient.

Subsequent to the above project, I have been working with the Southern California Coastal Water Research Project (SCCWRP) and the LA Regional Board in facilitating two workshops on TALU for the region. In the most recent stakeholder workshop (held June 2008), there was focused discussion on the issue of appropriate reference conditions, in which there was agreement that low gradient (rather than low elevation) was perhaps the most critical factor distinguishing stream biology in the region and that reference condition for low gradient streams (many but not all of which occur at low elevation) is a critical data gap (Schiff and Diamond, 2009). In fact, in the "road map" of projects developed from this workshop, defining reference condition for streams in this region was identified as one of the top priority needs.

Given the difficulty in identifying appropriate reference conditions for low gradient coastal streams in southern California, it is perhaps premature to set regulatory requirements based on biology observed at these types of sites. The TALU framework, as well as the regional stakeholder workshops (e.g., Schiff and Diamond, 2009) recognize that different hydrologic, geomorphic, and other habitat-related factors will dictate the biological characteristics that can be expected in a given stream. The type of aquatic life uses one can reasonably expect from a low gradient or modified stream in southern California, for example, are not the same as from a high gradient or natural stream, as our previous work has demonstrated. What is the expected biological condition for low gradient or modified streams in southern California is a question that needs more attention and, as noted by all stakeholders at the June 2008 workshop, incorporation of information using other assemblages (e.g., algae) in addition to macroinvertebrates.

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