revised: 31 Mar 2025 trf

Report on Resource Limitation Bioassays (Light, N, & P Limitation) April 2, 2025

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Outline of Report: 2 Apr 2025

- Overview of protocols
- Synthesis of previous bioassays (1990-2003)
- Comparison of 1990-2003 results with those of 2023-2025

- 30 minute presentation with 24 slides, ask questions!

Report to Modeling Subcommittee: 2 April 2025

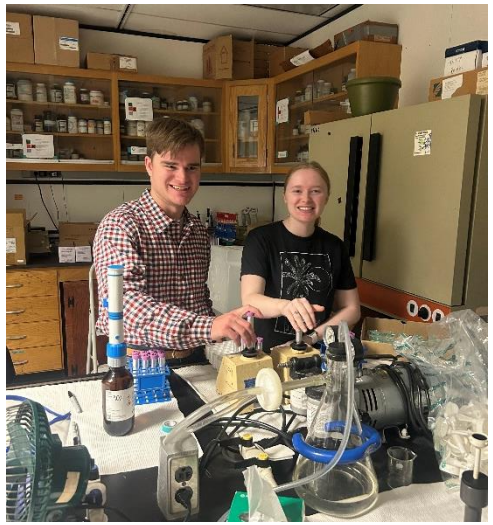
- Focus on bioassays during Sept 2023 – Nov 2024
  - 12 stations x 14 sampling periods with complete datasets
    - Some delays for nutrient analyses

Meeting the sampling teams at the dock on collection day.

Carboys of water are taken to HPL on the collection day.

Next morning: sample water from each carboy is transferred to 4 liter containers for controls and treatments in the lab and **incubated at the dock at 50% light** in flowing Choptank water

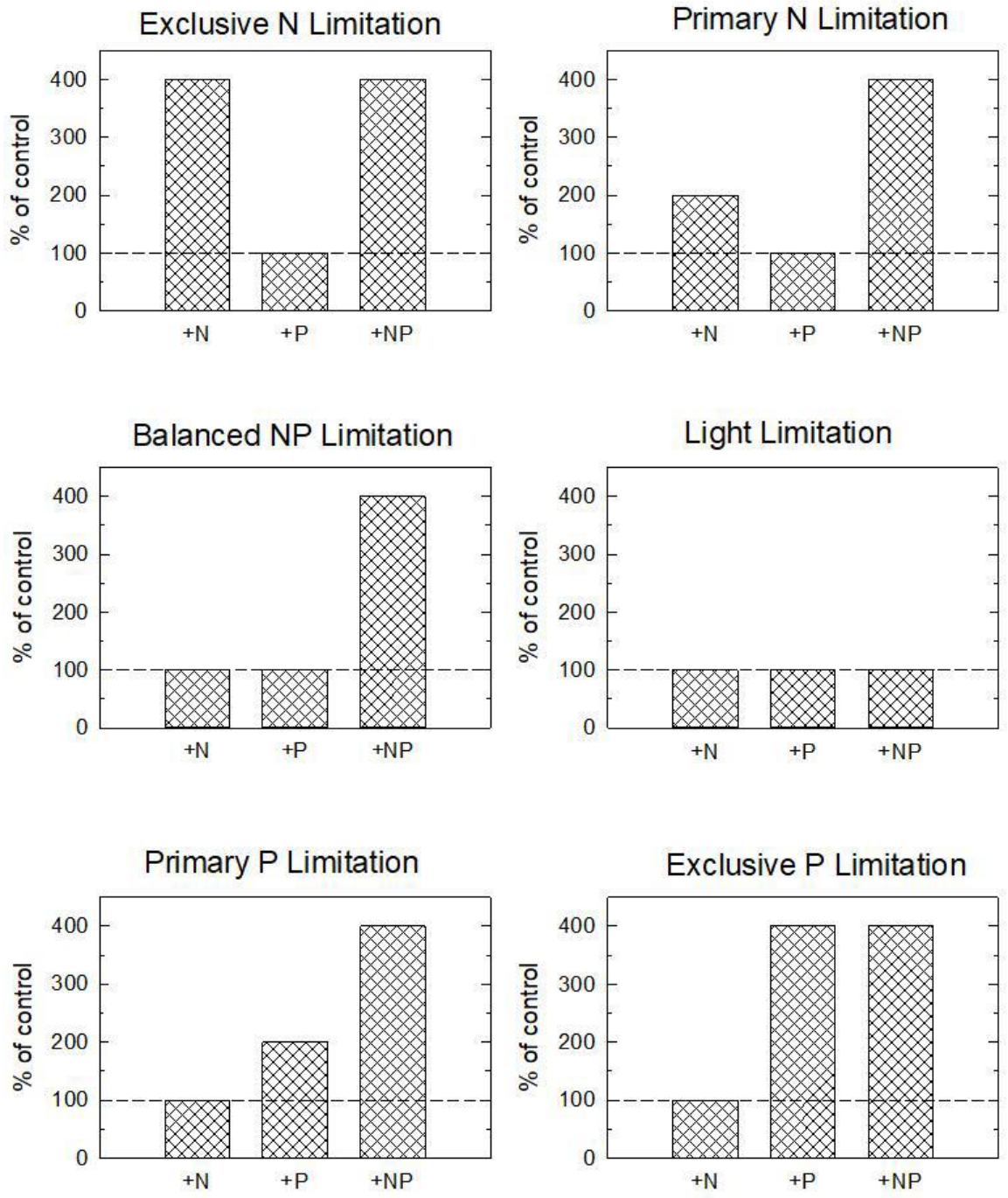
in all weather conditions at the HPL dock for the equivalent of **2 days of ave PAR for each month**. Paleena checking the incubator.

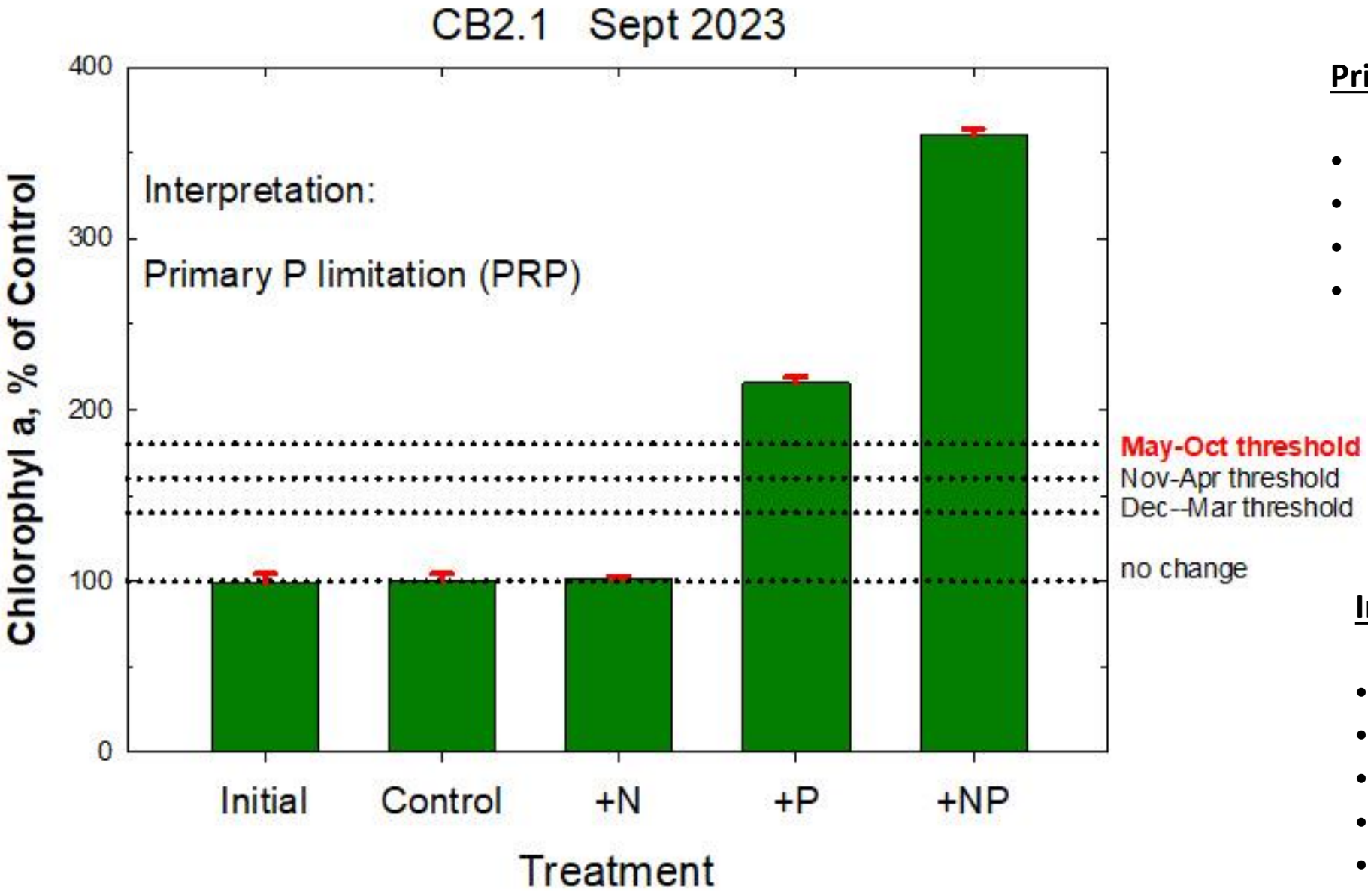


Two interns from Washington College helping with **sample processing** in the lab after the incubation.

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)





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

Paradigm of Resource Limitation of Phytoplankton

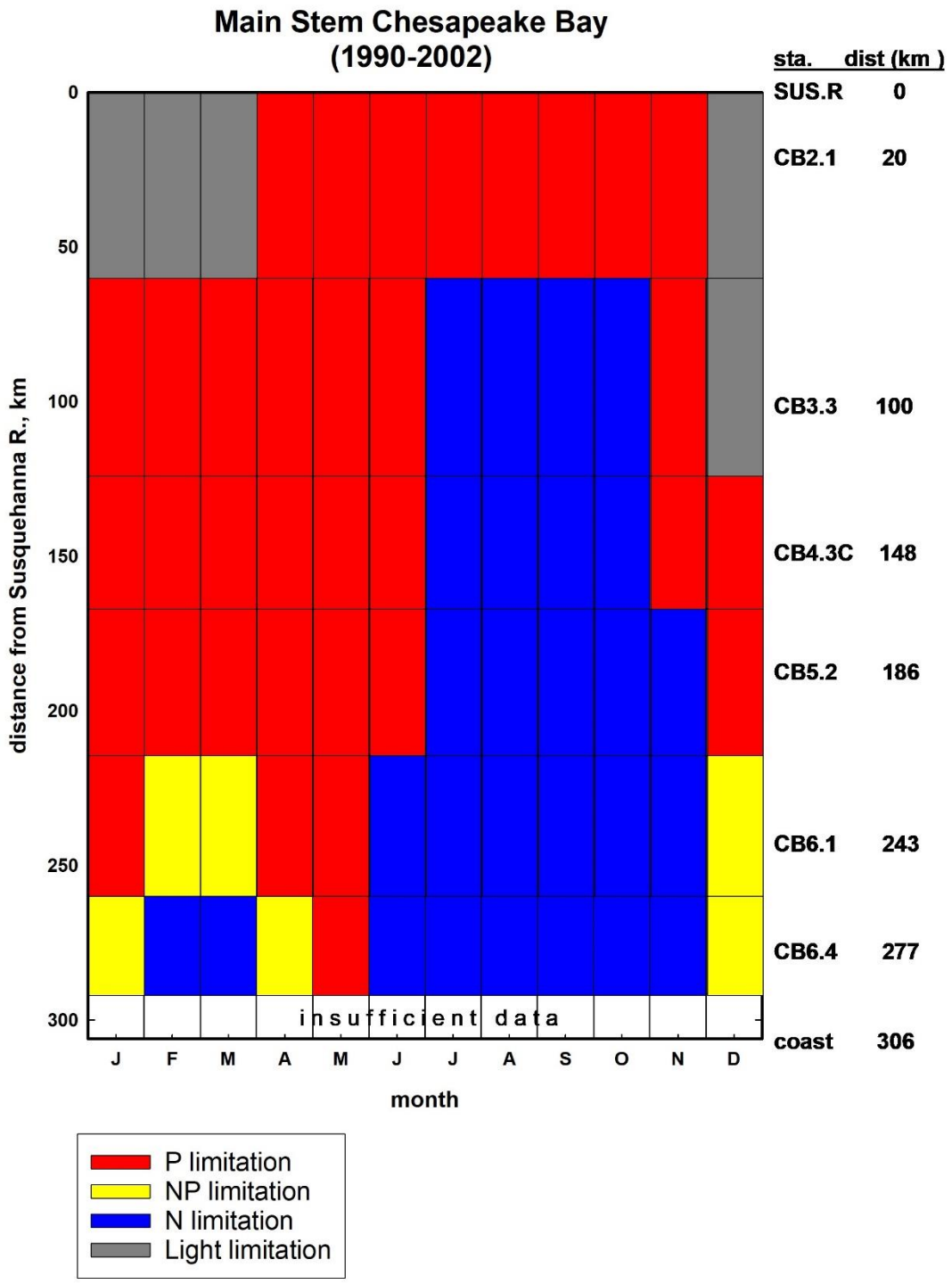
Resources needed for algal growth: **light**, **P**, and **N**

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** in spring and fall

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



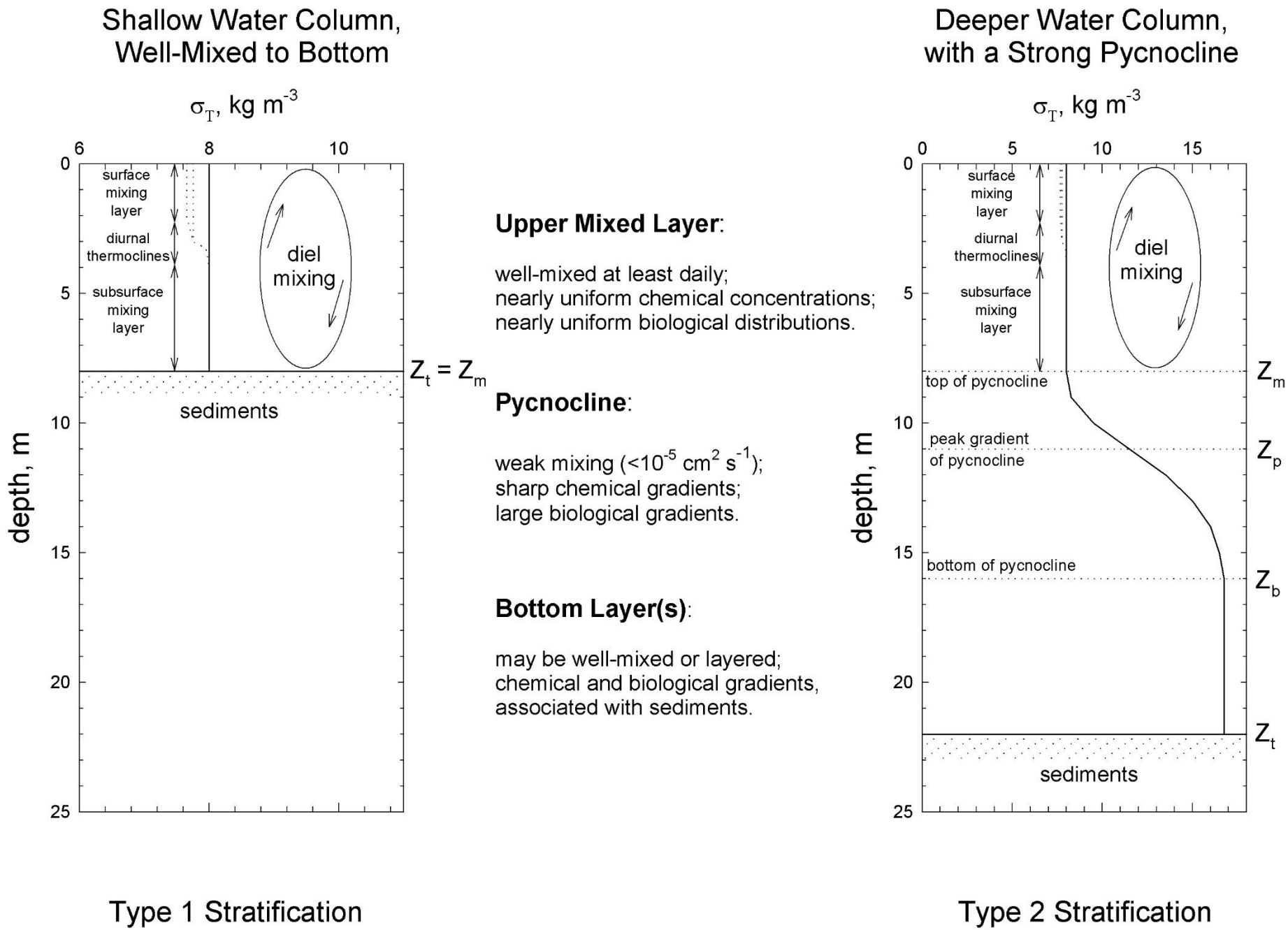
Report: 2 Apr 2025

- Original project goal: validation data for models based on earlier data
- Goals of this presentation
  - Can we detect changes in:
    - Stratification?
    - Nutrient levels?
    - N, P, or light limitation?
  - Compared to 1990-2003
  - Depth of mixing and incident PAR determine light energy for photosynthesis
- Caveats
  - We have complete data for only 14 of the 24 months
  - Last bioassays will be done in Aug 2025 for 24 bioassays per station
    - Only 12 for Pax and Rapp stations
  - 1990-2003 bioassays = 889 at 12 stations
    - Limited statistical power for detecting small changes in 2023-2025

Can we detect changes in stratification?

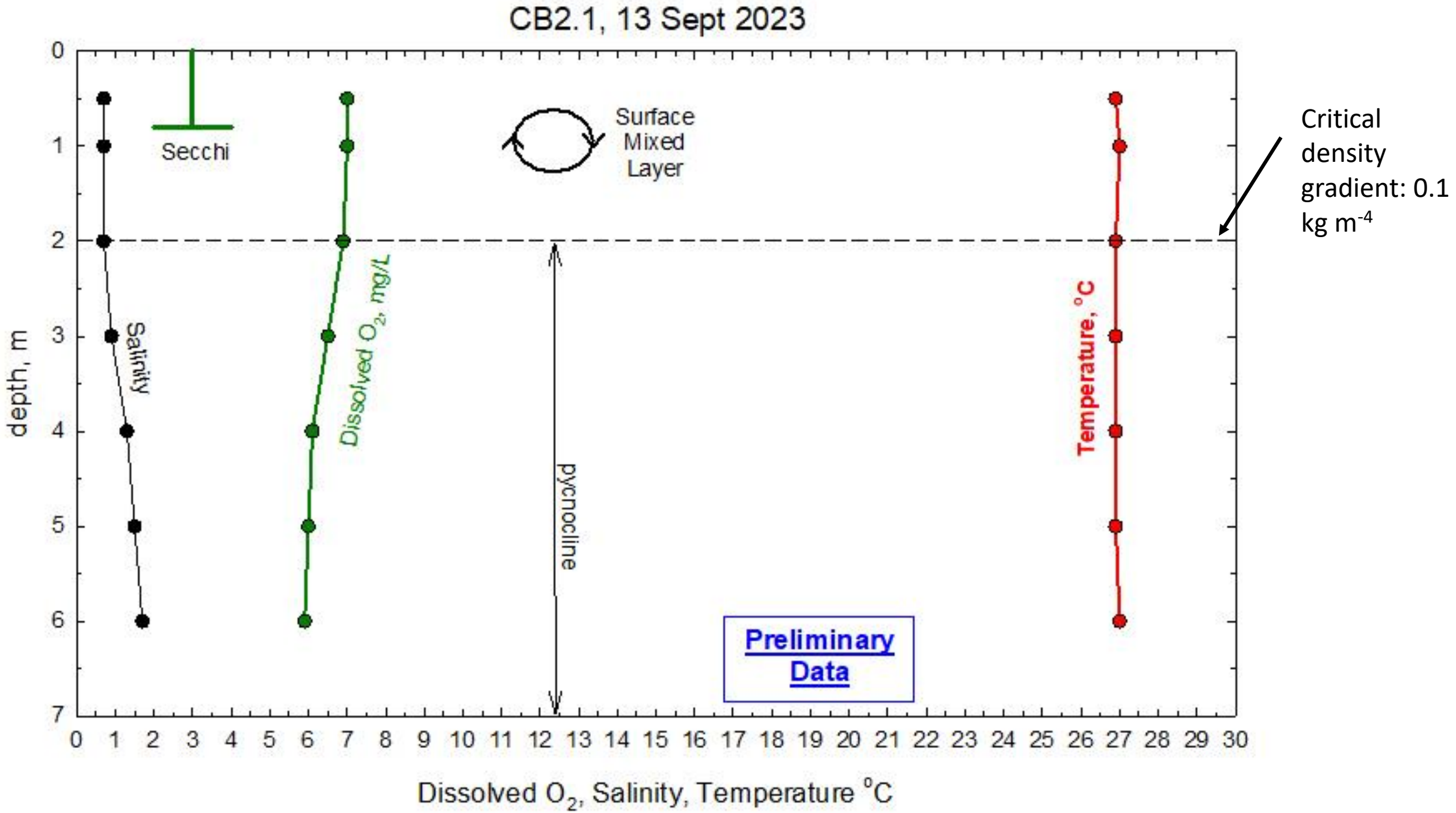
- Light energy for photosynthesis depends on stratification
- Depth of mixing (Z_m, meters) in 2023-2024 varied over:
  - 2 m (most shallow Z_m) to 10 m (unstratified at CB2.1)
- Goal of final report:
  - Compile Z_m at **all** stations in 2023-2025
  - Compile Z_m at all stations in 1990-2003 (available)
  - Statistical tests of changes in Z_m between the two periods

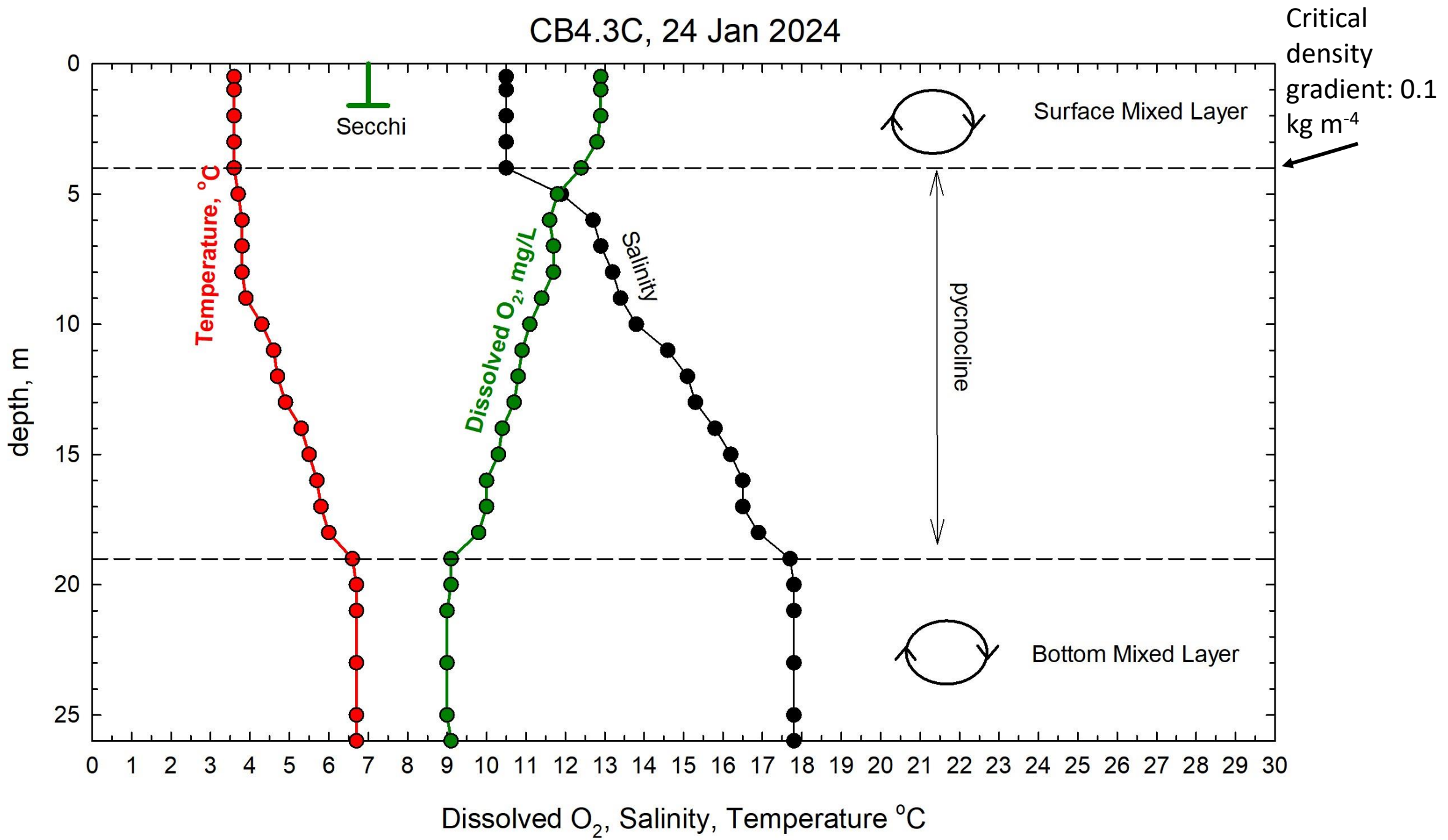
Fig. 1, Fisher et al.

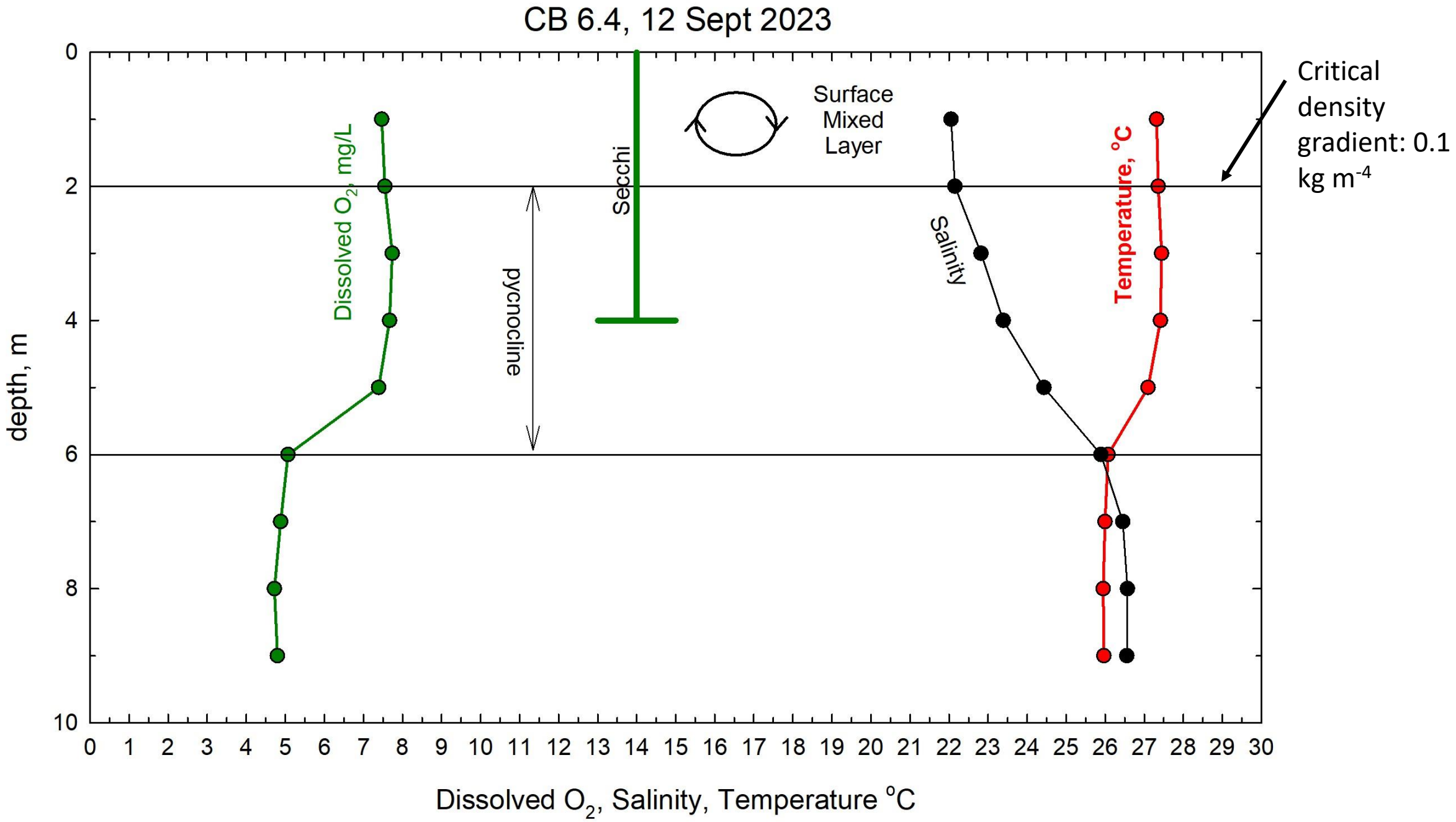


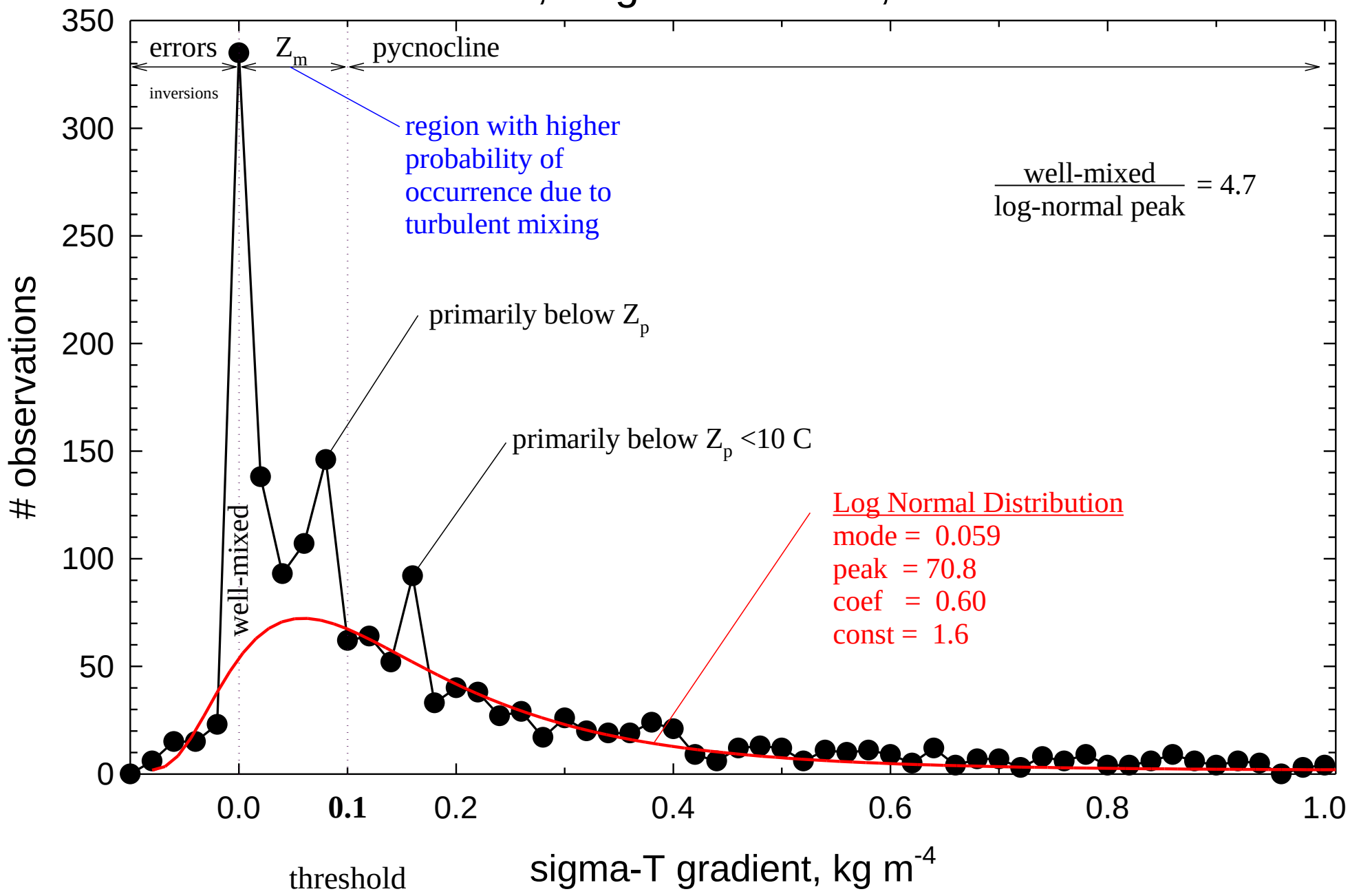
Type 1 Stratification

Type 2 Stratification

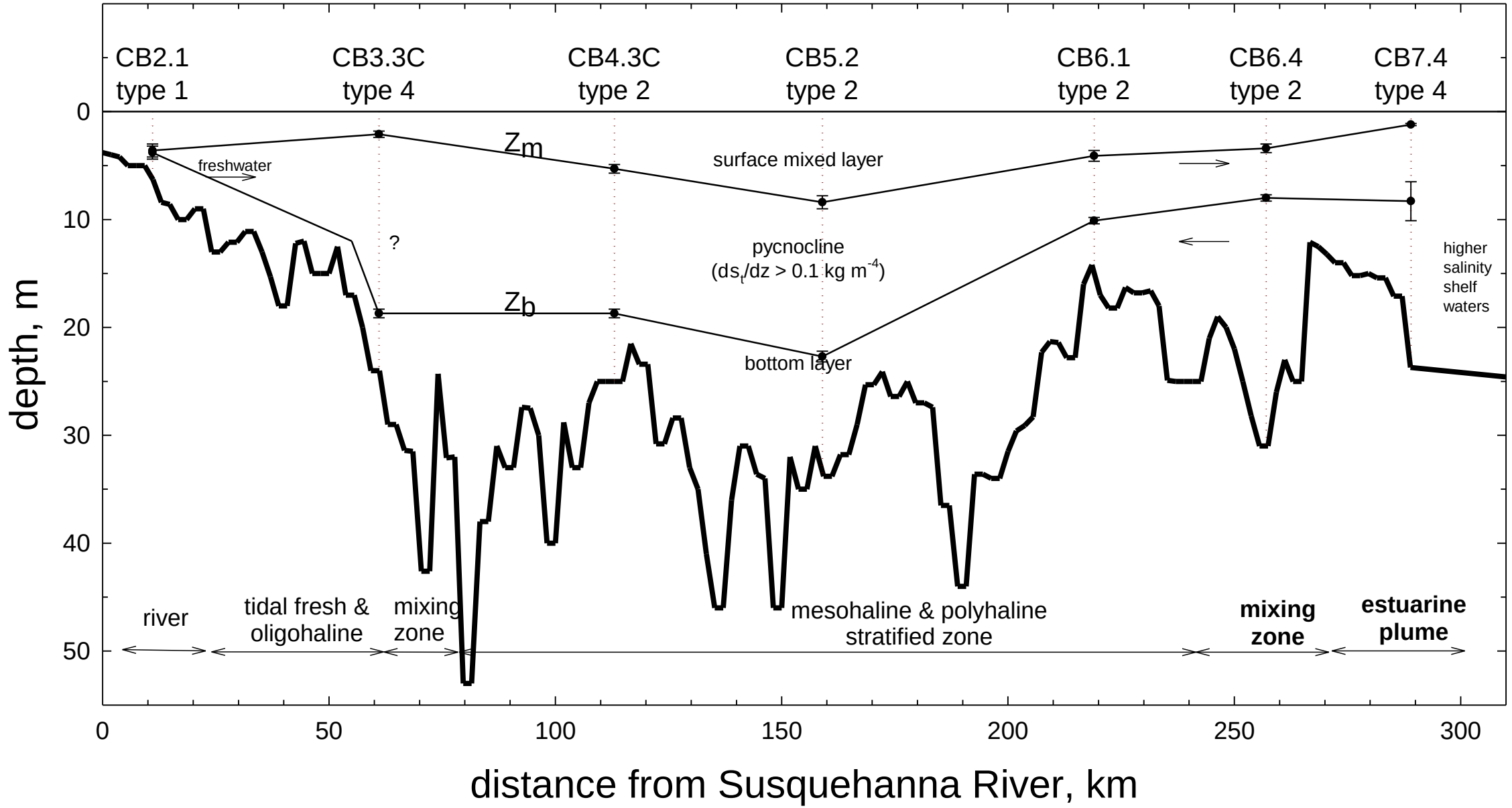

CB2.1, 13 Sept 2023
depth, m
Secchi
Surface
Mixed
Layer
Salinity
Dissolved O2, mg/L
pycnocline
Temperature, °C
Preliminary
Data
Dissolved O2, Salinity, Temperature °C
Critical
density
gradient: 0.1
kg m-4


CB4.3C, 24 Jan 2024
Critical density gradient: 0.1 kg m-4
Secchi
Surface Mixed Layer
Temperature, °C
Dissolved O2, mg/L
Salinity
pycnocline
Bottom Mixed Layer
depth, m
0
5
10
15
20
25
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


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
Critical
density
gradient: 0.1
kg m^{-4}
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


MCB5.2, Aug.90 - Dec.96, n = 1759
errors
inversions
Z_m
pycnocline
region with higher probability of occurrence due to turbulent mixing
well-mixed / log-normal peak = 4.7
primarily below Z_p
primarily below Z_p <10 C
Log Normal Distribution
mode = 0.059
peak = 70.8
coef = 0.60
const = 1.6
well-mixed
observations
0
50
100
150
200
250
300
350
0.0
0.1
0.2
0.4
0.6
0.8
1.0
threshold
sigma-T gradient, kg m^{-4}

Chesapeake Bay main axis (1990-1996)

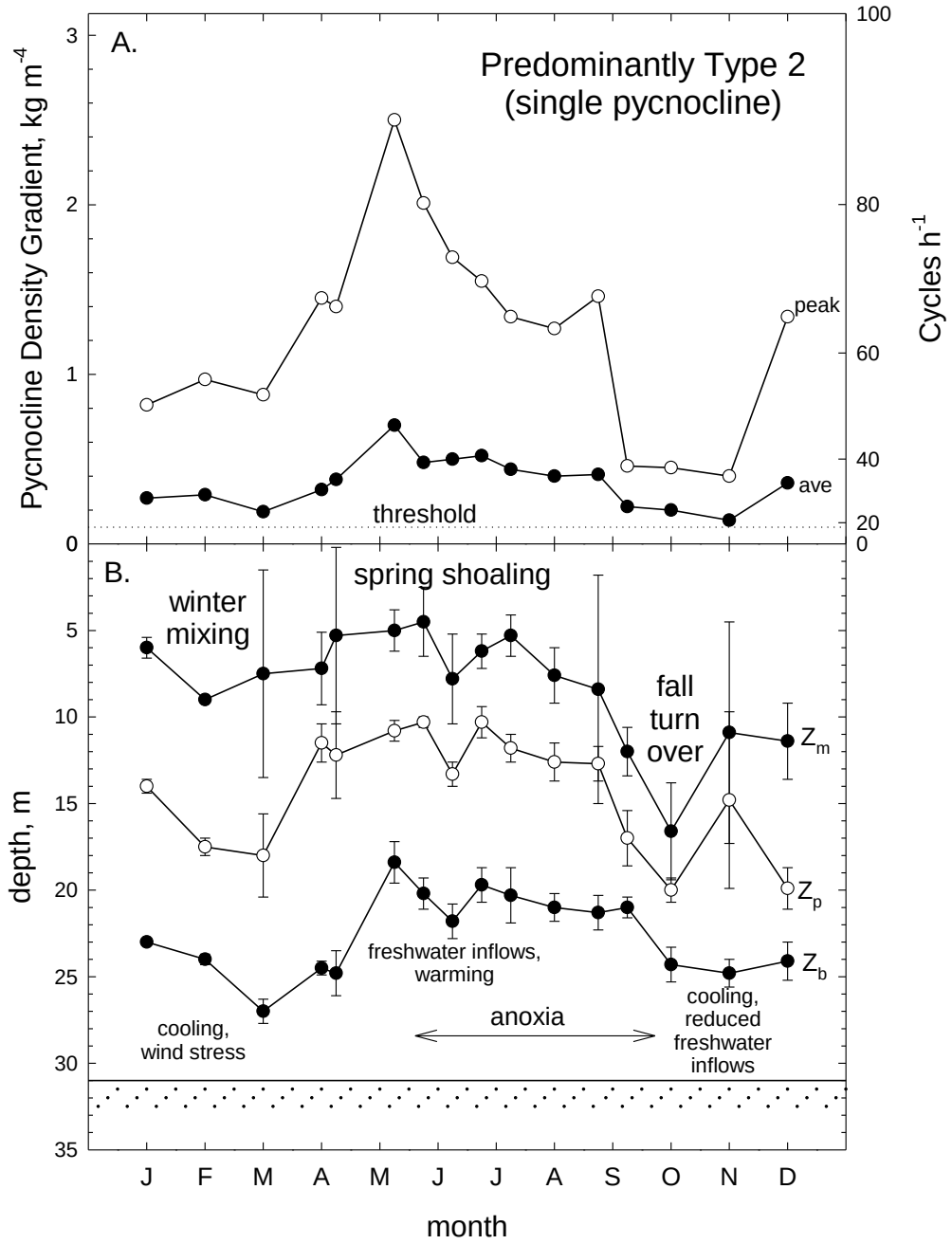


CB5.2, 1990 - 1996

<u>Pycnocline density gradients</u>:

Highest in Apr-May: high river flows
bay warms

Lowest in Sept-Oct: low river flows
bay cools



N^2 (Brunt-Vaisala Frequency)

Can we detect changes in stratification?

- Depth of mixing (Z_m, meters) in 2023-2024 varied over:
  - 2 m (most shallow Z_m) to 10 m (unstratified at CB2.1)
- One goal of final report:
  - Compile Z_m at **all** stations in 2023-2025
  - Compile Z_m at all stations in 1990-2003 (available)
  - Statistical tests of changes in Z_m between the two periods
    - Statistical advice from Elgin Perry?
- Bottom Line: not yet

Report: 2 Apr 2025

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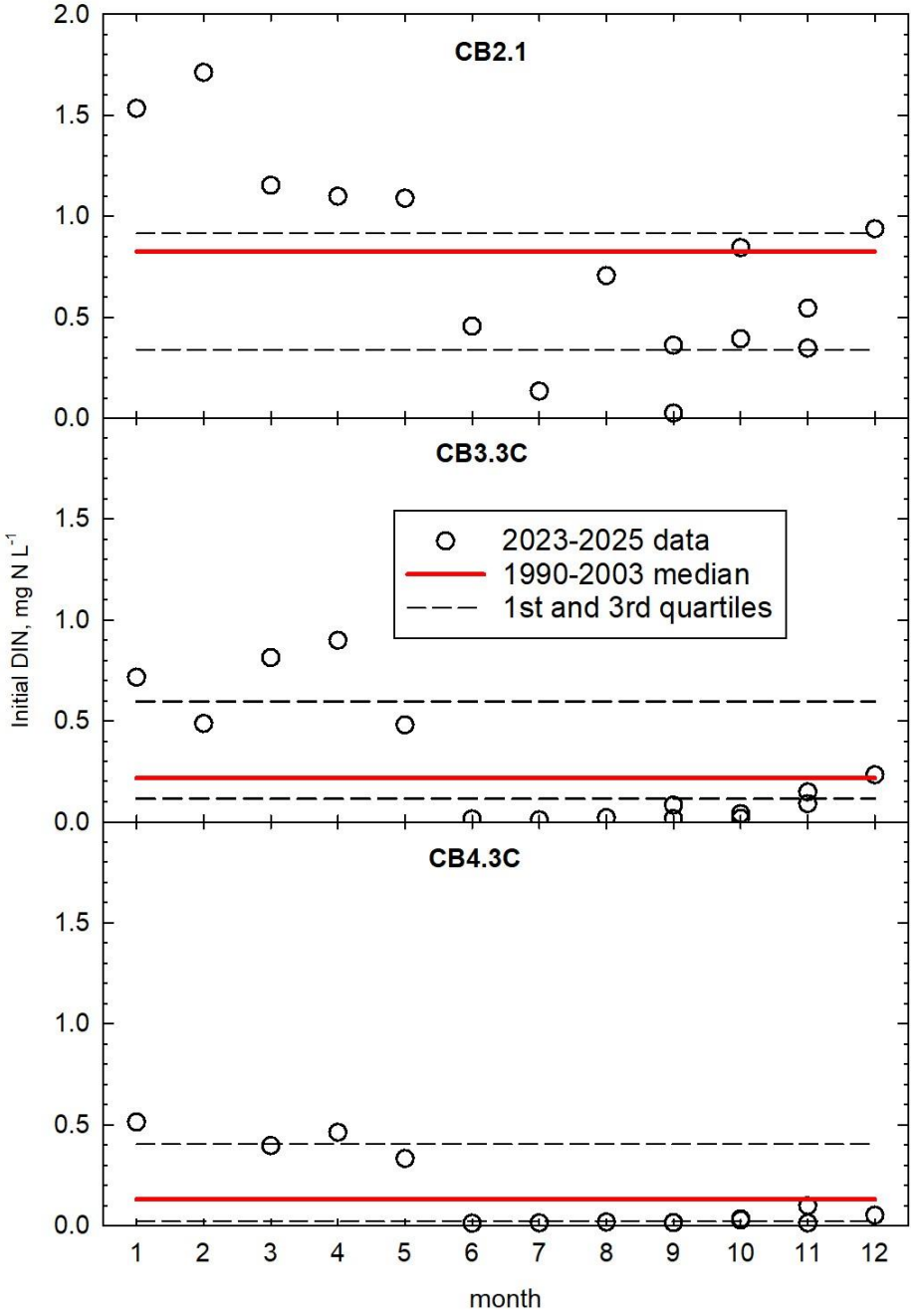
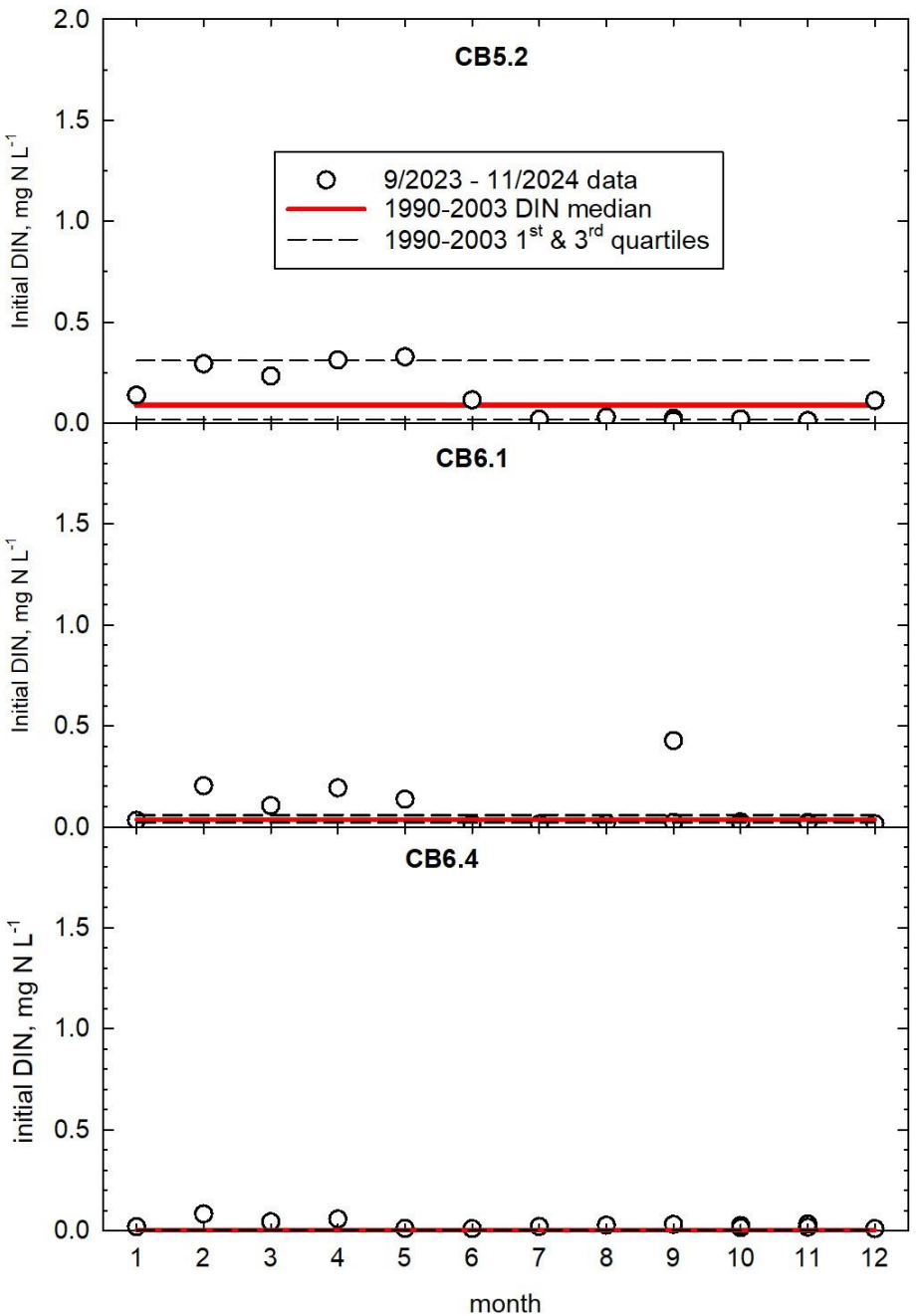
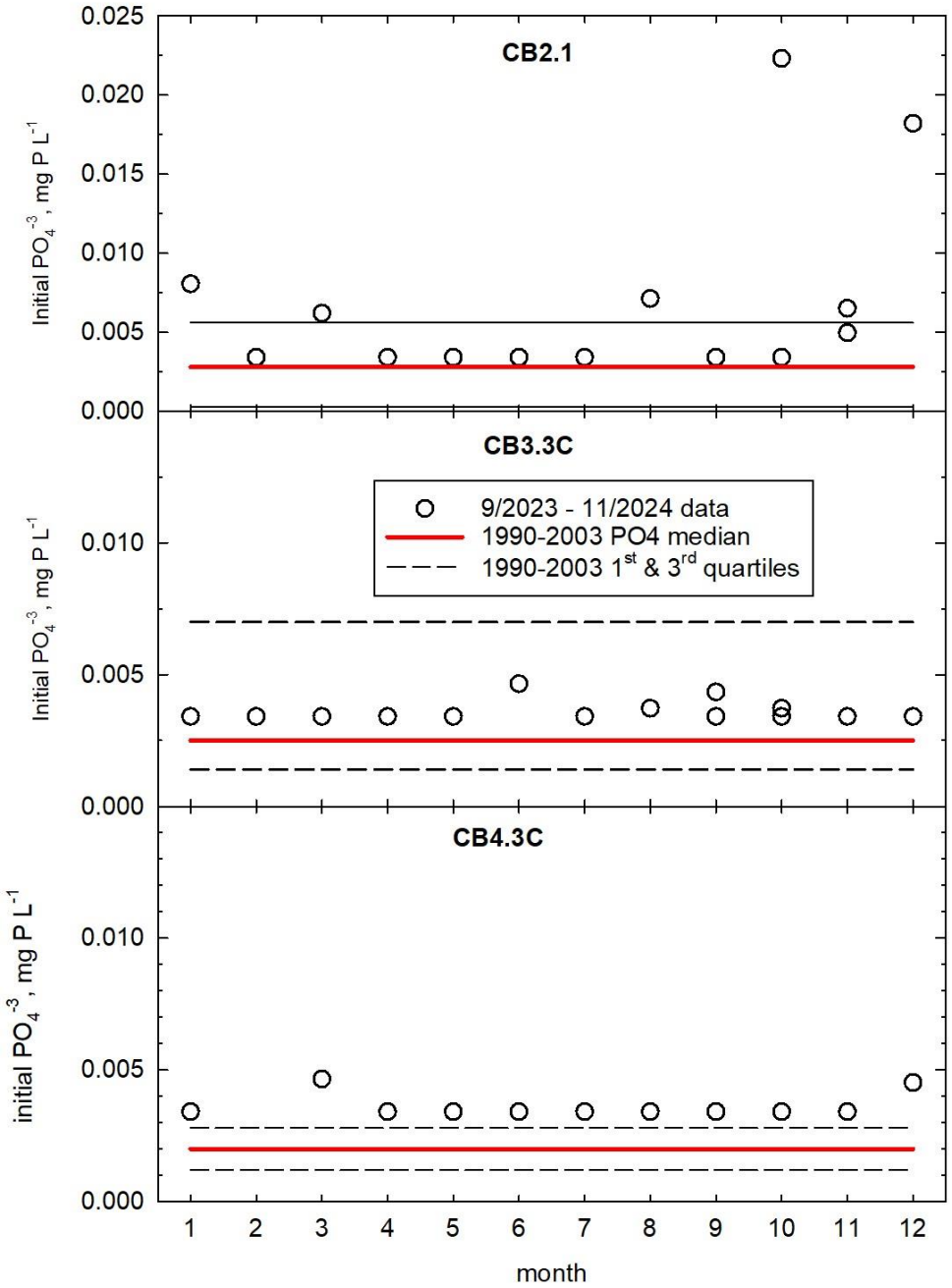
DIN is abundant in upper Ches Bay, and declines down bay. Seasonally, DIN is greatest in winter and lowest in summer. No major changes in DIN in water used for bioassays between 1990 –2003 and 2023 – 2024 (so far).

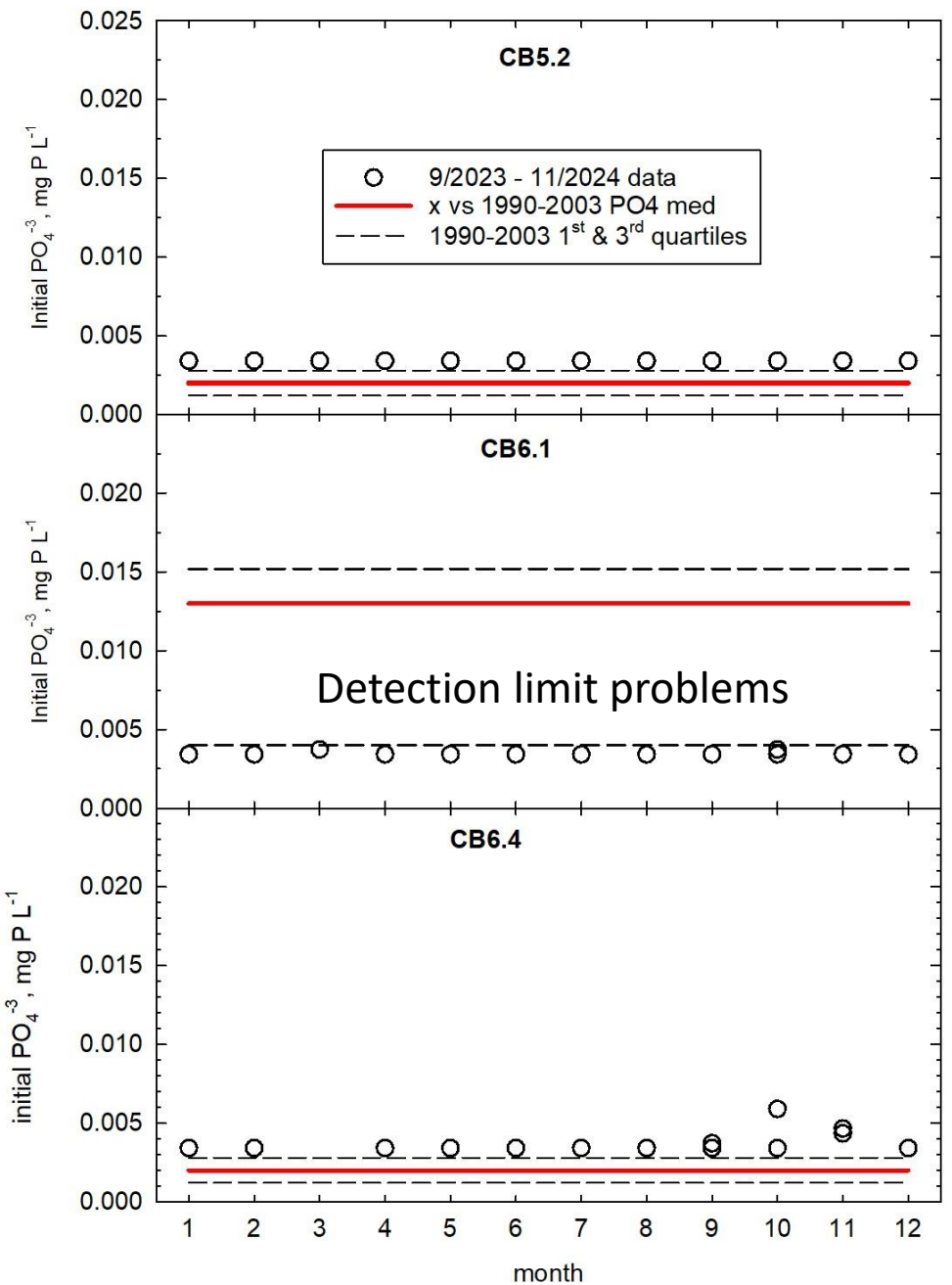
DIN = NO_3^- + NO_2^- + NH_4^+



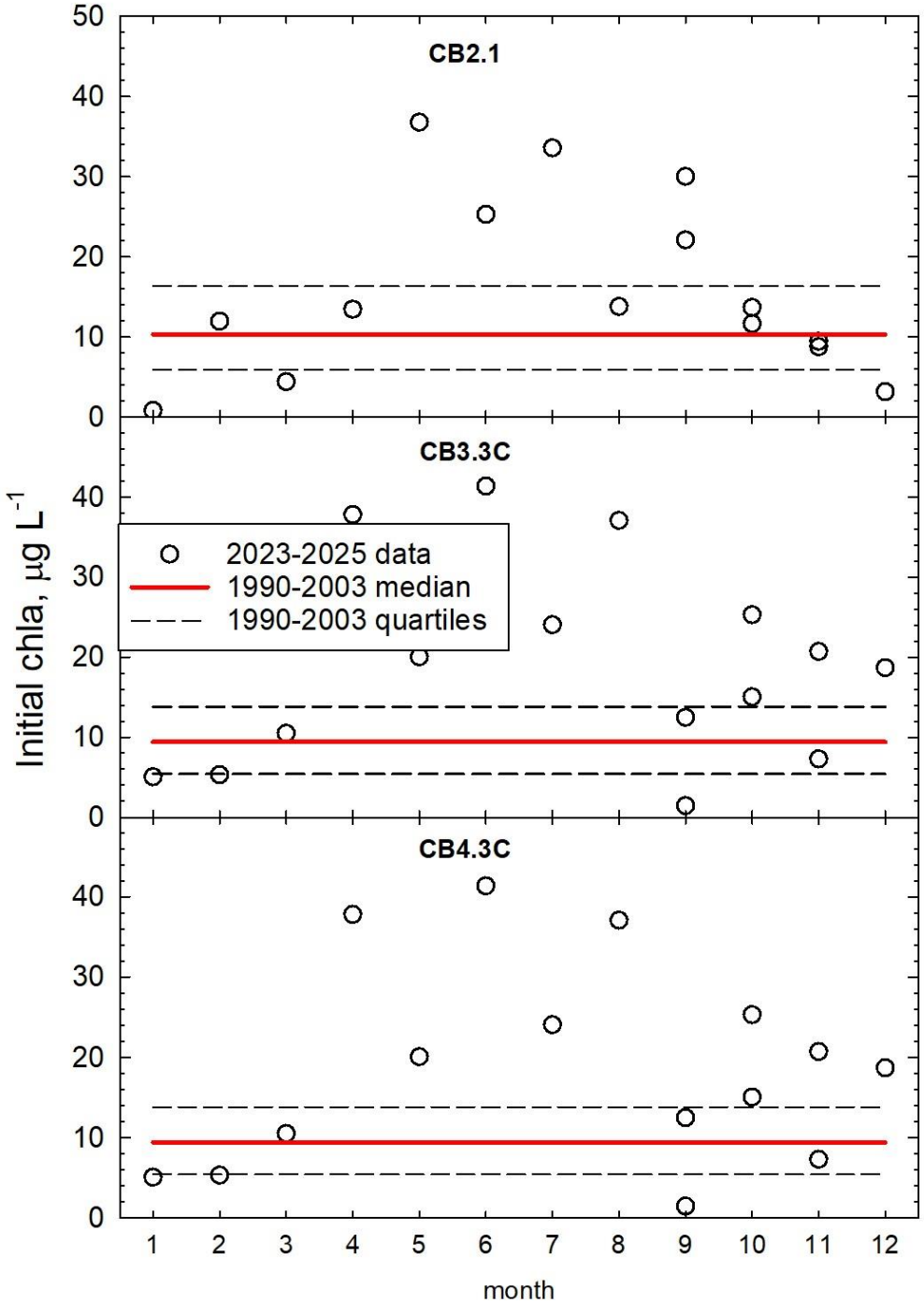


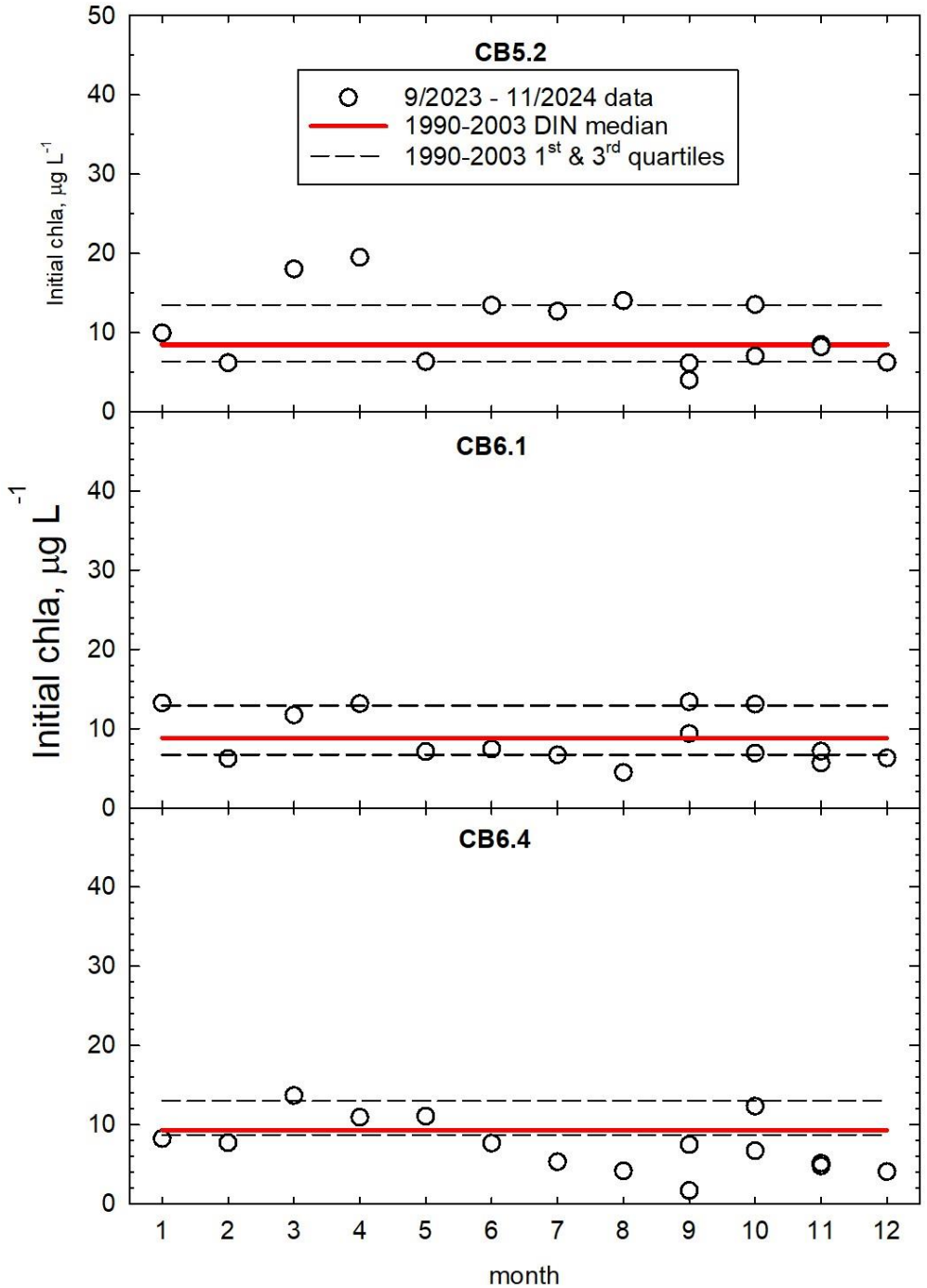
Phosphate is generally Low in Ches Bay, and there were detection limit changes in PO_4^{-3} at CB6.1. However, there may be slight increases in PO_4^{-3} in water used for bioassays between 2003 – 2024 at mid- and lower bay stations.





Chlorophyll a is generally higher in 2023-2024 in the upper bay than in 1990-2003, but the lower Bay in 2023 – 2024 is comparable to data from 1990-2003





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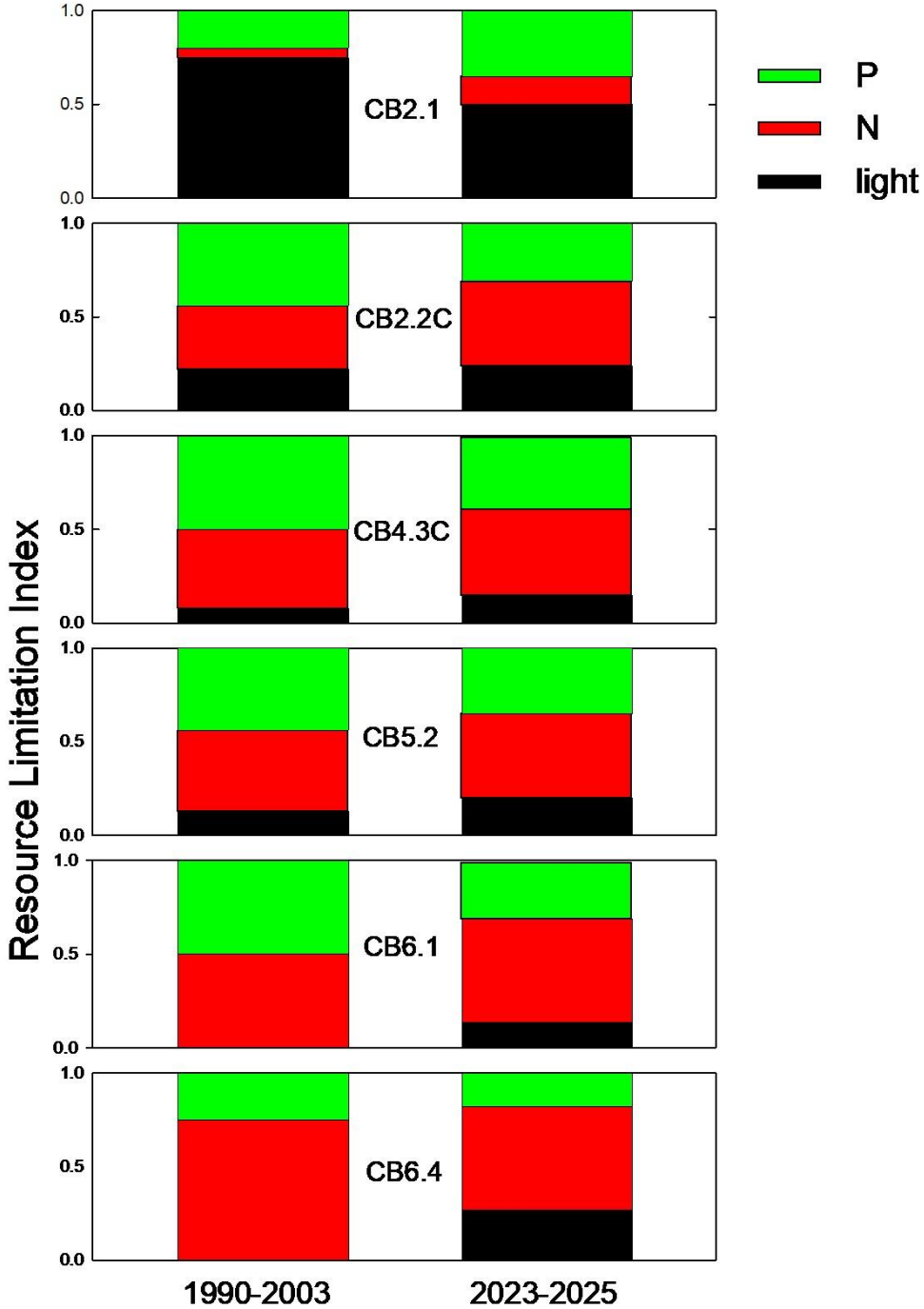
Light Limitation is high in upper bay

turbid

clear

But diminishes to near zero near the coast



N Limitation is low in upper bay

But increases in importance near the coast

P Limitation is high in upper bay

High DIN & PO4

depleted DIN & PO4

But decreases greatly near the coast

Report: 2 Apr 2024 (Continued)

- Last water samples will be collected August 2025
- We have requested a 9-month, no-cost extension to allow time for all analyses and reports to be completed.
- Final report and publications in 2026
  - Data for model validation
  - Manuscript of comparison of both data sets relative to Bay Program goals
  - Manuscript on vertical stratification in Chesapeake Bay

Summary

- Depth of mixing of surface waters typically varies from 2-8 m and determines the light energy available for phytoplankton growth
- DIN is abundant (>0.5 mg N/L) in the upper bay, decreasing to very low and limiting concentrations in the lower bay.
- Phosphate is abundant (>0.05 mg P/L) in the upper bay, decreasing to colorimetric detection limits in the lower bay
- Chlorophyll a (phytoplankton biomass) is higher in upper bay stations (up to 40 µg/L), decreasing to <15 µg/L in the lower bay
- DIN and phosphate are relatively unchanged in 2023-2024 compared to 1990-2003, but chlorophyll a appears to have increased somewhat in the upper bay
- Resource limitation of phytoplankton growth varies seasonally and spatially throughout the bay, and appears relatively unchanged

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