



Development of next generation molecular tools for assessing stream ecosystem function

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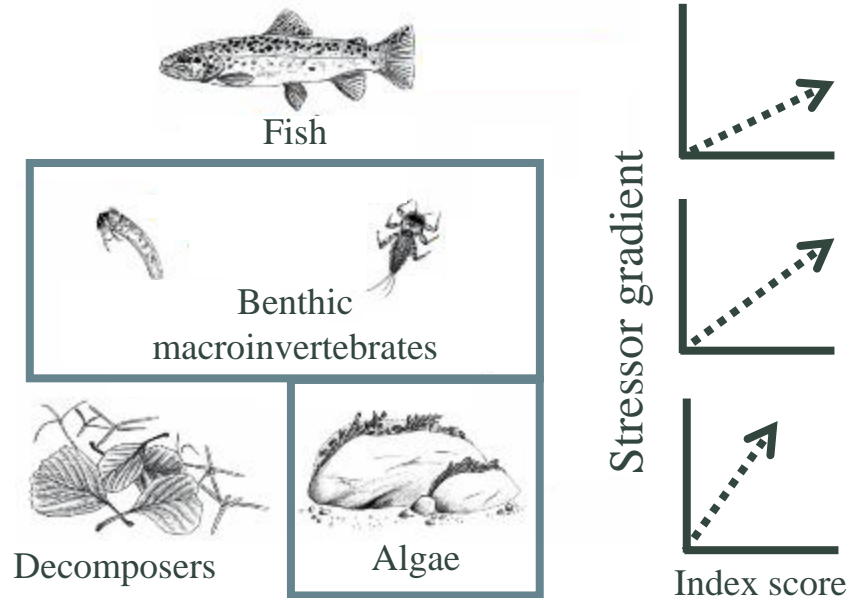
Traditional bioassessment and biomonitoring



- Bioassessment allows for the direct measure of resident biota to understand biological health
- Typically relies on fish, algae, or benthic macroinvertebrates (BMI)
- Calculate biological indices to determine biological integrity and impacts of stressors
- More taxa == multiple lines of evidence

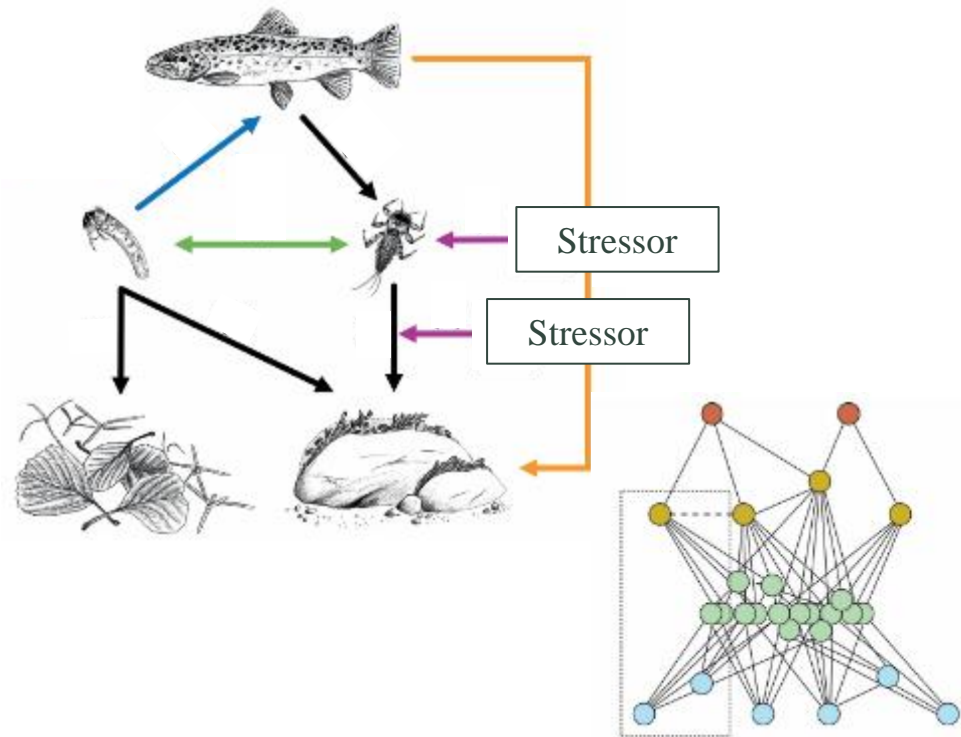
Biological indices

- Biological indices are sensitive tools that reflect species' responses to perturbations integrated over time
- In California, we have two main indices for streams
 - California Stream Condition Index (CSCI) for BMI
 - Algal Stream Condition Index (ASCI) for algae
- However, indices alone do not tell us about biotic interactions and how these are impacted by stress



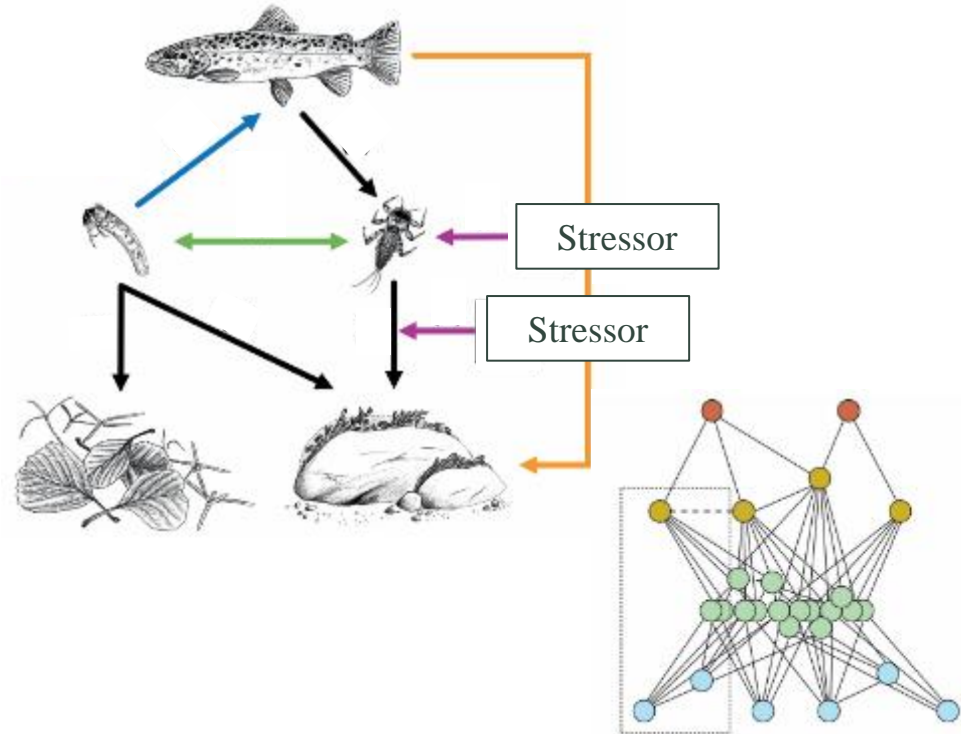
Exploring multitrophic networks

- Multitrophic networks allow us to better understand ecosystem health
 - Food web support is a critical component of a healthy ecosystem
 - By looking across multiple trophic levels we get a more holistic understanding of how the ecosystem is functioning
 - May also help us understand how stressors ripple through a community



Our approach

- Build capacity to assess multitrophic networks
 1. Identify species
 2. Quantify species interactions
 3. Develop novel index that leverages multitrophic networks to assess ecosystem health



Step 1. Identify species

Environmental
sample

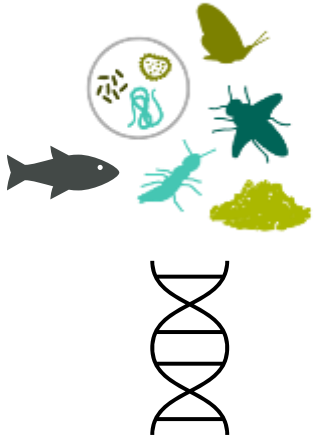


Microscopy

- Limited spatial and temporal resolution
- Miss key taxonomic groups
- Costly and long data turnaround times

Step 1. Identify species with DNA

Environmental
sample



DNA

- Expand the taxonomic groups that we can monitor
- Potential to be faster, cheaper, and more accurate

Step 1. Identify species with DNA

Environmental sample



Extract DNA



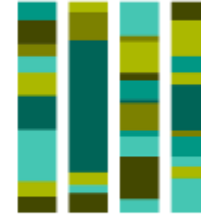
Amplify DNA barcode regions



Bioinformatics

```
tttgagtatacaact
ttcgagcatagact
aacgtccaaggagt
ttggagcatagact
aaggccaagagt
ttcgagcatagact
atcgccaatggagt
aaggccaacgagt
aacgtccaaggagt
tttgagtatacaact
```

Taxonomy assignment



Assessment



Step 1. Identify species with DNA

Which preservation method?

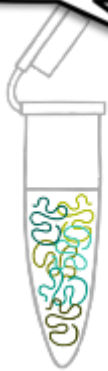
Are all kits created equal?

What DNA barcode primers should I use?

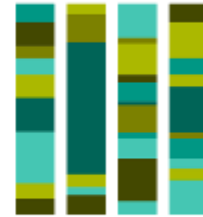
Does bioinformatics pipeline matter?

Which DNA reference library?

Are our assessment outcomes the same?



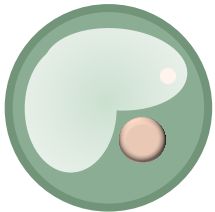
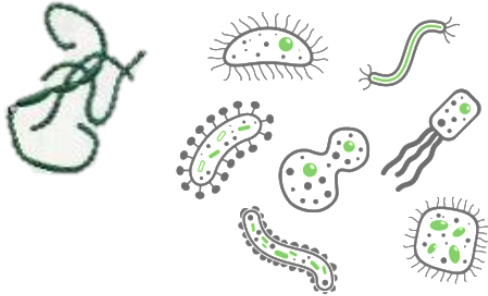
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ttagtatacaact
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atcgtccaatggagt
aagggtccaaacgagt
aacgtccaaaggagt
tttagtatacaact
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Step 1. Identify DNA barcodes to target

16S rRNA genes

Universal bacteria/archaea



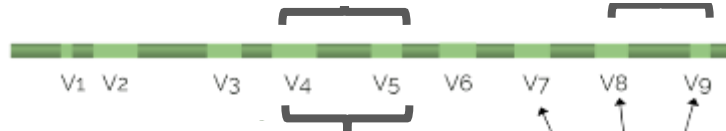
18S rRNA genes

Universal eukaryote



18S V4

18S V9

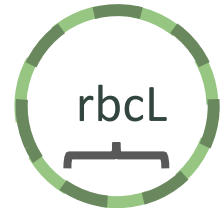


16S V4

Variable Regions

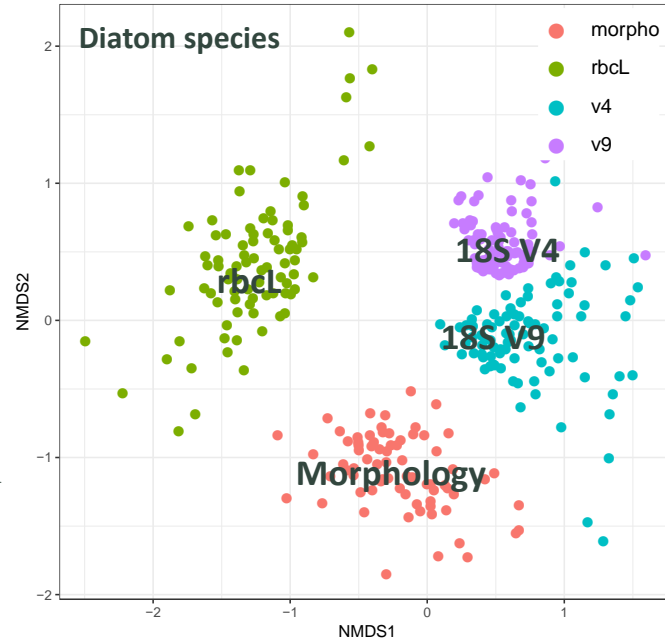
rbcL

Diatom-specific



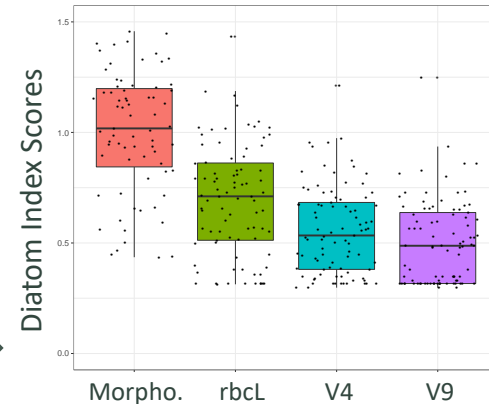
Results: High variability in DNA barcode performance

- DNA barcode influenced resulting taxonomic composition
- Biological index scores lower with DNA-based taxonomic data
 - Reflected many novel taxa with no trait attributes
 - Missing DNA reference sequences

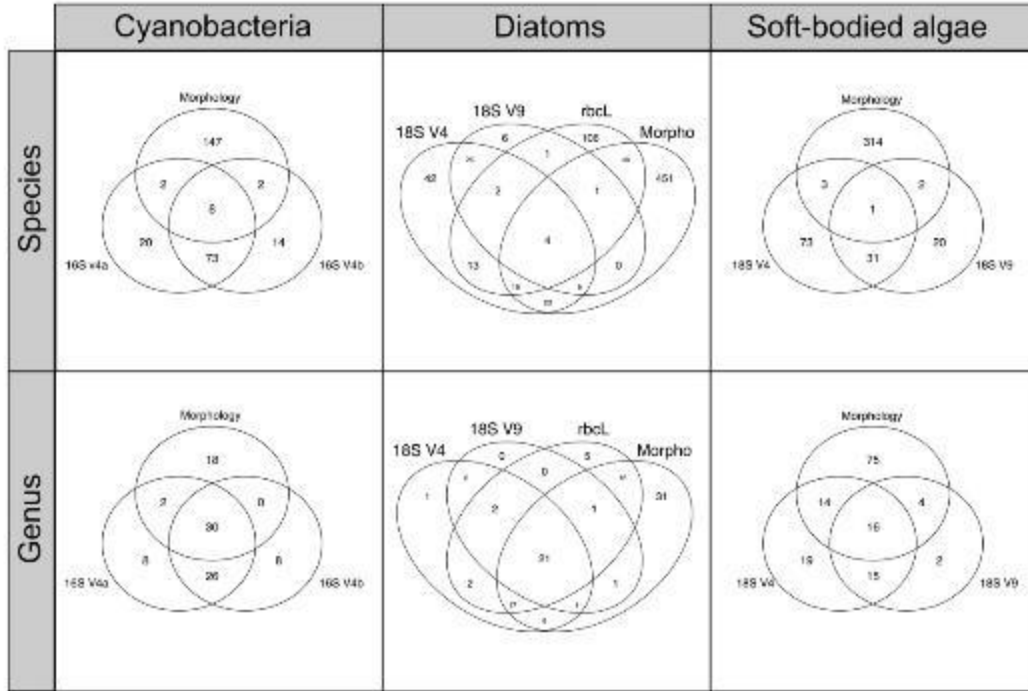


← Strong clustering in community composition

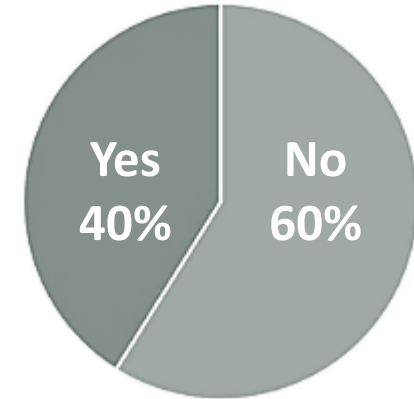
Index scores lower with DNA data →



Results: High variability in DNA barcode performance



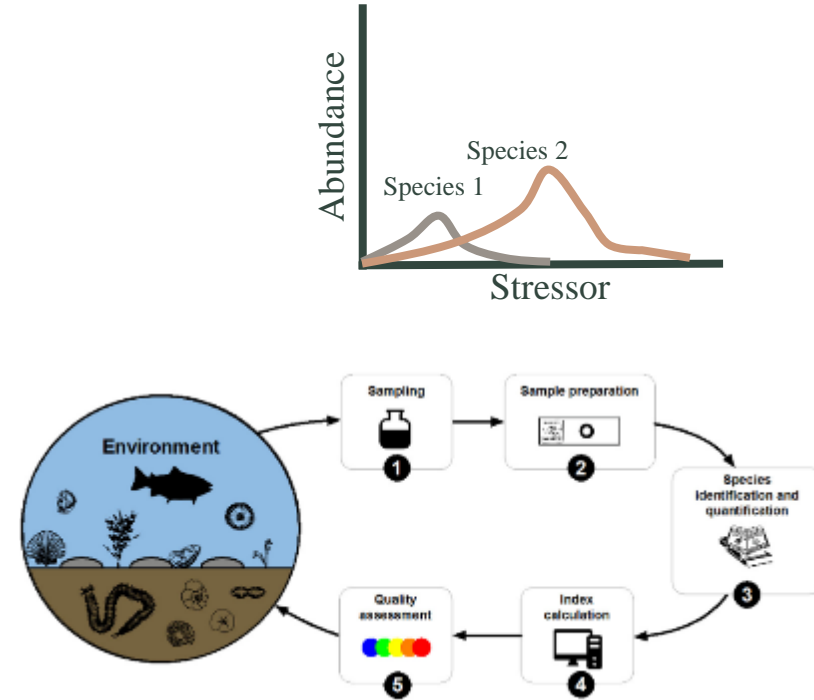
Algae species with reference sequence
(n = 1700)



Lack of DNA reference sequences limiting index performance with DNA-based taxonomy

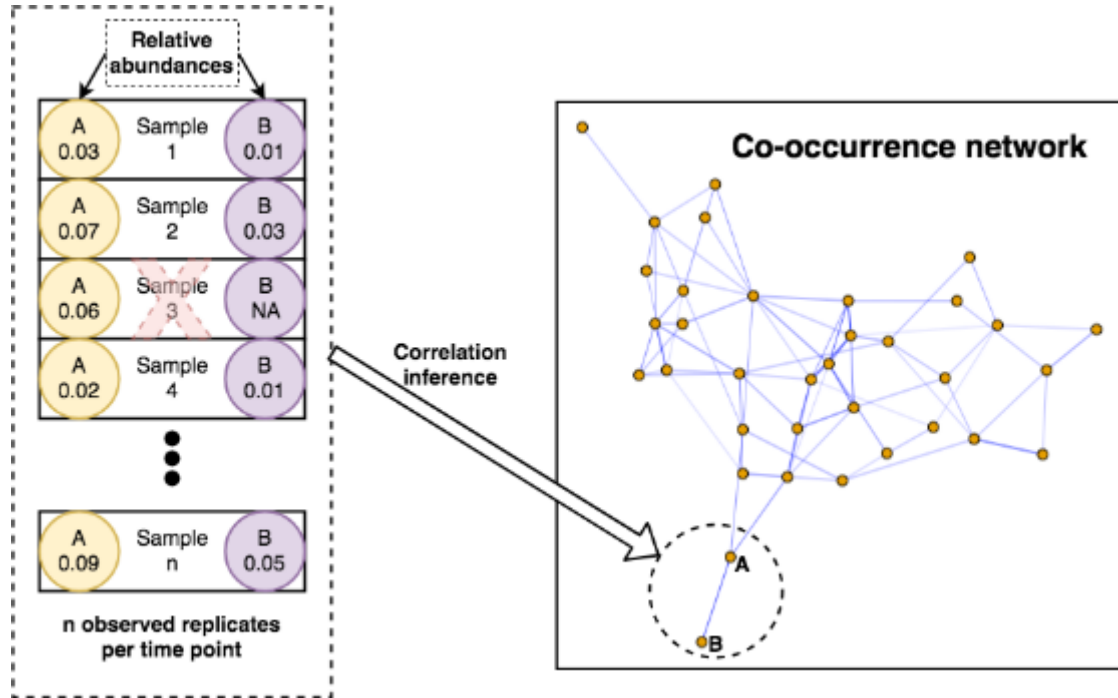
Results: High variability in DNA barcode performance

- Universal DNA barcode primers allowed for the identification of multiple taxonomic groups at one time
- DNA-based taxonomy resulted in orders of magnitude more taxa being identified, although many taxa still lack DNA reference sequences
- Improvements in DNA reference libraries will aid in biological index calculations
- Assigning trait attributes to novel sequences with “taxonomy-free” approaches will help circumvent this limitation



Step 2. Quantify species interactions

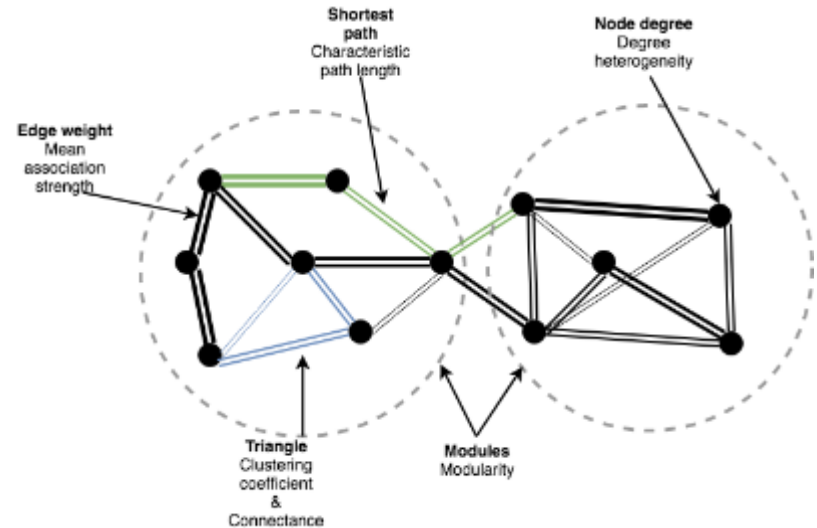
- Use a co-occurrence network approach to explore multitrophic networks
- Co-occurrence networks can help infer community patterns which are not captured by diversity measures
- Use modeling to predict species-species interactions
- Identify statistically significant correlations and responsiveness to stressors gradients



Step 2. Quantify species interactions

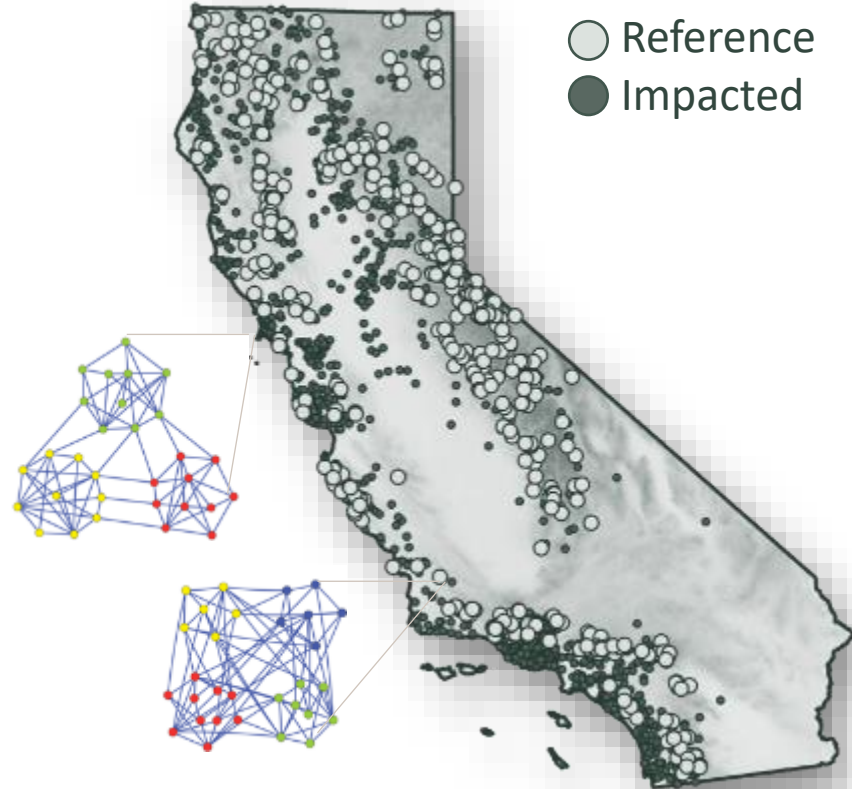
Identify network characteristics (“topologies”) that respond to stressor gradients

Topological measure	Ecological relevance	Hypothesized relationship with stress
Network size	The number of unique types of taxa across a set of communities.	(-)
Connectance	The fraction of significant co-occurrences realized compared to theoretical maximum for a network.	(+)
Mean co-occurrence strength	Correlation strength between unique types of taxa.	(+)
Modularity	How strongly patterns of co-occurrence are partitioned into subcommunities.	(+)
Degree heterogeneity	How skewed the distribution of the number of co-occurrences per unique type of taxa is in a community.	(-)



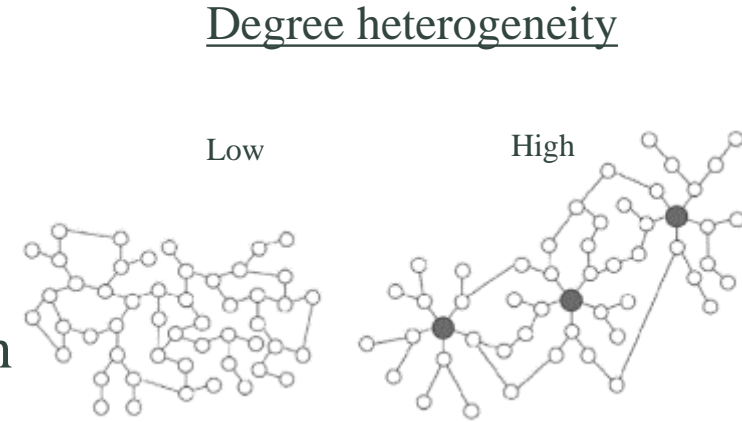
Step 2. Quantify species interactions

- Built co-occurrence networks using 10+ years of BMI monitoring data (> 4000 samples)
- Observed co-occurrences were compared against 100 randomized null communities with the same taxonomic richness as the observed community
- Identified statistically significant correlations
- Calculate topological measures



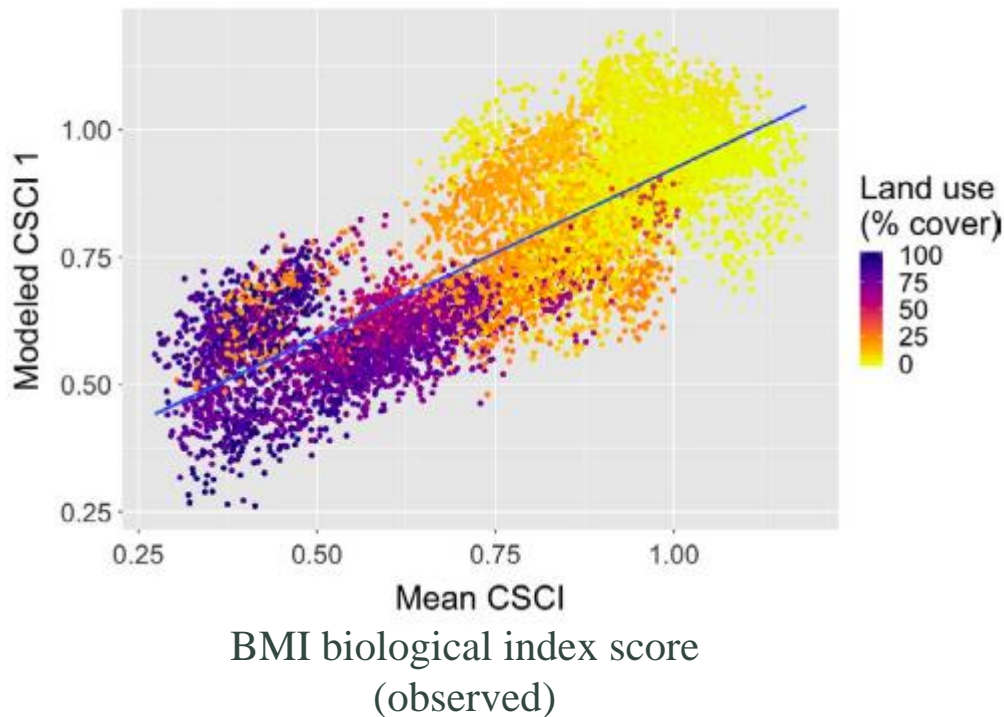
Results: Co-occurrence networks

- Size of co-occurrence networks declined with land use
 - Both number of genera and number of functional feeding groups declined
- Connectance, degree heterogeneity, and mean co-occurrence strength increased with stress
 - May indicate preferential loss of weak co-occurrences



More edges per node (right) may make networks more resilient to stress

Results: Co-occurrence networks



- A linear model composed of topological measures could describe a significant portion (~66%) of the observed variation in CSCI scores

$$\text{Mean CSCI} = 0.3 + 4.6 \times 10^{-3} \times N - 1.2 \times C - 1.8 \times 10^{-2} \times S + 0.3 \times M + 0.1 \times \zeta$$

- Remove network size, and model still explains ~40% of variation
- These results suggest that we are not simply observing a decline in local diversity in response to stress but a change in landscape diversity as well

Results: Co-occurrence networks

ORIGINAL RESEARCH

Ecology and Evolution WILEY

Using co-occurrence network topology in assessing ecological stress in benthic macroinvertebrate communities

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Abstract

Ecological monitoring of streams has often focused on assessing the biotic integrity of individual benthic macroinvertebrate (BMI) communities through local measures of diversity, such as taxonomic or functional richness. However, as individual BMI communities are frequently linked by a variety of ecological processes at a regional scale, there is a need to assess biotic integrity of groups of communities at the scale of watersheds. Using 4,615 sampled communities of streambed BMIs, we investigate this question using co-occurrence networks generated from groups of communities selected within California watersheds under different levels of stress due to upstream land use. Building on a number of arguments in theoretical ecology and network theory, we propose a framework for the assessment of the biotic integrity of watershed scale groupings of BMI communities using measures of their co-occurrence network topology. We found significant correlations between stress, as described by a mean measure of upstream land use within a watershed, and topological measures of co-occurrence networks such as network size ($r = -0.1, p < 10^{-3}$), connectance ($r = -0.1, p < 10^{-3}$), linear co-occurrence strength ($r = 0.25, p < 10^{-3}$), degree heterogeneity ($r = -0.1, p < 10^{-3}$), and modularity ($r = -0.1, p < 10^{-3}$). Using these five topological measures, we constructed a linear model of biotic integrity, here a composite of taxonomic and functional diversity known as the California Stream Condition Index, of groups of BMI communities within a watershed. This model can account for 66% of among-watershed variation in the mean biotic integrity of communities. These observations imply a role for co-occurrence networks in assessing the current status of biotic integrity for BMI communities, as well as their potential use in assessing other ecological communities.

KEYWORDS

co-occurrence network, ecological index, ecological stress, landscape ecology, stream ecosystems, topology

1 | INTRODUCTION

Humanity can be considered a global scale force for ecosystem engineering (Gentry et al., 2015; Laranas, Seewi, & Casman, 2004; Woodbury et al., 2008). Subsequent to the rise of anthropogenic

stressors on the environment, there has been the recognition of the need for ecological monitoring, which can track the scale of human activity (Bergelson, Russ, & Olson, 2019; Ewers, 2016; Mollaret, Winer, & Ostank, 2011; Foley et al., 2013; Schneider et al., 2012; Steiner et al., 2017).

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www.intellect.org | 1

- A linear model composed of topological measures could describe a significant portion (~66%) of the observed variation in CSCI scores

$$\text{Mean CSCI} = 0.3 + 4.6 \times 10^{-3} \times N - 1.2 \times C - 1.8 \times 10^{-2} \times S + 0.3 \times M + 0.1 \times \xi$$

- Remove network size, and model still explains ~40% of variation
- These results suggest that we are not simply observing a decline in local diversity in response to stress but a change in landscape diversity as well

Results: Co-occurrence networks

- Co-occurrence networks have demonstrated utility as a potential biological indicator
- We can assess biotic interactions previously ignored by traditional biomonitoring approaches
- Use of molecular methods will help us to expand the taxonomic groups we can assess, and will be more sensitive than traditional approaches



Step 3: Develop an index

- Combine Steps 1 & 2: build co-occurrence networks using DNA-based taxonomy
- Analyze DNA samples from > 400 sites across California
- Reference sites (> 50) will help us to calibrate our index
- Build co-occurrence networks for bacteria, archaea, algae, BMI, fish
- Assess network characteristics and responses to stressor gradients



Step 3: Develop an index



Interested in this project?
Please email me to get involved!

Thank you!
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A screenshot of the website for the California Water Quality Monitoring Council. The header includes the CA.GOV logo, the "WATER QUALITY" logo, and the text "California Water Quality Monitoring Council" and "My Water Quality". Below the header is a navigation bar with links for "Home", "Portals", "About Us", and "Workgroups". The main content area features the title "California Water Quality Monitoring Council (CA Senate Bill 1070)" and a bullet point: "New! Molecular Methods Workgroup". A URL is provided: https://mywaterquality.ca.gov/monitoring_council/mmw.html.