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Preliminary Report: Resource Limitation Bioassays (Light, N, & P Limitation) ITAT June 26, 2024

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Paleena Amy, Douglas Bell

Talk Outline (Sept 2023 – June 2024)

(interrupt and ask questions as needed)

- **(5 min) Context – Resource limitation of phytoplankton in Chesapeake Bay**
  - **Fisher et al. 1992, 1999, Kemp et al. 2005, Zhang et al. 2021, 2022**
  - **Spatial and seasonal differences in light, P, and N limitation of phytoplankton**
  - **Development of empirical models (Elgin Perry, Qian Zhang)**
- (5 min) Bioassay Methodology in the Bay, Sampling Scheme, Observations
  - Excellent cooperation from sampling teams
  - Occasional challenges with sampling vessels
- (10 min) Results
  - Nutrient/Biomass vs. Bioassay Data.
  - Examples of Light, N, P limitation
- (5 min) Current Interpretation of Bioassays
  - *Acknowledge the big question - what's different!?*
- (5 min) Additional research projects and year 2 station changes
  - eDNA, Pigments, zooplankton
  - September transition of tributary stations
- Open for Q/A

Paradigm of Resource Limitation of Phytoplankton

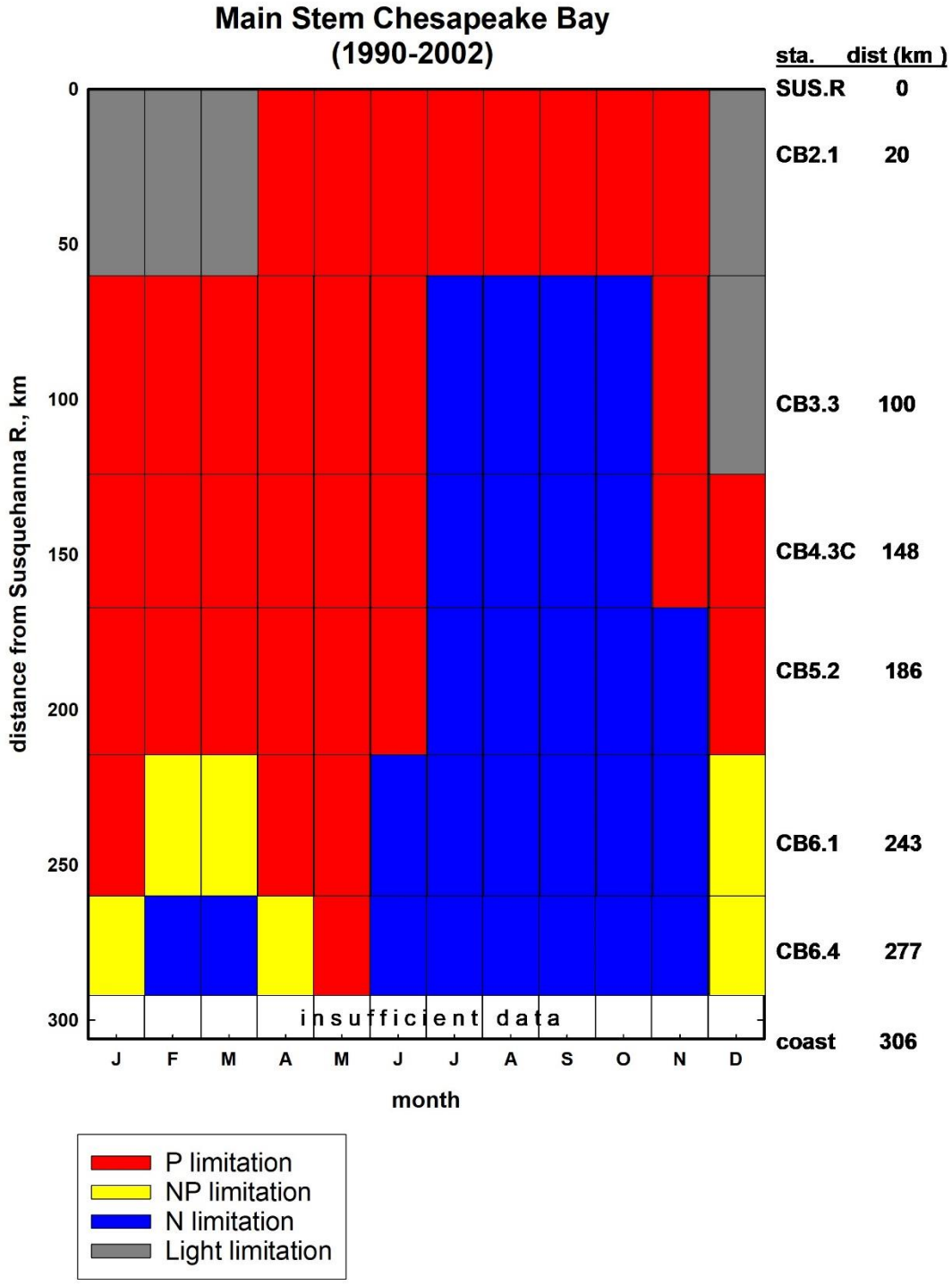
Resources needed for algal growth: **light**, P, and N

Light Limitation occurs in upper bay in winter: high river flow, turbid, nutrient-rich, deeply mixed (little salinity)

Riverine inputs have high N/P (>100:1 molar, excess N)
increased salinity downbay leads to vertical stratification

Stratification reduces mixing depth, increases light,
Stimulates algal growth
Phytoplankton consume N and P in a 16:1 ratio
P is depleted prior to N in spring, leading to **P limitation**

Coastal seawater has a low N/P (<16:1 molar)
River flow decreases in summer, salinity increases
Hypoxic sediments remove N and release P
N depleted, P enriched: **N limitation**



Development of Empirical Models of Resource Limitation

Fisher, Gustafson, and Perry (reports, excel algorithm, not published)
Multi-parameter models: Cal 1990-1996, Val 1997-1999

Light limitation: depth of mixing, mixed layer depth/Secchi?
P limitation: DIN/PO4, TN/TP, POC/PP (92% precision)
N limitation: DIN, TN/TP, chla/seston (97% precision)

Zhang et al 2021, 2022 (trib and mainstem predictions, published)
CART models: modest improvements in mainstem bay and tribs.
CART models need validation data

Transition from concepts to applications

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  - Development of empirical models (Elgin Perry, Qian Zhang)
- **(5 min) Bioassay Methodology in the Bay, Sampling Scheme, Observations**
  - **Profile information**
  - **Excellent cooperation from sampling teams**
  - **Occasional challenges with sampling vessels**
- (10 min) Results
  - Vertical profiles
  - Examples of Light, N, P limitation
- (5 min) Current Interpretation of Bioassays
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Bioassay Stations

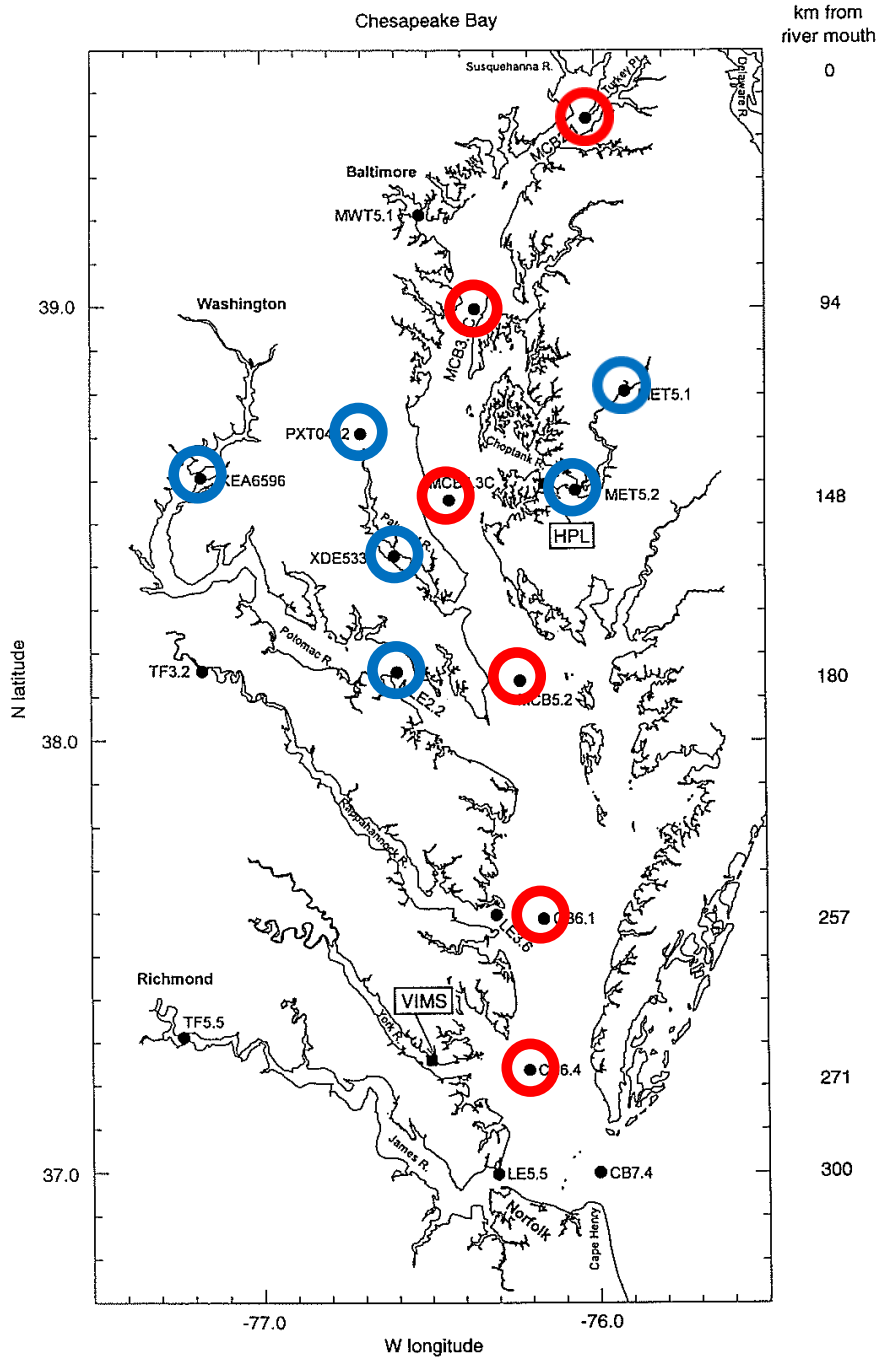
monthly sampling at 12 stations (year 1)

6 main stem

- CB2.1 example
- CB3.3C
- CB4.3C example
- CB5.2
- CB6.1
- CB6.4 example

6 tributary (changes in year 2)

- ET5.1 Choptank
- ET5.2 Choptank
- TF2.3 Potomac
- LE2.2 Potomac
- TF1.5 Patuxent
- LE1.1 Patuxent



Bioassay Protocols: sample collection and handling

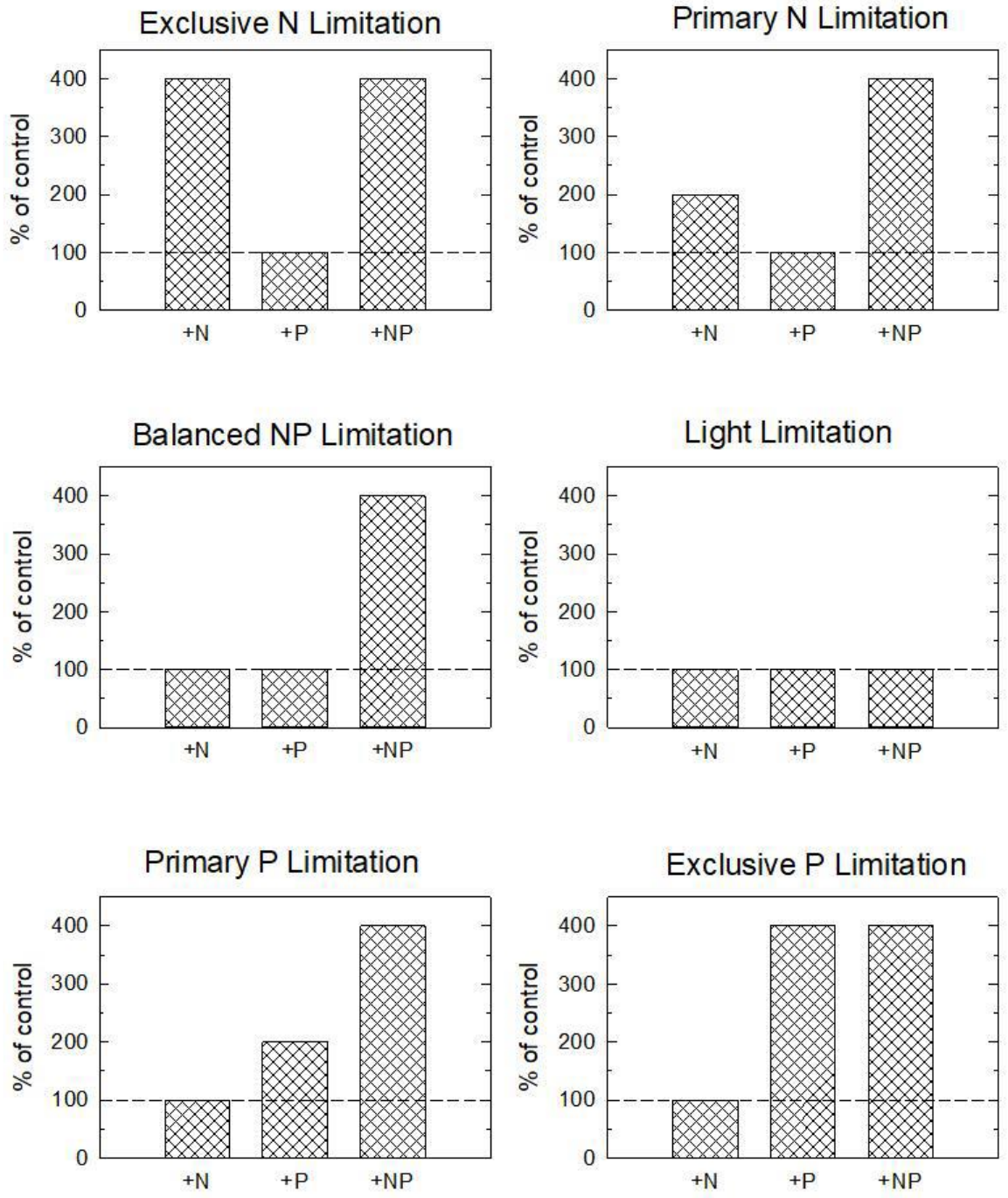
- MD and VA monitoring teams collect **surface** water samples for us
  - Same time/location as normal monitoring
  - Typically 3 groups of 2-5 stations each month
  - Provide data on **vertical profiles**
  - Excellent cooperation from monitoring teams
- We pick up samples in screened carboys from the monitoring teams
  - reduces light and temperature shock
- Samples are transported to HPL on the sampling day
  - Stored overnight in an incubator at bay temperature and 20% ambient light
  - Goal is minimal temperature change, moderate light exposure
- Bioassays are started the next morning

Bioassay protocols: treatments and incubations

- Treatments: all unscreened (no zooplankton removal, intact plankton)
  - Initial samples of chlorophyll a and nutrients (test for handling artefacts)
  - 3 L subsamples of original station sample go into 4 L cubitaners
    - 2 controls (no additions, but incubated at 50% light
    - N treatment (+0.42 mg NH_4^+ L^{-1})
    - P treatment (+0.061 mg PO_4^{-3} L^{-1})
    - NP treatment (both N and P)
- Incubation conditions:
  - 50% light (PAR) in an incubator at HPL dock with running Choptank water
  - Maintained at Choptank River temperatures for 2-4 days
  - Duration depends on monitored incident light (PAR)
    - Goal: equivalent PAR of 1 average day for the month under the screen
  - End: samples of chlorophyll a from each cubitainer (test for responses)

Bioassay interpretation:

- Treatment response is measured by chlorophyll a at the end of the incubation
- Normalized to average chlorophyll a of the controls
- Classified as:
  - Exclusive N (EXN)
  - Primary N (PRN)
  - Balanced NP (BNP)
  - Light limitation (NOR)
  - Primary P (PRP)
  - Exclusive P (EXP)



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- (5 min) Bioassay Methodology in the Bay, Sampling Scheme, Observations
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- **(10 min) Results**
  - **Vertical profiles**
  - **Examples of Light, N, P limitation**
- (5 min) Current Interpretations of Bioassays
  - Physical conditions.
  - *Acknowledge the big question - what's different!?*
- (5 min) Additional research projects and year 2 station changes
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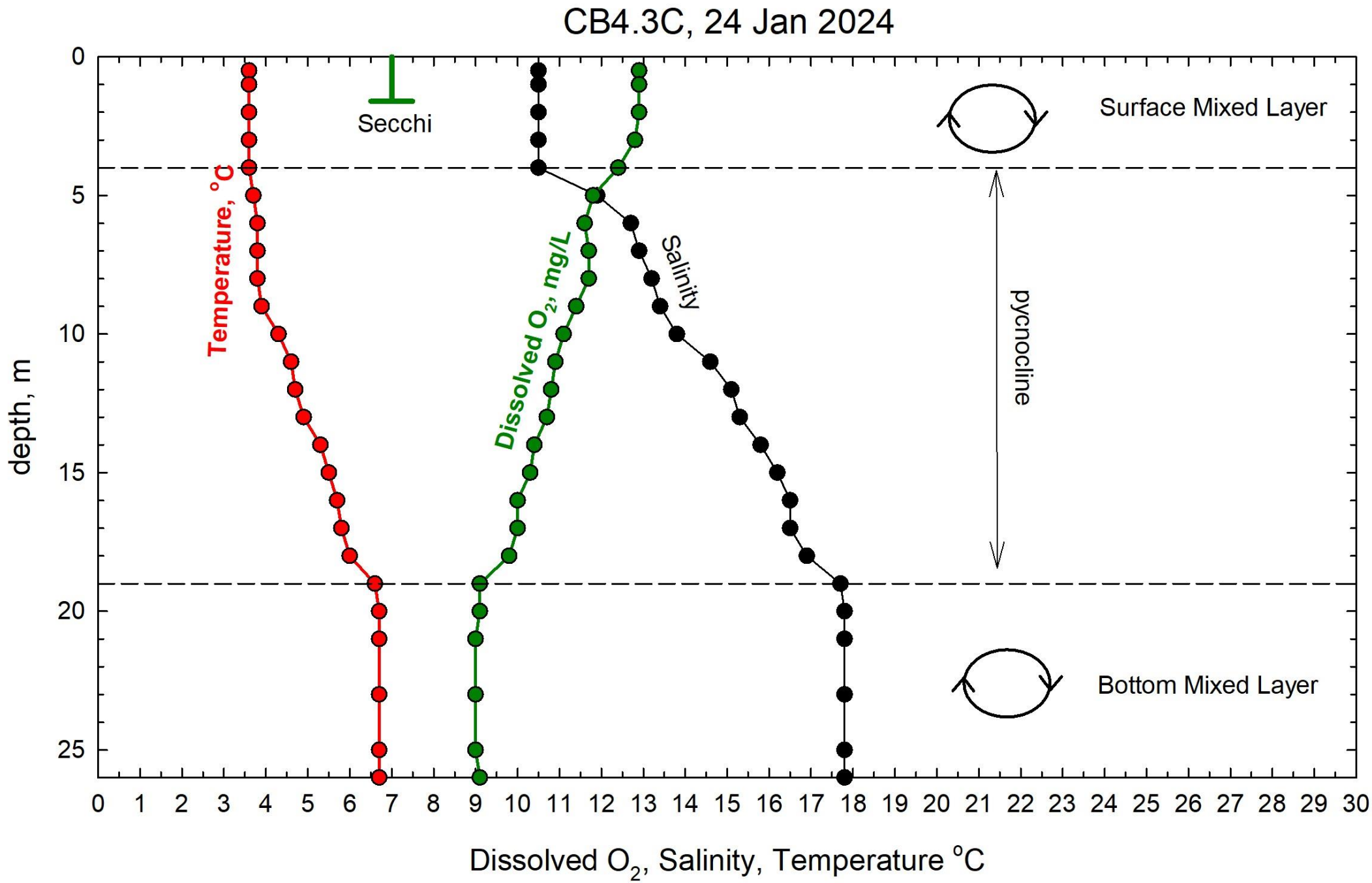
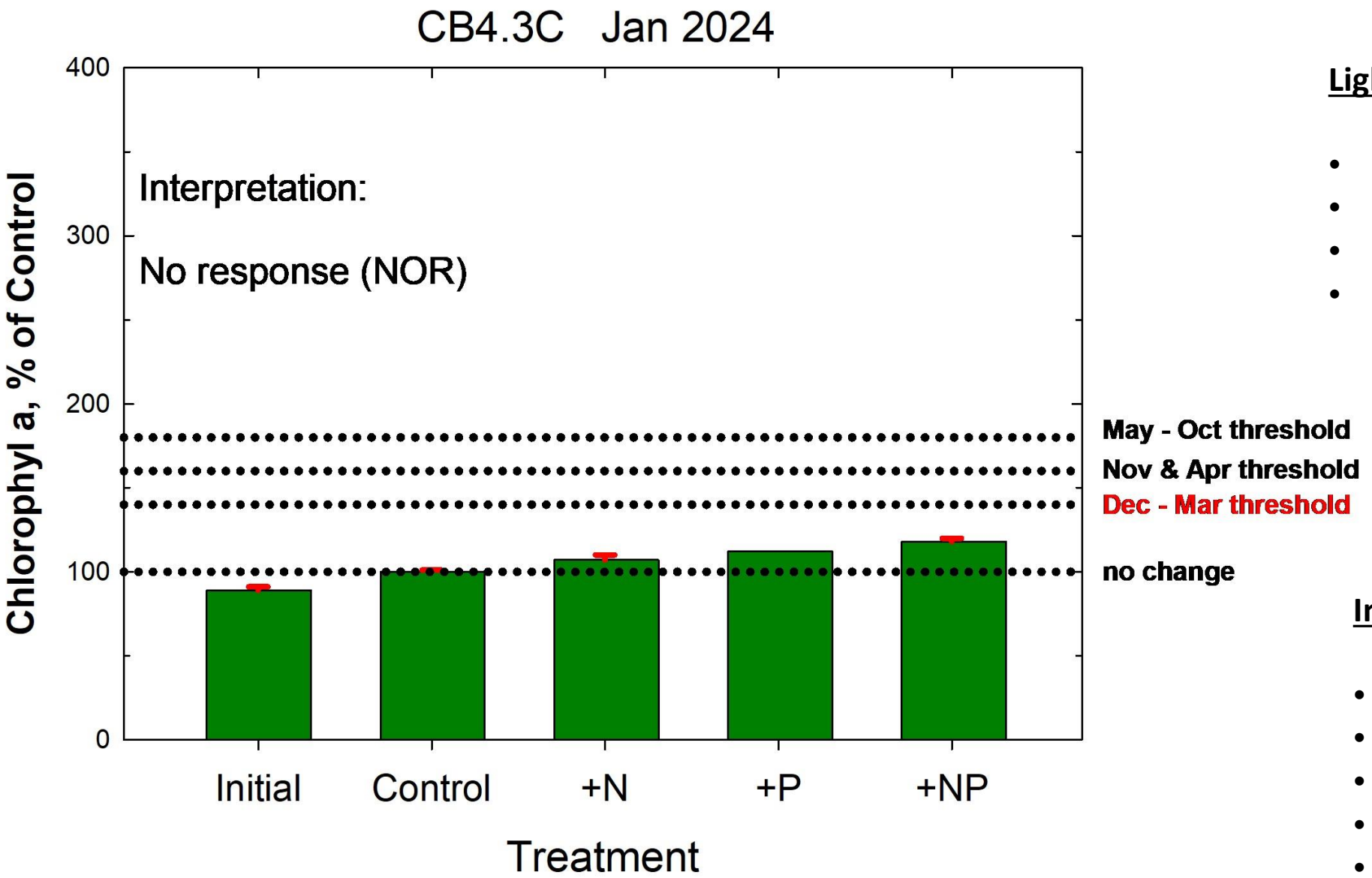

CB4.3C, 24 Jan 2024
depth, m
0
5
10
15
20
25
Secchi
Temperature, °C
Dissolved O2, mg/L
Salinity
Surface Mixed Layer
pycnocline
Bottom Mixed Layer
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Dissolved O2, Salinity, Temperature °C



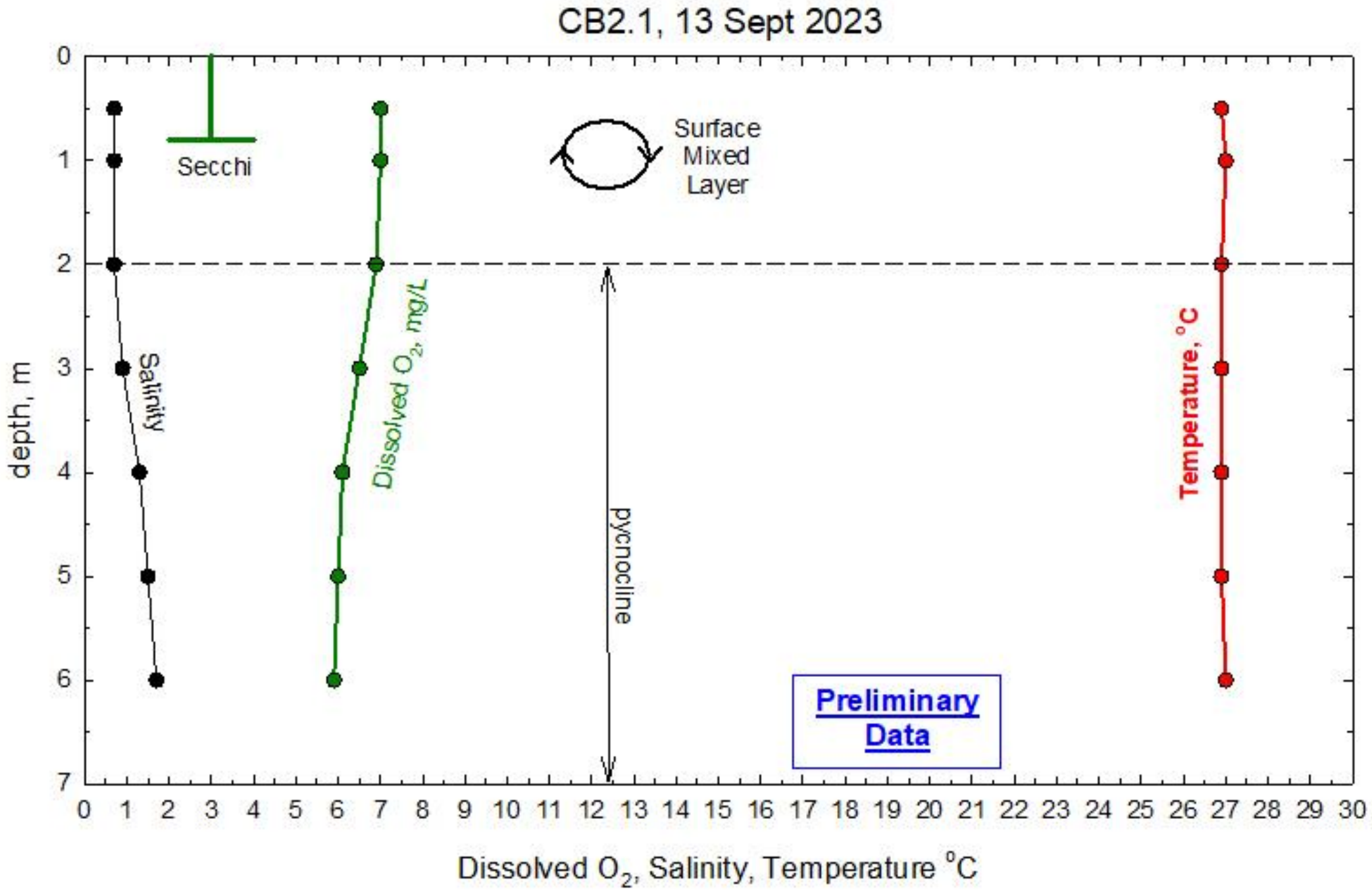
Light Limitation (NOR)

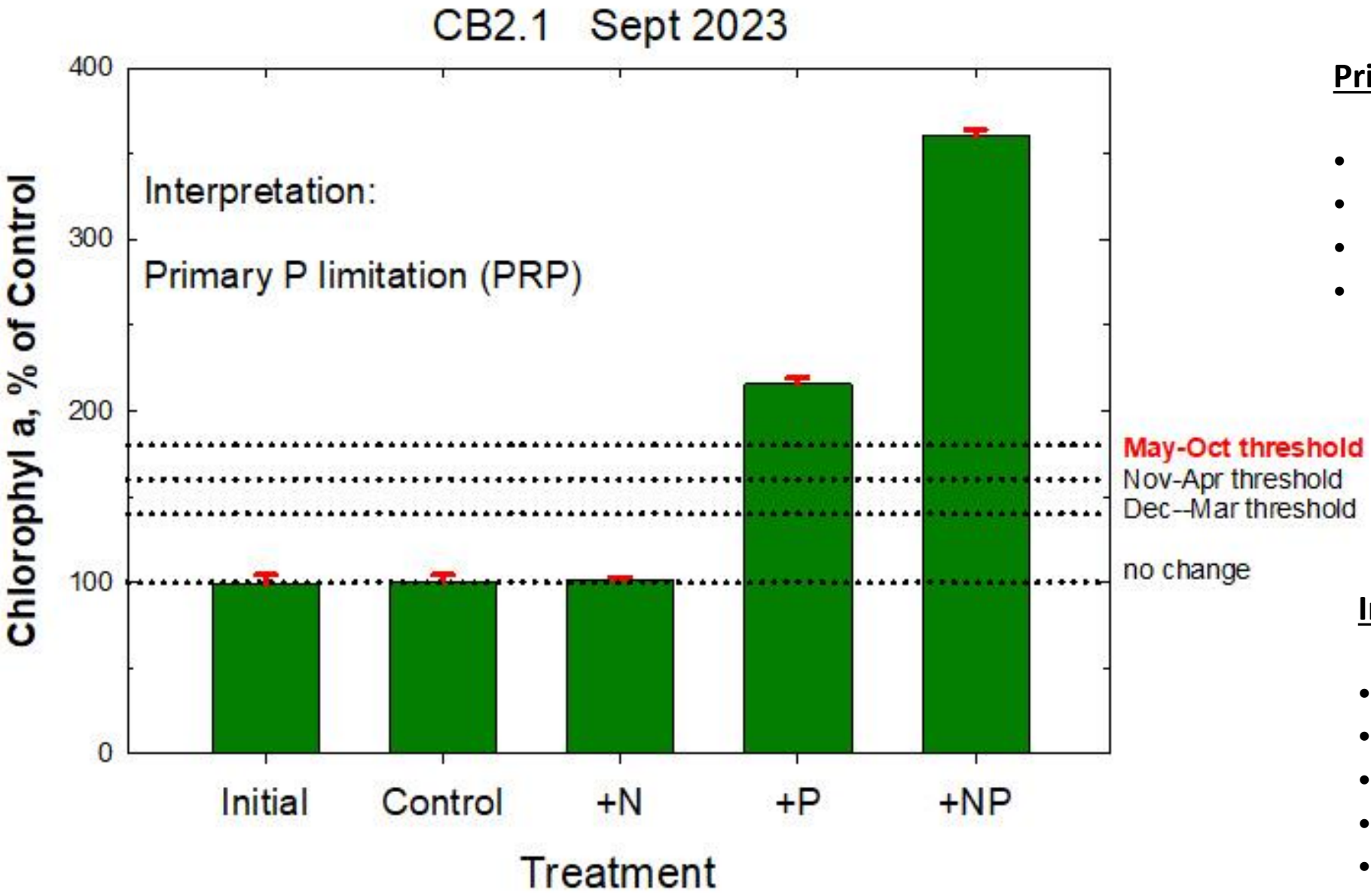
- Initial < Control
- No significant response to +N
- No significant response to +P
- No significant response to +NP

Error propagation:
handling,
sub-sampling,
analytical, etc.

Initial ambient nutrients

- NH_4^+ = 0.013 mg N L^{-1}
- NO_3- = 0.500 mg N L^{-1}
- PO_4^{-3} = 0.113 mg P L^{-1}
- Depth of mixing = 4 m
- Water column depth = 27 m


CB2.1, 13 Sept 2023
depth, m
Secchi
Surface
Mixed
Layer
Salinity
Dissolved O2, mg/L
pycnocline
Temperature, °C
Preliminary
Data
Dissolved O2, Salinity, Temperature °C

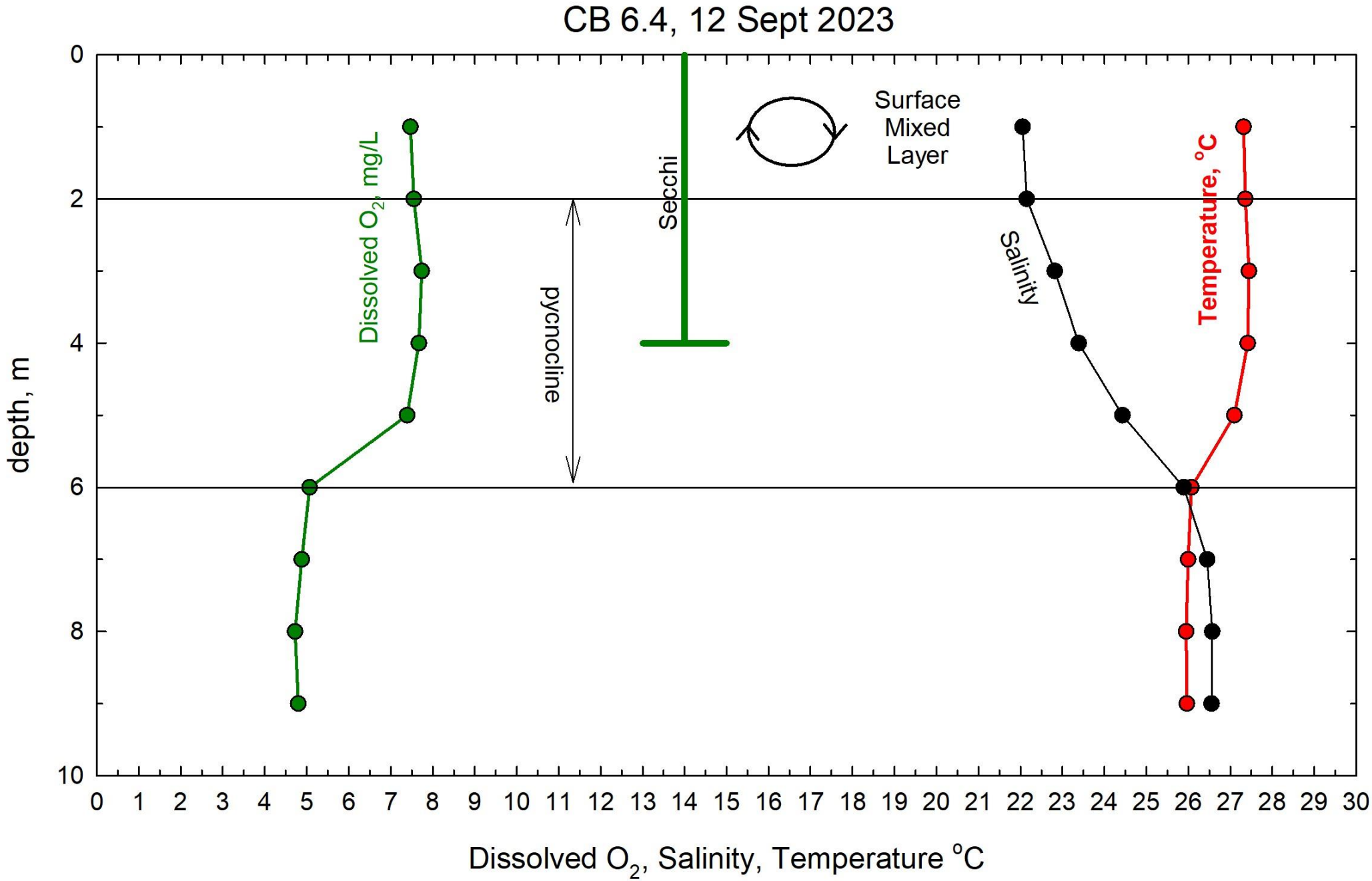


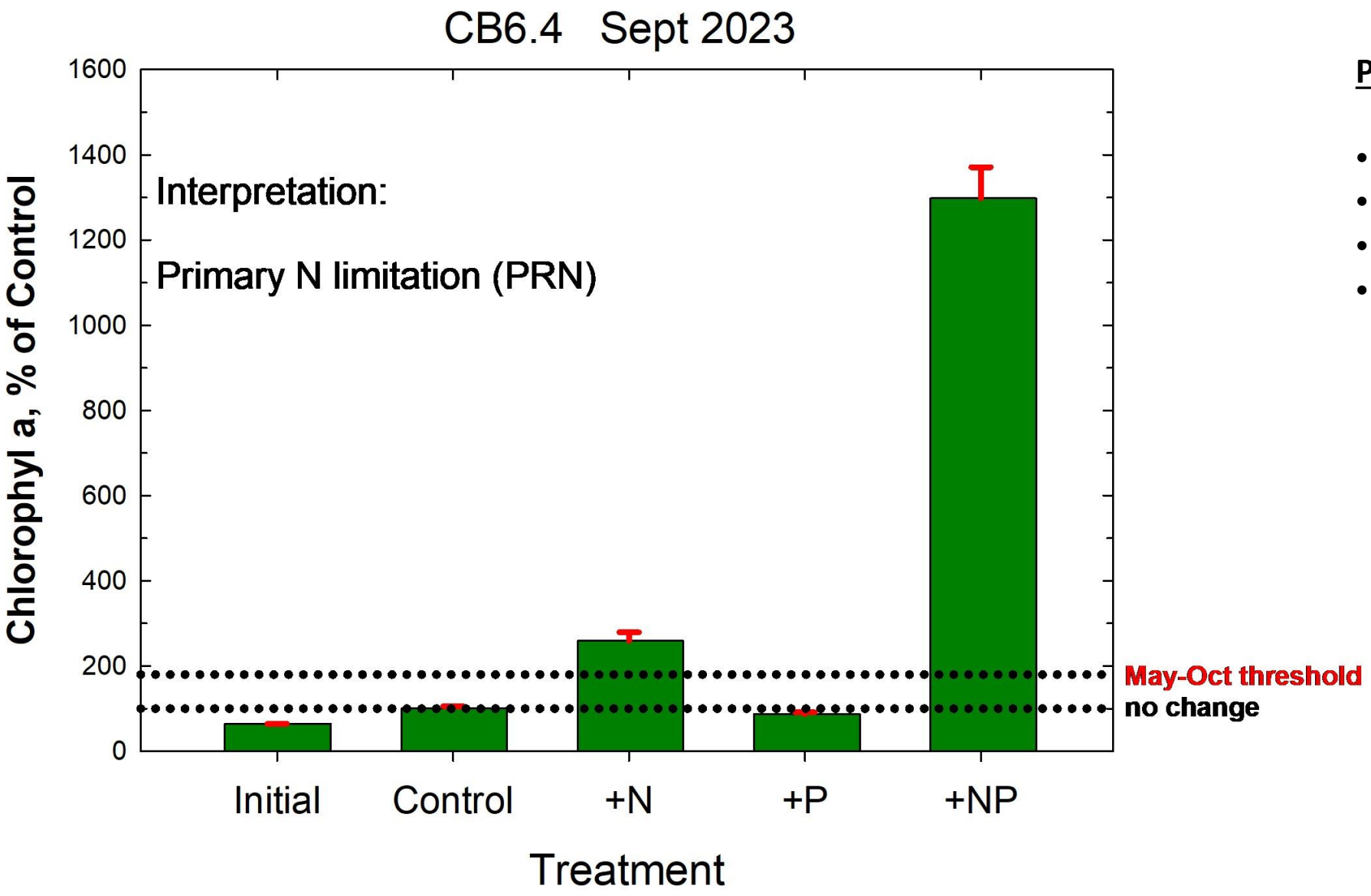
Primary P Limitation (PRP)

- Initial = Control
- No response to +N alone
- Doubling of chla due to +P
- Greatest response to +NP

Initial ambient nutrients

- NH_4^+ = 0.020 mg N L^{-1}
- NO_3- = 0.006 mg N L^{-1}
- PO_4^{-3} = 0.003 mg P L^{-1}
- Depth of mixing = 2 m
- Water column depth = 7 m


CB 6.4, 12 Sept 2023
depth, m
0
2
4
6
8
10
Dissolved O_2, mg/L
pycnocline
Secchi
Surface
Mixed
Layer
Salinity
Temperature, °C
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30
Dissolved O_2, Salinity, Temperature °C



Primary N Limitation (PRN)

- Control > Initial
- Doubling of chla to +N alone
- No response to +P alone
- 14 X response to +NP

Initial ambient nutrients

- NH_4^+ = 0.025 mg N L^{-1}
- NO_3- = 0.006 mg N L^{-1}
- PO_4^{-3} = 0.003 mg P L^{-1}
- Depth of mixing = 2 m
- Water column depth = 10 m

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Big Question: Do we see differences so far?

- To date we have done bioassays monthly since last September
  - Sept 2023 to June 2024
    - Total bioassays since Sept 2023 = 113
    - Total bioassays during 1990-2002 = 1058
  - All data components are currently available for Sept 2023 – Feb 2024
- Not enough data yet for us to see changes relative to 1990-2002
- Main goal is NOT to see changes in 2023-2025 relative to 1990-2002
- 2023-2025 data are for validation of empirical models
- However, we will do a comparison of 2023-2025 and 1990-2002 data
  - Low statistical power due to sample sizes?

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Side Projects:
taking advantage of our water samples

1- Phytoplankton Taxonomic Diversity - Greg Silsbe (HPL)

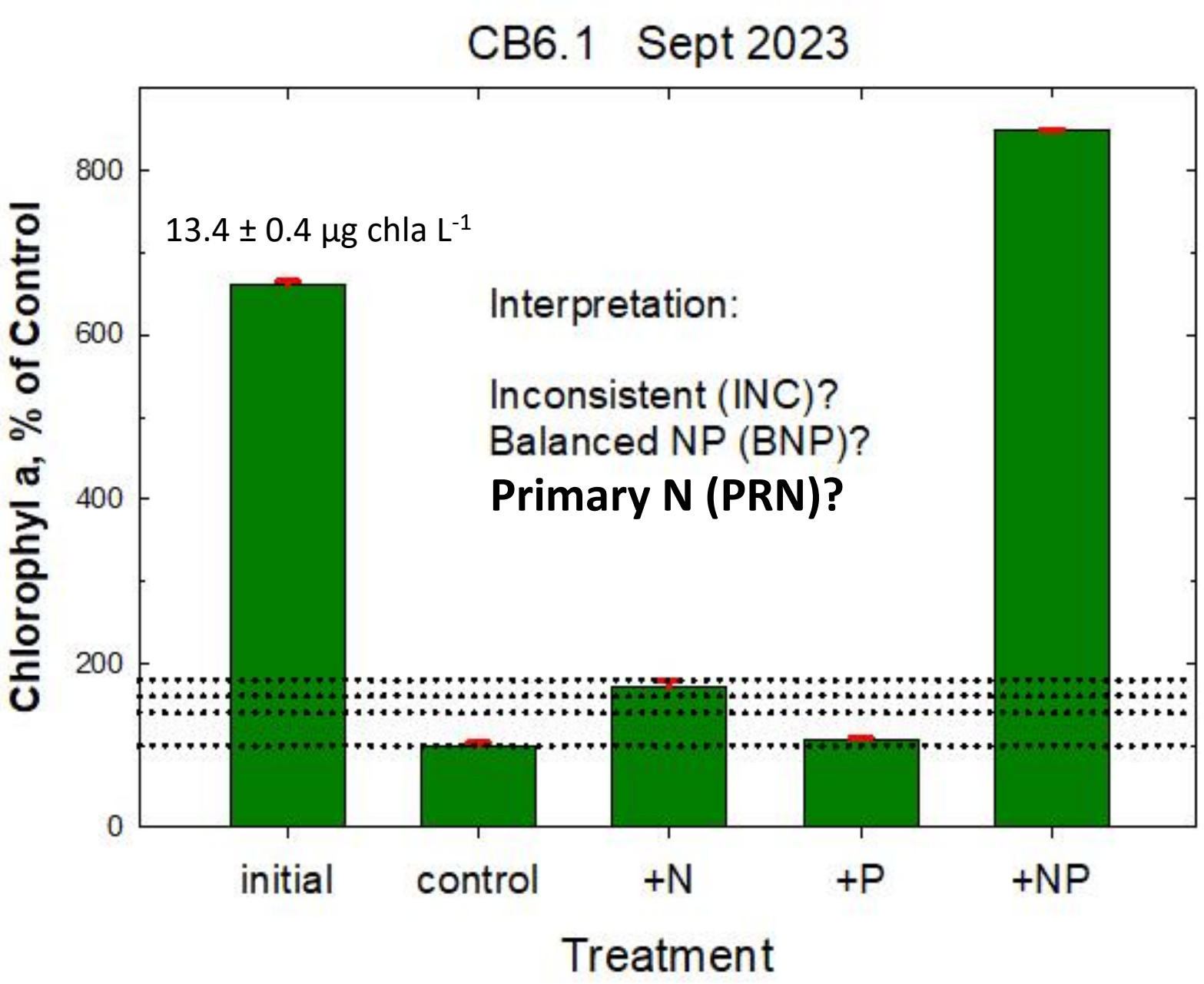
IFCB imaging for HPLC Community composition based on photosynthetic pigments.

2- Cyanobacteria eDNA - Judy O'Neil (HPL), Feng Chen (IMET)

Molecular quantification of >3 µm, >1 - 3 µm and <0.22 µm plankton size fractions

3- Zooplankton grazing during bioassays - Judy O'Neil, Sarah Gasko (HPL)

Zooplankton grazing (>200 µm) and quantification of copepod species



Occasional large decreases in chla in the control compared to the initial now and during 1990-2002

Effect of zooplankton grazing during the bioassay incubation?

Low initial nutrients and growth rates?

Grazing effect?

Initial/control = grazing index?

September transition of tributary stations:

- Proposed for Year 2:
  - Same mainstem stations
  - Change tributary stations
    - Year 1 tribs: Choptank, Patuxent, Potomac (all MD tribs)
      - One tidal fresh, one mesohaline station in each tributary
    - Proposed for year 3 tribs: two MD tribs, 1 VA trib
      - One tidal fresh, one mesohaline station in the VA tributary
- Need decision by August 1 to set up logistics
- Options
  - Change one MD trib to a VA trib (longer pickup logistics)
  - No changes
  - Other?
- Format of long-term database at end of project

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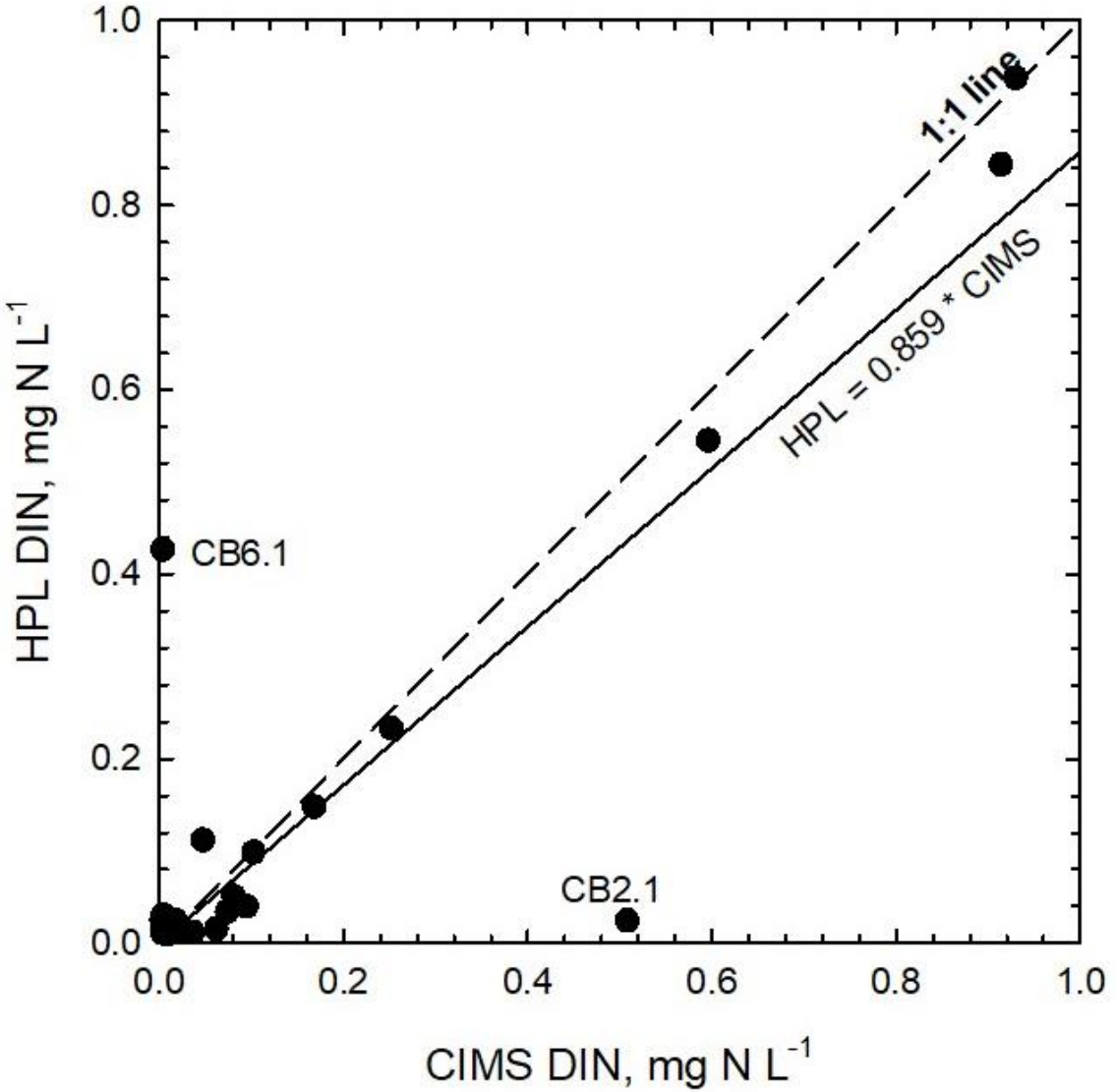
Nutrients and chla data (Sept – Dec 2023)

- Two sources of water:
  - Bay Program samples taken on the ship, analyzed at CBL
  - Samples taken at HPL 24 h later (initial samples for bioassays)
    - Nutrients analyzed at CBL
    - Chla measured at HPL
- Differences:
  - Bay Program samples taken first on ship, then our carboys are filled (30 min?)
    - Potentially a real spatial/temporal difference, not split samples
  - Some differences in analysis of chla (CBL grinds, we don't)
    - Potentially a real analytical difference
  - 24 h time difference between ship samples and our initial samples for bioassays
    - Potentially real changes in plankton over 24 h (e.g., growth or grazing)

Nutrients: DIN (nitrate + ammonium)



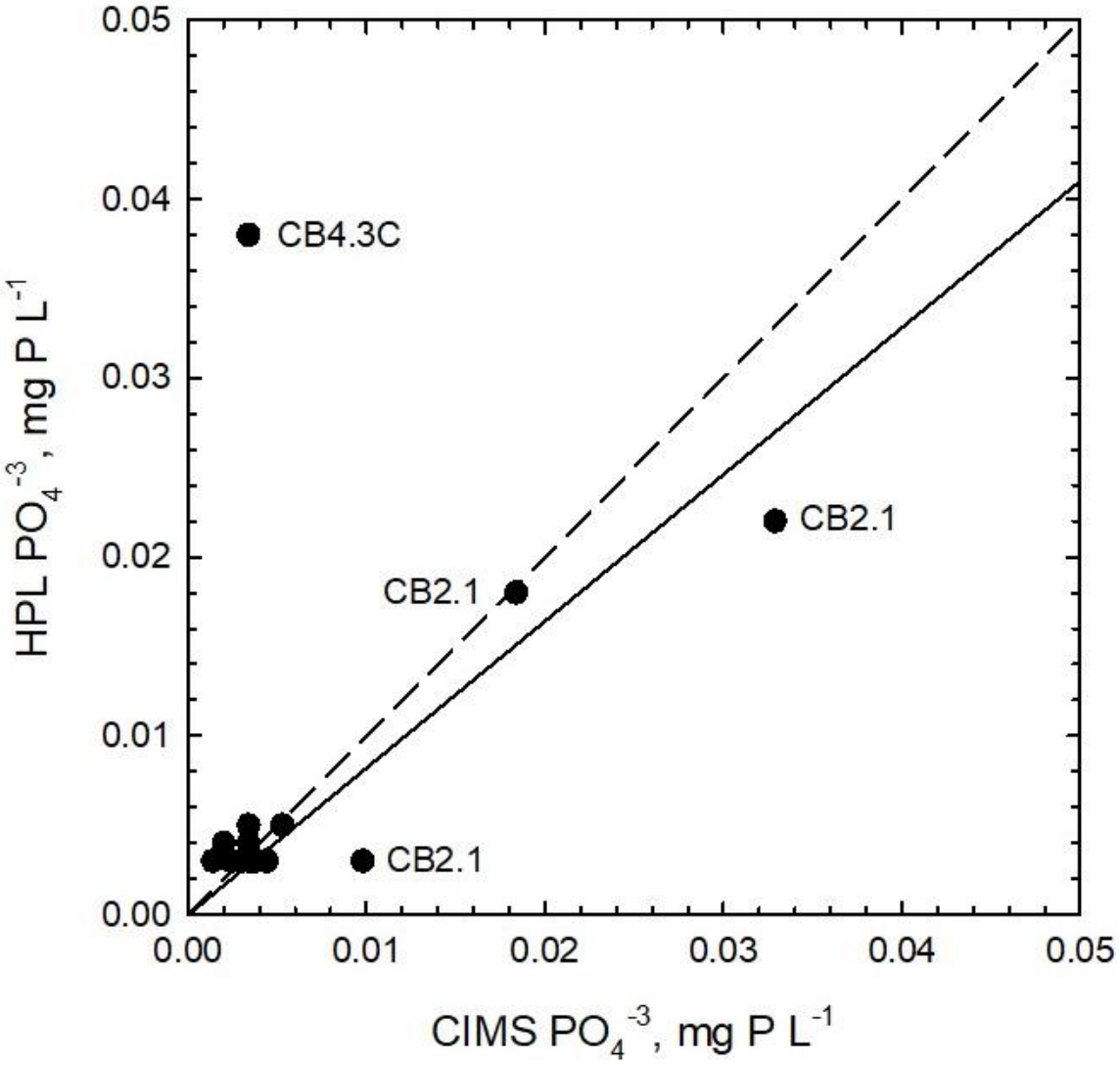
data analysis (DIN)

r^2 = 0.822
intercept defined = 0
slope = 0.858 ± 0.077
95% CL = 0.70 - 1.02

HPL = no bias, probable sample mislabeling

Possible causes:
- sample mislabeling (CB2.1, CB6.1)
- NO3 in Sept unlikely at CB6.1

Nutrients: phosphate



data analysis (PO4)

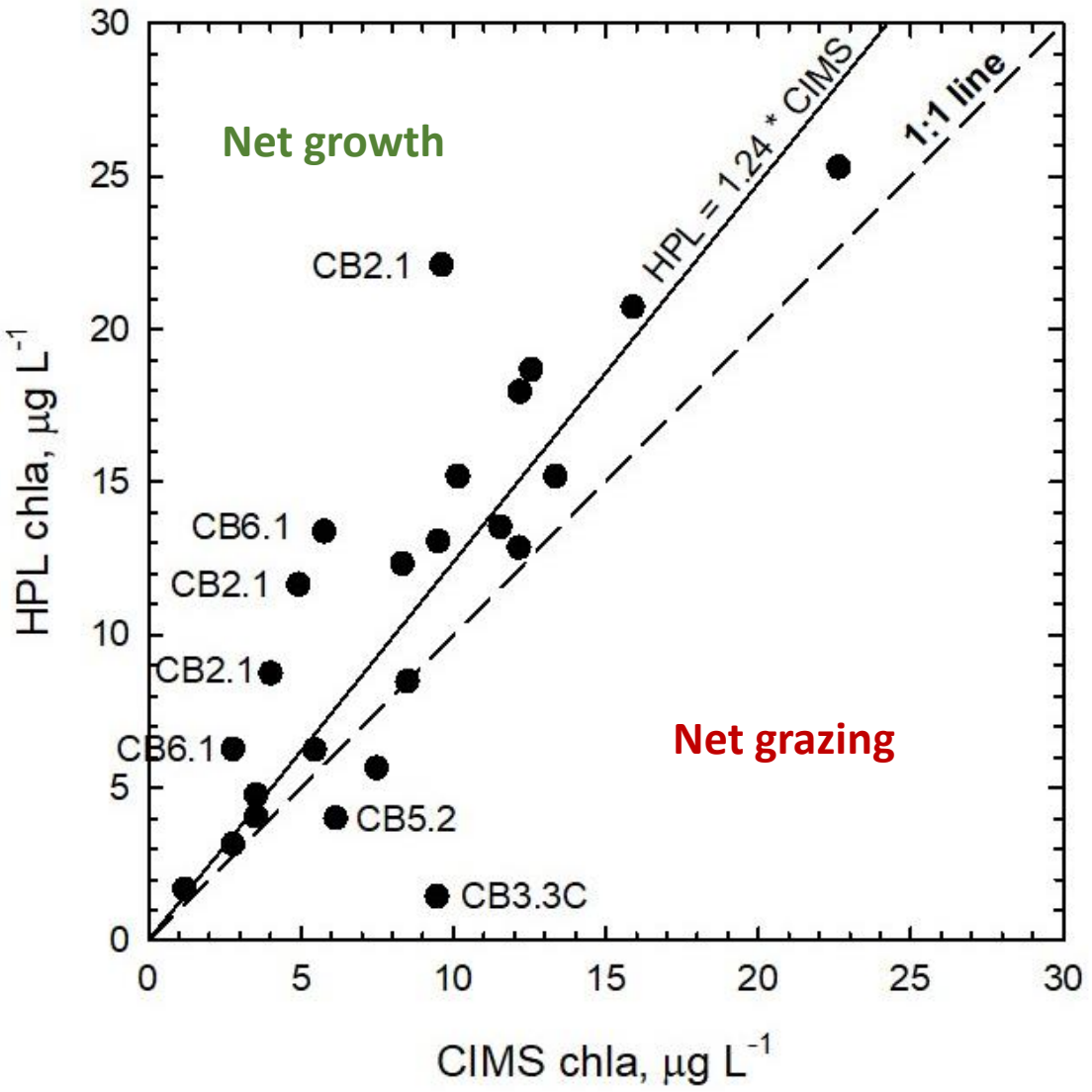
r^2 = 0.472
intercept defined = 0
slope = 0.820 ± 0.167
95% CL = 0.478 - 1.162

HPL = no bias, large scatter

Possible causes:
analytical bias
handling contamination

Although not statistically significant, both DIN and PO4 may be decreasing during the 24 h from collection to start of the bioassays. Does chla increase during the same time?

Chlorophyll a



data analysis (chla)

r^2 = 0.908
intercept defined = 0
slope = 1.238 ± 0.076
95% CL = 1.083 - 1.394

HPL = +24% bias, scatter

Possible causes:
- analytical bias
- net growth during transport (CB2.1, CB6.1)
- net grazing during handling (CB3.3C)

With more data after Dec 2023, we may see that plankton growth and grazing can be detected during the 24 h handling period prior to the start of the bioassays.

Incubator temperature vs Choptank River temperature – QA check

both loggers in incubator						
n =	104					
ave =	-0.197		incubator logger has -0.20^{o} bias			
stdev =	0.173					
se =	0.017					
min =	-0.197					
max =	0.500					
one logger in incubaor, one in water at dock, incubater - dock						
n =	228					
ave =	-0.357		incubator is 0.4 ^{o}C cooler			
stdev =	0.404					
se =	0.027		correcting for bias: -0.2 ^{o}C			
min =	-1.3					
max =	0.9					
Incubator is always within 1 ^{o}C of Choptank River, with a -0.2 ^{o}C bias						