The Impact of Seston on the Susceptibility of Black Fly Larvae to *Bti*

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Re-suspended Seston

- Water taken from the North Branch of the Susquehanna River - 2009 and 2010
- A portion of the water filtered through a 0.2µm filter
- Seston re-suspended in DI water
- 4 larval media - river water, river filtrate, DI water, and DI with re-suspended seston

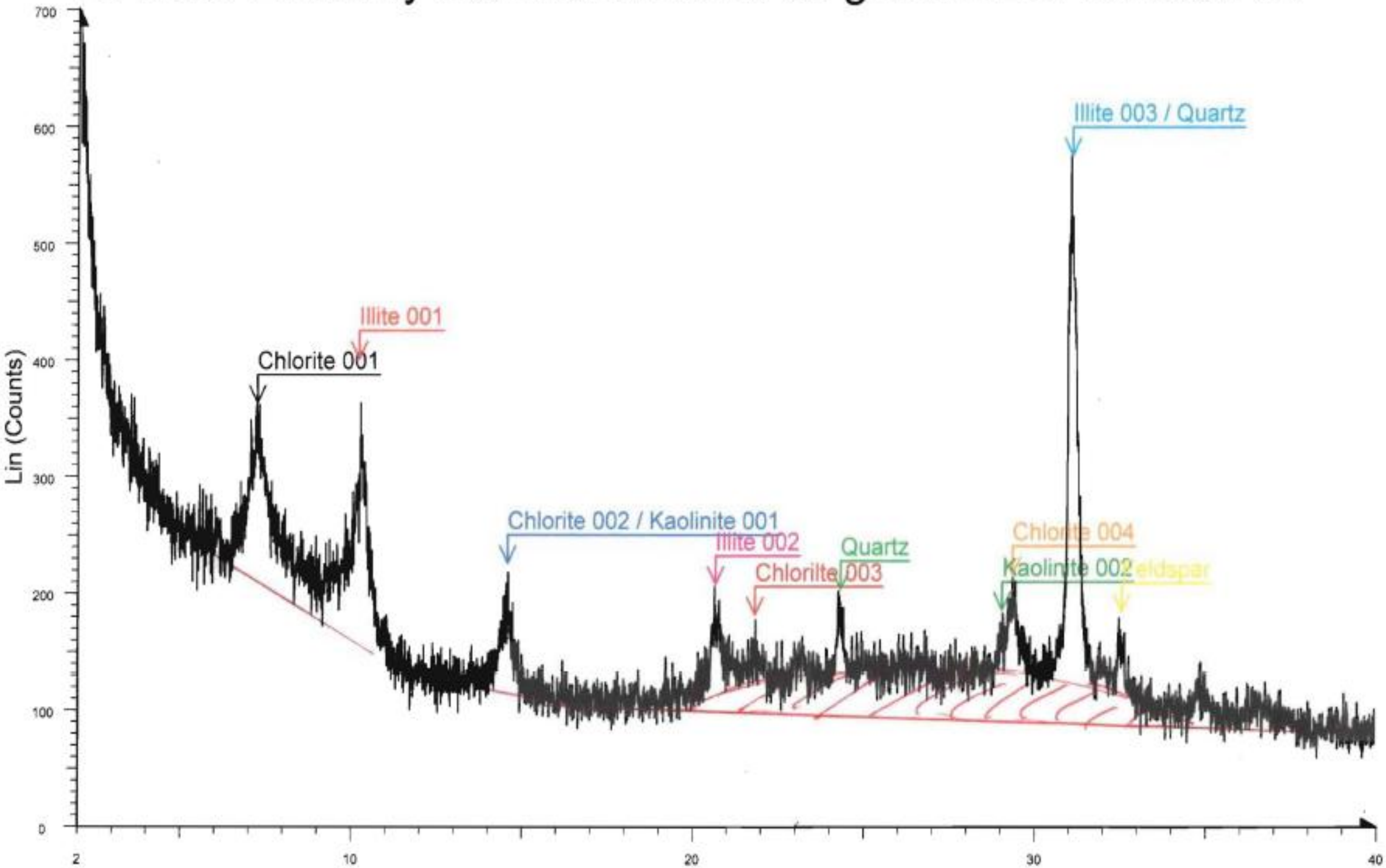
Controlled Current Toxicity Test (CCTT)

The Mortality of *Simulium vittatum* Larvae after Exposure to *Bti* ICPs in Various Media

Medium	Experiment 1 Turbidity (NTU)	Experiment 1 2009 *Bti* (%)	Experiment 2 Turbidity (NTU)	Experiment 2 2010 *Bti* (%)
Water	0.05	65.5 ± 3.7 a	0.03	58.3± 2.4 a
River water filtrate	0.06	71.3 ± 4.3 a	0.03	59.6 ± 3.5 a
Unfiltered river water	17.3	44.6 ± 2.7 b	9.5	30.0 ± 2.3 b
Re-suspended seston	18.5	48.5 ± 6.0 b	8.6	37.1 ± 3.5 b

- 2009- 17.3 NTU, 37% reduction in mortality
- 2010- 9.5 NTU, 49% reduction in mortality

S. River Black fly sediment mount on glass H2O2 treated air



S. River Black fly sediment mount on glass H2O2 treated air - File: BF_Oriented_AD.raw - Type: 2Th/Th locked - Start: 2.000 ° - End: 40.005 ° - Step: 0.011 ° - Step time: 15.4 s - Temp.: 25 °C (Room) - Tim
Operations: Import

The Mortality of *S. vittatum* Larvae from *Bti* ICPs After Exposure to 10 ppm of Selected Clay Minerals

Medium	Turbidity (NTU)	Control (%)	*Bti* Exposure (%)
Water	0.03	0.6 ± 0.6 a	88.8 ± 1.8 b
Chlorite	3.9	2.4 ± 1.5 a	89.8 ± 2.0 b
Illite	6.4	2.8 ± 1.8 a	89.0 ± 2.2 b
Kaolinite	7.7	0.0 ± 0.0 a	87.8 ± 3.0 b
Feldspar	2.9	1.7 ± 0.7 a	89.0 ± 2.7 b

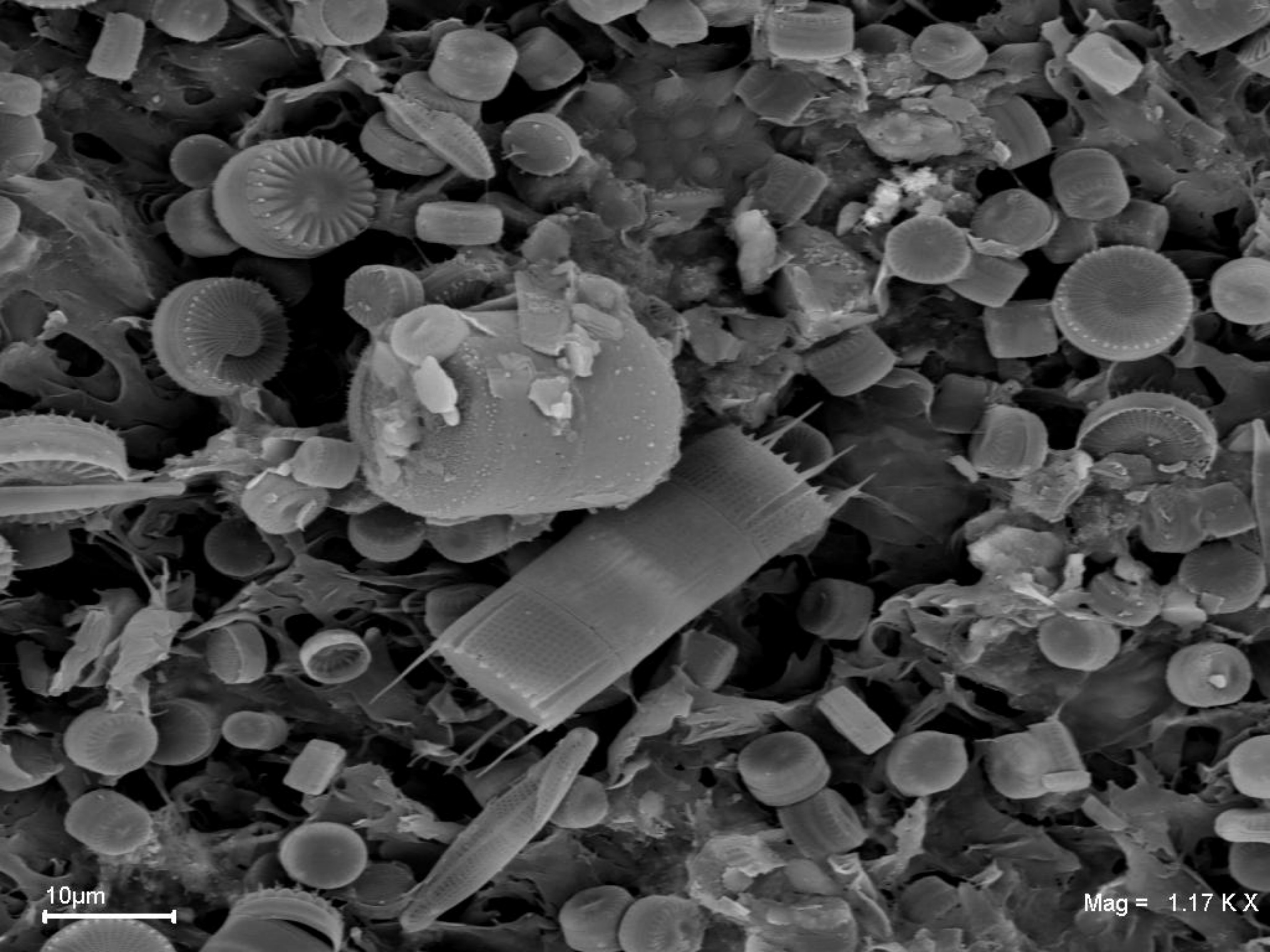
Cellulose

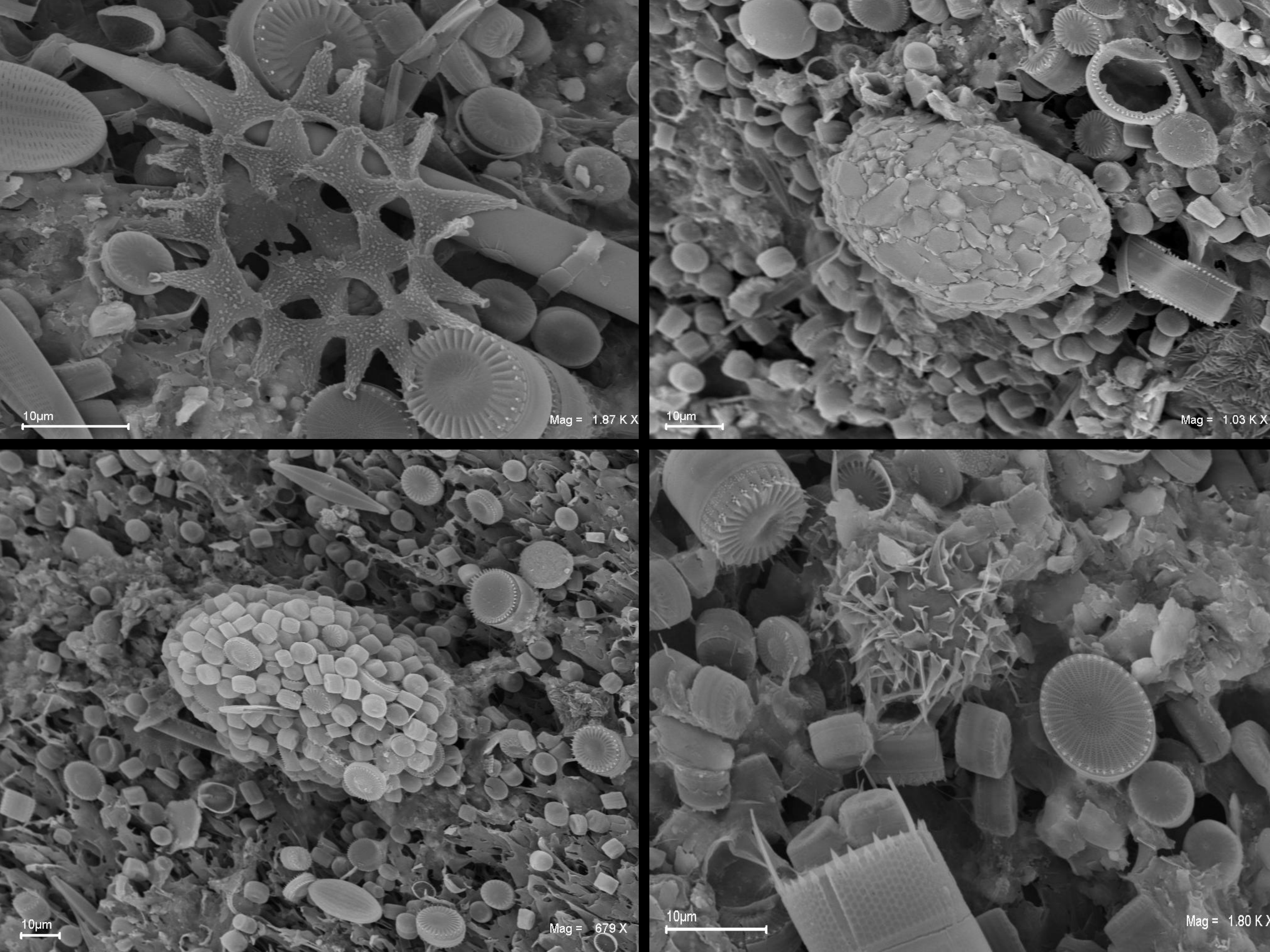
- Secondary waste fibers often discharged in waterways from paper mills
- Multiple paper mills are located on the Susquehanna River
- Cellulose in the literature
- Cessation of feeding?

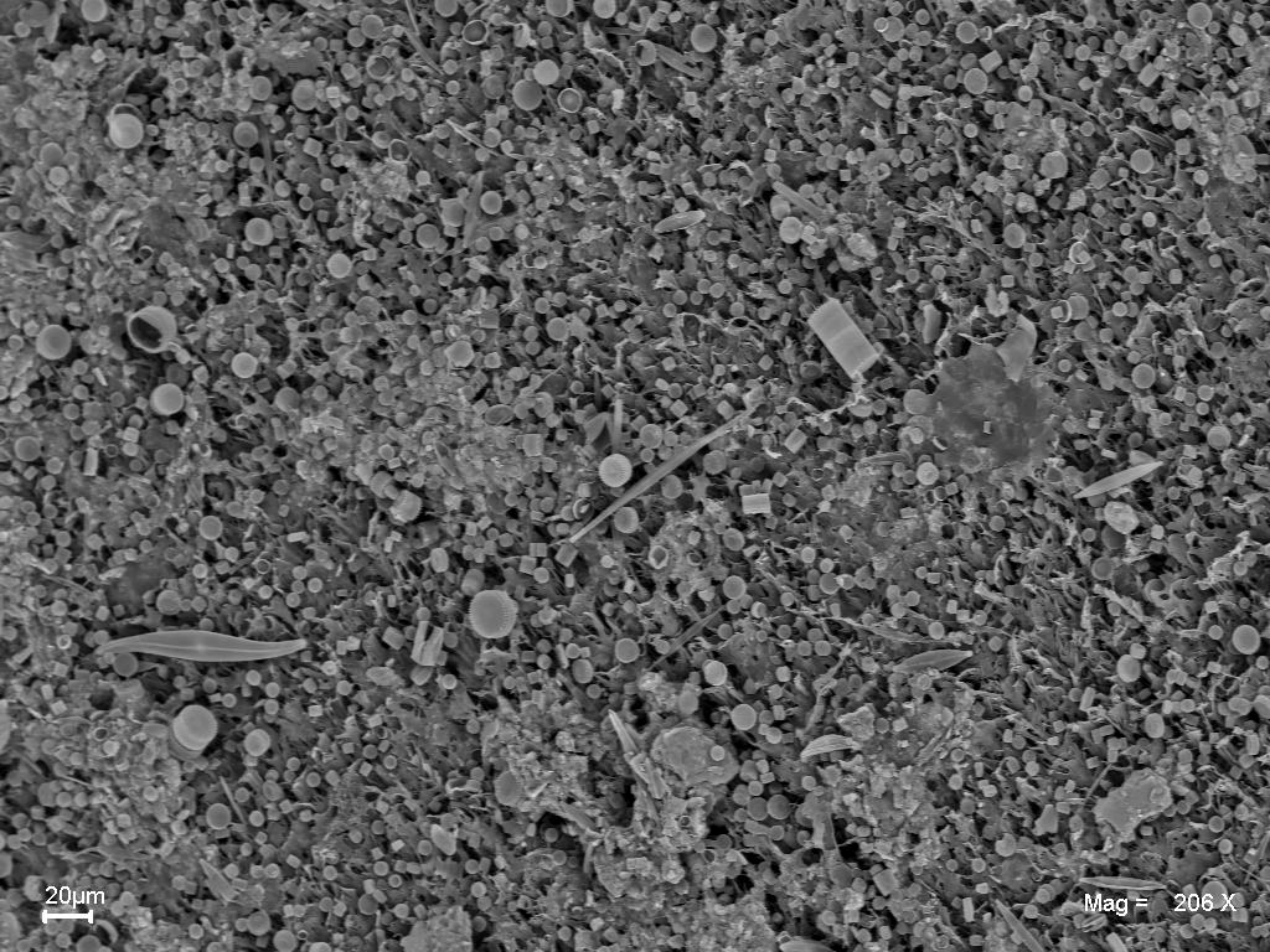
- **Illesova, D. 1988.** Reaction of blackfly larvae (Diptera: Simuliidae) to paper-mill wastewaters. Biológia 43: 535-541.

The Mortality of *S. vittatum* Larvae from *Bti* ICPs after Exposure to 10 ppm of Cellulose or Silicon Dioxide

Medium	Turbidity (NTU)	*Bti* (%)
Water	0.04	80.3 ± 1.1 a
Cellulose	1.9	61.8 ± 1.9 b
Silicon dioxide	0.8	14.2 ± 1.4 c

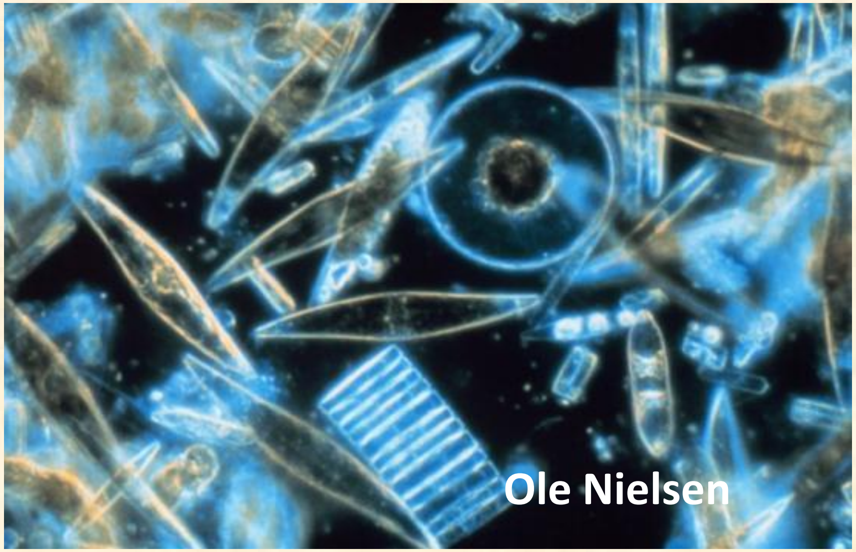

10µm
Mag = 1.17 K X

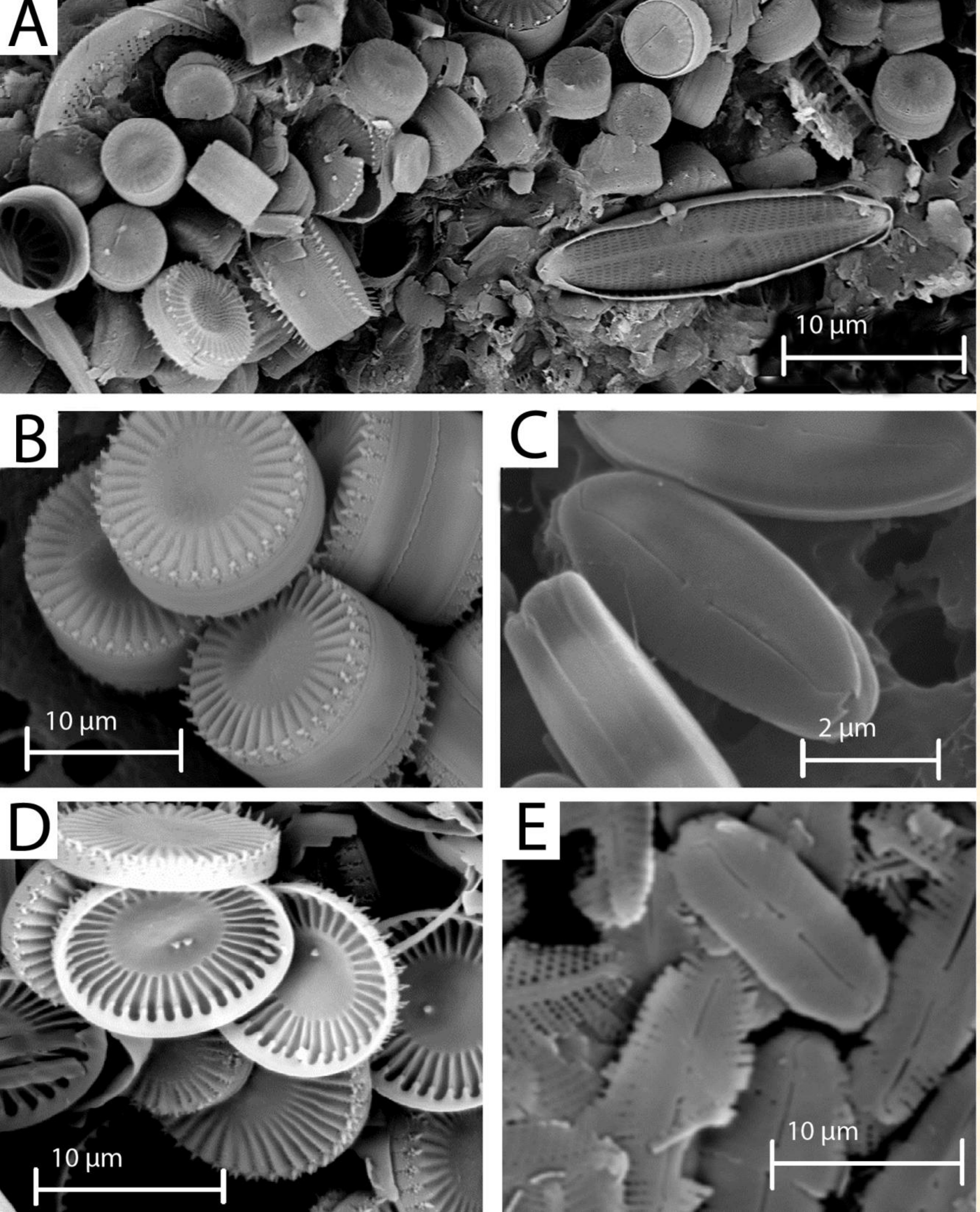

10µm
Mag = 1.87 K X
10µm
Mag = 1.03 K X
10µm
Mag = 679 X
10µm
Mag = 1.80 K X


20µm
Mag =   206 X

Diatom Ecology

- Extremely sensitive to environmental conditions
- Population size and composition can be very dynamic
- Part of Simuliidae diet, but how much is too much
- Multiple sewage treatment plants near Lime Ridge
- Algae needed for experimentation
- Diatoms:
  - Radial Centric
  - Pennate





A. Seston from the Susquehanna River containing radial centric and pennate diatoms

B. Viable *Cyclotella meneghiniana*

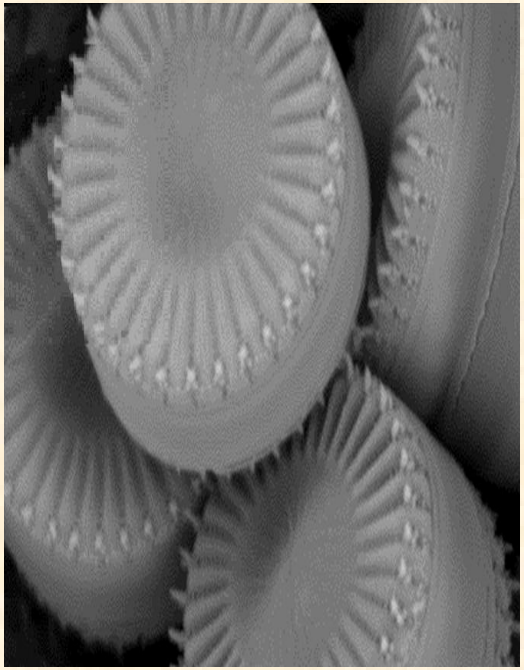
C. Viable *Navicula pelliculosa*

D. Purified *C. meneghiniana* frustules

E. Purified *N. pelliculosa* frustules

The Mortality of *S. vittatum* Larvae from *Bti* ICPs after Exposure to Viable *Cyclotella meneghiniana* Cells

Medium	Turbidity (NTU)	Control (%)	*Bti* (%)
Water	0.03	1.0 ± 0.5 a	68.0 ± 1.7 b
C. meneghiniana	2.3	1.3 ± 0.5 a	29.9 ± 3.1 c

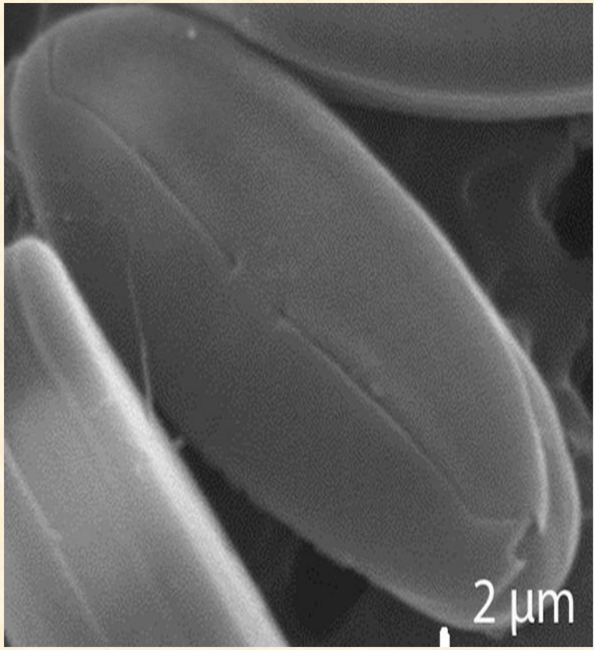


Viable cells at an *in vitro* concentration of 9,000 cells/ml.

- Mortality reduced 56%

The Mortality of *S. vittatum* Larvae from *Bti* ICPs after Exposure to Viable *Navicula pelliculosa* Cells

Medium	Turbidity (NTU)	Control (%)	*Bti* (%)
Water	0.03	2.4 ± 0.9 a	75.2 ± 2.7 b
N. pelliculosa	0.86	1.1 ± 0.5 a	63.0 ± 3.4 c



Viable cells at an *in vitro* concentration of 102,000 cells/ml.

- Mortality reduced 16%

The Mortality of *S. vittatum* from *Bti* ICPs after Exposure to Selected Diatom Frustules

Medium	Turbidity (NTU)	Control (%)	*Bti* (%)
Water	0.04	0.8 ± 0.6 a	58.8 ± 1.8 b
2 ppm *N. pelliculosa*	1.3	1.9 ± 0.8 a	68.5 ± 3.1 c
5 ppm *N. pelliculosa*	2.7	1.2 ± 0.8 a	18.0 ± 4.3 d
2 ppm *C. meneghiniana*	1.5	1.0 ± 1.0 a	**5.6 ± 1.3 a**
5 ppm C. *meneghiniana*	3.4	0.3 ± 0.3 a	**3.2 ± 1.2 a**

- Mortality reduced from 90 to 94 %!

What is happening?

- Altered feeding behavior in the presence of some materials but not others
- High turbidity would likely cause a decrease in probability of capture
- How do we explain low turbidity along with altered feeding behavior
- Using the CCTT, expose the larvae to DayGlo fluorescent particles

DayGlo®

- DayGlo® Neon Red (15 ppm)
- Triton® X-100 (2.5 ppm)

The Effect of Triton® X-100 on *Bti* Toxicity

Media	Mortality (%)
DI	1.3 ± 3.7 A
DI + *Bti*	54.9 ± 0.7 B
Triton® X-100 (2.5 ppm)	3.5 ± 0.8 A
Triton® X-100 (2.5 ppm) + *Bti*	52.3 ± 2.9 B

The Effect of DayGlo® on *Bti* Toxicity

Media	Mortality (%)
DI	2.0 ± 1.1 A
DI + *Bti*	83.7 ± 2.0 B
DayGlo® (15 ppm)	0.0 ± 0.0 A
DayGlo® (15 ppm) + *Bti*	86.5 ± 1.9 B

Methods of Measuring Consumption

- Dissection
- Length of DayGlo band/full length of gut X100= % gut occupied by DayGlo
- Clearing method
- Experimentation with multiple concentrations of KOH
- 1% KOH for 1 Hr

The Response of *S. vittatum* Larvae to *Bti* ICPs and the Passage of DayGlo® after Exposure to Various Larval Media

Media	Turbidity (NTU)	*Bti* Induced Mortality (%)	Gut containing DayGlo® (%)
Water	0.03	88.8 ± 1.8 A	40.7
Kaolinite	7.7	87.8 ± 3.0 A	37.4
Cellulose	1.9	61.8 ± 1.9 B	30.6
C. meneghiniana frustules	3.4	3.2 ± 1.2 C	19.1

DayGlo concentration was 15 ppm/flask

Correlation coefficient (r) = 0.98

Possible Explanations

- Retraction of cephalic fans after contact with certain materials (cellulose,frustules)
- Slower rate of feeding due to high nutrient loads
- Cessation of feeding when gut is full of diatoms
- Any or all of the above