

ALGAE-REDUCING ECOSYSTEM SERVICES OF A NATIVE BIVALVE IN AN URBAN ESTUARY

ALLY KIDO
APRIL 4, 2024

OUTLINE

Background

Study System

Research Aims

Experiments and Results

Conclusions and Future Directions





THE CAPITAL CENTER

Marshalls

Marshalls

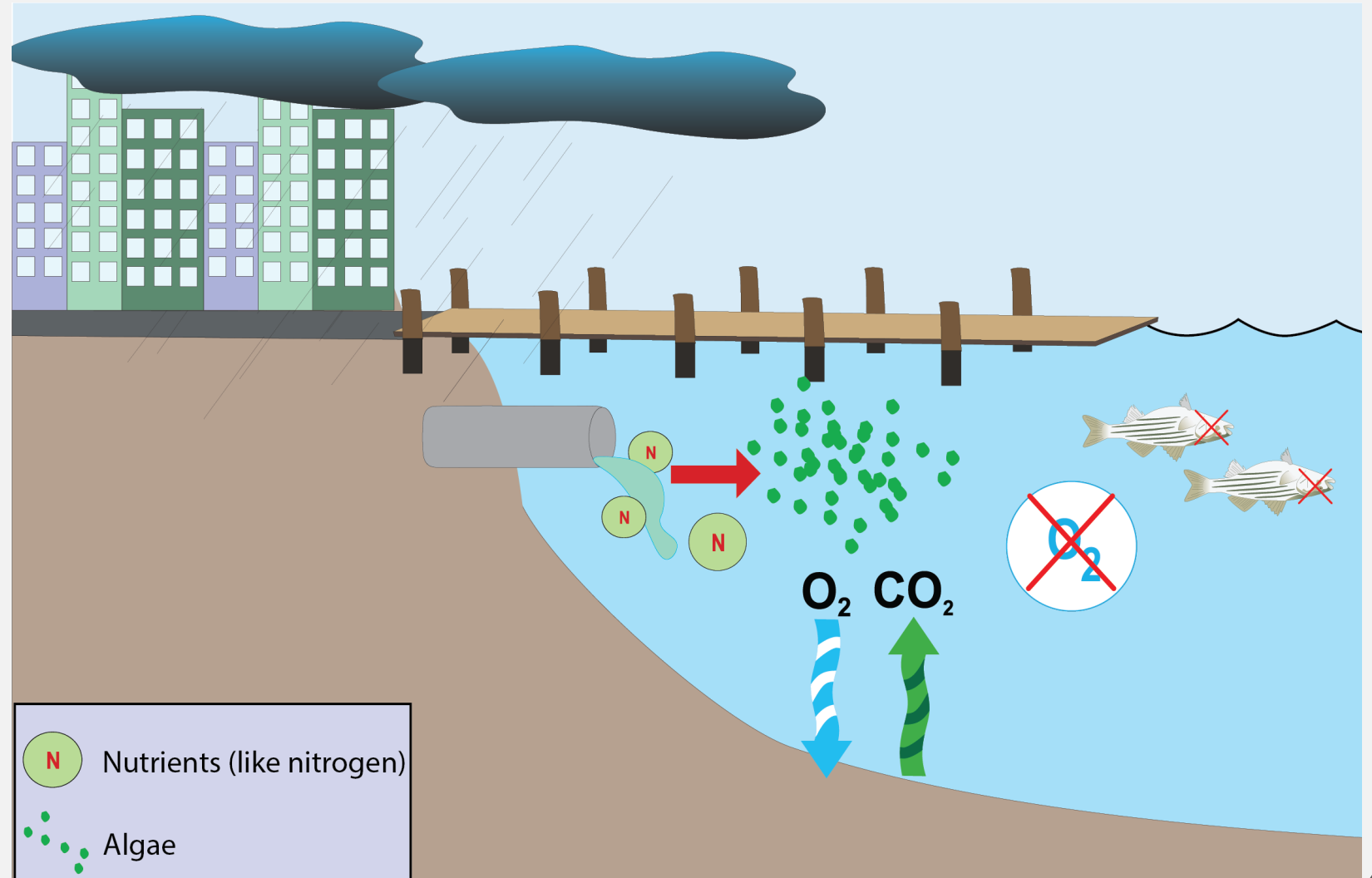
WARREN & NOBLE

SEAFOOD

WHY ARE EXCESS NUTRIENTS BAD?

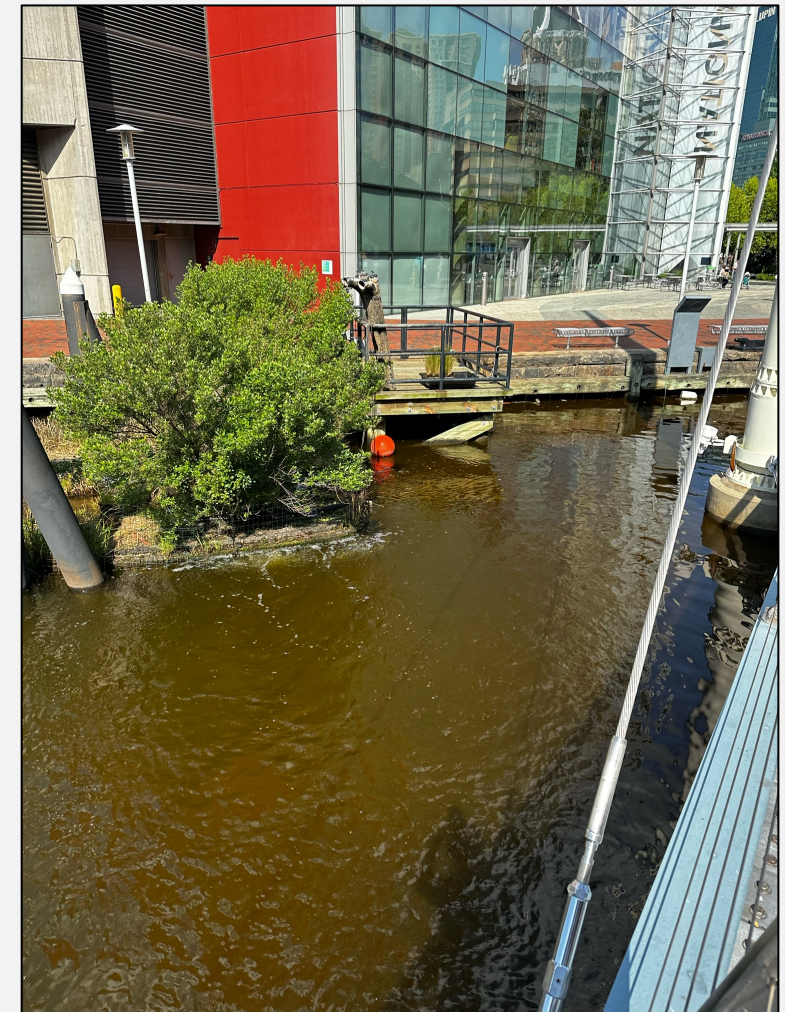
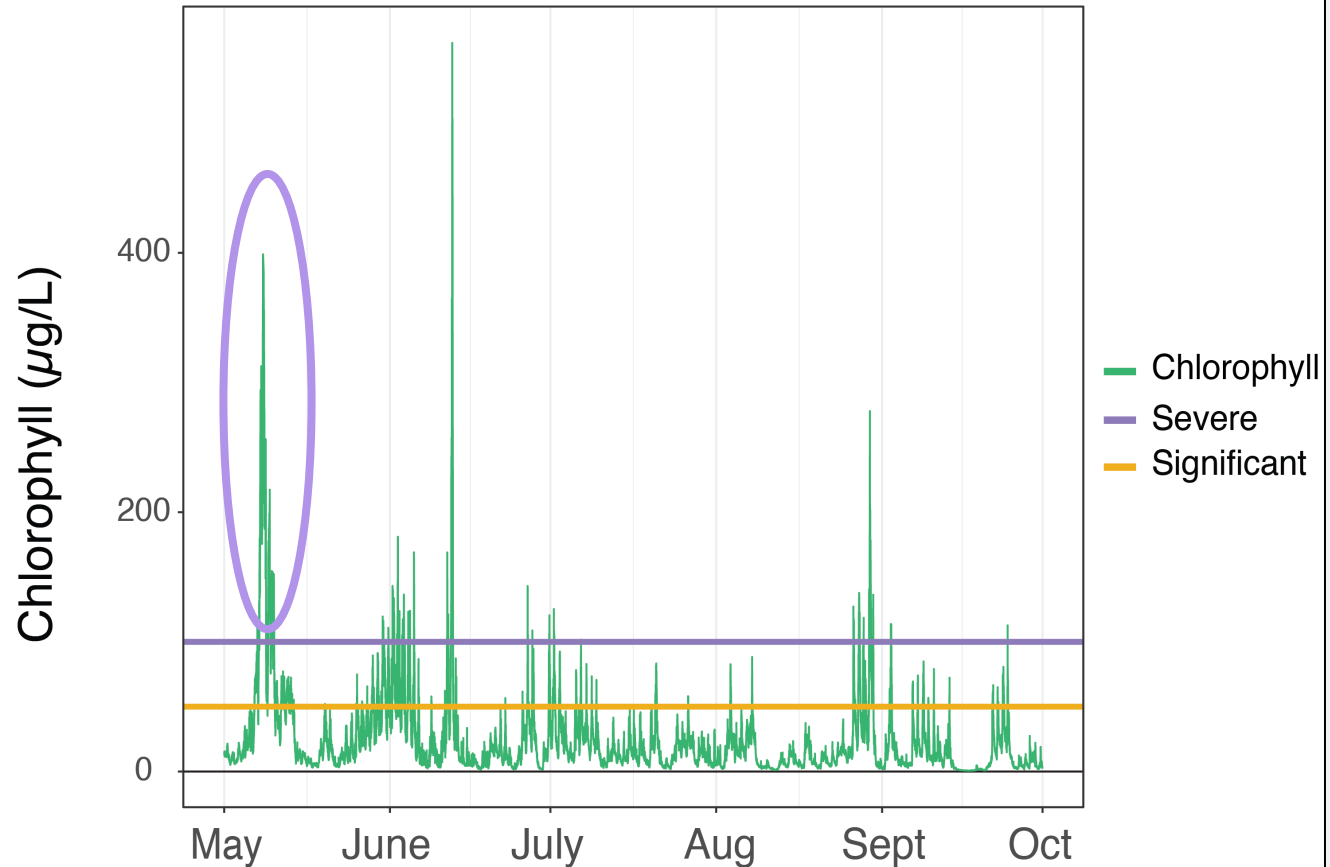
Algae Blooms

- Reduces oxygen
- Creates hypoxic and anoxic zones
- Not good for other animals in the water



ALGAE BLOOMS ARE FREQUENT IN BALTIMORE HARBOR

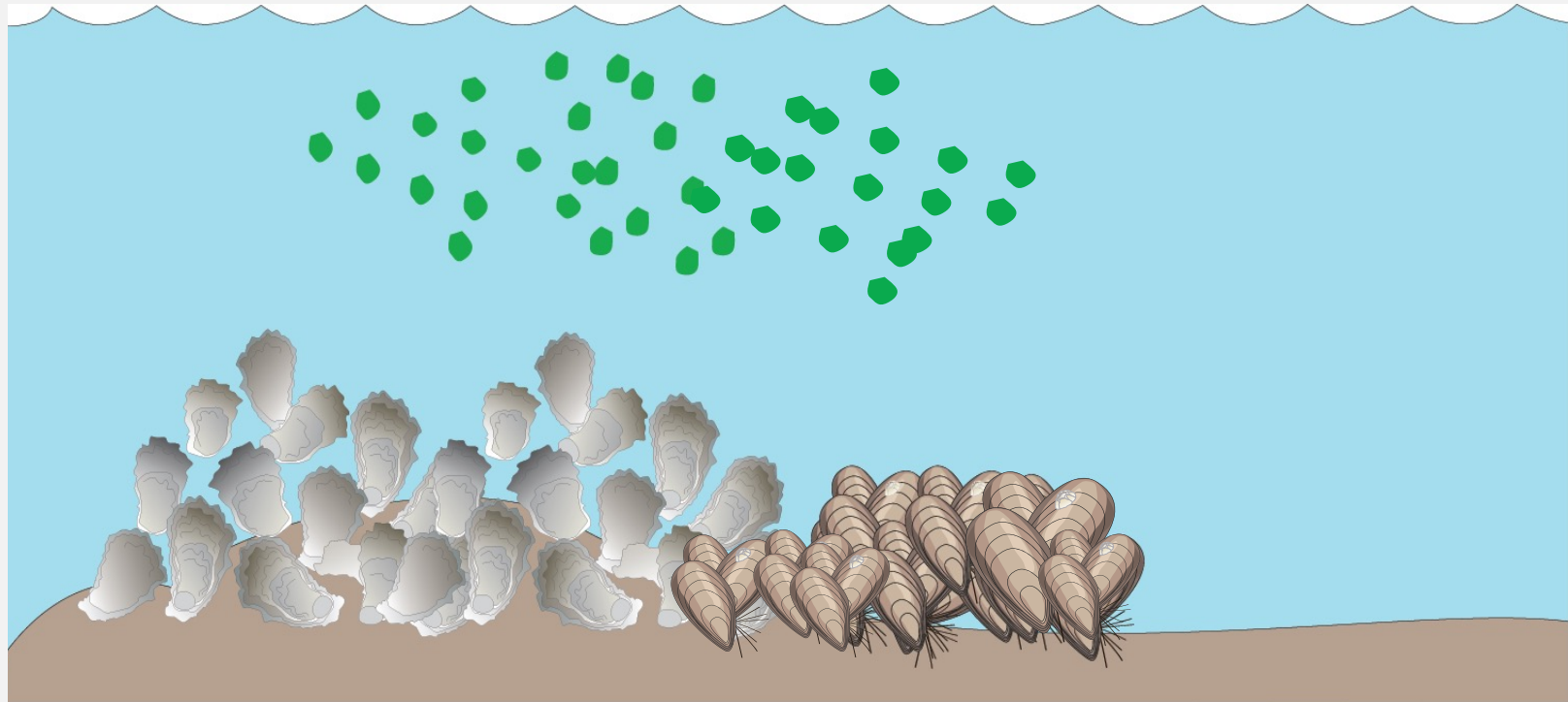
Chlorophyll Levels ($\mu\text{g/L}$) in Baltimore Harbor



WAYS TO REDUCE ALGAE IN THE WATER

HOW CAN BIVALVES CLEAR THE WATER?

- Bivalves are filter feeders
- Sort through particles and use nutrients for growth
- Unused particles are returned as:
 - Feces/Pseudofeces
 - Dissolved nutrients
- This is an ecosystem service provided by the bivalve

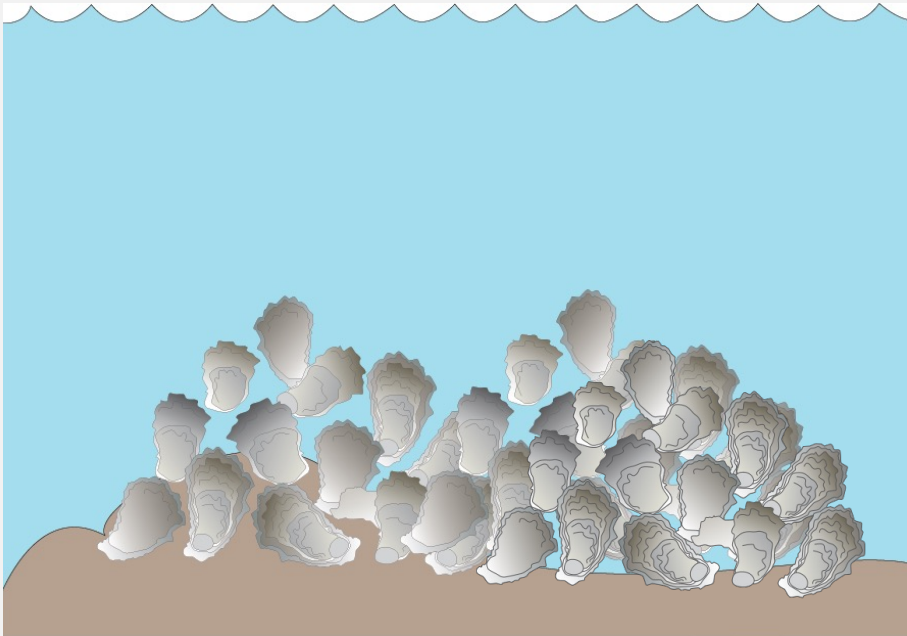


OYSTER NUTRIENT CREDIT TRADING

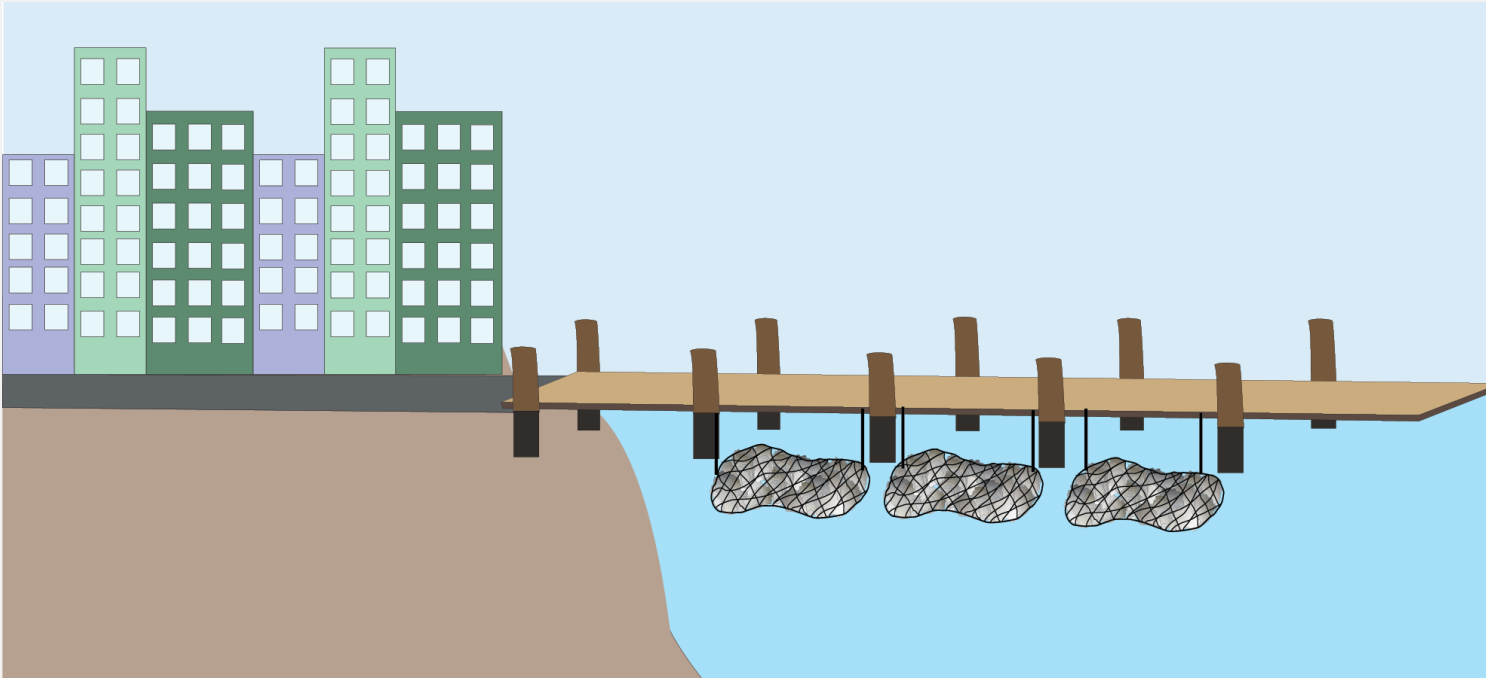
Oyster Growers

Nutrient Market

Groups that need to
reduce nutrient input



DO OYSTERS GROW IN BALTIMORE HARBOR?



- Oyster gardening does occur, but oysters are moved to restoration sites
- No natural oyster populations
- Bacteria contamination
- Low salinity water

DARK FALSE MUSSEL (*MYTILOPSIS LEUCOPHAEATA*)



DARK FALSE MUSSEL (*MYTILOPSIS LEUCOPHAEATA*)



CAN *M. LEUCOPHAEATA* REMOVE ALGAE AND NUTRIENTS FROM BALTIMORE HARBOR?

RESEARCH AIMS

1. Determine if *M. leucophaeata* can reduce cultured algae

2. Examine the effect of water temperature on clearance rate

3. Examine the effect of water salinity on clearance rate

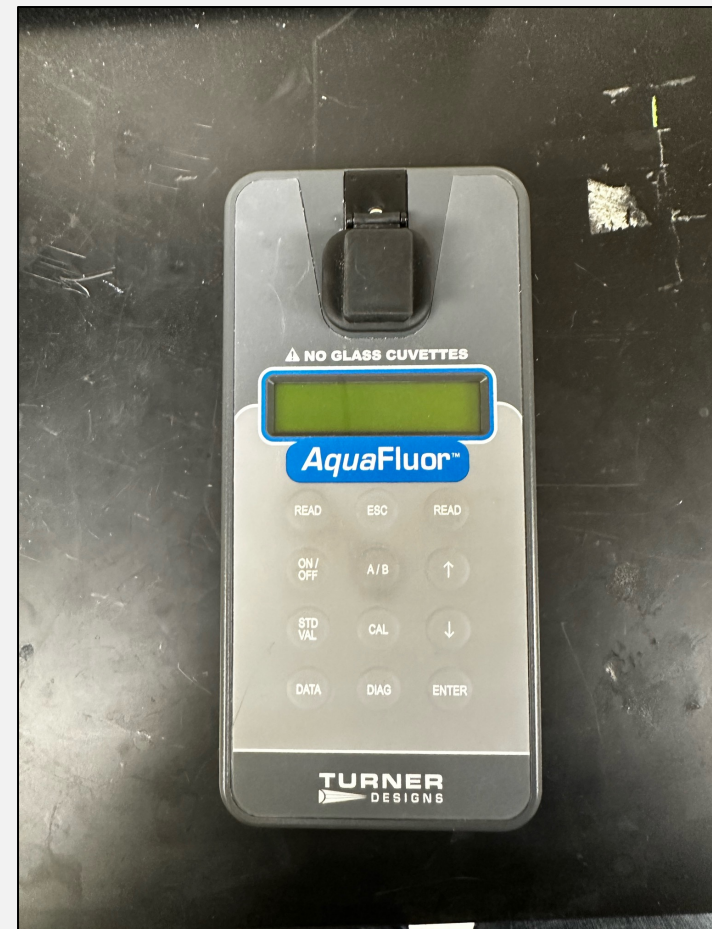
4. Determine if *M. leucophaeata* can reduce natural algae blooms

METHODS

- In vitro chlorophyll (IVCH)
- Algae counts
- Carbon and nitrogen analysis
- Extracted total chlorophyll

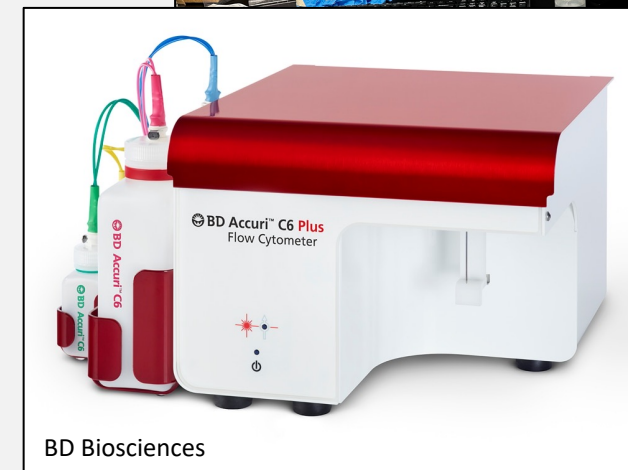
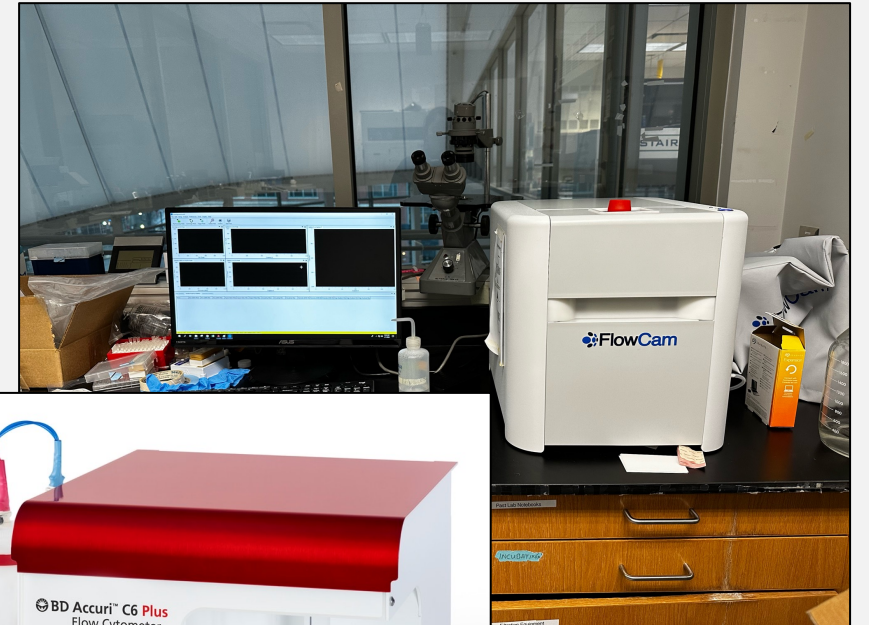
METHODS

- In vitro chlorophyll (IVCH)
- Algae counts
- Carbon and nitrogen analysis
- Extracted total chlorophyll



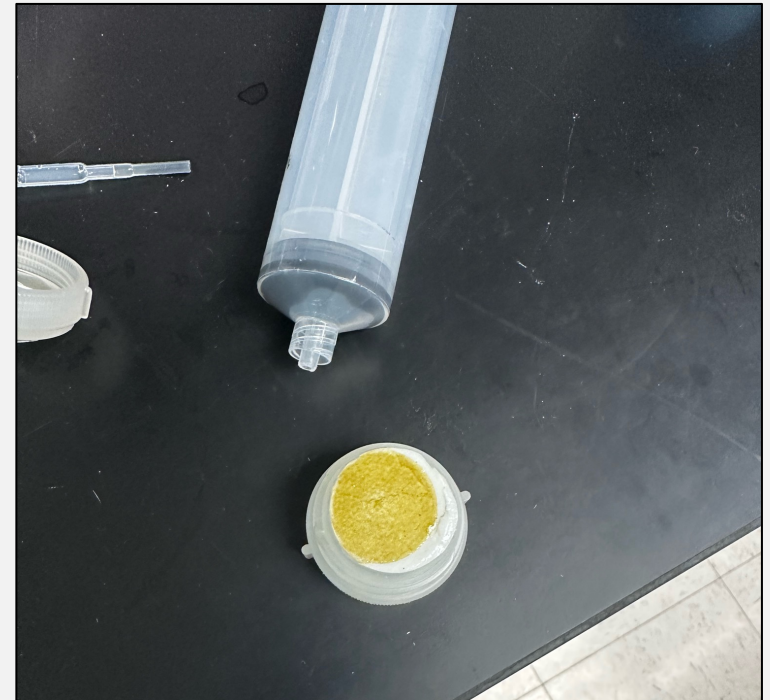
METHODS

- In vitro chlorophyll (IVCH)
- Algae counts
- Carbon and nitrogen analysis
- Extracted total chlorophyll



METHODS

- In vitro chlorophyll (IVCH)
- Algae counts
- Carbon and nitrogen analysis
- Extracted total chlorophyll (fluorometer)



RESEARCH AIMS

1. Determine if *M. leucophaeata* can reduce cultured algae

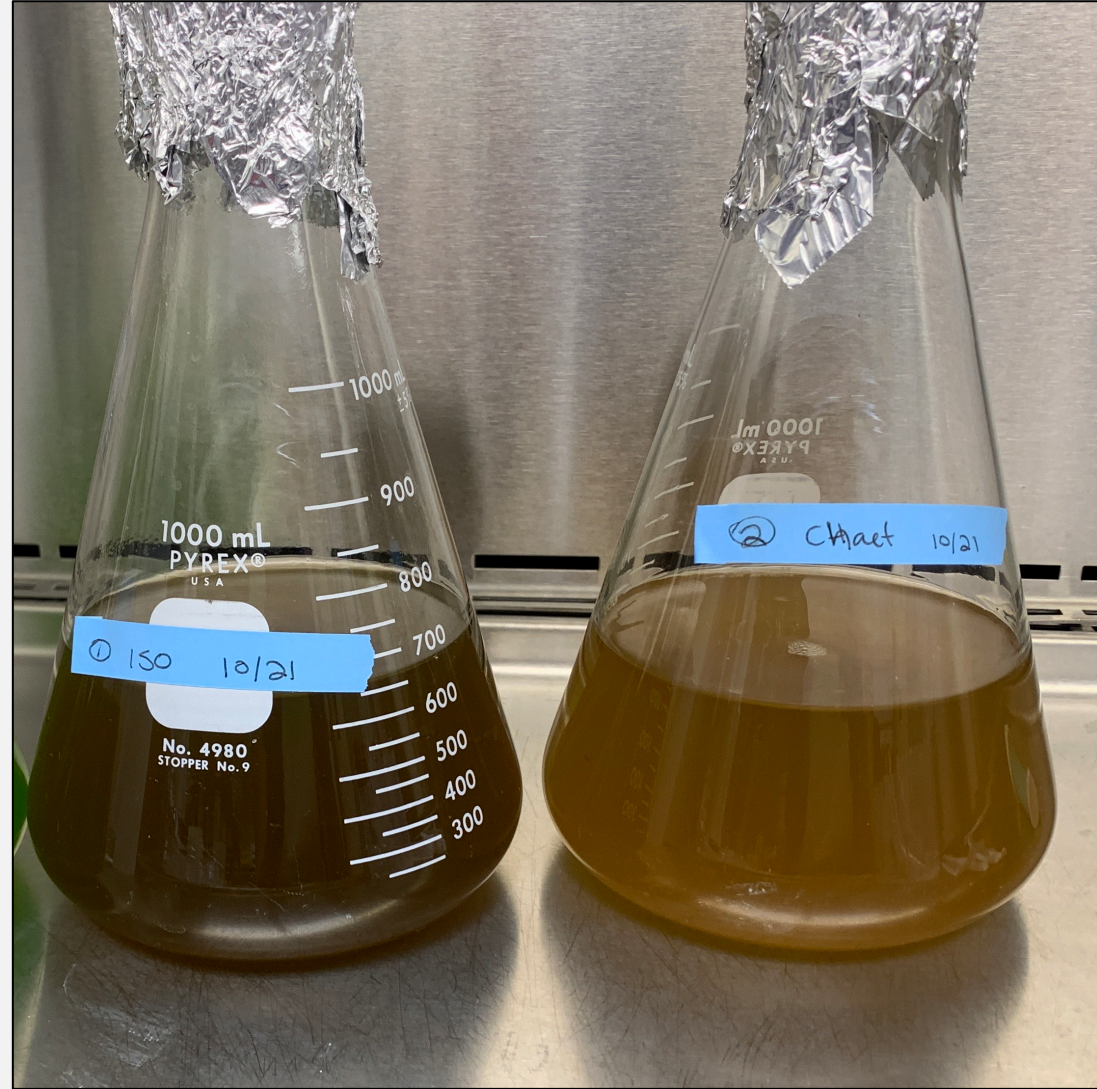
2. Examine the effect of water temperature on clearance rate

3. Examine the effect of water salinity on clearance rate

4. Determine if *M. leucophaeata* can reduce natural algae blooms

AIM 1 METHODS – ALGAE SPECIES

- 1000 ml of water
- ~20 mussels per container
- 2 algae species:
 - *Isochrysis*
 - *Chaetoceros*
- Water collections: 0, 2, 4, 6, and 22.5 hours



Isochrysis

Chaetoceros

TYPES OF SAMPLES COLLECTED



Mussel Treatment

No-Mussel Treatment

- In vitro chlorophyll (IVCH)
- Chlorophyll extractions

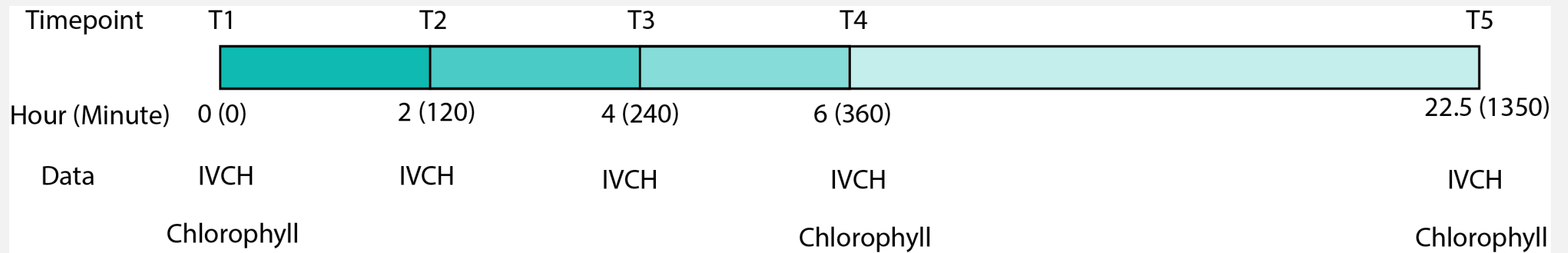
AIM 1: Algae Species

AIM 2

AIM 3

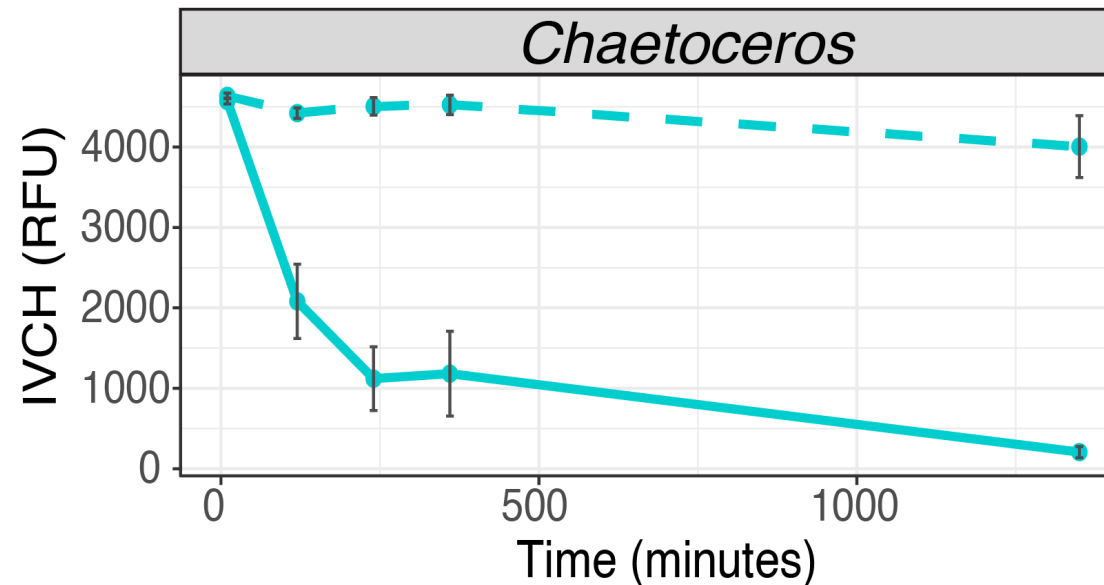
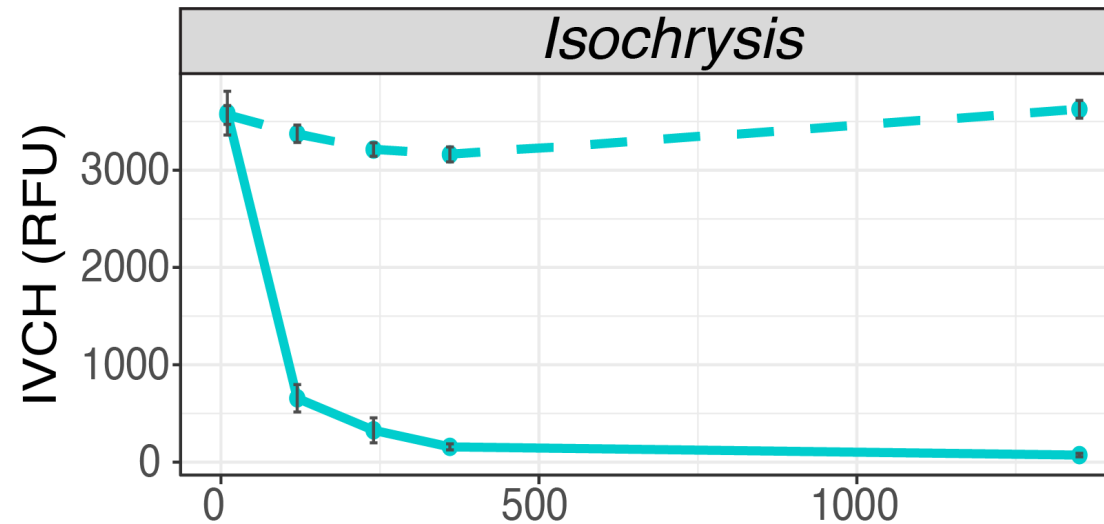
AIM 4

COLLECTION TIMELINE



MUSSELS CAN REDUCE CULTURED ALGAE IVCH LEVELS

Effect of Treatment:
Isochrysis – $p < 0.001$
Chaetoceros – $p < 0.001$

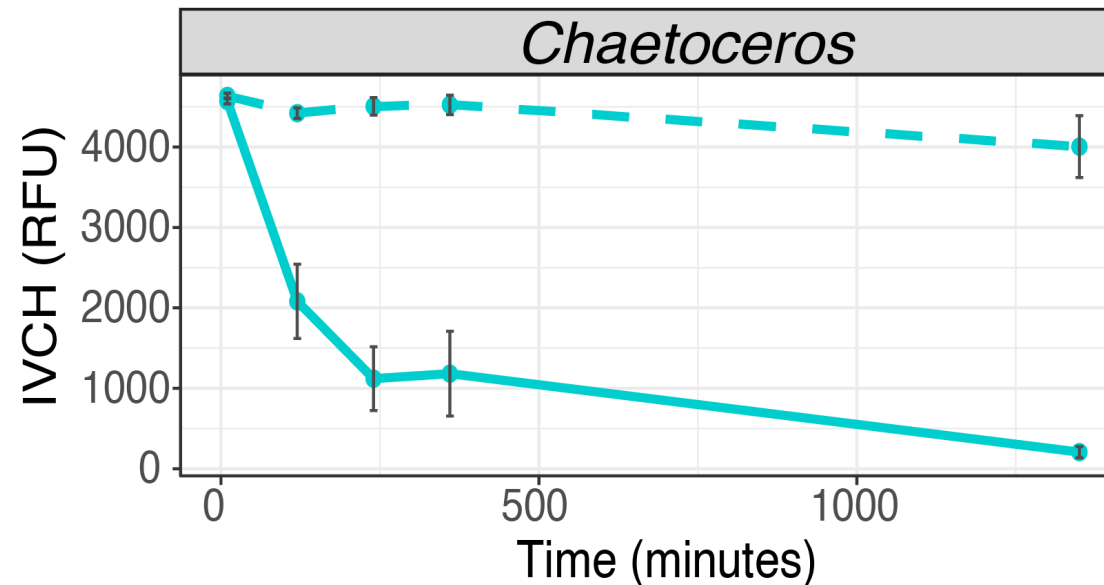
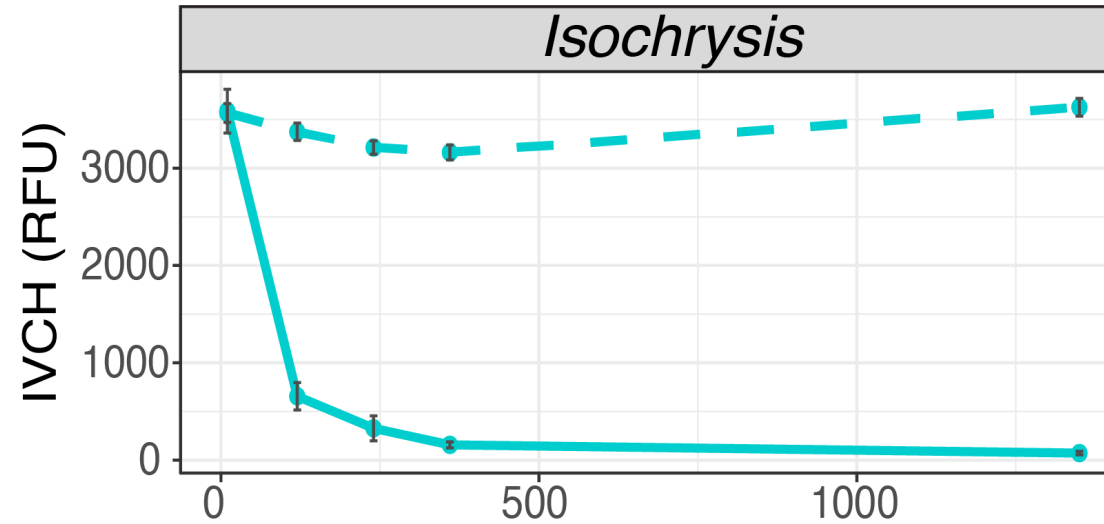


— Mussels
- - - No Mussels

Error bars are SEM

MUSSELS CAN
REDUCE
CULTURED
ALGAE IVCH
LEVELS

| Effect of Time | Iso. | Chaet. |
|----------------|---------------------|---------------------|
| Mussels | p < 0.001 | p < 0.001 |
| No Mussels | p < 0.001 | 0.088 |

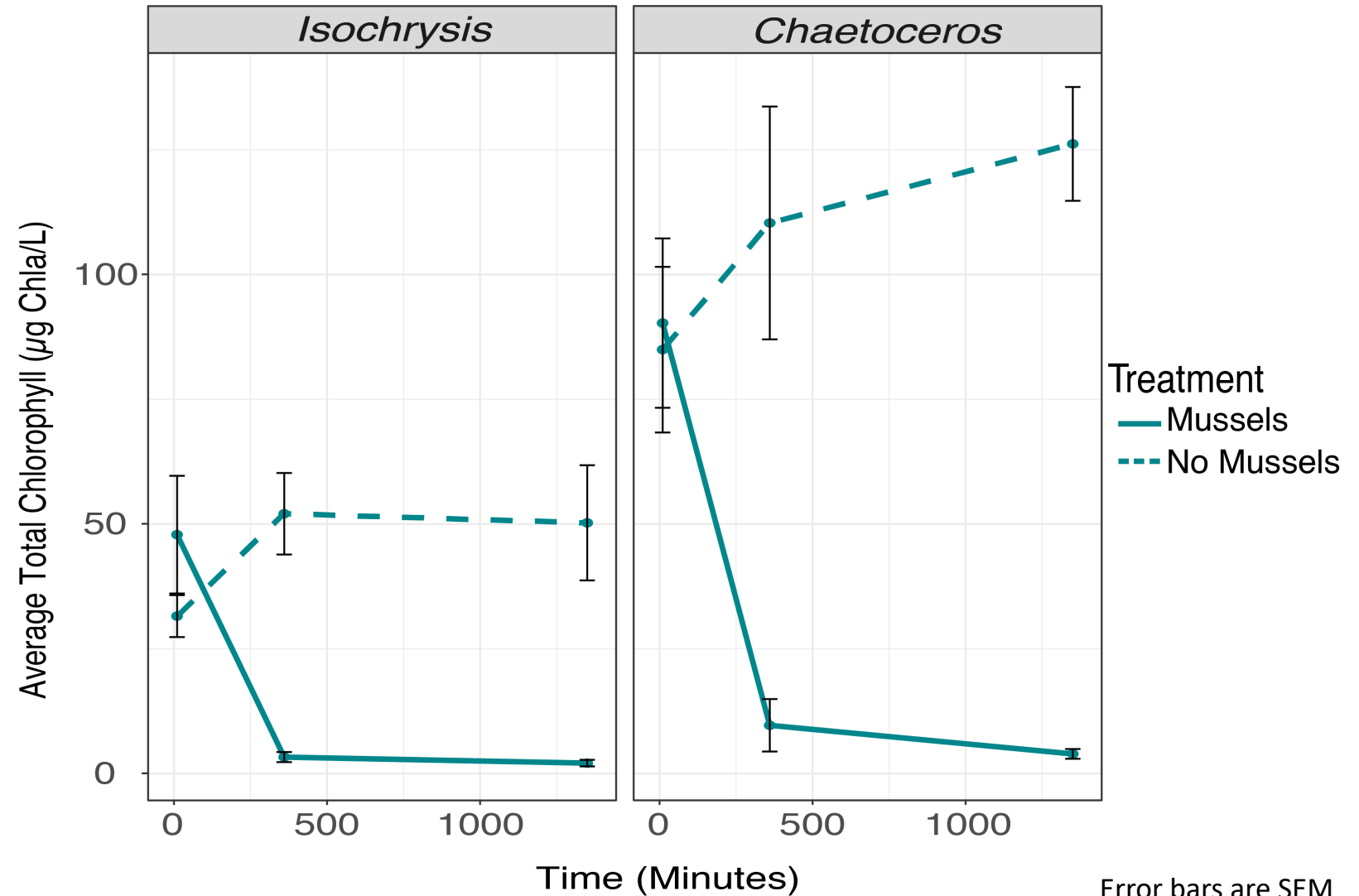


— Mussels
- - - No Mussels

Error bars are SEM

MUSSELS CAN REDUCE EXTRACTED CHLOROPHYLL

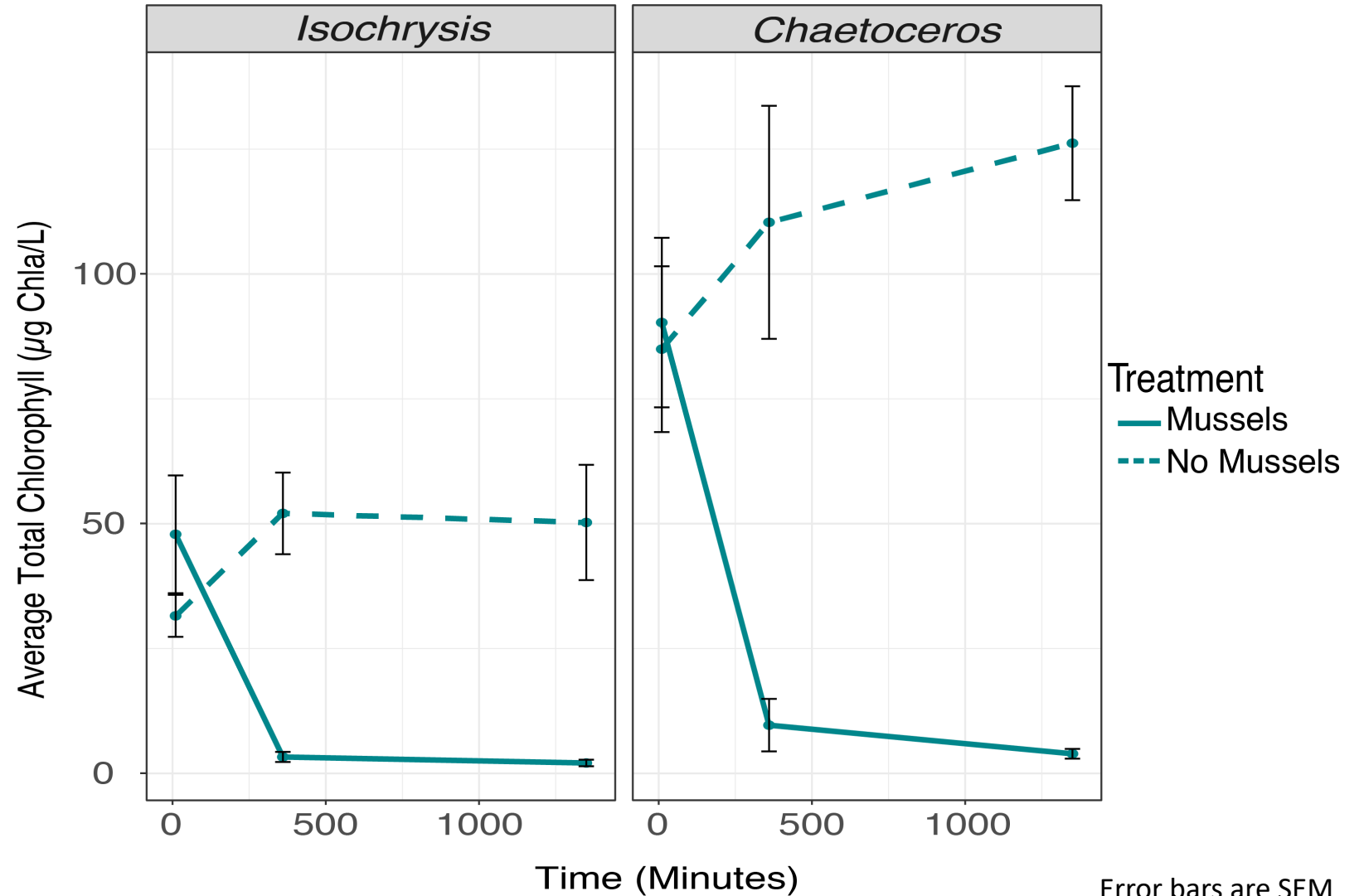
Effect of Treatment:
Isochrysis – $p = 0.011$
Chaetoceros – $p = 0.002$



Error bars are SEM

MUSSELS CAN REDUCE EXTRACTED CHLOROPHYLL

| Effect of Time | Iso. | Chaet. |
|----------------|--------------|--------------|
| Mussels | 0.039 | 0.001 |
| No Mussels | 0.717 | 0.135 |



Error bars are SEM

CONCLUSIONS AIM 1

- Mussels can reduce cultured algae
- Time has a significant effect on chlorophyll (IVCH and extracted) levels



RESEARCH AIMS

1. Determine if *M. leucophaeata* can reduce cultured algae

2. Examine the effect of water temperature on clearance rate

3. Examine the effect of water salinity on clearance rate

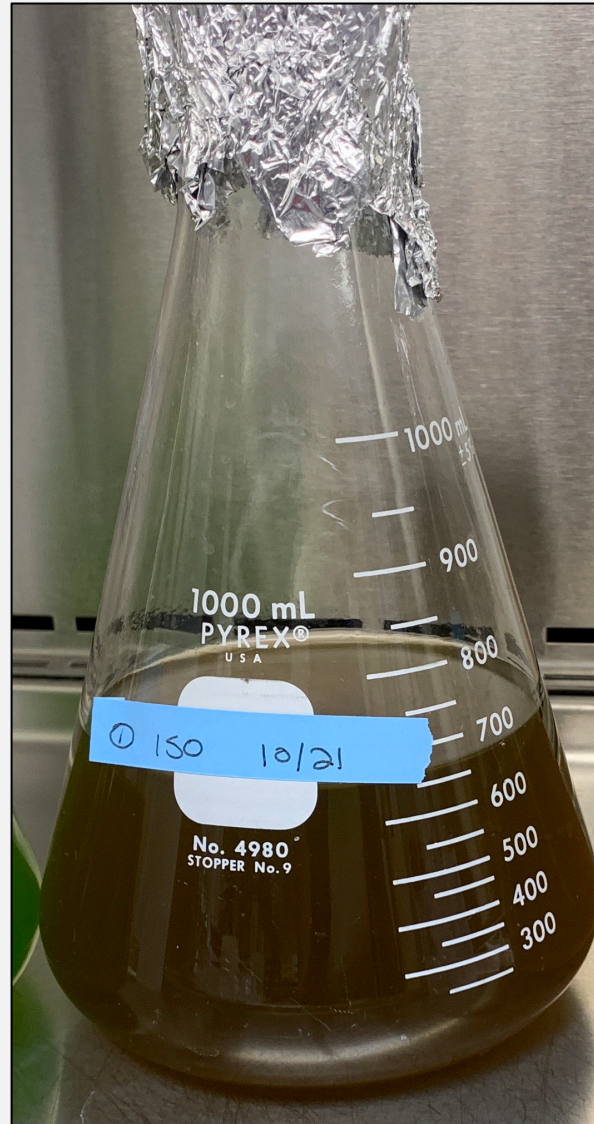
4. Determine if *M. leucophaeata* can reduce natural algae blooms

AIM 2 METHODS - TEMPERATURE

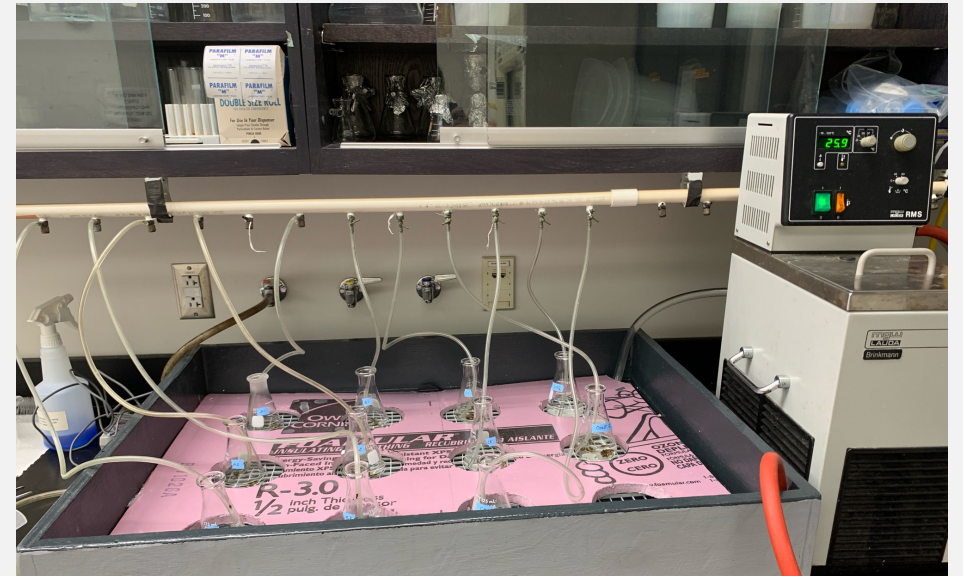
- 500 or 800 ml of water
- ~10 mussels per container
- Temperatures: 10, 20, and 30°C
- Water collections: 0, 2, 4, and 6 hours



Noah Mansfield

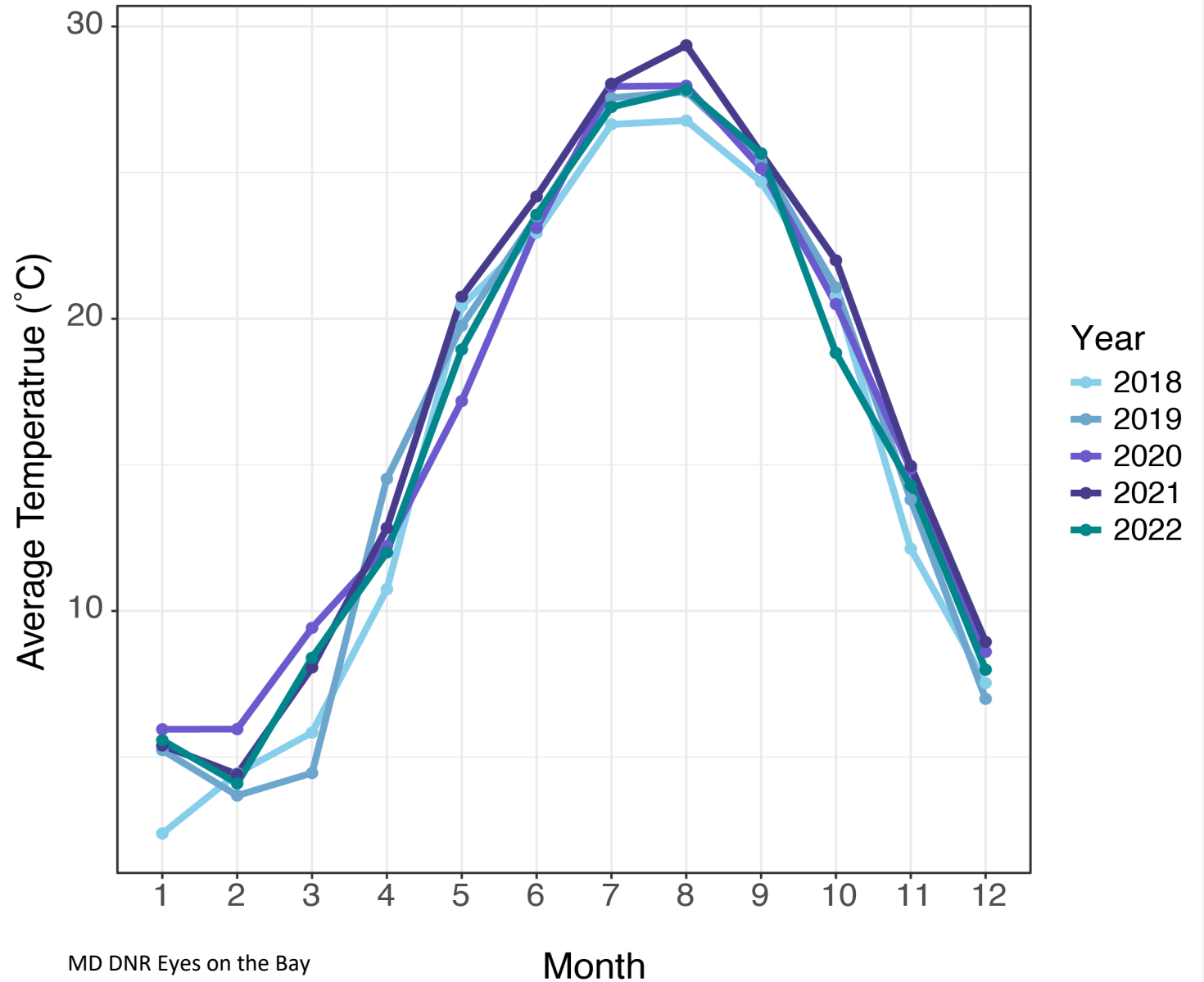


Isochrysis algae culture



Example set up using water baths to alter temperature

WATER TEMPERATURES IN BALTIMORE HARBOR



TYPES OF SAMPLES COLLECTED



Mussel Treatment

No-Mussel Treatment

- In vitro chlorophyll (IVCH)
- Algae counts

| Timepoint | T1 | T2 | T3 | T4 |
|---------------|-------------|---------|---------|-------------|
| Hour (Minute) | 0 (0) | 2 (120) | 4 (240) | 6 (360) |
| Data | IVCH | IVCH | IVCH | IVCH |
| | Cell Counts | | | Cell Counts |

TYPES OF SAMPLES COLLECTED



Mussel Treatment

No-Mussel Treatment

- In vitro chlorophyll (IVCH)
- Algae counts

| Timepoint | T1 | T2 | T3 | T4 |
|---------------|-------------|---------|---------|-------------|
| Hour (Minute) | 0 (0) | 2 (120) | 4 (240) | 6 (360) |
| Data | IVCH | IVCH | IVCH | IVCH |
| | Cell Counts | | | Cell Counts |

CLEARANCE RATE EQUATION

$$R_C = \frac{V}{nt} \left\{ \ln \left(\frac{C_0}{C_t} \right) - \ln \left(\frac{C_{0'}}{C_{t'}} \right) \right\}$$

R_C = Clearance Rate

V = Volume (liters)

n = Number of mussels

t = Time (hours)

C_0 = Concentration of treatment at start

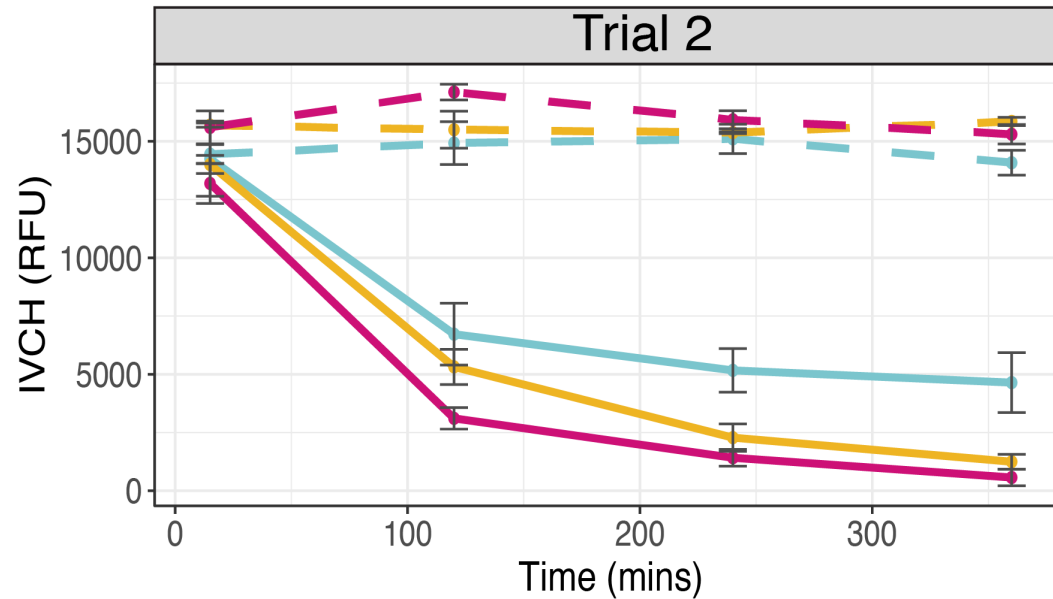
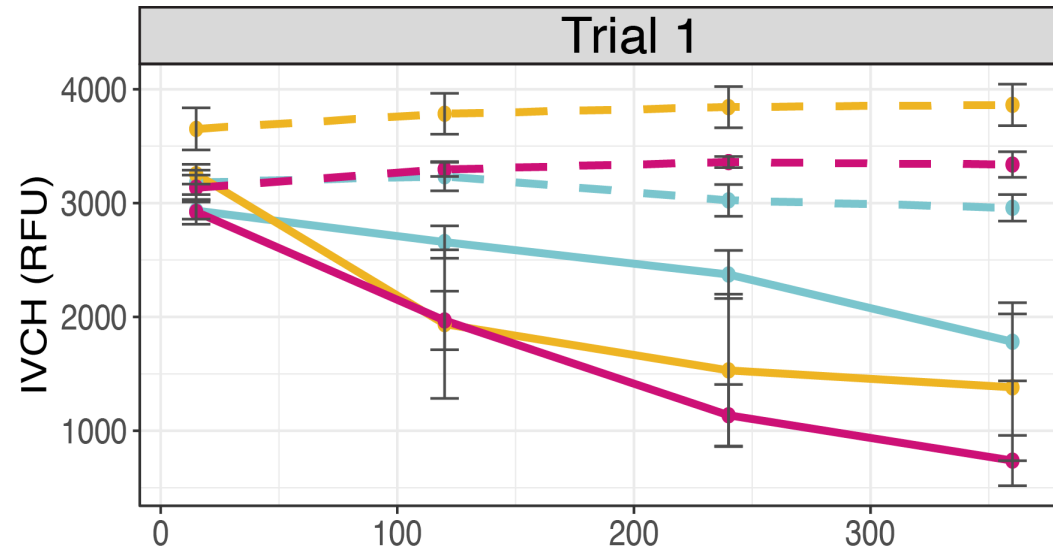
C_t = Concentration of treatment at end

$C_{0'}$ = Concentration of control at start

$C_{t'}$ = Concentration of control at end



AT ALL TEMPERATURES TESTED, MUSSELS REDUCE IVCH LEVELS



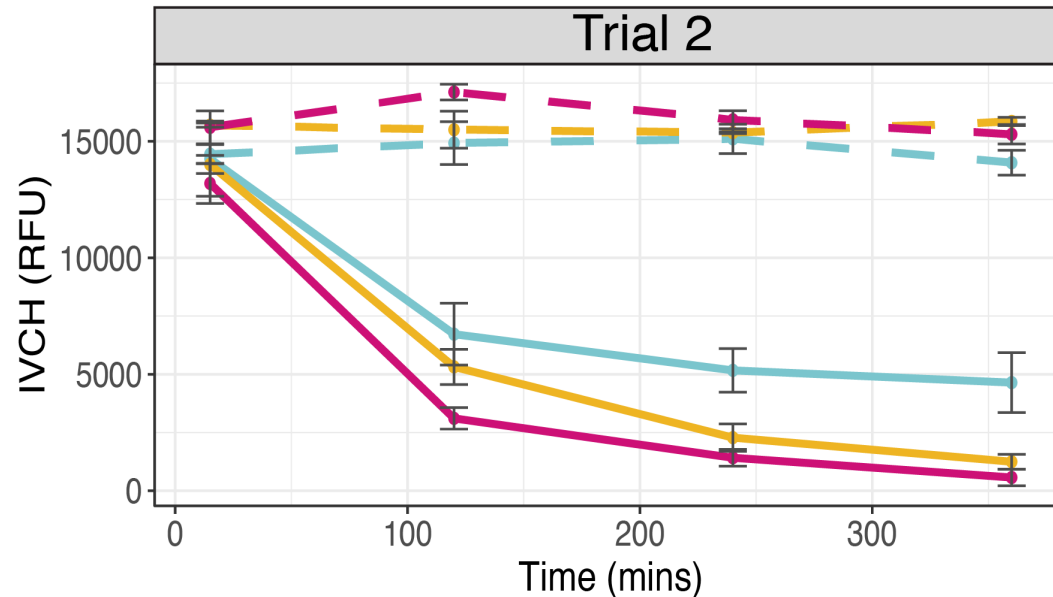
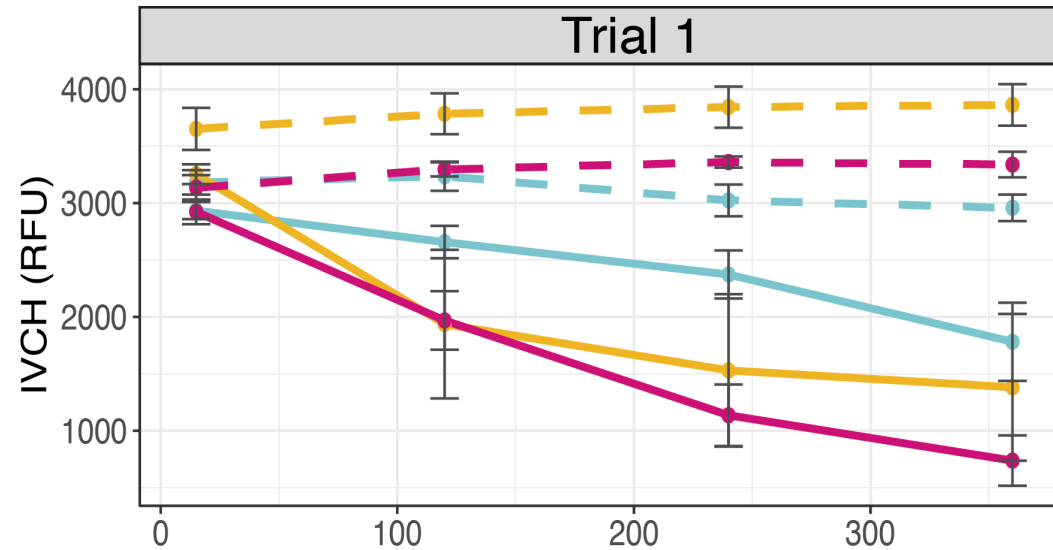
Temperature
 10°C
 20°C
 30°C

Treatment
 — Mussels
 - - - No Mussels

| | Effect of Treatment |
|---------|---------------------|
| Trial 1 | p < 0.001 |
| Trial 2 | p < 0.001 |

AT ALL TEMPERATURES TESTED, MUSSELS REDUCE IVCH LEVELS

| Effect of Time | Trial 1 | Trial 2 |
|----------------|---------------------|---------------------|
| Mussels | p < 0.001 | p < 0.001 |
| No Mussels | 0.348 | 0.06 |



MUSSELS HAVE LOWER
IVCH-BASED
CLEARANCE RATES AT
LOWER TEMPERATURES

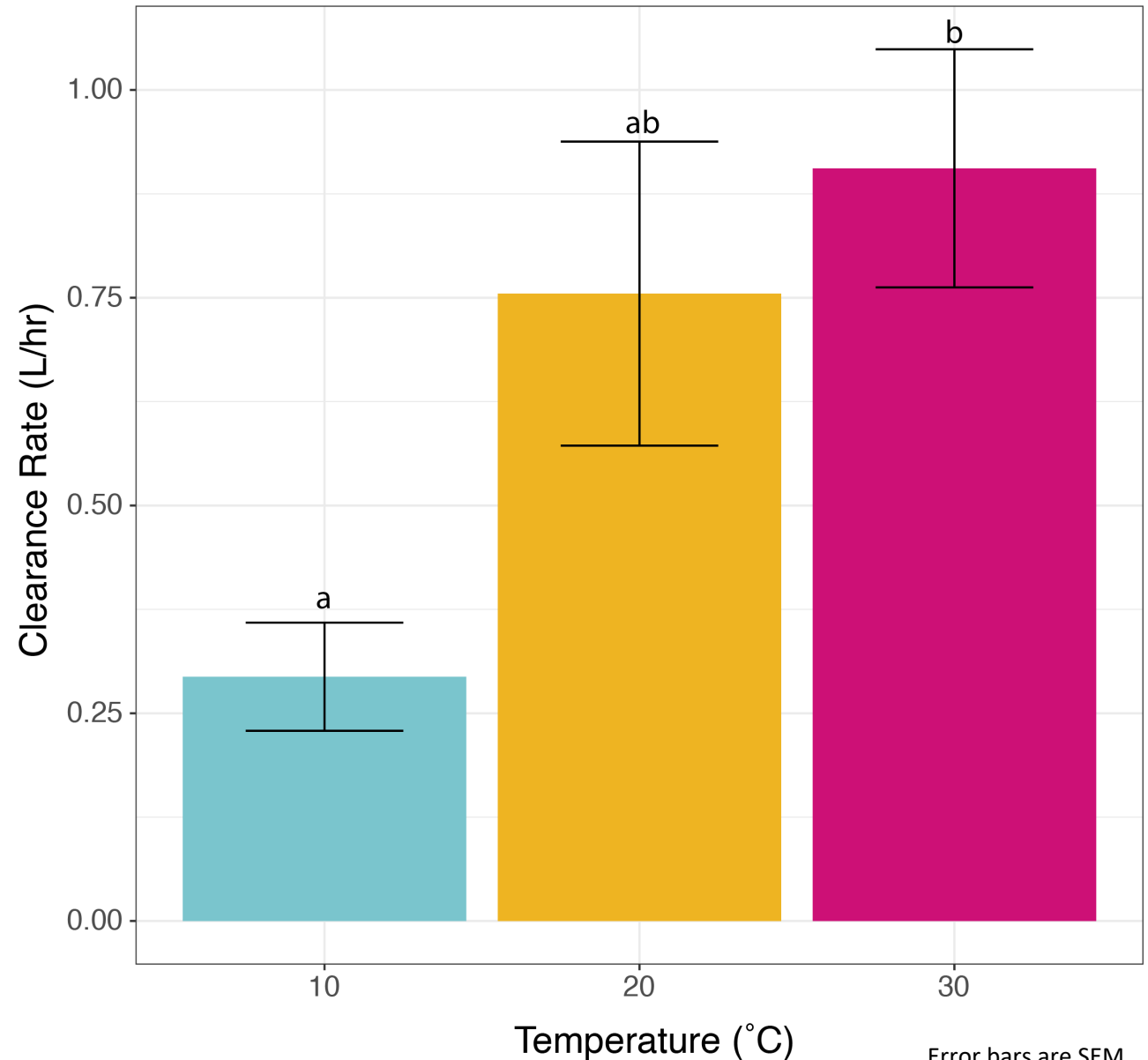
One way ANOVA: $p = 0.014$

Pairwise Comparisons:

10°C – 20°C: 0.079

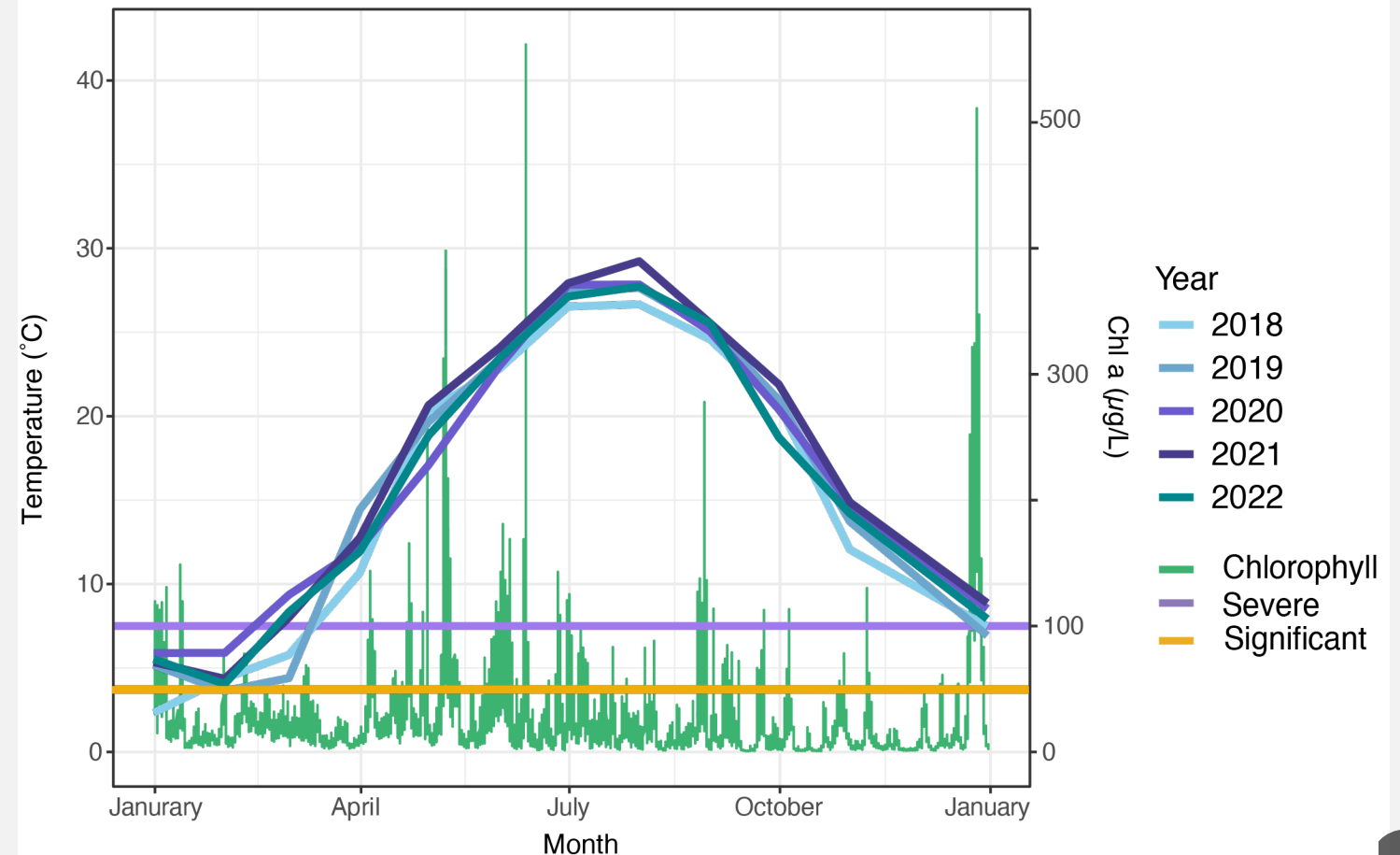
10°C – 30°C: 0.014

20°C – 30°C: 0.728



CONCLUSIONS AIM 2

- Mussels have a lower clearance rate at lower temperatures
- At all temperatures, the mussels were able to reduce the IVCH levels



RESEARCH AIMS

1. Determine if *M. leucophaeata* can reduce cultured algae

2. Examine the effect of water temperature on clearance rate

3. Examine the effect of water salinity on clearance rate

4. Determine if *M. leucophaeata* can reduce natural algae blooms

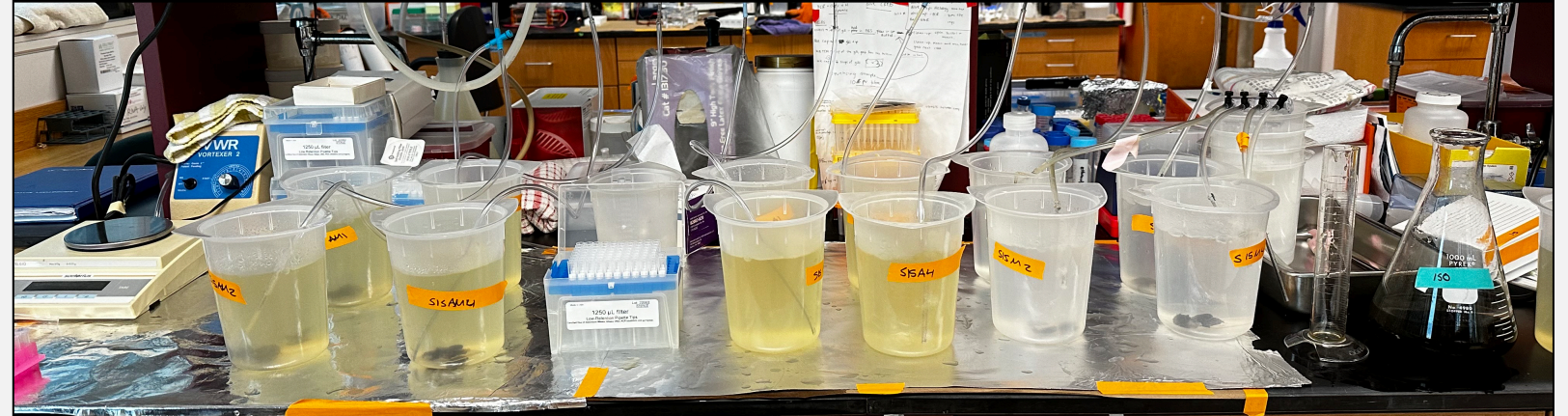
AIM 3 METHODS - SALINITY

- 500 or 800 ml of water
- ~10 mussels per container
- Salinities: 5, 10, and 15 ppt
- Water collections: 0, 2, 4, and 6 hours



Noah Mansfield

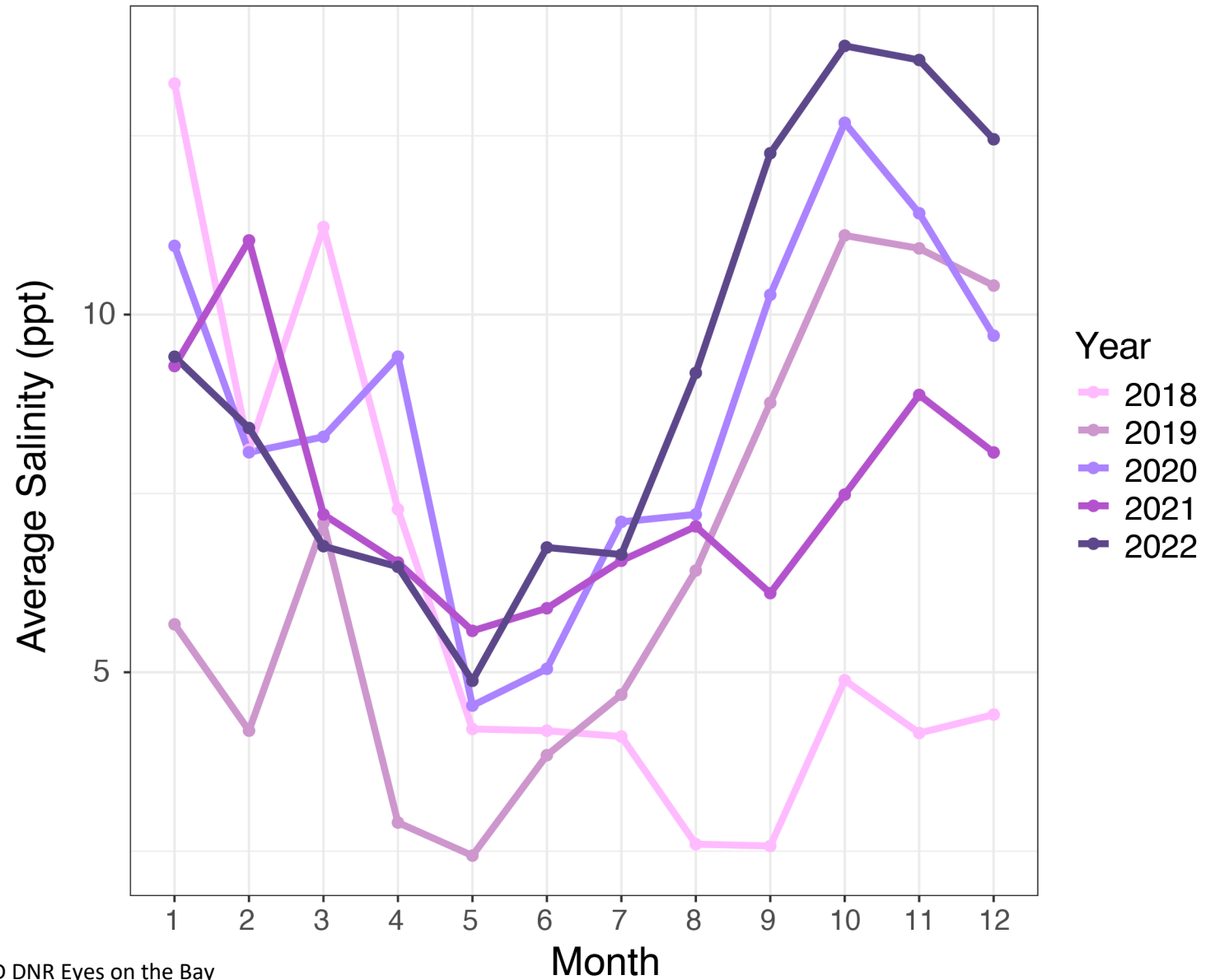
0 Hours



6 Hours



SALINITIES IN
BALTIMORE
HARBOR



TYPES OF SAMPLES COLLECTED



Mussel Treatment

No-Mussel Treatment

- In vitro chlorophyll (IVCH)
- Algae counts

| Timepoint | T1 | T2 | T3 | T4 |
|---------------|-------------|---------|---------|-------------|
| Hour (Minute) | 0 (0) | 2 (120) | 4 (240) | 6 (360) |
| Data | IVCH | IVCH | IVCH | IVCH |
| | Cell Counts | | | Cell Counts |

TYPES OF SAMPLES COLLECTED



Mussel Treatment

No-Mussel Treatment

- In vitro chlorophyll (IVCH)
- Algae counts

| Timepoint | T1 | T2 | T3 | T4 |
|---------------|-------------|---------|---------|-------------|
| Hour (Minute) | 0 (0) | 2 (120) | 4 (240) | 6 (360) |
| Data | IVCH | IVCH | IVCH | IVCH |
| | Cell Counts | | | Cell Counts |

CLEARANCE RATE EQUATION

$$R_C = \frac{V}{nt} \left\{ \ln \left(\frac{C_0}{C_t} \right) - \ln \left(\frac{C_{0'}}{C_{t'}} \right) \right\}$$

R_C = Clearance Rate

V = Volume (liters)

n = Number of mussels

t = Time (hours)

C_0 = Concentration of treatment at start

C_t = Concentration of treatment at end

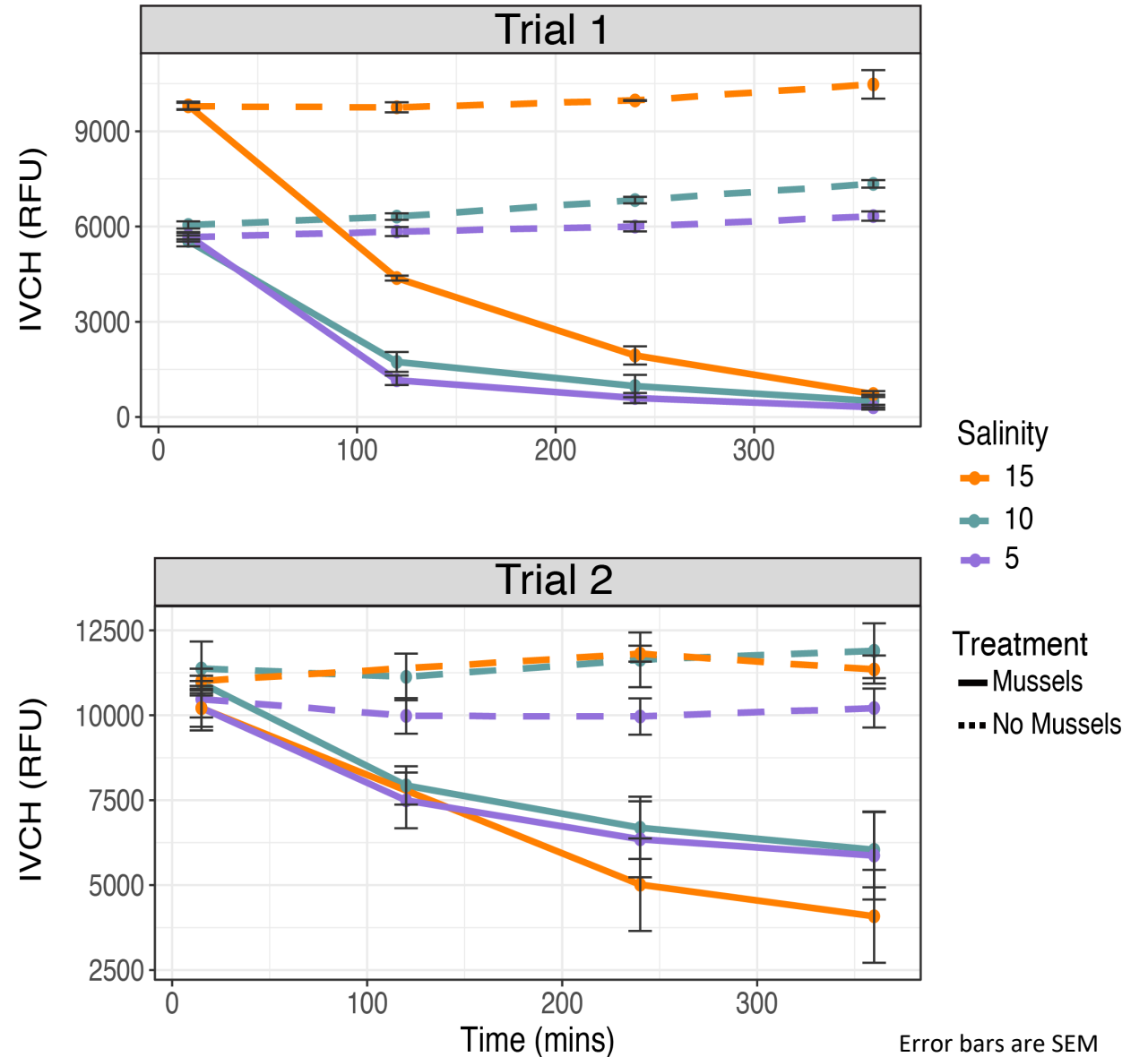
$C_{0'}$ = Concentration of control at start

$C_{t'}$ = Concentration of control at end



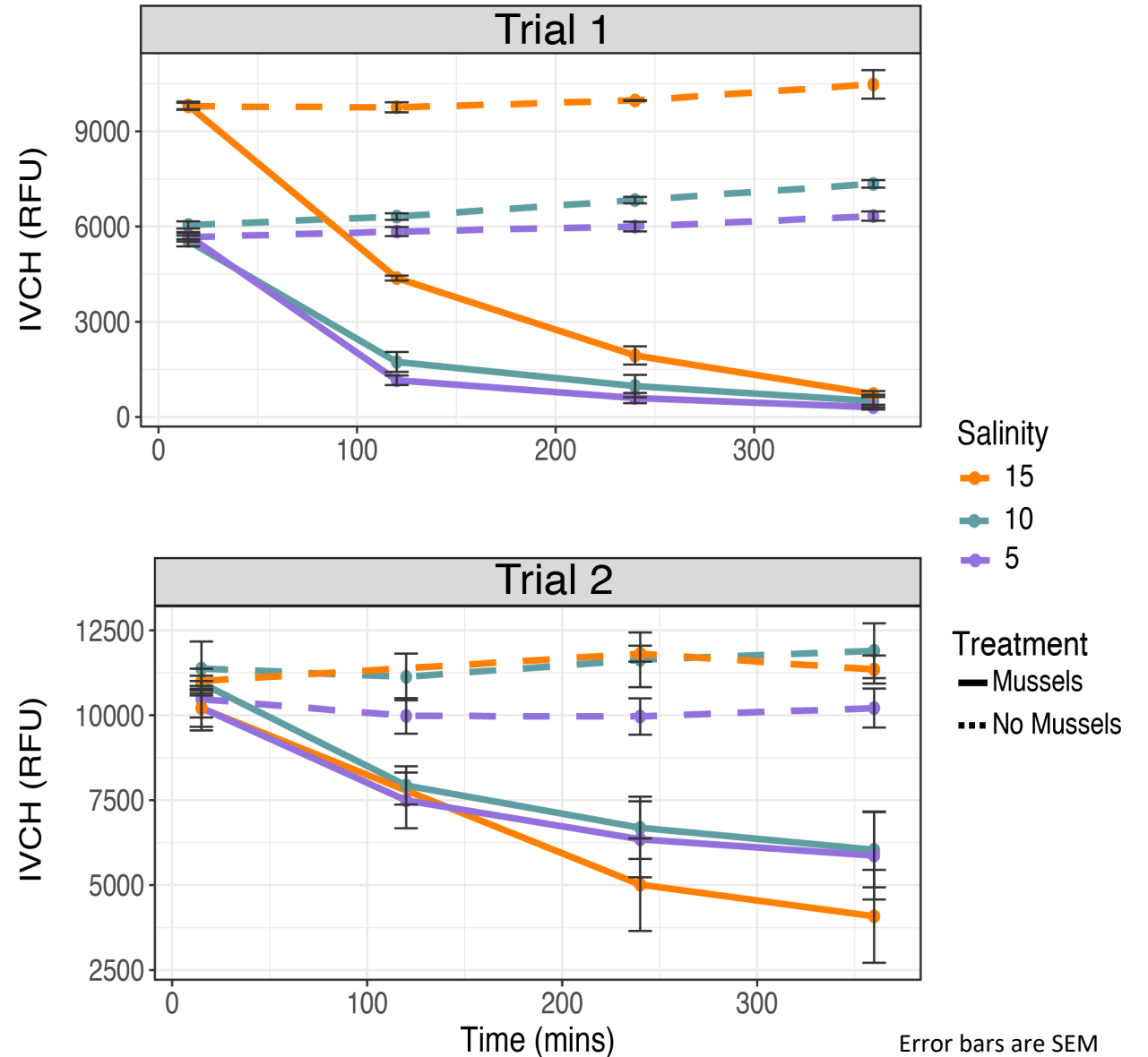
AT ALL SALINITIES TESTED, MUSSELS REDUCE IVCH LEVELS

| | Effect of Treatment |
|---------|---------------------|
| Trial 1 | p < 0.001 |
| Trial 2 | p < 0.001 |



AT ALL SALINITIES TESTED, MUSSELS REDUCE IVCH LEVELS

| Effect of Time | Trial 1 | Trial 2 |
|----------------|---------------------|---------------------|
| Mussels | p < 0.001 | p < 0.001 |
| No Mussels | p < 0.001 | 0.43 |



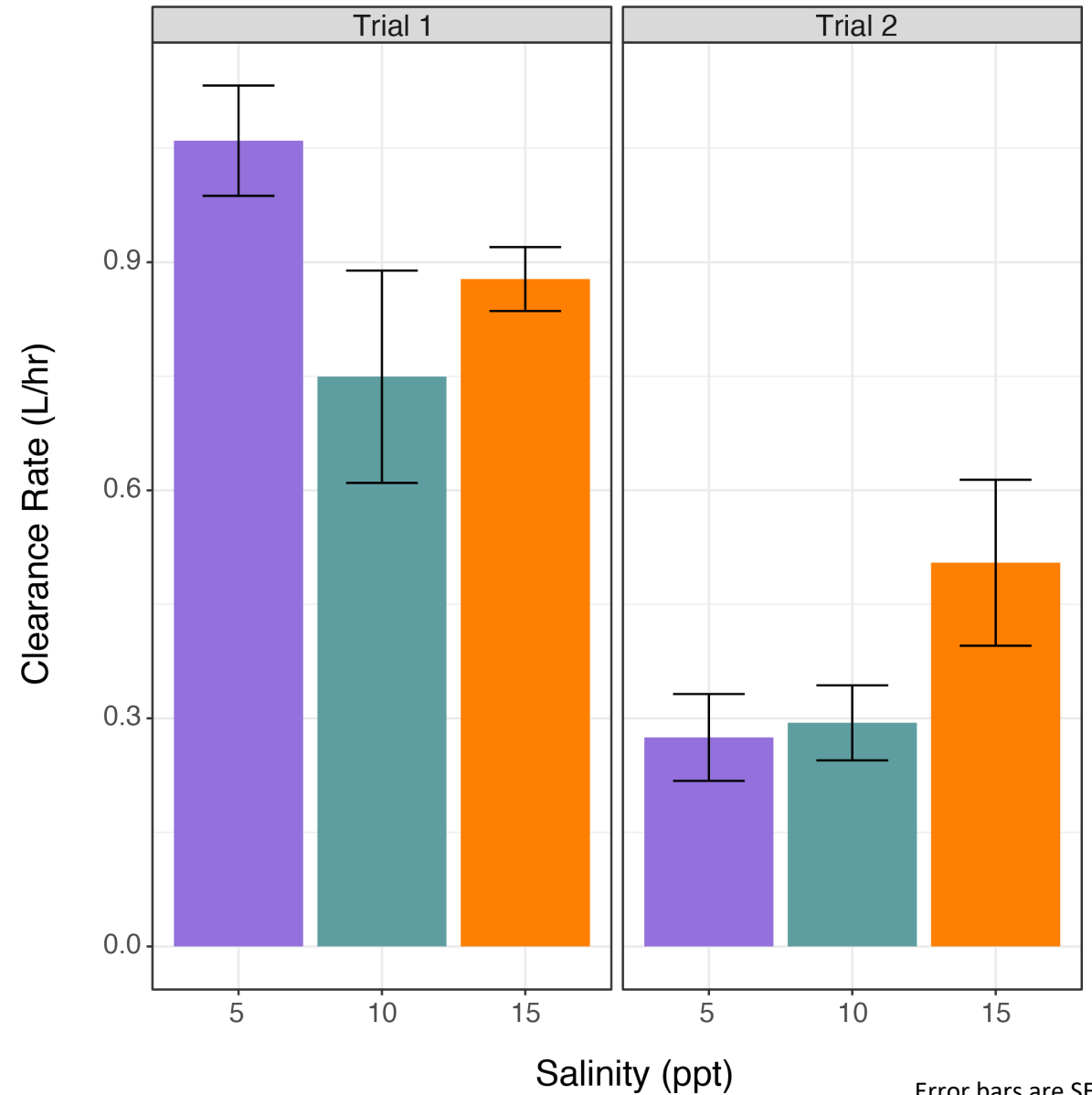
SALINITY DOES NOT AFFECT CLEARANCE RATES IN MUSSELS

One-Way ANOVA:

Trial 1 – $p = 0.162$

Trial 2 – $p = 0.116$

| Pairwise comparisons | Trial 1 | Trial 2 |
|----------------------|---------|---------|
| 5-10 | 0.141 | 0.983 |
| 5-15 | 0.455 | 0.140 |
| 10-15 | 0.621 | 0.182 |



Error bars are SEM

CONCLUSIONS AIM 3



- Salinity does not affect clearance rates of mussels
- Mussels are still able to reduce the IVCH levels
- Still more environmental variables to consider:
 - DO
 - pH
- Stage of mussels

RESEARCH AIMS

1. Determine if *M. leucophaeata* can reduce cultured algae

2. Examine the effect of water temperature on clearance rate

3. Examine the effect of water salinity on clearance rate

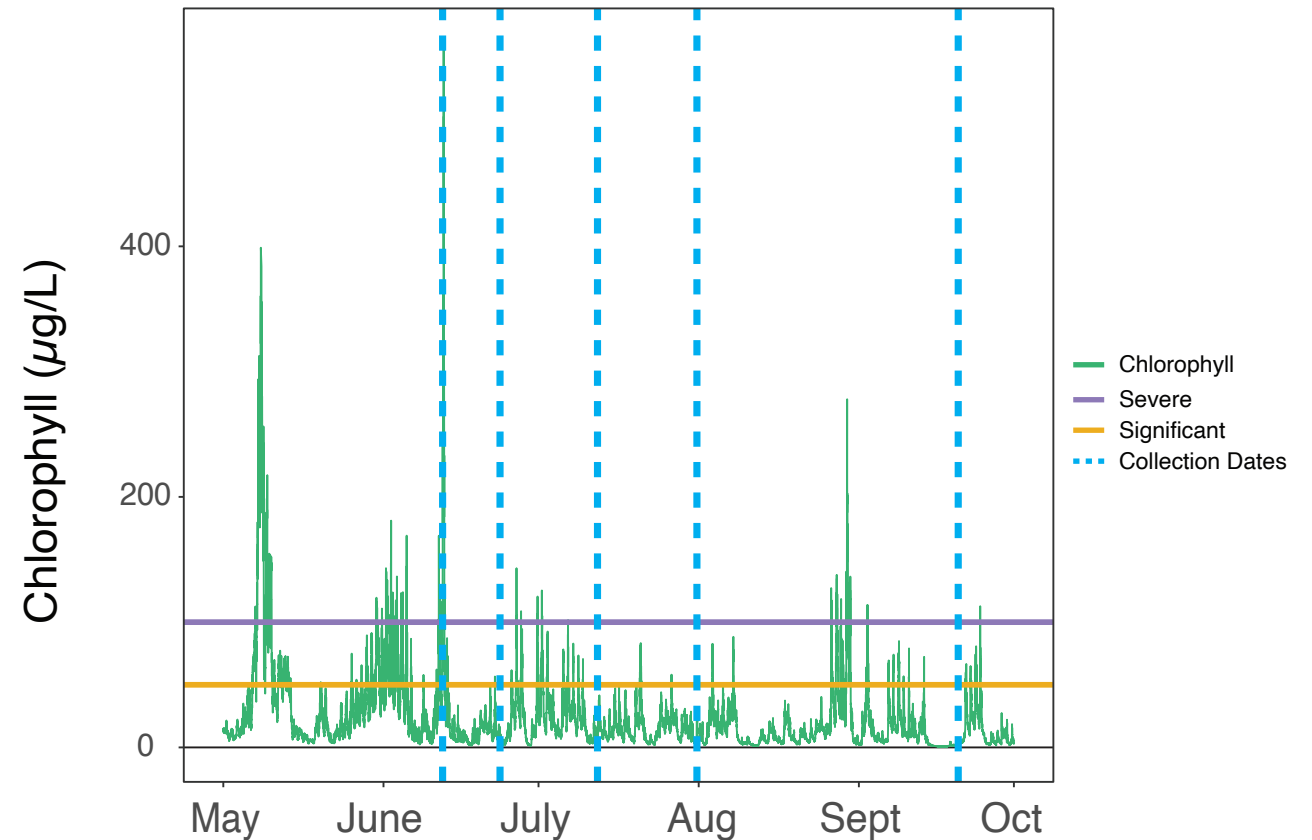
4. Determine if *M. leucophaeata* can reduce natural algae blooms

AIM 4 METHODS – NATURAL ALGAE BLOOMS

- 1,500 ml of algae bloom water
- ~20 mussels per container
- Water collections: 0, 2, 4, 6, and 24 hours
- June - September



ALGAE BLOOM COLLECTION DATES



TYPES OF SAMPLES COLLECTED



Mussel Treatment

No-Mussel Treatment

- In vitro chlorophyll (IVCH)
- Algae counts
- Carbon and nitrogen analysis
- Extracted total chlorophyll

TYPES OF SAMPLES COLLECTED



Mussel Treatment

No-Mussel Treatment

- In vitro chlorophyll (IVCH)
- Algae counts
- Carbon and nitrogen analysis
- Extracted total chlorophyll

AIM 1

AIM 2

AIM 3

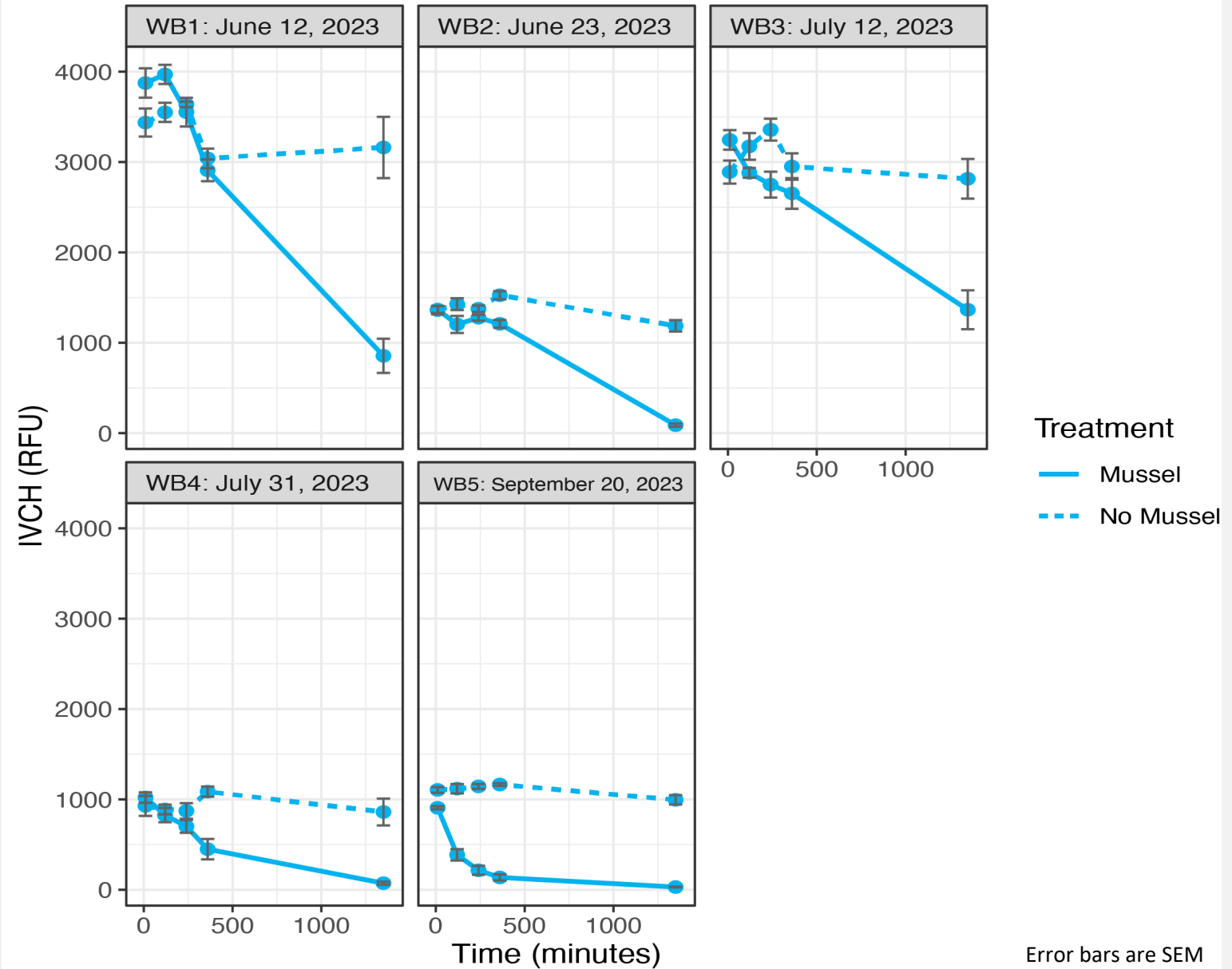
AIM 4: Natural Algae Blooms

COLLECTION TIMELINE

| Timepoint | T1 | T2 | T3 | T4 | T5 |
|---------------|---------------------|---------|---------|---------------------|---------------------|
| Hour (Minute) | 0 (0) | 2 (120) | 4 (240) | 6 (360) | 24 (1440) |
| Data | IVCH | IVCH | IVCH | IVCH | IVCH |
| | Chlorophyll | | | Chlorophyll | Chlorophyll |
| | Cell Counts | | | Cell Counts | Cell Counts |
| | Carbon and Nitrogen | | | Carbon and Nitrogen | Carbon and Nitrogen |

MUSSELS
REDUCE IN
VITRO
CHLOROPHYLL
(IVCH)

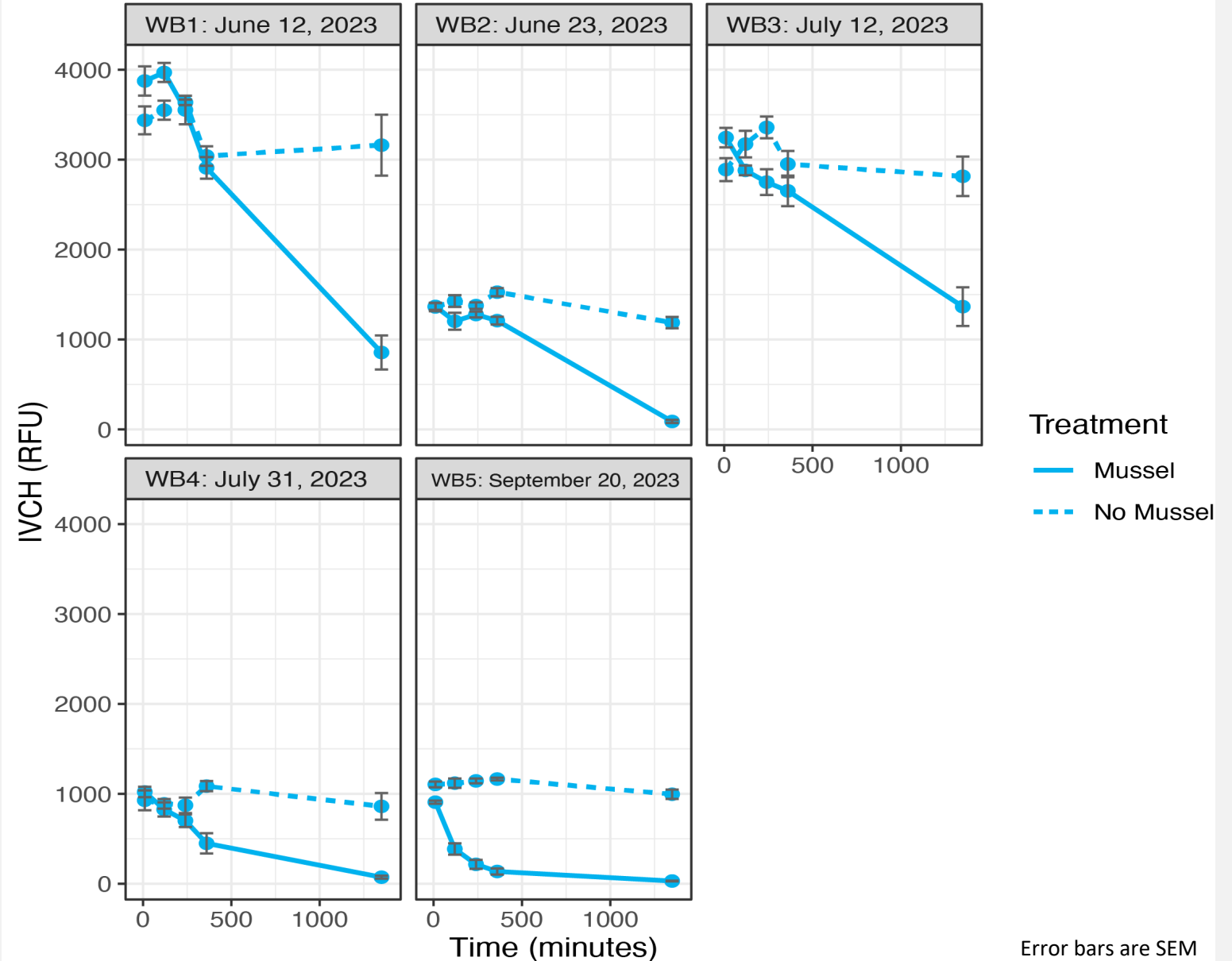
Effect of Treatment:
 WB1 – 0.877
 WB2 – **0.001**
 WB3 – **0.013**
 WB4 – **0.006**
 WB5 – **p < 0.001**



Error bars are SEM

MUSSELS REDUCE IN VITRO CHLOROPHYLL (IVCH)

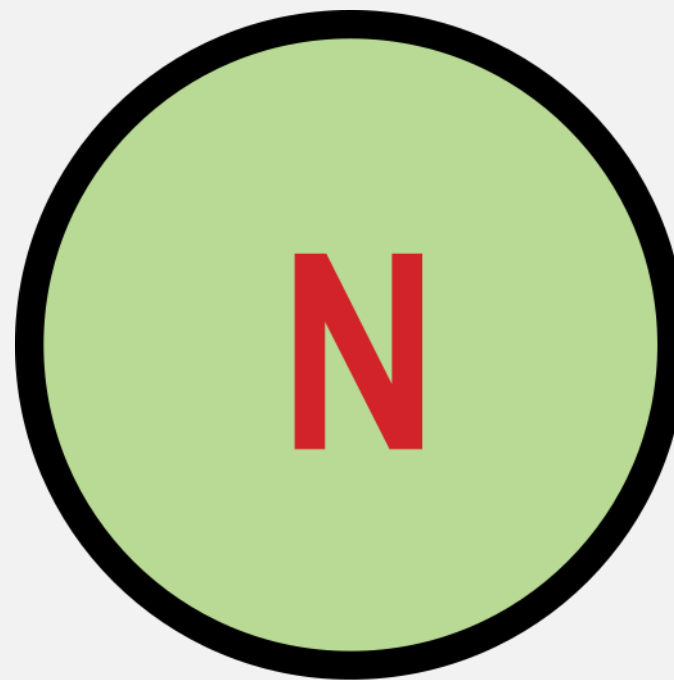
| Effect of Time | Mussel | No Mussel |
|----------------|---------------------|---------------------|
| WB1 | p < 0.001 | 0.297 |
| WB2 | 0.011 | p < 0.001 |
| WB3 | p < 0.001 | 0.148 |
| WB4 | p < 0.001 | 0.25 |
| WB5 | p < 0.001 | 0.071 |



OYSTER NUTRIENT CREDIT TRADING

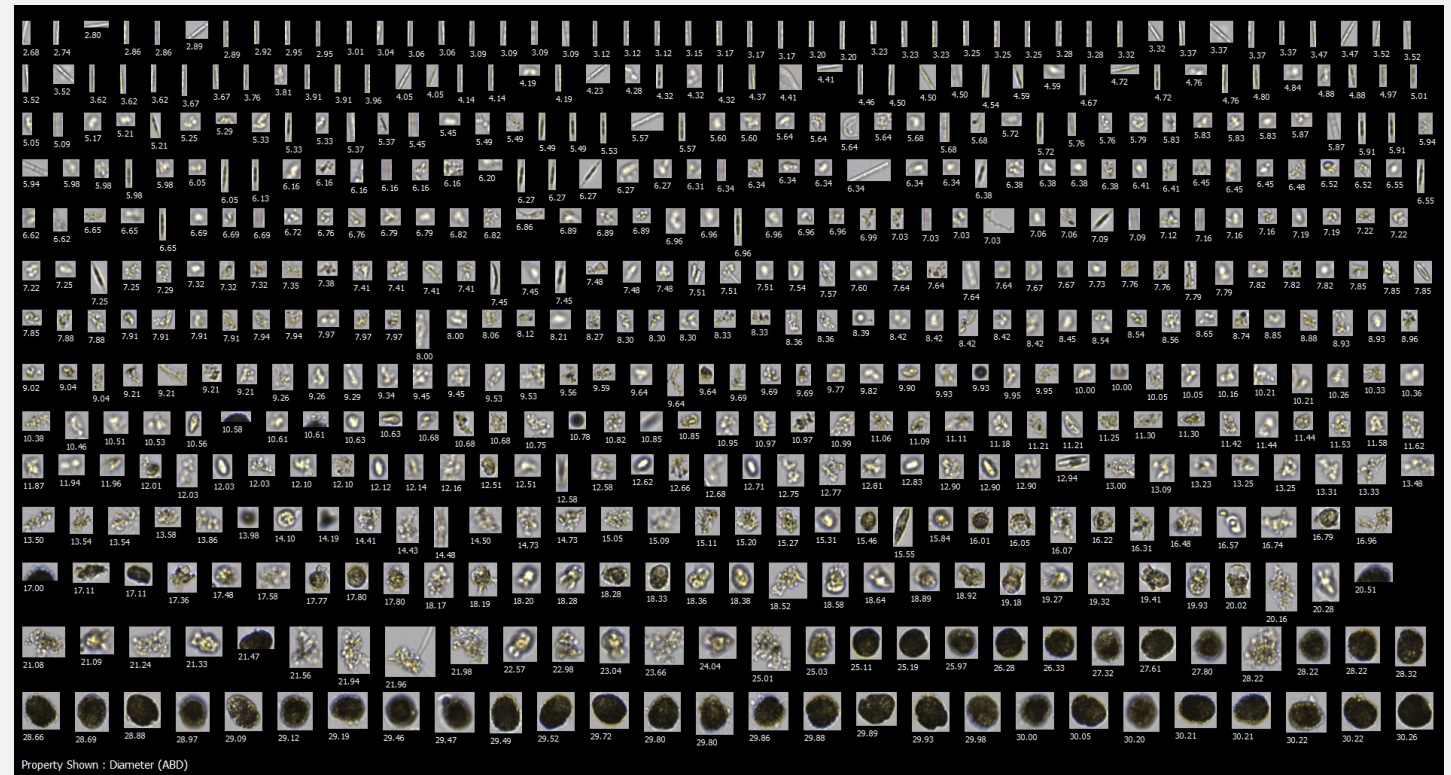


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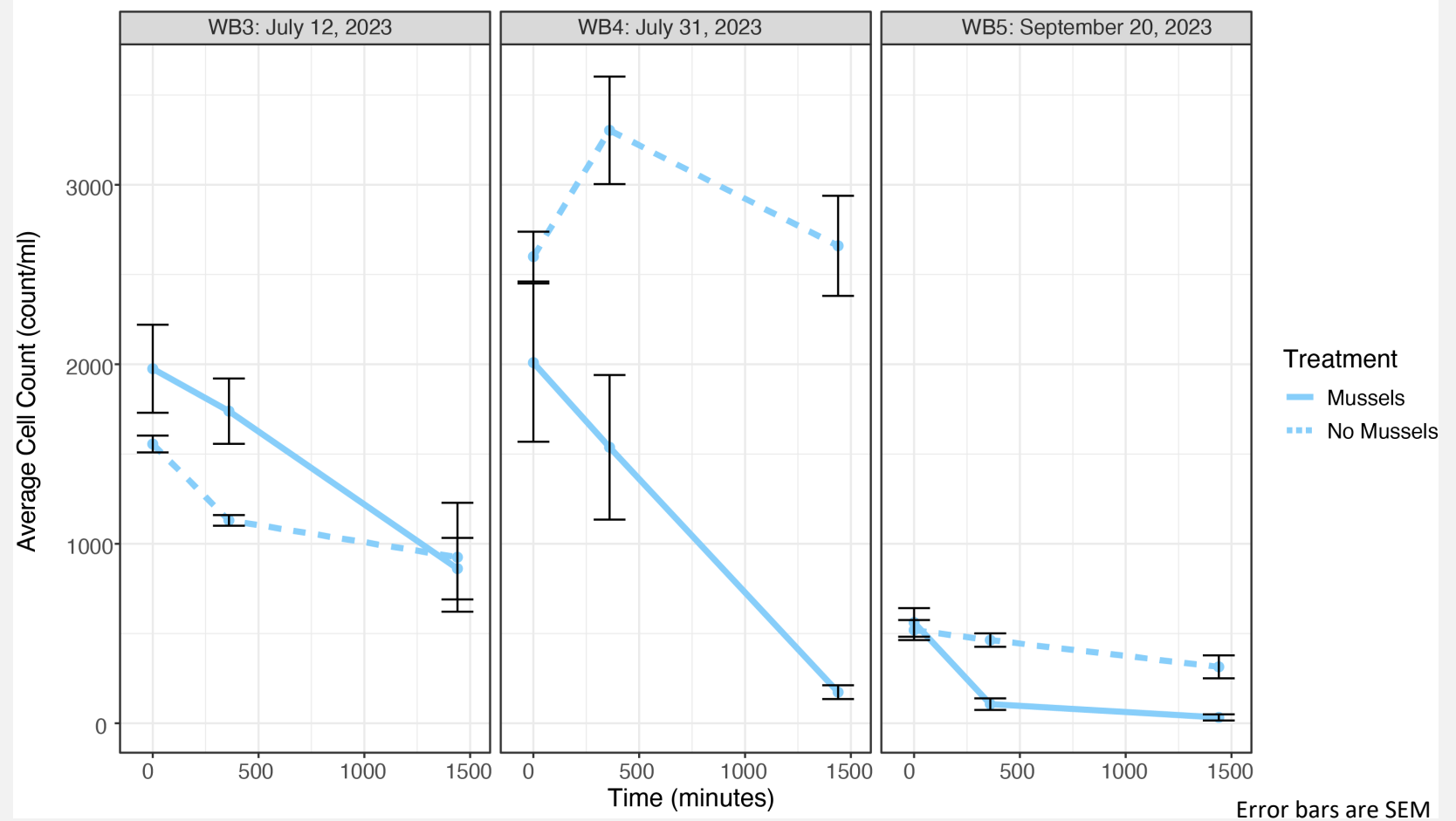
FLOWCAM IMAGES SHOW VARIATION IN ALGAE SPECIES

- Three experiments:
 - WB3 – July 12, 2023
 - WB4 – July 31, 2023
 - WB5 – September 20, 2023



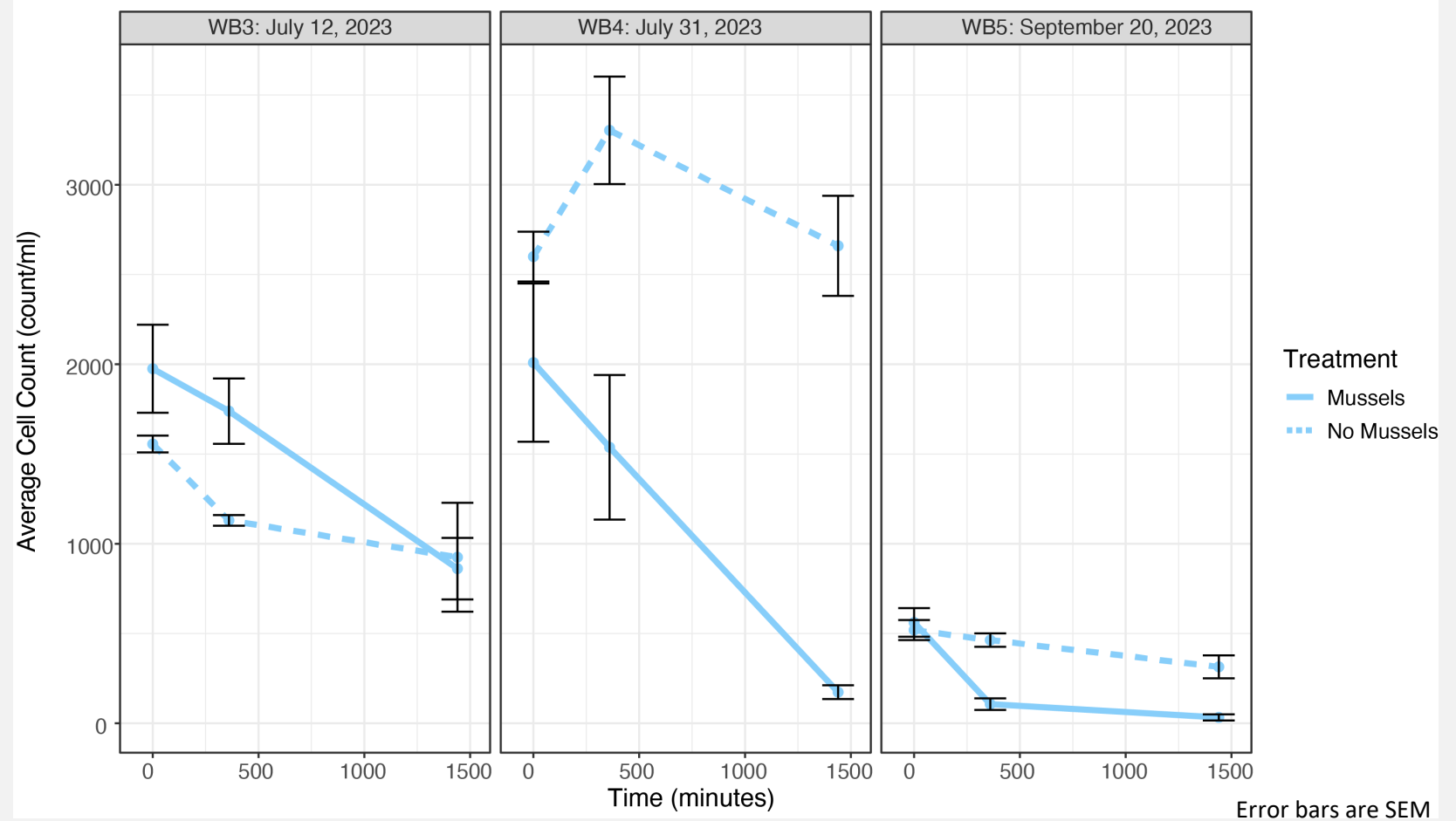
MUSSELS REDUCE CELL COUNTS

Effect of Treatment:
 WB3 – 0.152
 WB4 – **p < 0.001**
 WB5 – **0.042**

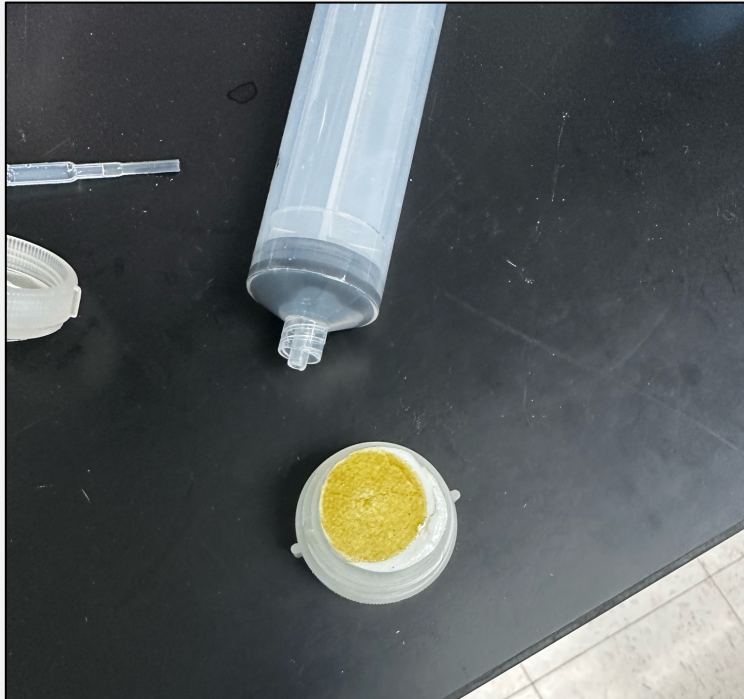


MUSSELS REDUCE CELL COUNTS

| Effect of Time | Mussel | No Mussel |
|----------------|--------------|--------------|
| WB3 | 0.036 | 0.111 |
| WB4 | 0.039 | 0.039 |
| WB5 | 0.039 | 0.368 |



NITROGEN



- Nitrogen analysis from particulate matter
- Samples sent to Nutrient Analytical Services Laboratory

MUSSELS REDUCE NITROGEN CONCENTRATIONS

Effect of Treatment:

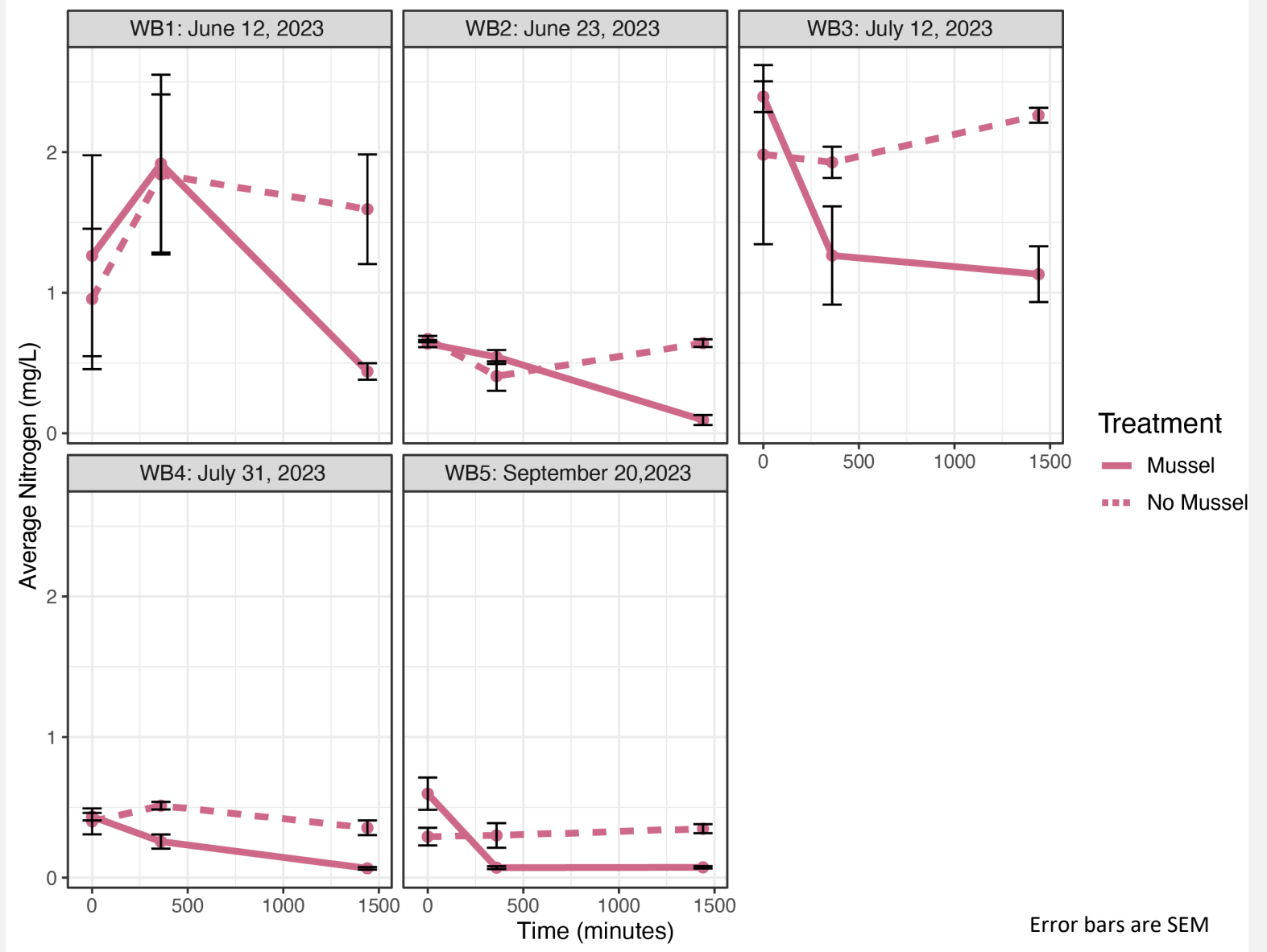
WB1 – $p = 0.686$

WB2 – $p = \mathbf{0.005}$

WB3 – $p = 0.129$

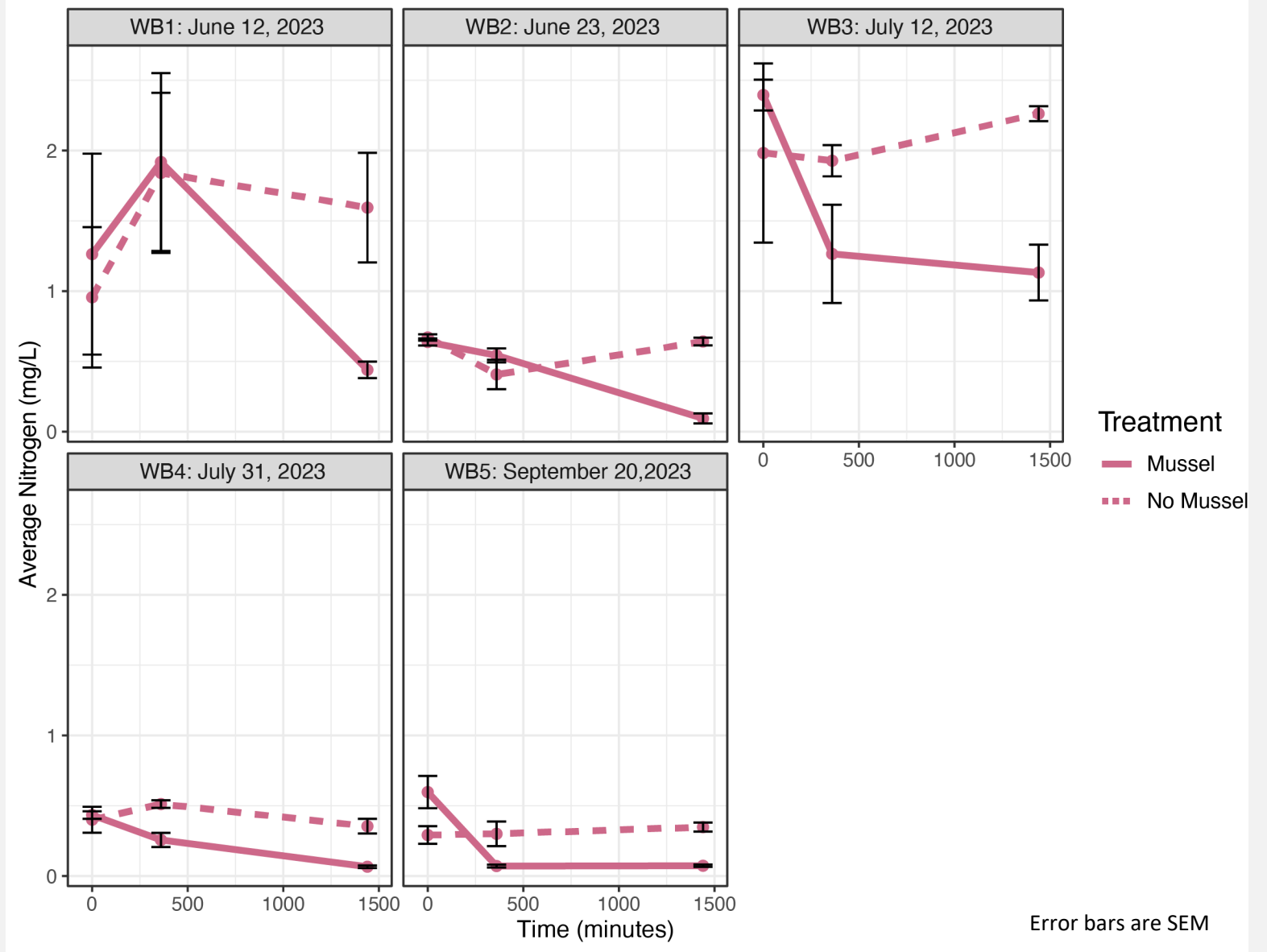
WB4 – $p = \mathbf{0.012}$

WB5 – $p = 0.254$



MUSSELS REDUCE NITROGEN CONCENTRATIONS

| Effect of Time | Mussel | No Mussel |
|----------------|---------------------|--------------|
| WB1 | 0.472 | 0.091 |
| WB2 | p < 0.001 | 0.033 |
| WB3 | 0.006 | 0.683 |
| WB4 | 0.014 | 0.105 |
| WB5 | p < 0.001 | 0.808 |



CONCLUSIONS AIM 4

- Mussels reduce IVCH levels of wild algae blooms
- Mussels can reduce nitrogen
- Numerous variables to consider:
 - Mussel stage
 - Algae bloom composition
 - Other filter feeders



SUMMARY OF RESULTS

1. Determine if *M. leucophaeata* can reduce cultured algae



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Chlorophyll



=



Clearance Rate

3. Examine the effect of water salinity on clearance rate



4. Determine if *M. leucophaeata* can reduce natural algae blooms

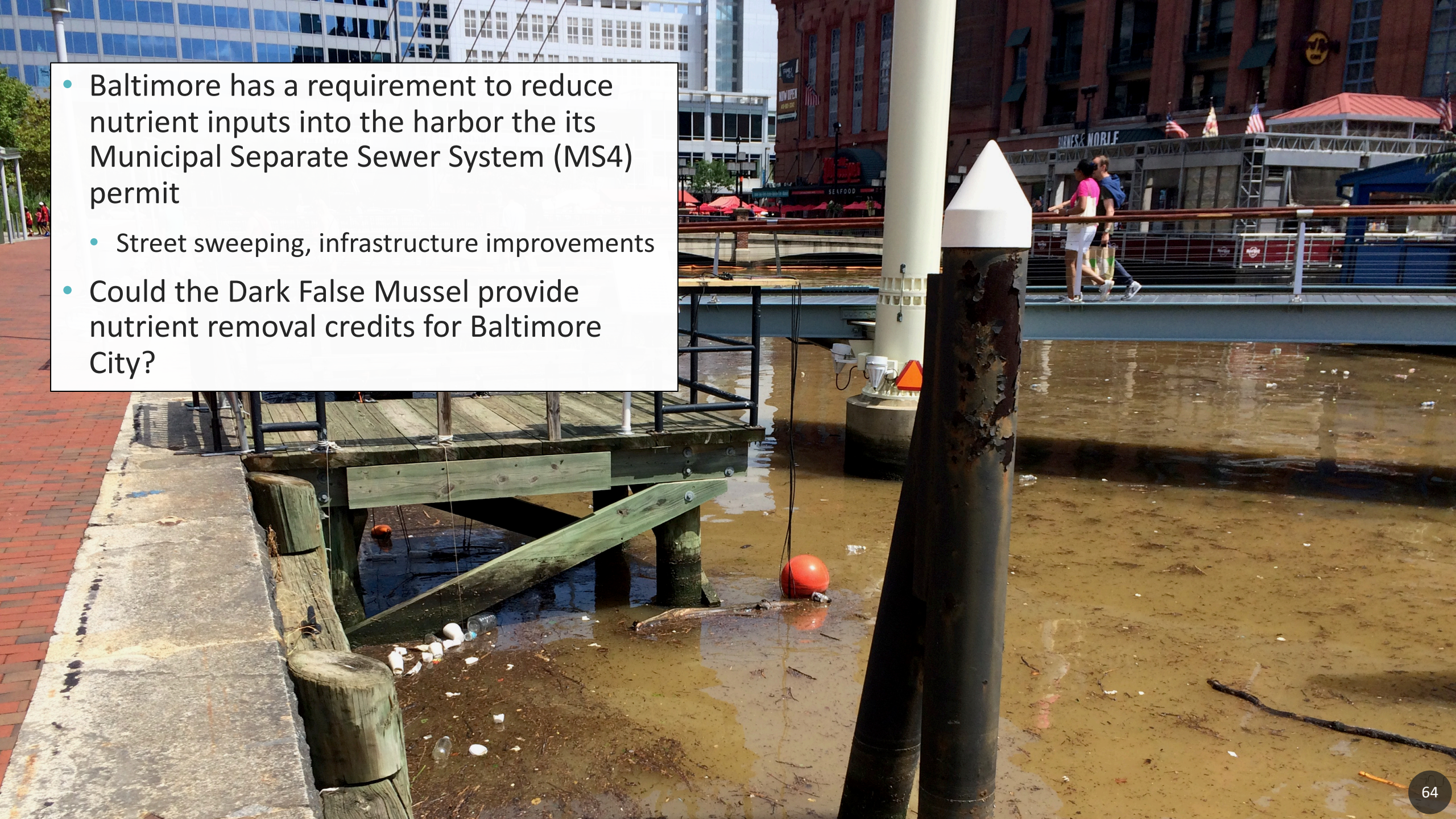


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IVCH

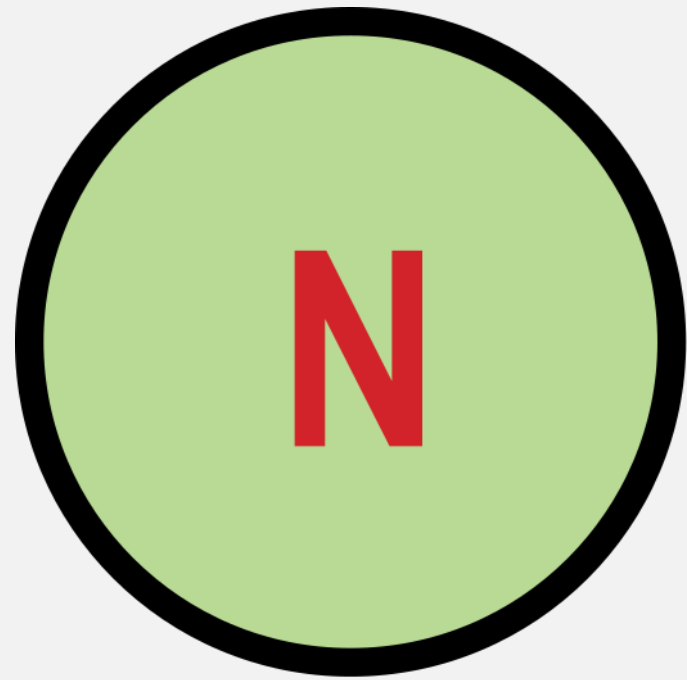
- Baltimore has a requirement to reduce nutrient inputs into the harbor the its Municipal Separate Sewer System (MS4) permit
 - Street sweeping, infrastructure improvements
- Could the Dark False Mussel provide nutrient removal credits for Baltimore City?



OYSTER NUTRIENT CREDIT TRADING

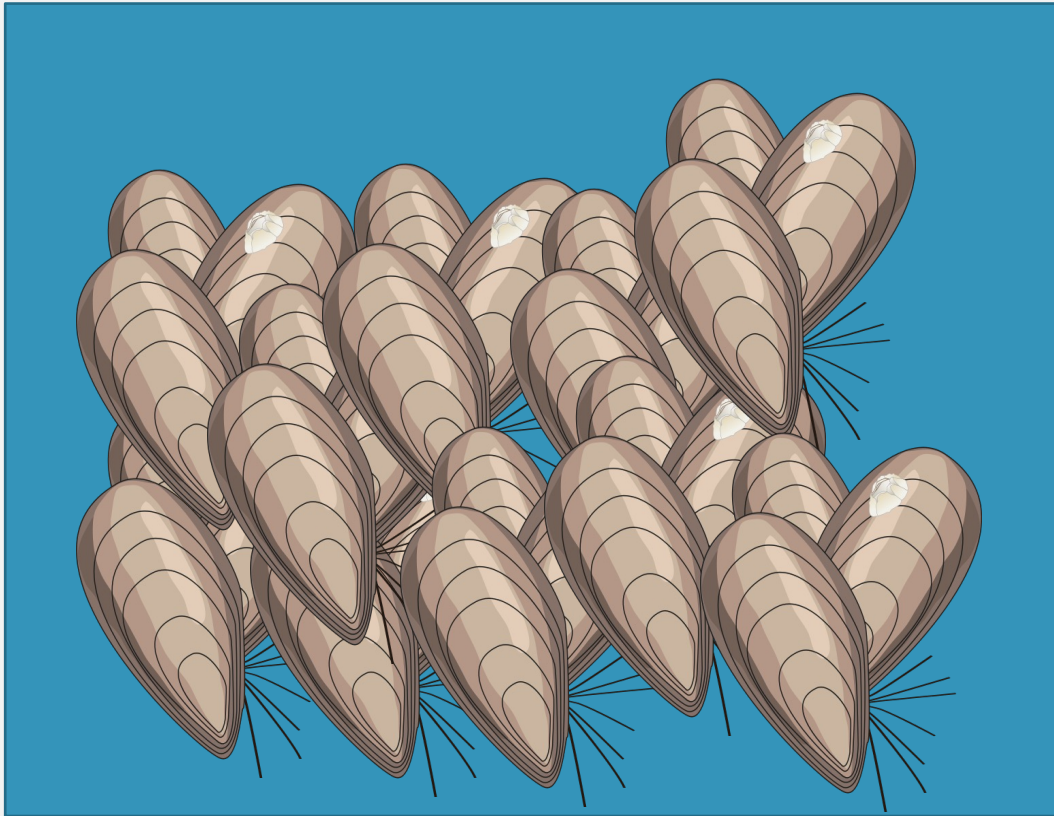


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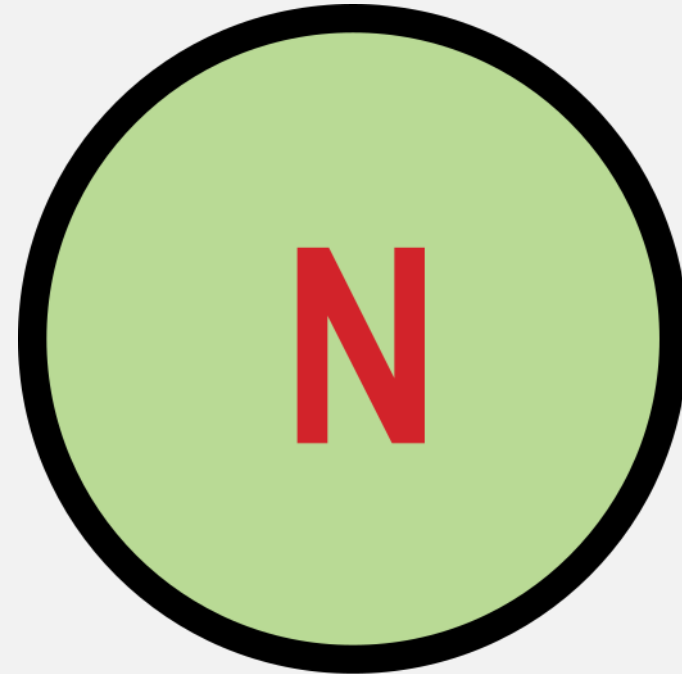


\$190/ lb of Nitrogen

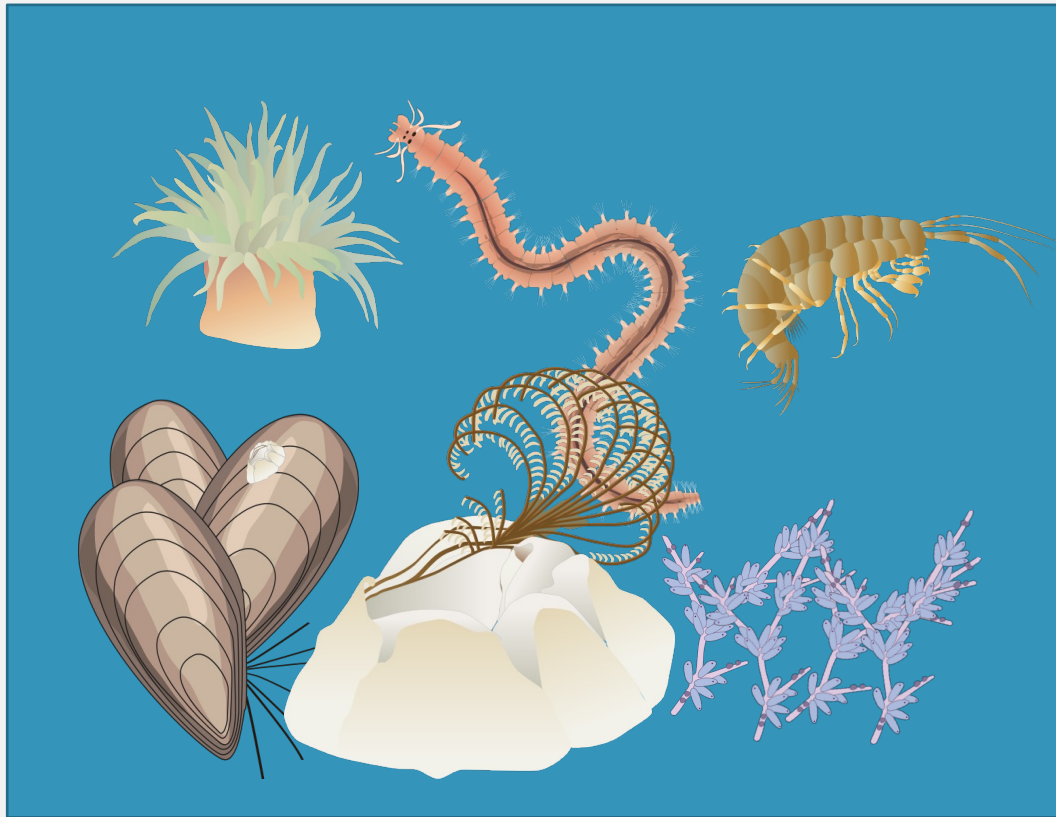
MUSSEL NUTRIENT CREDITS?



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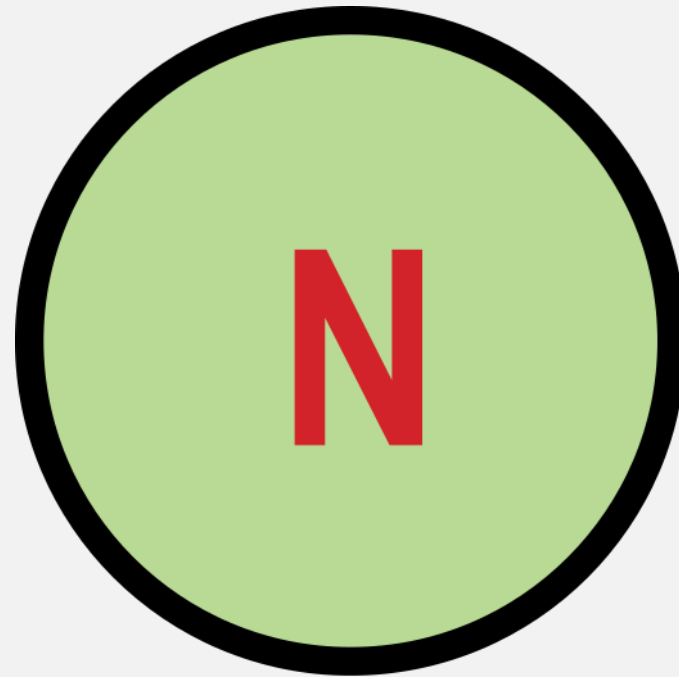


BIOFOULING NUTRIENT CREDITS?

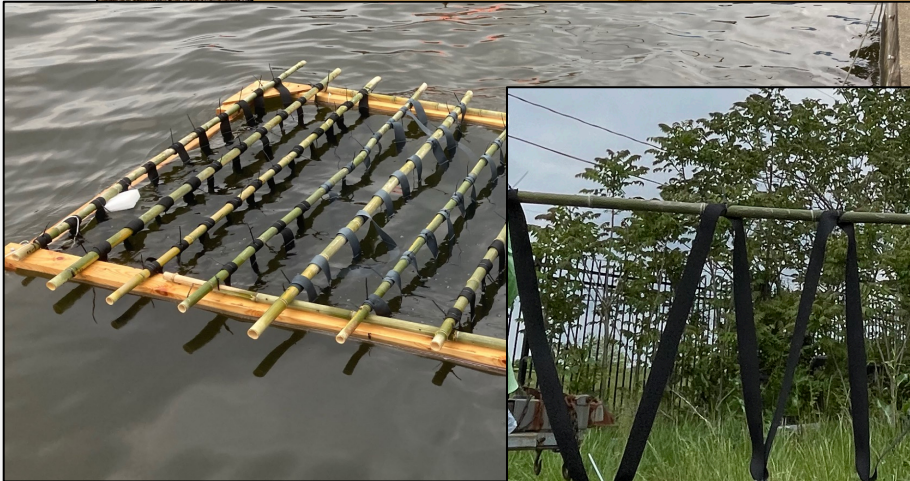
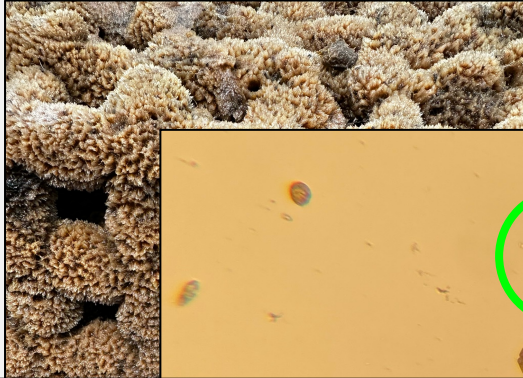


Biofouling community

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FUTURE DIRECTIONS



- More filter feeders to examine
- Other environmental factors to investigate:
 - Algae bloom composition
 - Stage and age of mussels
- Other ecosystem services to consider
- Create a model for total nutrients removed by this ecosystem service

CONCLUSIONS

- Mussels can reduce both lab grown and wild algae blooms
- Provides a promising alternative to oysters and a way to remove nutrients from Baltimore Harbor
- Future work should focus on the biofouling community and understanding other ecosystem services that the growing structures may provide



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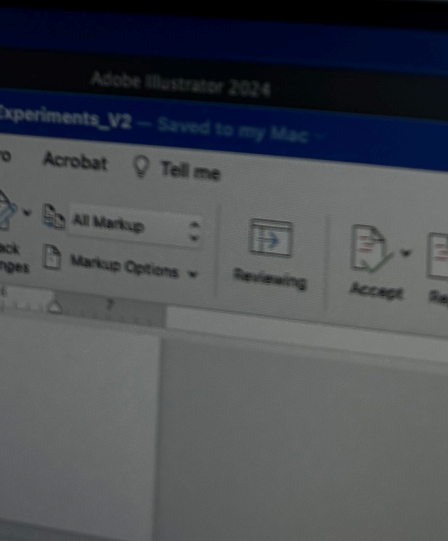


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UMBC

Email: akido1@umbc.edu



Questions?

