FTIR spectroscopy: analysis of light & nutrient stress on ice algae within the Northwest Passage of the Canadian Arctic

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Introduction

Ice algae

1º pulse of spring photosynthetic production

- Supports Arctic food-web
- Produce essential fatty acids
  - Poly-unsaturated (PUFA)
  - Saturated

Light & Nutrient Limited

- ↑Light & ↓Nutrients:↑Lipids & ↓ Protein

Diatoms

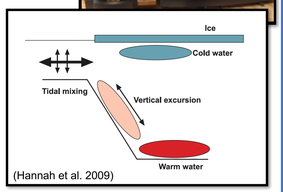
- Class Bacillariophyceae
- Encased in silica frustule
- Pennate & Centric

Fourier Transform Infrared (FTIR) Spectrochemical Analysis

- Measures biomolecular (biomass) composition
  - PUFA
  - Total Lipids (CH_2+CH_3)
  - Proteins (Amide I)
  - Silica (Si-O)

Tidal straits hypothesis

- Shallow, narrow water ways
- Increased water column mixing, Therefore: increased nutrient flux



Objectives

GOAL: Determine the influence of light & nutrient availability on biomass composition of individual Arctic diatom cells compared to bulk-community biomass & species composition

I. Use FTIR to examine biomass composition (PUFA, total lipids, & protein), in individual cells of different diatom taxa; compare to bulk algal community

II. Compare FTIR-derived biomass to bulk measurements (e.g. Chl *a*, organic C & N, etc.), & taxonomic composition

III. Relate changes in biomass to nutrient fluxes, location in tidal strait & penetration depth in bottom fine structure of sea ice

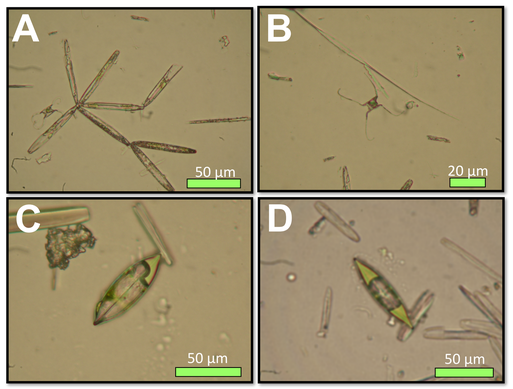


Figure 1: Target diatoms A) *Nitzschia frigida*, B) *Attheya septentrionalis*, C), *Navicula kariana*, & D) *Navicula transitans*

Methods

Field Work & Sample Collection

- Finlayson Islands, Dease Strait, near Cambridge Bay, NU, CA
  - 26 April to 12 May 2017
- Fine Structure (Sites #: 1 – 4)
  - Thin Snow Cover (< 8 cm)
  - Bottom: 0-2, 2-5, & 5-10 cm
- Cells filtered onto a poly-carbonate filter
- Store @ -80℃; prep on dry ice

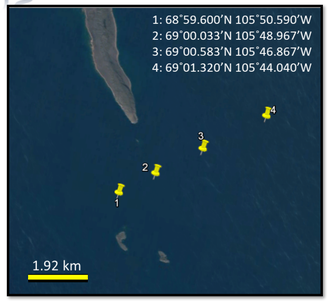


Figure 2: Finlayson Island site map (Google Earth 2017)

Transmission Mode FTIR – Individual Biomass

- Light passes through sample + substrate, BaF_2
- Wavelengths = vibrational energies of functional groups are absorbed
- IR spectrum: Abs= -log(%Transmittance) vs. wavenumber (cm^{-1})

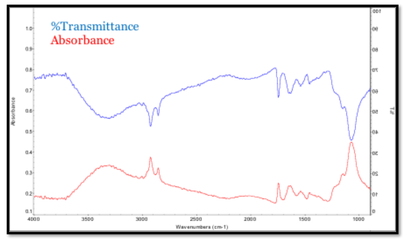


Figure 3: FTIR spectrum in %transmission versus absorbance

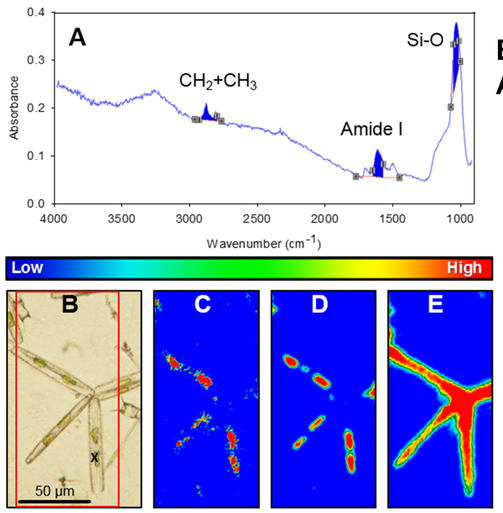


Figure 4: FTIR image analysis (A) spectrum (B) microscope image. False colour images: (C) lipid (CH_2+CH_3), (D) protein (Amide I), and (E) silica (Si-O; Pogorzelec et al. 2017).

Biomolecular Analysis

- Agilent Cary 670 IR Spectrometer & 620 IR Microscope:
  - Globar Light Source
  - 64 x 64 pixel FPA detector
  - 15x (0.62NA) optics
  - 1.1 x 1.1 µm² pixel (projection)
- Processed in MATLAB™

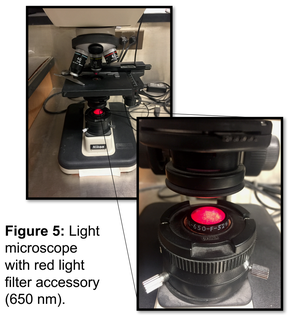


Figure 5: Light microscope with red light filter accessory (650 nm).

Sample Preparation for PUFA - *Lights Out!!*

- Filters sectioned in dark
- Cells released onto a BaF_2 windows, with Milli-Q water (4 µl drop)
- I.D. taxa under light microscope with red light filter (650 nm)
- Samples dried in desiccant chamber overnight (~12 hrs), in dark
- Analysed next day, in darkened room

Preliminary Results

Finding PUFA

- PUFA Band: 3006 cm^{-1} (red arrow)
- Low T + 650nm filter & dark room prevents photo-oxidation of PUFA
- *Navicula* genus has been observed to have the greatest quantity of PUFA

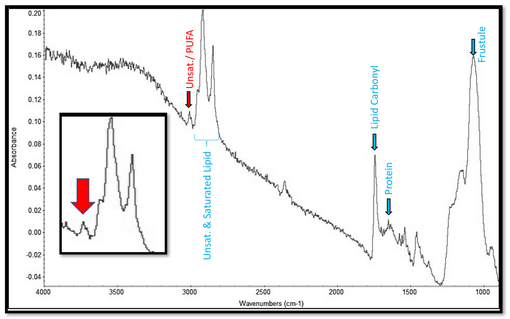
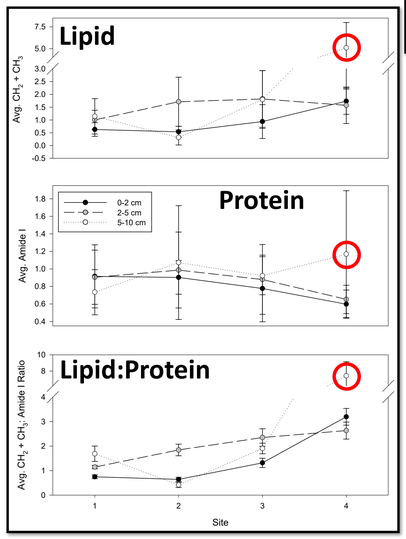


Figure 6: *Navicula kariana* FTIR spectrum collected in a dark cool environment. PUFA absorbance is showcased in red.

Figure 8: Average biomass composition for *Nitzschia frigida* *Note unusual Lipid & protein at site 4,* O



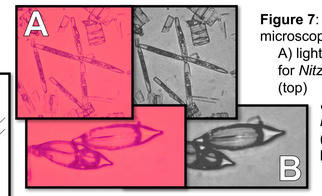


Figure 7: Light microscopic photos A) light filter image for *Nitzschia frigida* (top) & *Navicula kariana* (bottom) B) detailed grey scale

Nitzschia frigida Biomass Composition

Light = constant

Lipids increase (site 1 to 4)

- Variability between ice sections

Proteins decrease (site 1 to 4)

- Except site 4 (5-10cm)

Ratio Pattern

- Normalizes lipids & proteins across all sites
- Reflects increased nutrient stress, with high lipid & low protein
- Greatest, further from site 1

Next Steps…

Bulk-Community Biomass Composition Analysis

Attenuated Total Reflectance (FTIR-ATR)

- Light passes through crystal in contact with sample
- Creates an evanescent wave, penetrates sample by few microns

Bulk-Filter Based Measurement Analysis

- Chlorophyll *a*, HPLC, Particulate Organic C & N, & Fatty Acids

Acknowledgments

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