



Molecular methods for bioassessment

Susie Theroux

May 30, 2018



Background

- Bioassessment and biomonitoring play a critical role in evaluating and protecting California's waterbodies
- Our ability to assess biological communities to inform management decisions relies on generating taxonomy data quickly and reliably
 - Algae
 - Invertebrates
 - Fish
 - Endangered species
 - Invasive species



Background

- New DNA-based technologies are enabling us to generate taxonomy data faster, cheaper, and more reliably
- Multiple agencies across California are developing and actively using DNA-based approaches for biomonitoring and bioassessment
- As the popularity of DNA methods increases, so too does our need for coordinated approaches to generating DNA data

Goals of this talk

- Overview of DNA-based methods
- Examples of DNA-based approaches in monitoring and bioassessment
- Key elements of a DNA-based approach that require coordination to ensure comparable data
- Potential role of a DNA working group

DNA sampling: cell-bound DNA



eDNA sampling: free DNA



“environmental”
or “extracellular” DNA



Generating DNA data



```
Env. Barcode 1  
ATCGGGATGCCA  
Env. Barcode 2  
ATCGGGATGCCA  
Env. Barcode 3  
ATCGGAAACCA  
...
```

Species	%
<i>D.tenuis</i>	20
<i>N.palea</i>	10
<i>A.pediculus</i>	5
...	...



Sampling

DNA
extraction

DNA analysis

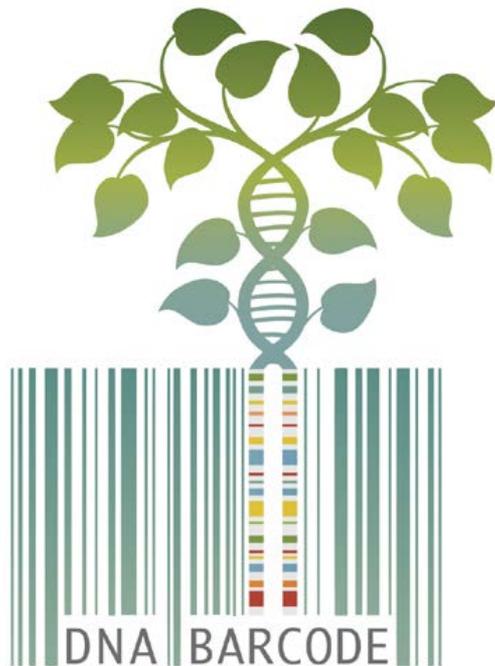
Bioinformatics

Taxonomy ID

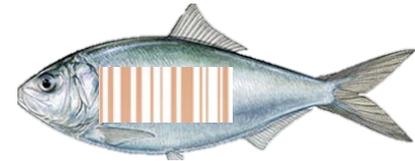
Biological
indices

What is DNA barcoding?

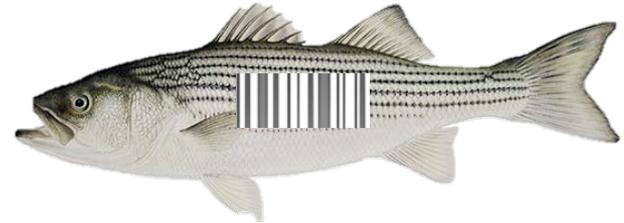
- **DNA barcode:** short region of DNA used for species identification
- Allows for rapid, efficient identification
- Scalable, increasingly affordable



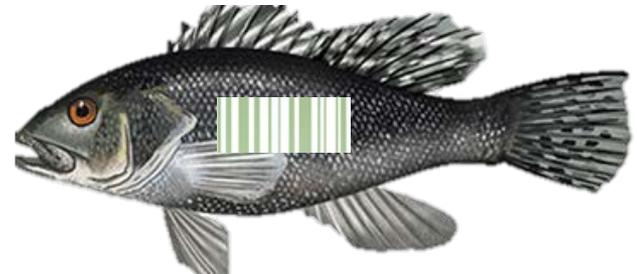
GCATAGGGACGAGCATAACG



GCATAGCTACAAGCACACG



GCATAGCTACGAGGAAACG



DNA approaches

Quantitative PCR (qPCR)

How many *Microcystis* cells are in my sample?

How many pathogen markers are present in my sample?



DNA barcode sequencing

What species of fish did I catch?

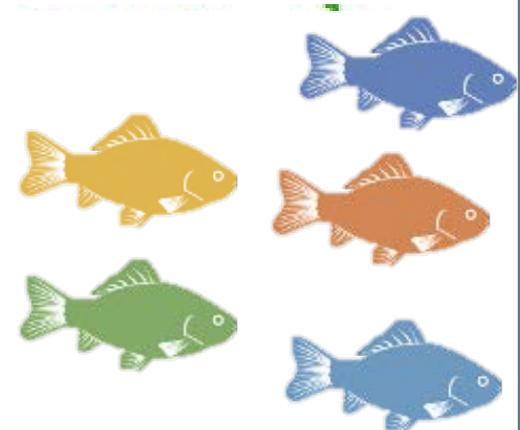
Is this a quagga or zebra mussel?



DNA metabarcode sequencing

What species of fish are in my sample?

How many different invertebrate species are in my sample?



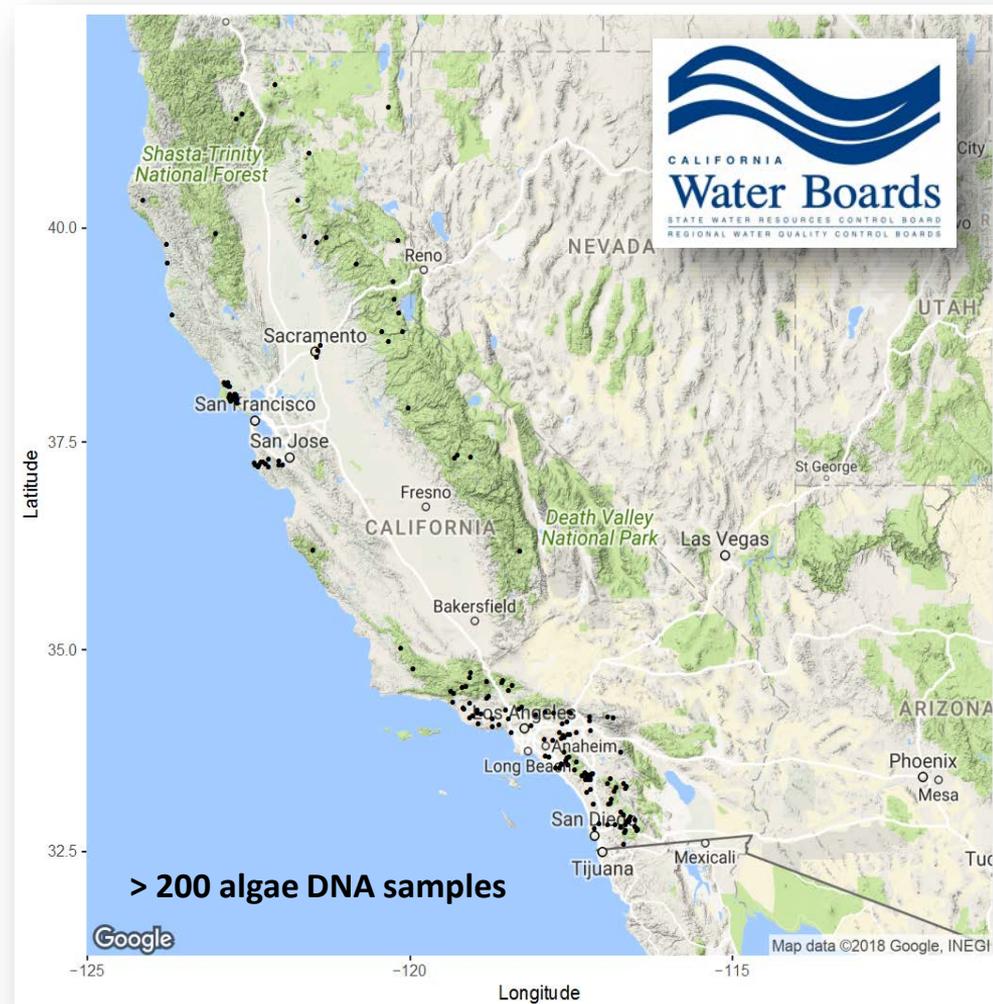
DNA methods in action

- Bioassessment
 - Algae
 - Invertebrates
 - Fish
- Human health
 - Pathogens
 - Toxic algae
- Endangered species
- Invasive species

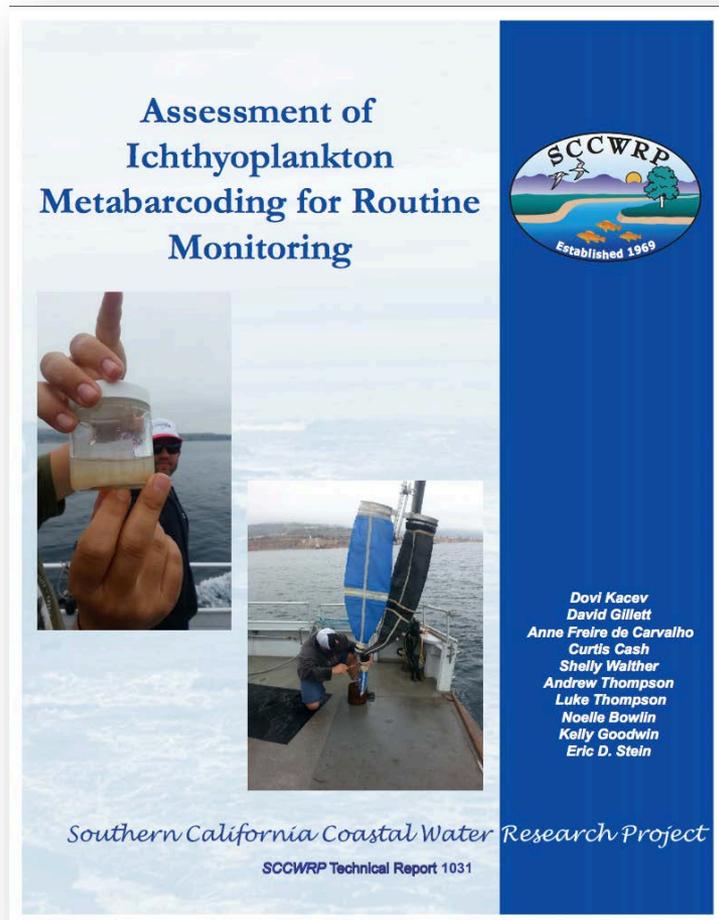


DNA approach: algal bioassessment

- State Water Board is supporting the development of a DNA-based approach to algal bioassessment
- 2016-2018 algae DNA sampling paired with traditional morphology sample



DNA approach: ichthyoplankton bioassessment



- Ichthyoplankton morphology can make species identification difficult and time-consuming
- Pilot studies DNA-based approaches



DNA approach: toxic algae

DNA-based approaches used in cyanobacteria monitoring

- Ability to detect low levels of toxic species
- Identify species responsible for toxic events



DNA approach: endangered species

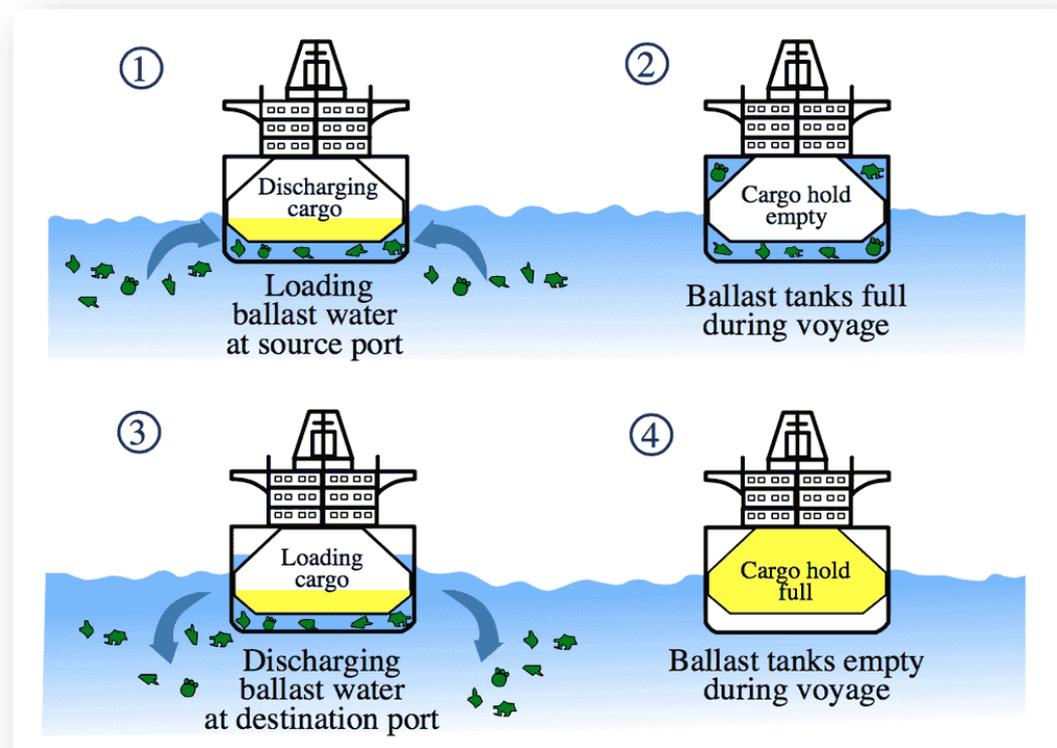
- CDFW and WB are pursuing eDNA-based approaches for endangered species monitoring in streams
- Non-destructive sampling
- Ability to detect organism at a distance



DNA approach: invasive species

The California Marine Invasive Species Program (MISP)

- Focus on ballast water introduction of non-indigenous species
- Paired morphology and DNA-based approaches to identify species



California
STATE LANDS
Commission

DNA approach: ready, set, go?

- DNA based methods are now routine and being integrated into agency programs
- Pilot studies are focused on adapting DNA-based approaches for the management community
- For DNA results to be comparable across agencies, we need a coordinated approach to generating DNA data



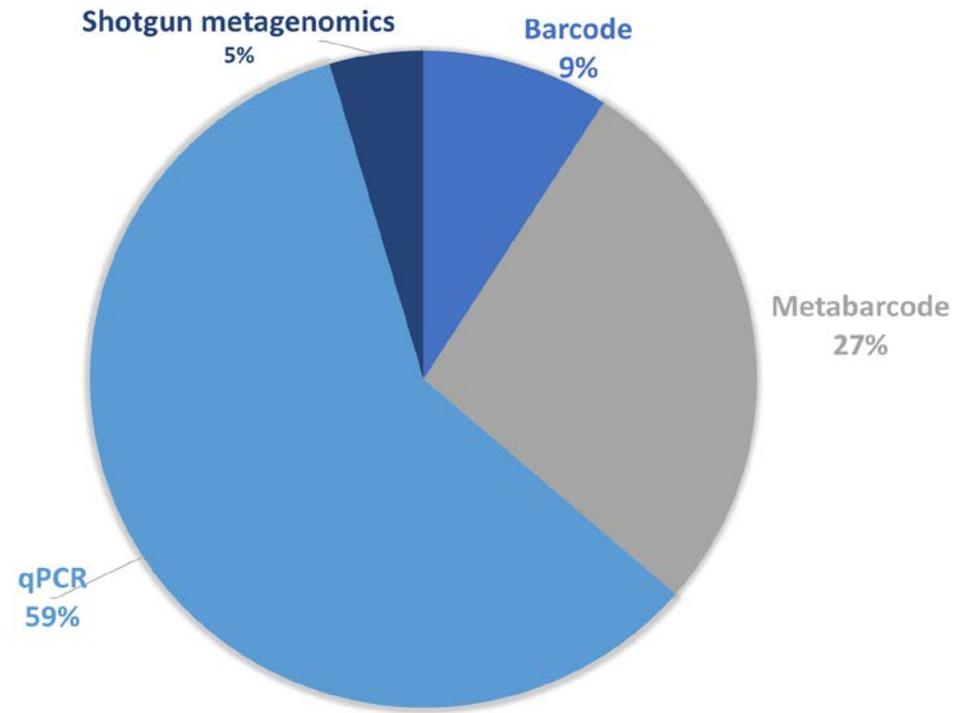
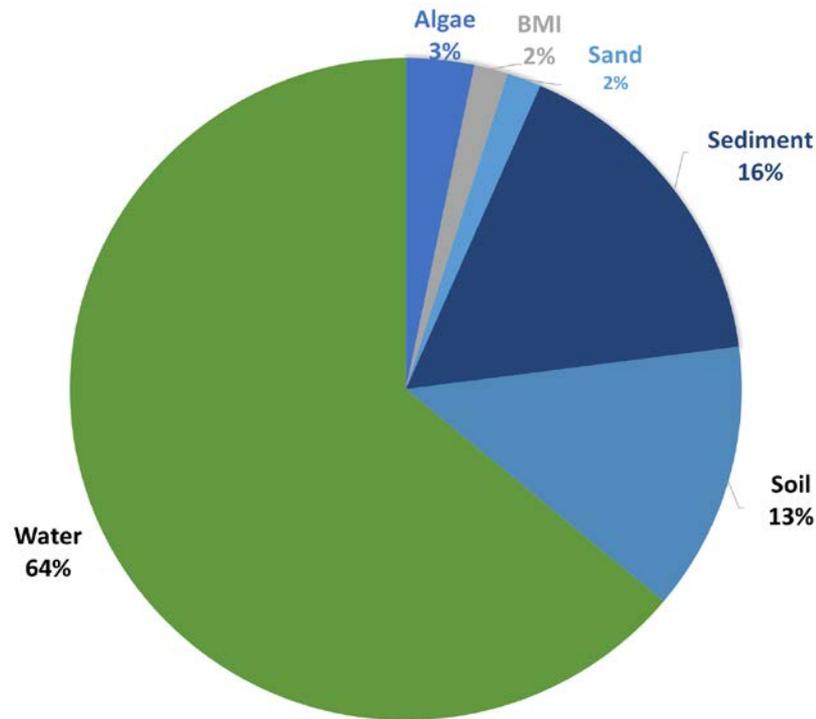
A need for coordination

- Water Board convened a planning meeting in February 2018 to discuss need/desire/capacity to form a DNA working group
- Organized by SWAMP members with OIMA, SCCWRP, and Cramer Fish Sciences participation
- Possible roles of DNA working group:
 - Establish guidelines and QA/QC recommendations for generating and interpreting DNA data
 - Facilitate collaboration between groups sampling and analyzing DNA samples



Survey: who is sampling DNA in California?

- Who/what/where DNA sampling is occurring



Survey: what sampling methods are used?



Rocky Mountain Research Station

Protocol for collecting eDNA samples from streams
Version 2.3- July 2015



Bull trout swimming exploring the complex habitat of a Montana stream. Photo Credit: Aubree Benson

Please cite as:
Carim, K. J., T. Padgett-Stein
(2015) Protocol for collecting
Genomics Center for Wildlife

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Methods in Ecology and Evolution

Methods in Ecology and Evolution 2017, 8, 635–645

doi: 10.1111/2041-210X.12683

Comparison of capture and storage methods for aqueous microbial eDNA using an optimized extraction protocol: advantage of enclosed filter

Johan Spens^{1,2*}, Alice R. Evans^{1†}, David Halfmaerten³, Steen W. Knudsen¹, Mita E. Sengupta⁴, Sarah S. T. Mak¹, Eva E. Sigsgaard¹ and Micaela Hellstrom^{1,5*}

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eDNA PROTOCOL
SAMPLE COLLECTION

Caren Goldberg and Katherine Strickler, Washington State University
Revised January 2017

USGS
science for a changing world

Prepared in cooperation with Washington State University

Environmental DNA Sampling Protocol—Filtering
Water to Capture DNA from Aquatic Organisms

Chapter 13 of

What are the key steps and decision points in generating DNA data?

MOLECULAR ECOLOGY
RESOURCES

Molecular Ecology Resources (2016) 16, 895–908

doi: 10.1111/1755-0998.12501

Using occupancy modelling to compare environmental DNA to traditional field methods for regional-scale monitoring of an endangered aquatic species

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Abstract

Environmental DNA (eDNA) monitoring approaches promise to greatly improve detection of rare, endangered and invasive species in comparison with traditional field approaches. Herein, eDNA approaches and traditional seining methods were applied at 29 research locations to compare method-specific estimates of detection and occupancy probabilities for endangered tidewater goby (*Eucyclogobius newberryi*). At each location, multiple paired seine hauls and water samples for eDNA analysis were taken, ranging from two to 23 samples per site, depending upon habitat size. Analysis using a multimethod occupancy modelling framework indicated that the probability of detection using eDNA was nearly double (0.74) the rate of detection for seining (0.39). The higher detection rates afforded by eDNA allowed determination of tidewater goby occupancy at two locations where they have not been previously detected and at one location considered to be locally extirpated. Additionally, eDNA concentration was positively related to tidewater goby catch per unit effort, suggesting eDNA could potentially be used as a proxy for local tidewater goby abundance. Compared to traditional field sampling, eDNA provided improved occupancy parameter estimates and can be applied to increase management efficiency across a broad spatial range and within a diversity of habitats.



Generating DNA data



Env. Barcode 1	ATCGGGATGCCA
Env. Barcode 2	ATCGGGATGCCA
Env. Barcode 3	ATCGGAAACCA
...	...

Species	%
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Sampling

DNA
extraction

DNA analysis

Bioinformatics

Taxonomy ID

Biological
indices

Generating DNA data



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How are you sampling?
How quickly do you
process samples?
What filters do you use?

Generating DNA data



Env. Barcode 1	ATCGGGATGCCA
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Env. Barcode 3	ATCGGAAACCA
...	...

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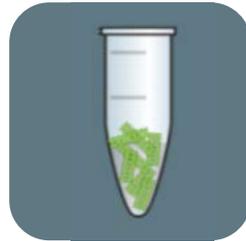
Bioinformatics

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Which DNA extraction
method do you use?
How do you prevent
contamination?

Generating DNA data



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ATCGGGATGCCA
Env. Barcode 3
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...
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Which DNA barcode
region do you sequence?
Which qPCR probes do
you use?

Generating DNA data



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Env. Barcode 2	ATCGGGATGCCA
Env. Barcode 3	ATCGGAAACCA
...	...

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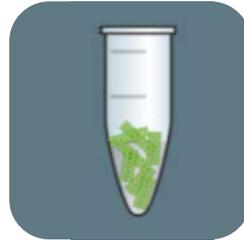
Bioinformatics

Taxonomy ID

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indices

Do you use custom or published bioinformatic scripts?
What QA/QC protocols do you follow?

Generating DNA data



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Env. Barcode 2	ATCGGGATGCCA
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...	...

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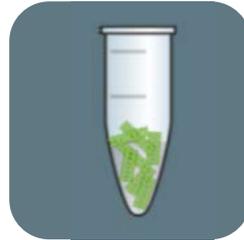
Bioinformatics

Taxonomy ID

Biological
indices

What DNA reference database do you use?
What taxonomy assignment method do you use?

Generating DNA data



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Env. Barcode 3	ATCGGAAACCA
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Results may vary widely!

The need for coordination

- Each step in the DNA process can influence the resulting DNA data
- Standardization of methods and QA/QC guidelines help ensure all users are generating comparable data
- European consortium is working to establish guidelines for generating and interpreting DNA data
- Monitoring Council can play a similar role for California



Research Ideas and Outcomes 2: e11321
doi: 10.3897/rio.2.e11321



Grant Proposal

DNAqua-Net: Developing new genetic tools for bioassessment and monitoring of aquatic ecosystems in Europe

Florian Leese^{‡§}, Florian Altermatt[‡], Agnès Bouchez[‡], Torbjørn Ekrem[#], Daniel Hering[‡], Kristian Meissner[‡], Patricia Mergen[‡], Jan Pawłowski[‡], Jeremy Jay Piggott[‡], Frédéric Rimeit[‡], Dirk Steinke[‡], Pierre Taberlet[‡], Alexander M. Weigand[‡], Kessy Abarenkov[‡], Pedro Beja[‡], Lieven Bervoets[‡], Snaedis Björnsdóttir[‡], Pieter Boets[‡], Angela Boggero[‡], Atle Magnar Bones[‡], Ángel Borja[‡], Kat Bruce[‡], Vojislava Bursić[‡], Jens Carlsson[‡], Fedor Ciampor[‡], Zuzana Ciamporová-Zatovičová[‡], Eric Coissac[‡], Filipe Costa[‡], Marieta Costachell[‡], Simon Creer[‡], Zoltán Csabai[‡], Kristy Deiner[‡], Ángel DelValls[‡], Stina Drakare[‡], Sofia Duarte[‡], Tina Eleršek[‡], Stefano Fazi[‡], Cene Fišer[‡], Jean-François Flot[‡], Vera Fonseca[‡], Diego Fontaneto[‡], Michael Grabowski[‡], Wolfram Graf[‡], Jóhannes Guðbrandsson[‡], Yaron Hershkovitz[‡], Peter Hollingsworth[‡], Bella Japoshvili[‡], John I. Jones[‡], Maria Kahlert[‡], Belma Kalamujic Strojil[‡], Panagiotis Kasapidis[‡], Martyn G Kelly[‡], Mary Kelly-Quinn[‡], Emre Keskin[‡], Urmas Kóljalg[‡], Zrinka Ljubešić[‡], Irena Maček[‡], Elvira Mächler[‡], Andrew Mahon[‡], Marketa Marečková[‡], Maja Mejdandžić[‡], Georgina Mircheva[‡], Matteo Montagna[‡], Christian Moritz[‡], Vallo Mulik[‡], Andreja Naumski[‡], Ion Navodaru[‡], Judit Padisák[‡], Snejbörn Pálsson[‡], Kristel Panksep[‡], Lyubomir Penev[‡], Adam Petrušek[‡], Martin Andreas Pfannkuchen[‡], Craig R Primmer[‡], Baruch Rinkevich[‡], Ana Rotter[‡], Astrid Schmidt-Kloiber[‡], Pedro Segurado[‡], Arjen Speksnijder[‡], Pavel Stoev[‡], Malin Strand[‡], Sigita Šulčius[‡], Michael Traugott[‡], Costas Tsigenopoulos[‡], Xavier Turon[‡], Alice Valentini[‡], Berry van der Hooft[‡], Gábor Várbiro[‡], Marlen Ines Vasquez Hadjilyra[‡], Javier Viguri[‡], Irma Vitonyte[‡], Alfred Vogler[‡], Trude Vrålstad[‡], Wolfgang Wägele[‡], Roman Wenne[‡], Anne Winding[‡], Guy Woodward[‡], Bojana Zegura[‡], Jonas Zimmermann[‡]

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[#] NTNU University Museum, Trondheim, Norway

[□] Finnish Environment Institute, Jyväskylä, Finland

⁻ Royal Museum for Central Africa, Tervuren, Belgium

⁼ Botanic Garden Meisei, Meisei, Belgium

^{*} University of Geneva, Geneva, Switzerland

[^] University of Otago, Dunedin, New Zealand

[;] INRA, Thonon-les-Bains cedex, France

[^] University of Guelph, Guelph, Canada

[^] Université Joseph Fourier, Grenoble, France

[©] Centre for Water and Environmental Research (CWE), Essen, Germany

[†] University of Tartu, Tartu, Estonia

Potential roles for working group

1. Sampling method standardization
 - Best practices for sterile sampling technique
2. QA/QC protocols for sample processing and DNA sequencing
 - Negative controls/field blanks
3. Bioinformatic guidelines and data interpretation thresholds
4. Reporting requirements
 - Minimum information about a marker gene sequence (MIMARKS)
5. **Coordinate researchers and agencies pursuing DNA approaches**

Bonus slides

Who could be on a working group?

1. State Water Board
2. CA Dept of Fish and Wildlife
3. Coastal Commission
4. Coastal Conservancy
5. Any group that does monitoring and assessment!

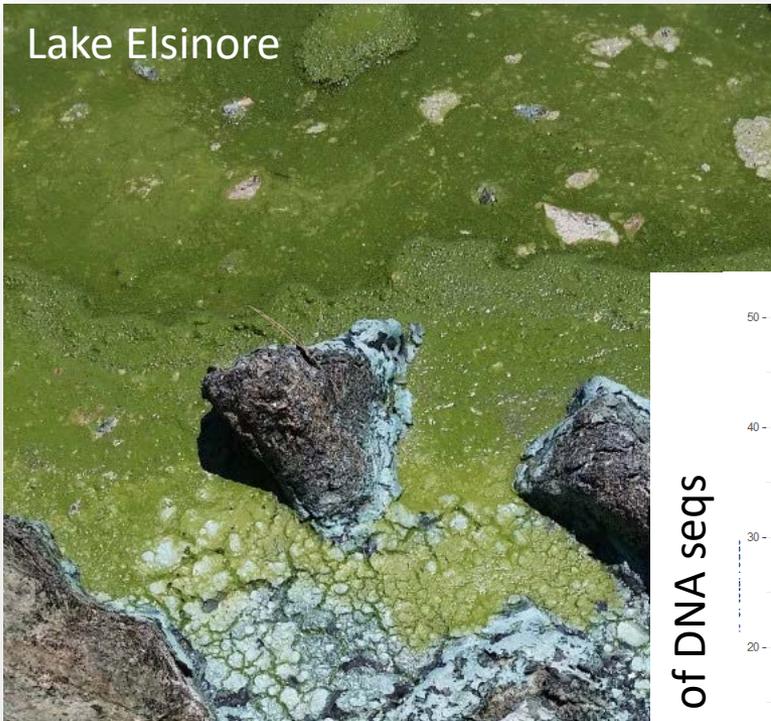
DNA-AquaNet



- **WG1 – DNA Barcode References**
lead by Torbjørn Ekrem, Fedor Čiampor
- **WG2 – Biotic Indices & Metrics**
Jan Pawlowski, Maria Kahlert
- **WG3 – Field & Lab Protocols**
Kat Bruce, Emre Keskin
- **WG4 – Data Analysis & Storage**
Diego Fontaneto, Alain Franc
- **WG5 – Implementation Strategy & Legal Issues**
Patricia Mergen, Daniel Hering

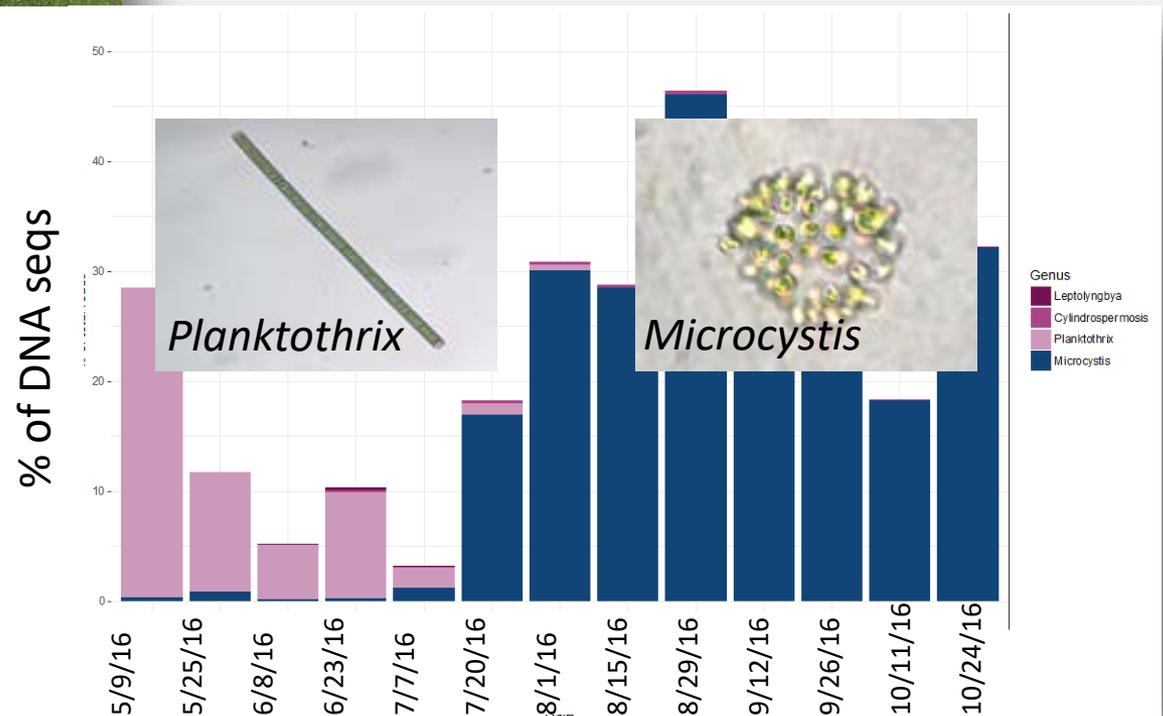
DNA approach: toxic algae

Lake Elsinore



DNA-based approaches used in cyanobacteria monitoring

- Ability to detect low levels of toxic species
- Identify species responsible for toxic events



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

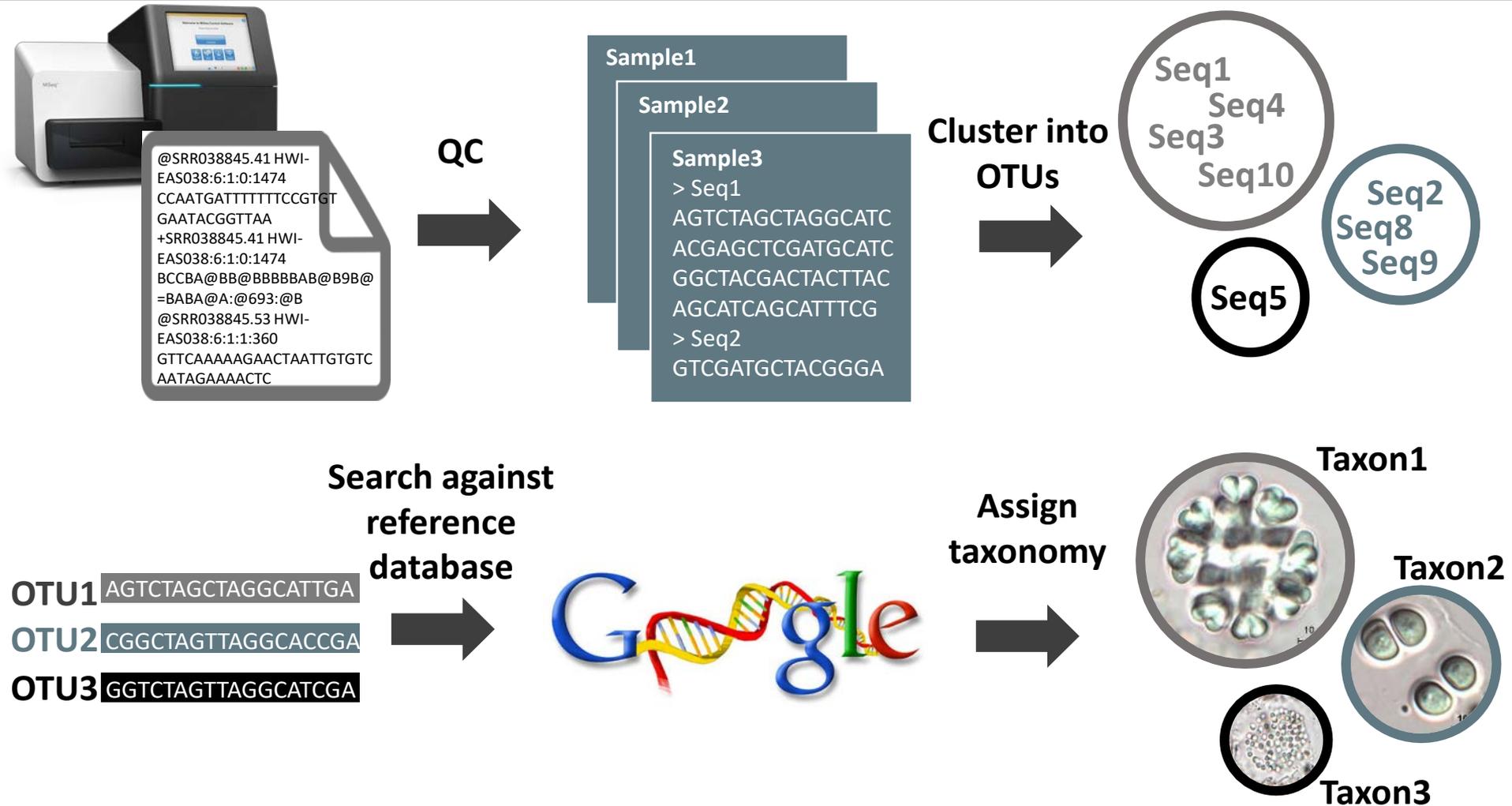


eDNA monitoring

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



Example bioinformatics pipeline



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



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