EPILITHIC DIATOMS AS BIOINDICATORS OF PACIFIC NORTHWEST STREAM HEALTH:
A BIOASSESSMENT USING SCANNING ELECTRON MICROSCOPY

By Jared Kerman and Sarah Hatcher

Purpose

The purpose of this study is to design and conduct a bioassessment using a scanning electron microscope to analyze epilithic diatoms (*Bacillariophyceae*). The goal of this experiment is twofold: 1) To assess the effectiveness of using epilithic diatoms as biological indicators of stream health within natural, industrial, and agricultural localities within the Pacific Northwest (N=10); 2) To advocate for the use of studying epilithic diatoms in tandem with scanning electron microscopy in statewide environmental monitoring and management plans for aquatic ecosystems. Our multimetric approach includes the following biological indices:

- **Genus-level diversity compared across each locality.**
- **Abundance of diatom indicator taxa: % pollution sensitive; indifferent; tolerant.**
- **Developing a metric for water quality testing using diatom saprobity values and relative abundances (weighted average autecological index for nutrients).**

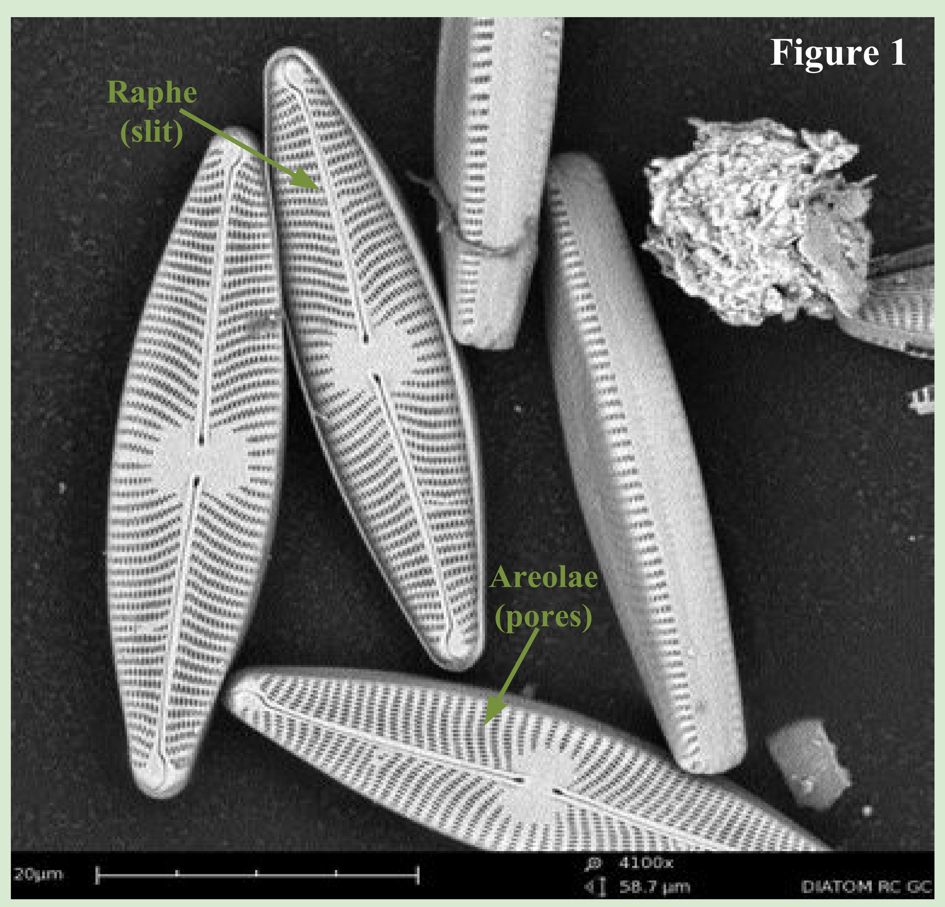


Figure 1: The genus *Navicula*, which includes motile, pennate, and cosmopolitan diatoms, is one of the more common taxa imaged from our samples via the scanning electron microscope. One of the characteristic morphological features of *Navicula* include its central raphe, or slit, located along the long axis (above in green). The raphe is critical to *Navicula*'s ability to secrete mucilage (polysaccharide strands), which allow *Navicula* to move freely in the water column, or attach to a substrate.

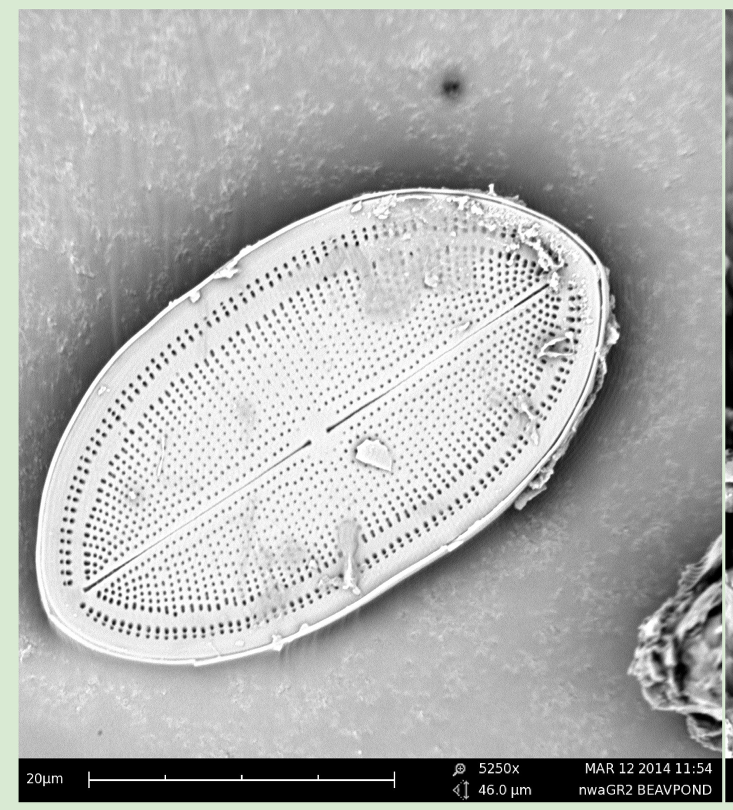
What Are Diatoms?

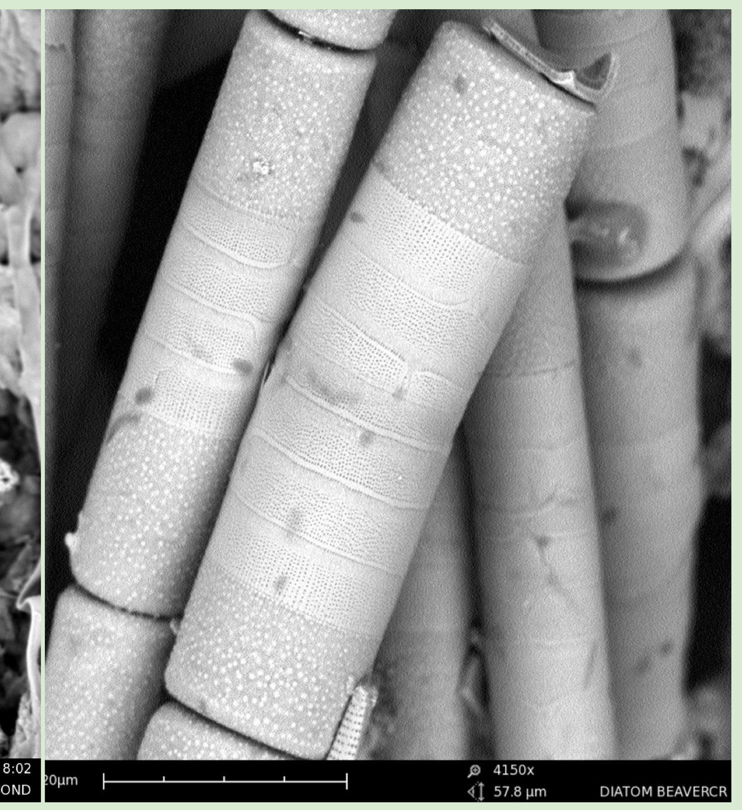
Diatoms, a group of unicellular, microscopic eukaryotic protists, are excellent biological indicators of stream health.

- **High taxonomic diversity with 200,000 unique species.**
- **Taxa respond predictably to ecological change, stream health, and anthropogenic disturbance.**
- **Easy collection due to abundance in areas with light and moisture. Collection is non-invasive and inexpensive.**
- **Silica frustules are decay-resistant and serve as a proxy of past climate patterns and environmental conditions.**
- **Primary producers occupy the base of aquatic food webs, playing an important role in nutrient cycling, silica sequestration, carbon fixation, and primary productivity.**

Background: Using the SEM to Understand Diatoms As Bioindicators

Diatom taxa were scored as
1= pollution tolerant
2= pollution-indifferent
3= pollution sensitive
after *Bahls 1993*

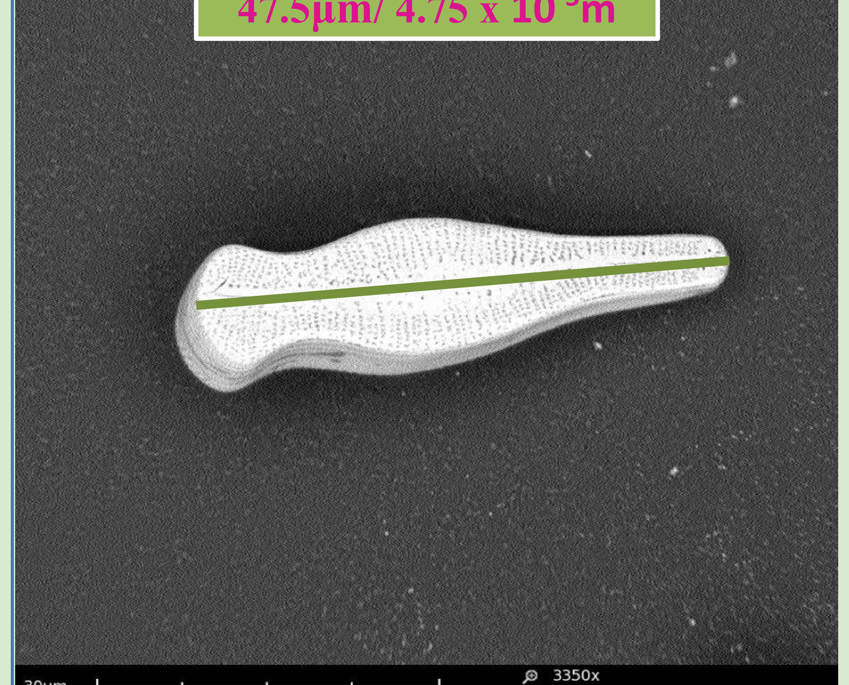




TAXON:	COCCONEIS	EPITHEMIA	MELOSIRA
pH TOLERANCE	Fundamental niche: 4.7-9.0 Optimum: 8.0	Fundamental niche: 4.7-9.0 Optimum: 8.3-8.5	Fundamental niche: 6.4-9.0 Optimum: 8.5
POLLUTION TOLERANCE:	Pollution Sensitive (3)	Pollution Sensitive (3)	Pollution Indifferent (2)
SAPROBIEN (NUTRIENTS):	Saprophytic/ Alpha-Mesosaprobic	Saproxenous/ Beta-Mesosaprobic Thrives in eutrophic environments	Beta-Mesosaprobic Thrives in eutrophic environments
GENERAL HABITAT:	Cosmopolitan; found in pristine lakes, ponds, springs, and ditches	Cosmopolitan + Aerophilous; can live in extreme environmental conditions, in soil, on hydrophytes/other substrates	Cosmopolitan; found in lakes, ponds, springs, ditches
SEASONALITY:	Maximum growth in fall	No season-based fluctuation of growth	Maximum growth in summer
TROPHIC LEVEL:	Photoautotrophic	Contains nitrogen-fixing endosymbiotic cyanobacteria; can live in minimally eutrophic microenvironments	Obligate nitrogen heterotroph
MOTILITY:	Non-motile; attaches to submerged substrate	Non-motile	Non-motile colonizer

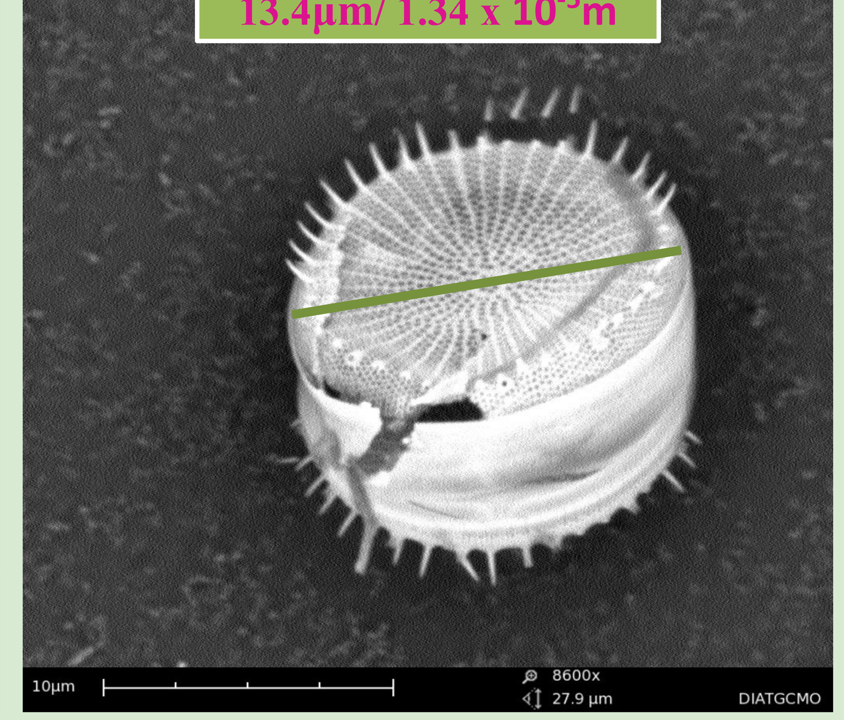
- **Morphological differences in diatom shape influence how they interact with their environment**
- **Shape is an important adaptation, influencing surface area-to-volume ratio and subsequently influencing nutrient availability, light absorption, and predator avoidance**
- **While diatoms vary widely in morphology, they range, on average, from 2µm to 500µm**

Gomphonema:
Frustule Length:
47.5µm/ 4.75×10^{-5}m



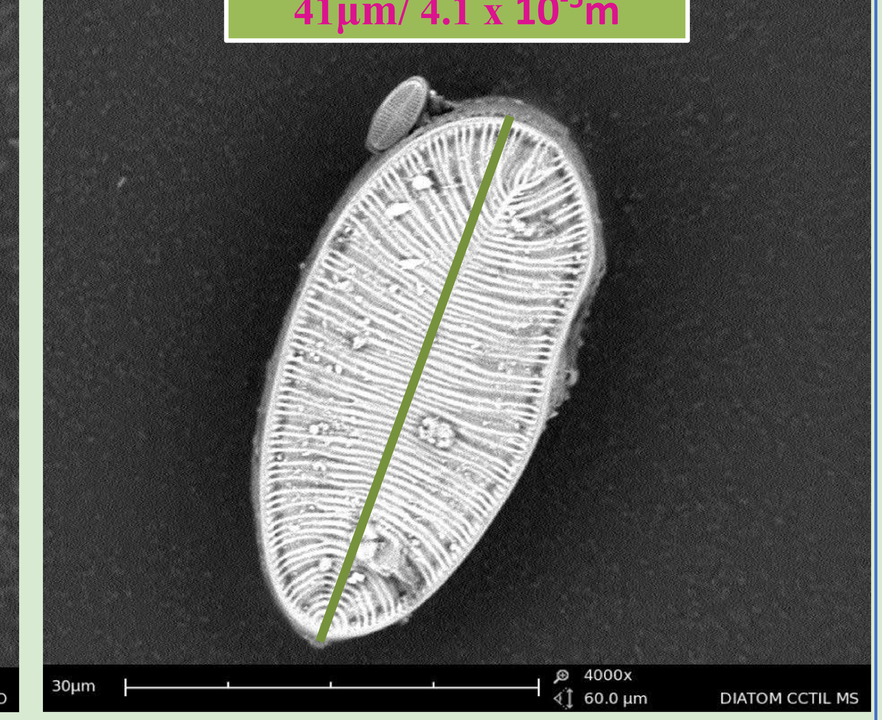
Shape: Clavate (club-shaped)

Stephanodiscus
Frustule Length:
13.4µm/ 1.34×10^{-5}m



Shape: Circular

Surirella:
Frustule Length:
41µm/ 4.1×10^{-5}m



Shape: Elliptical

Field Site Selection

Diverse field sites were chosen *a priori* based on differences in land use (agricultural, industrial, and pristine), watershed location, and proximity to pollution. Utilizing digital maps (Fig. 2), historical data, and government records (EPA, DEQ, ODFW), a pollution profile was constructed for each field site, which documented both historic and present point and non-point sources of pollution. We imaged diatom community assemblage data in the lab to analyze stream health, and make inferences about the types of anthropogenic stressors and abiotic conditions that characterize each site.

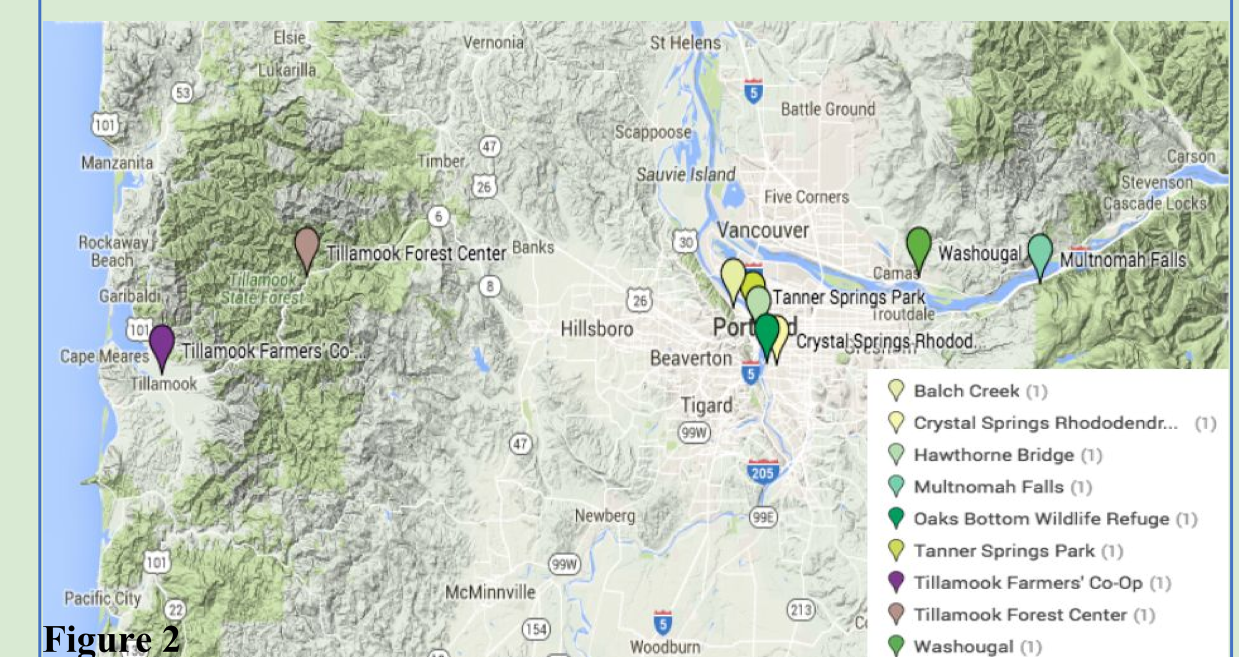


Figure 2: A map of the ten localities used in this study as viewed on Google Maps.

Figure 3: Natural site: Balch Creek at the Portland Audubon Society: The stream was oligotrophic with little erosion on the stream banks. There were variable microhabitats for diatoms. The water quality class for this site was 2.75, representing polluted water.

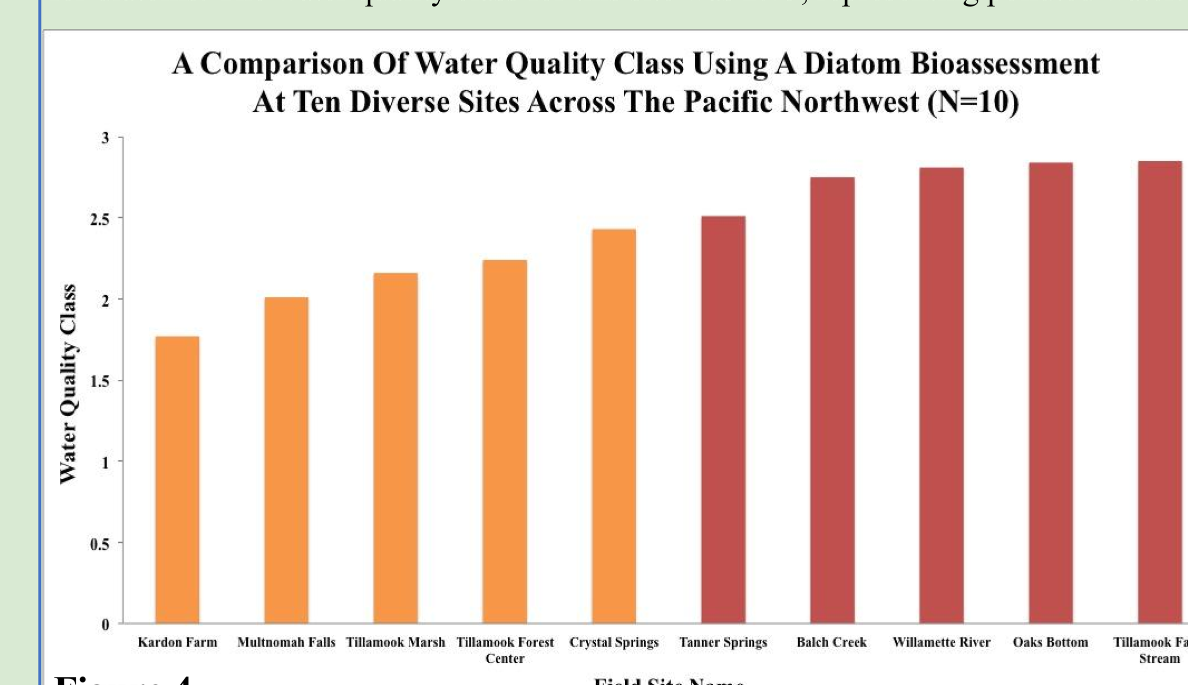
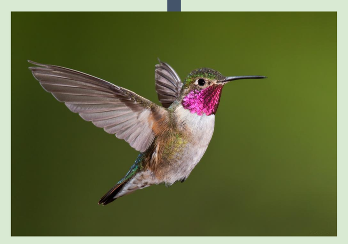
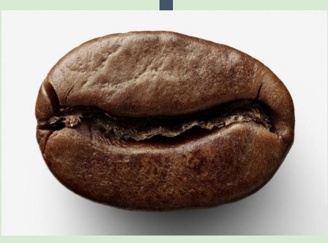
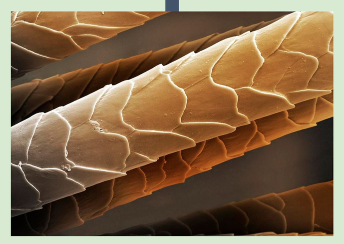
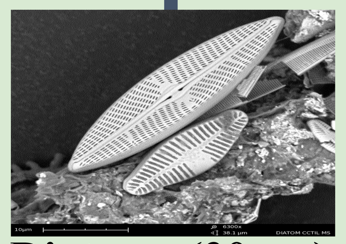
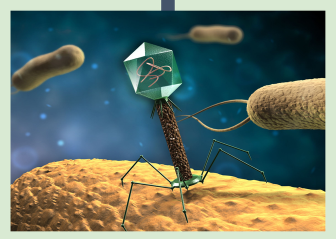


Figure 4: We calculated water quality using a diatom bioassessment. We multiplied the relative abundances of each genus by its saprobity index, creating a sum for all taxa present at a site, and dividing that sum by the total sample size (N). Five of the ten field sites were classified as slightly polluted, and five out of ten field sites were classified as polluted. The former and latter both include agricultural, urban, and industrial localities.

How Large Are Diatoms? A Biological Comparison Using SI Units In the Metric System

10^0m *meter*	1×10^{-1}m *deci*	1×10^{-2}m *centi*	1×10^{-3}m *milli*	1×10^{-4}m *micro*	1×10^{-5}m *micro*	10^{-6}m *micro*	10^{-7}m *nano*	
								The smallest object observable to an optical microscope
King Penguin (100cm)	Hummingbird (10cm)	Coffee Bean (1cm)	Ant (4mm)	Width of Hair (100µm)	Diatoms (30µm)	*E. coli* (2µm)	*Bacteriophage* (200nm)	

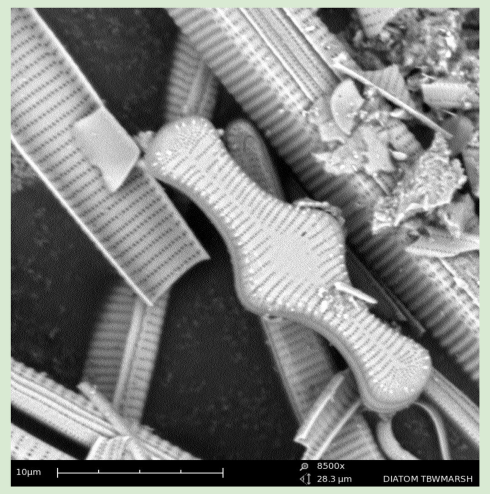
Hypotheses

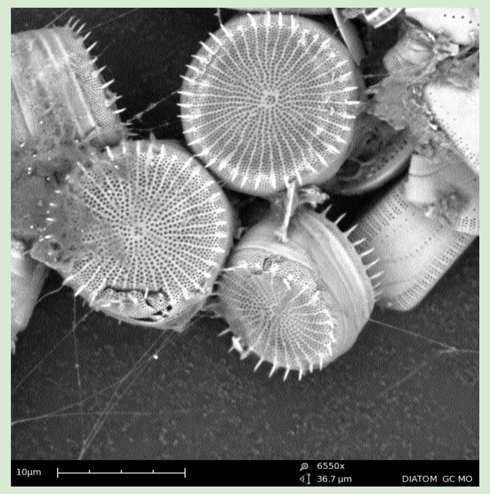
The Null Hypothesis (H_0)

H_0 assumes diatom taxa will be equally and randomly distributed across all field sites regardless of pollution sensitivities, autecological preferences, and the stream's degree of impairment.

The Alternate Hypothesis (H_a)

H_a assumes SEM analysis of diatom community assemblages will show differing distributions of genera across all field sites based on genera diversity and pollution tolerances, supporting their ideal role as bioindicators.





Images captured on the SEM that display the morphological differences between pennate (*Tabellaria*, left side) and centric (*Stephanodiscus*, right side) diatoms.

Flow Chart of Field Methodology & Lab Protocol

Find a safe access point to the stream

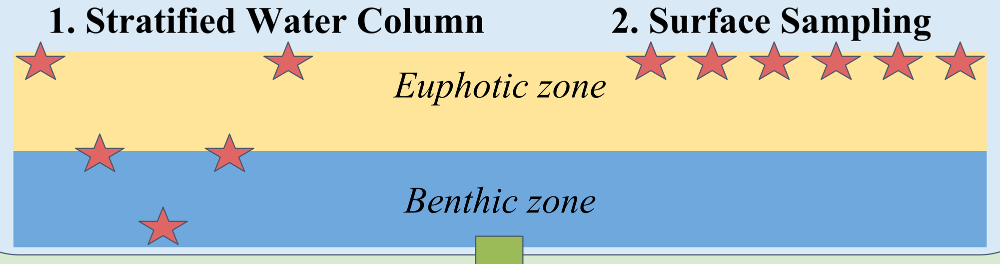
Select your substrate:

Select a small sample of hydrophytes and place in falcon tube. → Shake vigorously, then extract hydrophyte and return it to the stream.

Select a stone or rock from the stream and hold over a falcon tube. → Vigorously scrub with a toothbrush and apply stream water until biofilm washes off into a tube.

Collect six equidistant diatom samples following one of the patterns below. If the thalweg of the stream is inaccessible, choose pattern #2:

1. Stratified Water Column
2. Surface Sampling



Aspirate samples and then combine them with samples of similar collection procedures. Pipette 0.5 mL of sample onto a plate covered with plastic wrap.

Dry biofilm, adhere to carbon sticky-tape, blow off debris with canned air, and view on SEM to image diatom community assemblages.

Results, Key Findings & Statistical Analysis

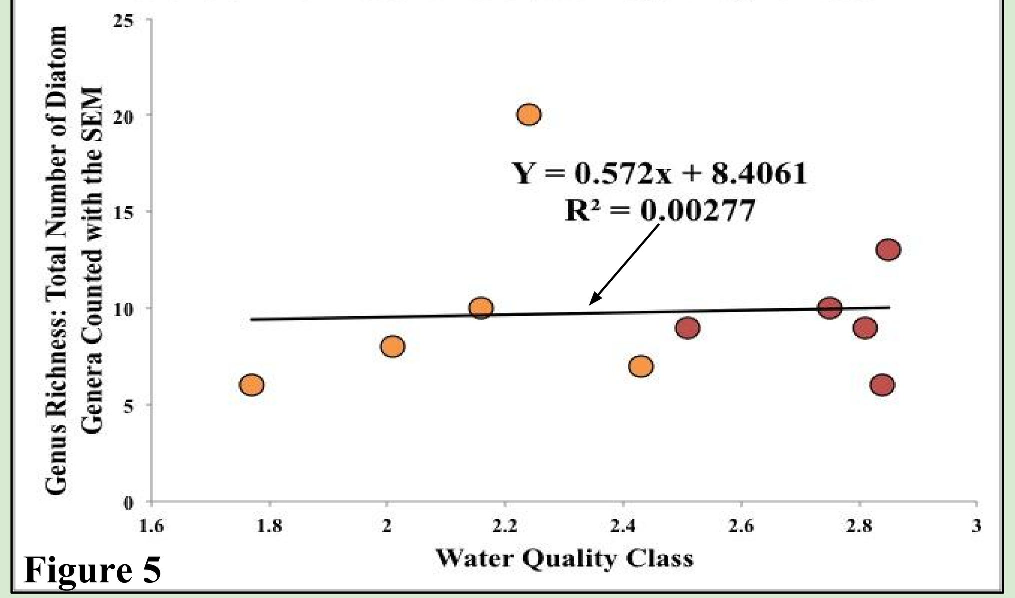


Figure 5

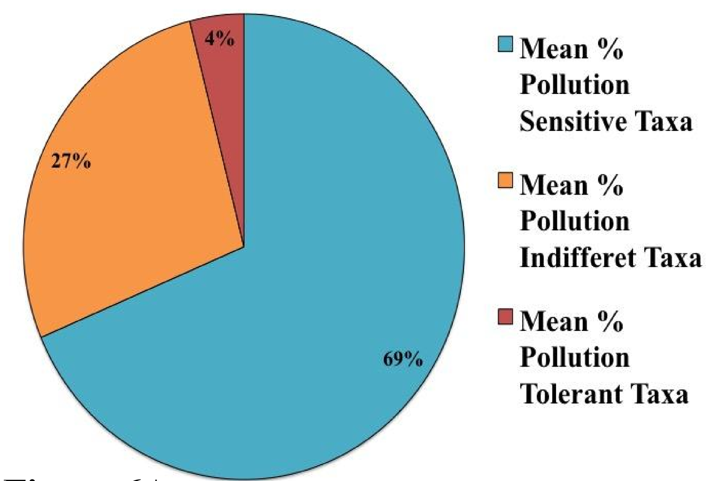


Figure 6A

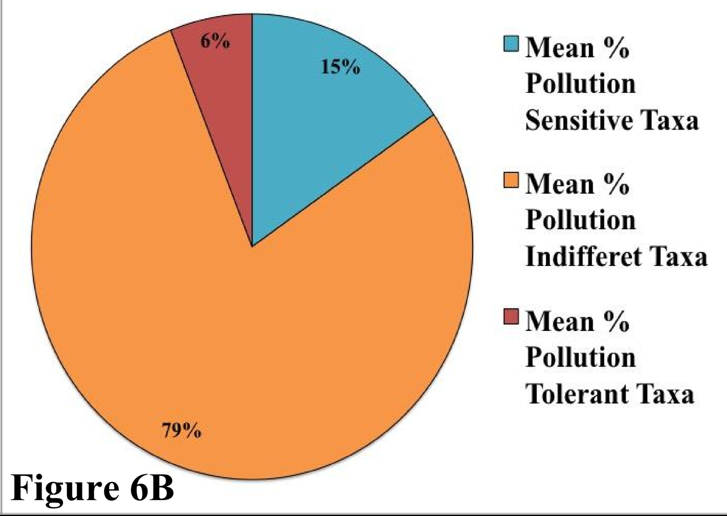


Figure 6B

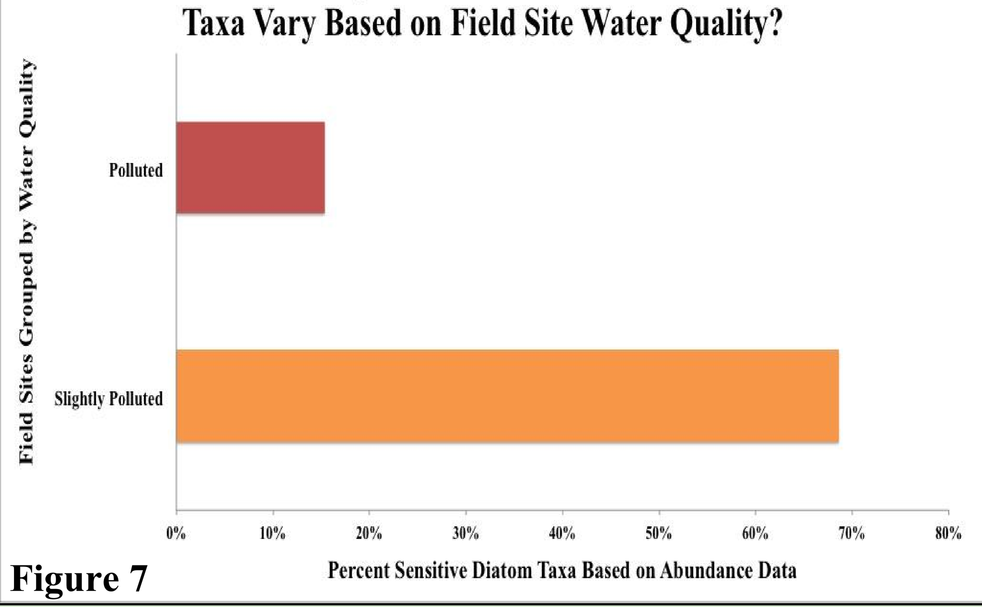


Figure 7

Diatom Genus	Abundance (N)	Pollution Tolerance Class	Saprobic Value(S)	Sum of N x S
Eunotia	2	3	1	2
Staurosira	534	2	2.5	1335
Navicula	5	2	3	15
Achnanthidium	22	3	2	44
Nitzschia	87	1	1	87
Cymbella	4	2	2	8
Staurosirella pinnata	47	3	2	94
Synedra	8	2	3	24
Epithemia	6	3	2	12

Sum total of N x S column	Sum total abundance (N)	Water Quality Class Calculation: (N X S)/(N)
1621	715	2.267132867

Figure 8

- **Figure 5: R^2 value (R^2 = 0.00277) shows weak, positive linear correlation between diatom genus richness (H) and water quality class.**
- **Figure 6: Chi-squared tests reveal extremely statistically significant differences in mean distribution of diatom indicator species for pollution across five slightly polluted localities (6a: $C^2(2, N=1509)=65.19$, $p<0.0001$) and five polluted localities (6b: $C^2(2, N=1438)=95.023$, $p<0.0001$), falsifying H_0.**
- **Figure 7: Supports H_a; strong negative correlation between degree of water pollution and proportion of pollution sensitive diatoms.**
- **Figure 8: Demonstrates mathematical equation used to calculate water quality class adapted from table on the relationship between saprobity index & water quality (Mayama 2011).**

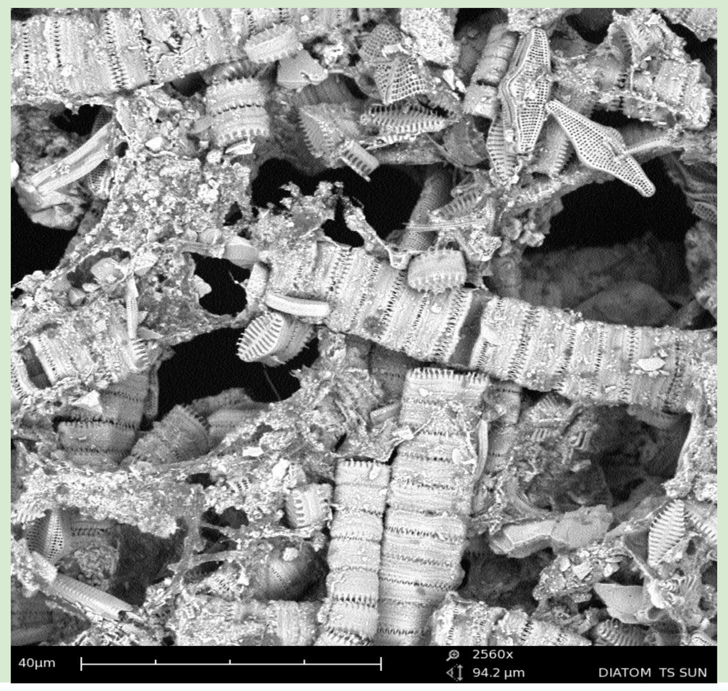
Creating a Stream Scorecard: Site-Specific Results

SLIGHTLY POLLUTED WATER
Kardon Farm, In Washougal, WA
Water quality class category 2
Water quality value: 1.77

- **Genus Richness:** 6 genera of diatoms sampled (N=220 individuals)
- **Pollution Tolerances:** 70% pollution sensitive taxa, 30% indifferent, 0% tolerant **($C^2(2, N=220) = 73.975$, $p<0.0001$)**
- **Motile Taxa:** 0.4% motile (includes *Surirella*)
- High proportion of ***Eunotia*** (46% of taxa; pollution tolerant; thrives in low pH, dystrophic environments)

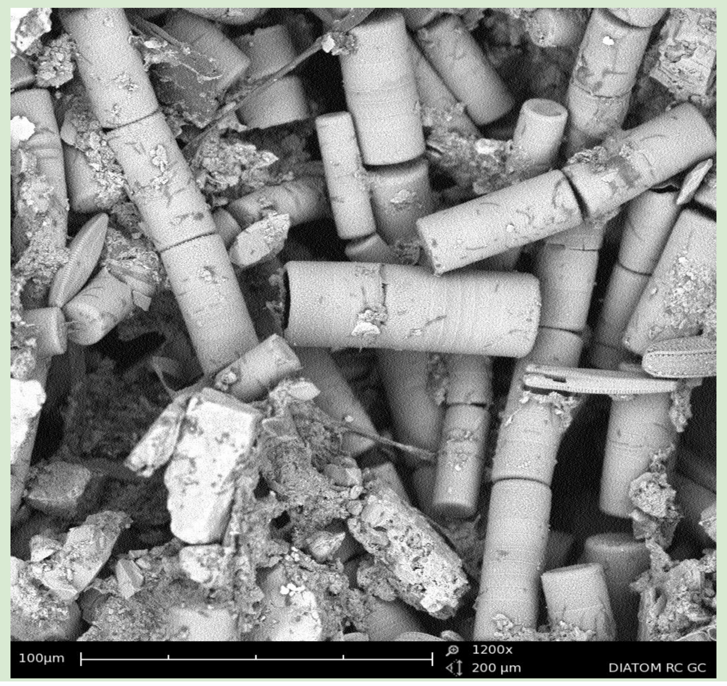
SLIGHTLY POLLUTED WATER
Tanner Springs Industrial Park
Water quality class category 2
Water quality value: 2.48

- **Genus Richness:** 9 genera of diatoms sampled (N=715 individuals)
- **Pollution Tolerances:** 11% pollution sensitive taxa, 77% indifferent, 12% tolerant **($C^2(2, N=715) = 85.785$, $p<0.0001$)**
- **Motile Taxa:** 0.7% motile (includes *Navicula*)
- High proportion of ***Staurosira*** (75% of taxa; pollution indifferent)



POLLUTED WATER
Willamette River,
Near Hawthorne Blvd.
Water quality class category 3
Water quality value: 2.8

- **Genus Richness:** 9 genera of diatoms sampled (N=224 individuals)
- **Pollution Tolerances:** 9% pollution sensitive taxa, 89% indifferent, 2% tolerant **($C^2(2, N=224) = 140.133$, $p<0.0001$)**
- **Motile Taxa:** 22% motile (includes *Nitzschia*)
- High proportion of ***Melosira*** (60% of taxa; pollution indifferent, highly eutrophic)



Why Are Diatoms Ideal Bioindicators?

- **Collection is non-invasive, inexpensive, and easy to conduct in the field without ecological harm.**
- **Indices and ecological tolerances are well-established; identification of specific stressors is therefore streamed.**
- **As primary producers, diatoms are respond quickly to disturbances (bottom-up trophic cascade).**
- **Monitoring diatom community assemblages allows for quick and efficient restoration before stressors disrupt ecosystem services or harm charismatic megafauna.**
- **Strengths of a rapid bioassessment include a lower cost, a streamlined collection process encompassing multiple sites, and efficiency in achieving results.**

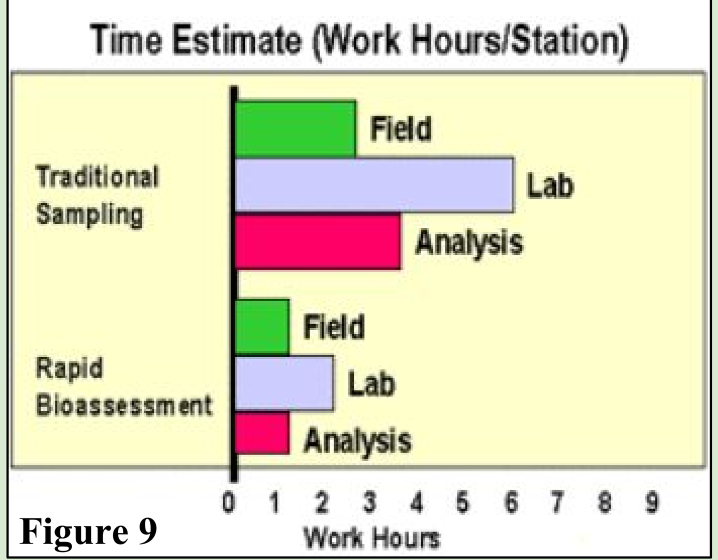


Figure 9

(Figure 9, image credit to EPA 2016)

Conclusions

Healthy Streams Provide Valuable Ecosystem Services
- Clean air and water
- Wildlife diversity
- Wetlands act as biological "sponge" filtering chemicals from waterways

Land-Use Changes Disrupt Streams
- Sedimentation harms all trophic levels, from diatoms to salmon
- Higher turbidity impedes diatom photosynthesis and reduces water clarity

Key Findings
- 10 out of 10 sites were polluted or slightly polluted
- Lack of pollution sensitive diatoms should be viewed as a call to action
- Identifying specific diatom genera was more precise using scanning electron microscopy

Environmental Awareness
- Our study provides supporting evidence for cultural eutrophication
- Integrating SEM studies with environmental protection should be a conservation priority (Stevenson et. al. 2016)

Diatom Bioassessment is a Powering Monitoring Tool
- Goals include improving regional diatom identification and increasing awareness of their ecological importance and value as bioindicators of stream health