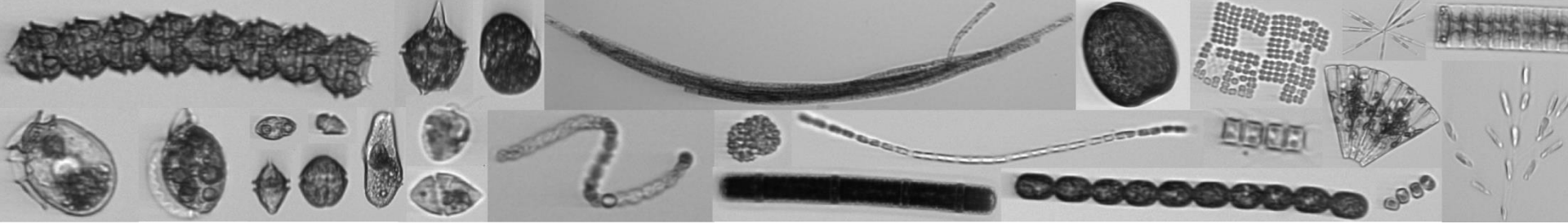
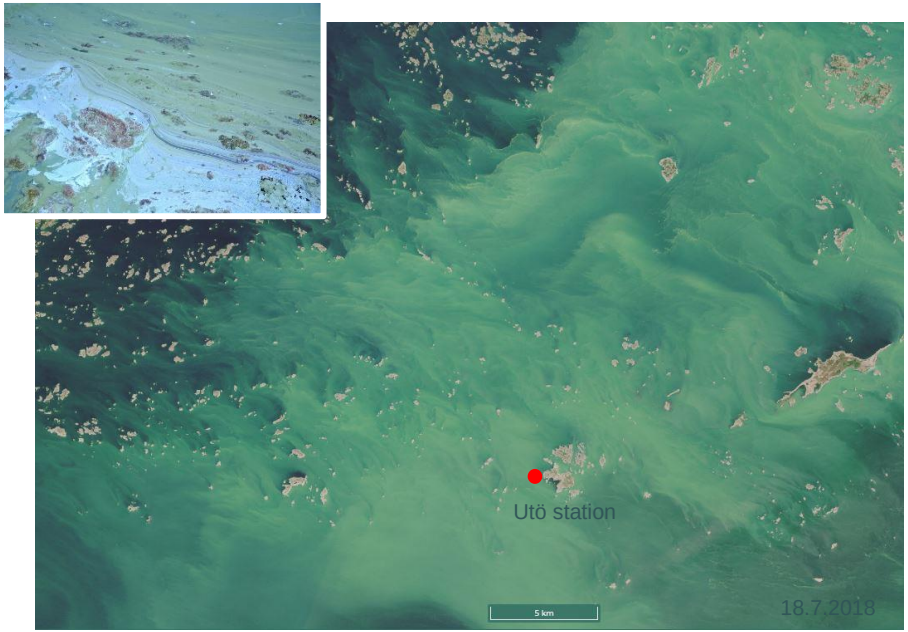


Detection of filamentous cyanobacteria blooms using imaging and pulse shape flow cytometry, and optical sensors

Kaisa Kraft
Finnish Environment Institute SYKE
Marine Ecological Measurements unit
01.10.2024

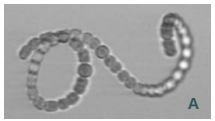
Cyanobacteria blooms in the Baltic Sea

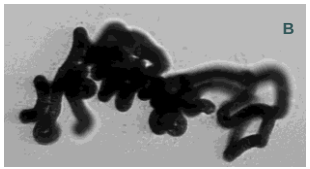


- Cyanobacteria are important component of the ecosystem
  - O_2-production, nutrient cycling, N_2-fixation
- Basin-wide and recurrent phenomena
- Bloom development initiated mainly by
  - phosphorus availability of the surface water
  - calm, warm weather period

  -> warming and shallowing of the surface layer above a seasonal thermocline
- Species composition varies -> some toxic
- Formation and dynamics cannot be resolved with traditional monitoring methods (light microscopy samples 1-2 times/month)
- Satellite images and optical sensors cannot resolve species composition

Dolichospermum spp. (A)
Nodularia spumigena (B)
Aphanizomenon flosaquae (C)

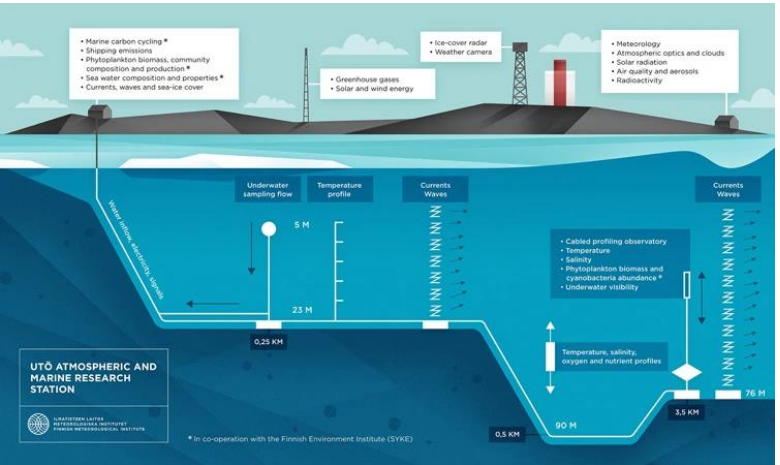




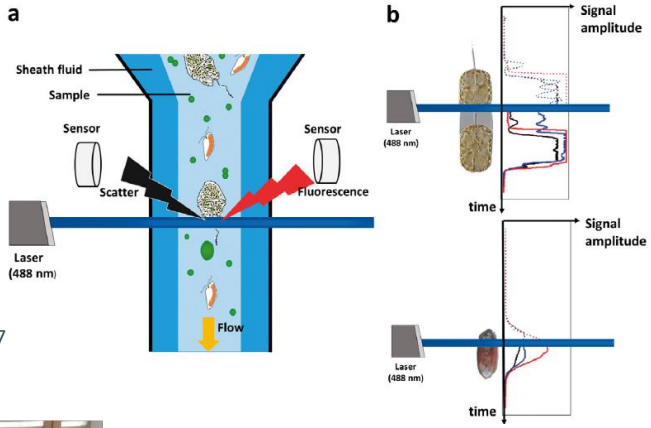
How do different methods compare in how they describe the blooms?

UTÖ MRS
joint station with **FMI**

- Underwater pump with inlet at 5 m depth, 250 m offshore
- Water distributed to different channels inside the station
- Represents pelagial community of a mixed surface layer
- Multiple parallel measurements from sea to atmosphere
- Imaging and pulse shape recording flow cytometry observations sporadically since 2017
  -> operationally since early 2020



CytoSense (CS) – Pulse-shape recording flow cytometer



Haraguchi et al., 2017

Records optical properties for each particle as they cross the laser beam, generating particle specific optical ”fingerprints”

Sensors:

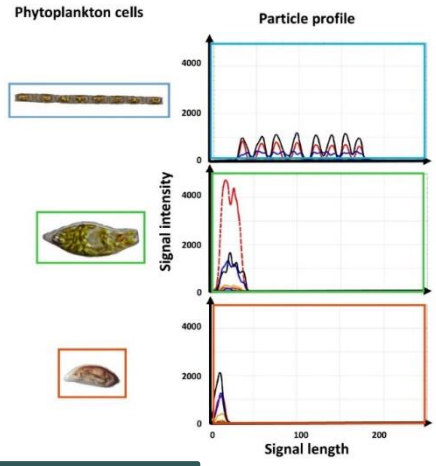
- FWS (R+L)
- SWS
- FLR (Chla; 668-726 nm)
- FLO (PC; 604-644 nm)
- FLY (PE; 553-577 nm)
- FLG (FITC; 502-538 nm)

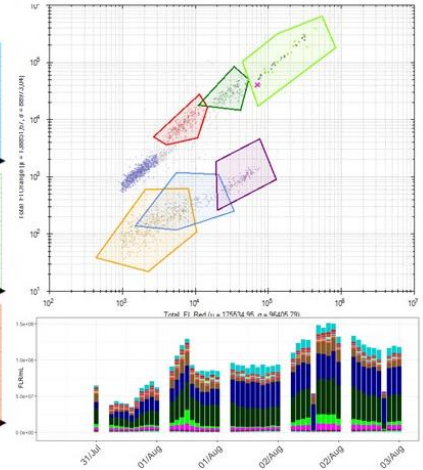
Imaging FlowCytobot (IFCB)

- Imaging flow cytometer
- Images particles inside size range ~10-150µm
- Sample of 5ml with approx. 20 min interval
- Camera triggered by chlorophyll-*a* or scatter
- Even ~30 000 images / hour
- 150 µm mesh in IFCB inlet to prevent it from clocking

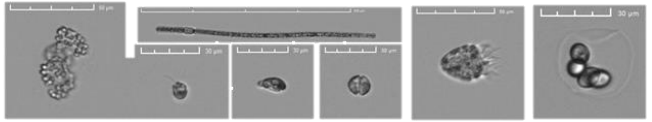
Collection of particle profiles

Populations: ID + abundance





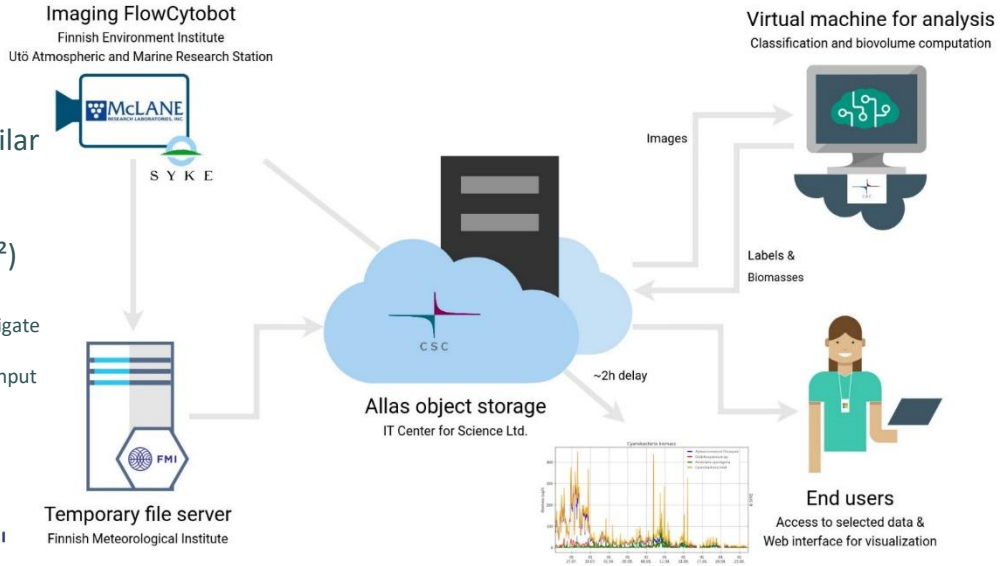
Images support population’s identitification



Automated data pipeline and classification with Convolutional Neural Networks (CNN)

- Automated data pipeline and classification system[2,3]
- Information to end users in near-real-time
- Biovolumes[4] computed automatically -> information in µg/l
- Weighted F1-score of test data of our labeled image data set **0.95**[2]
- Weighted F1-score of our evaluation data (59 natural samples annotated entirely) **0.83**[2]
  - 11 classes F1 of 0.7-0.79
  - 6 classes F1 of 0.8-0.89
  - 10 classes F1 of 0.9-1.0
- For this study, an updated classifier version was used with similar performance for filamentous cyanobacteria
- Class-specific thresholds
- CNN architecture: pre-trained Resnet-18 (detailed description[2])

- Biovolume calculations described in Moberg & Sosik 2012, github.com/hsosik/ifcb-analysis
- Broblem with loop forming filaments
- -> a conversion is done for Dolichospermum sp. [1] and Nodularia spumigena loop forms (seperate classes from ”straight” forms)

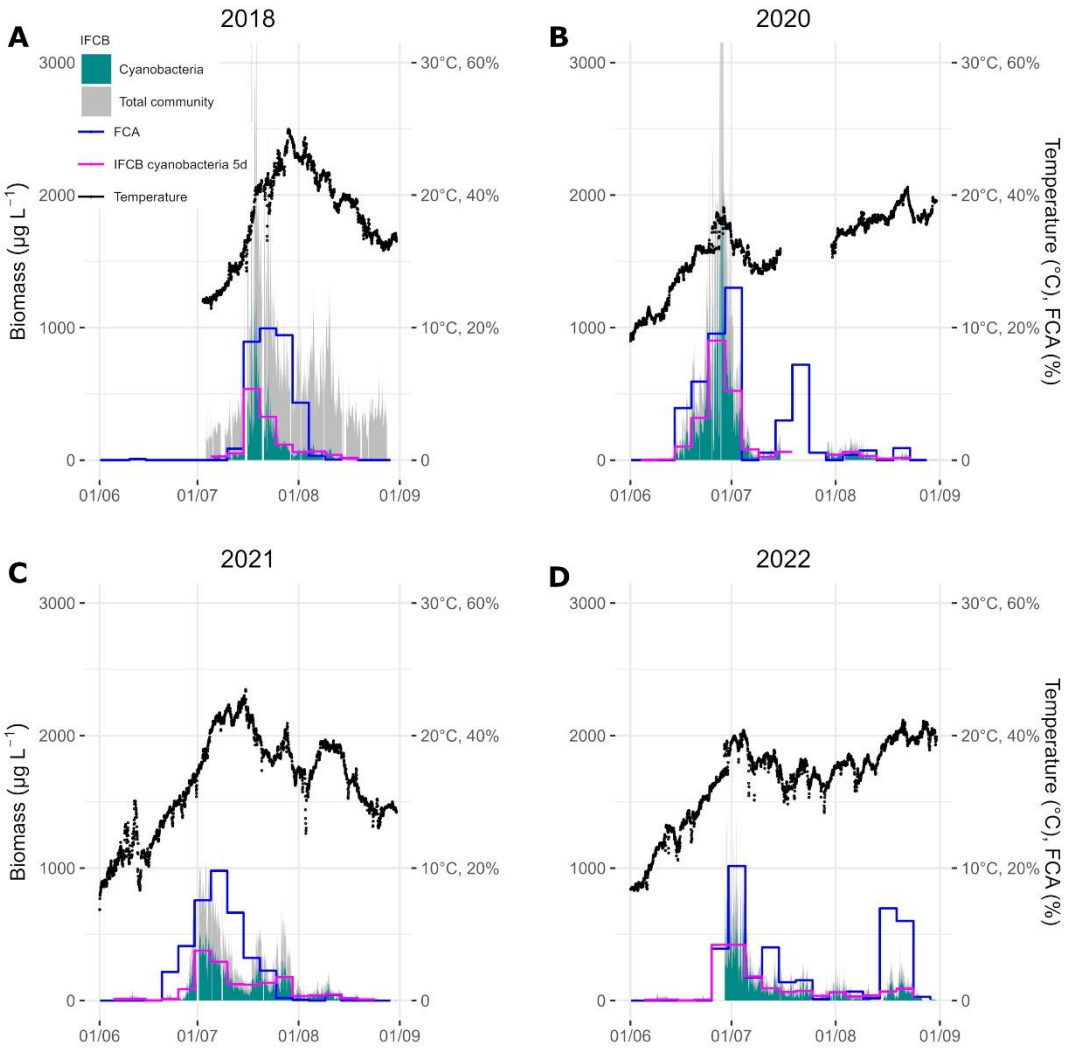


[1]Kraft et al. (2021). First Application of IFCB High-Frequency Imaging-in-Flow Cytometry to Investigate Bloom-Forming Filamentous Cyanobacteria in the Baltic Sea. Frontiers in Marine Sciences
[2]Kraft et al. (2022). Towards operational phytoplankton recognition with automated high-throughput imaging, near real-time data processing, and convolutional neural networks. Front. Mar. Sci., 9
[3]https://github.com/veot/syke-pic

Suomen ympäristökeskus
Finlands miljöcentral
Finnish Environment Institute
JERICO RI
TIINA JA ANTTI HERLININ SÄÄTIÖ
Academy of Finland project
FASTVISION
FMI

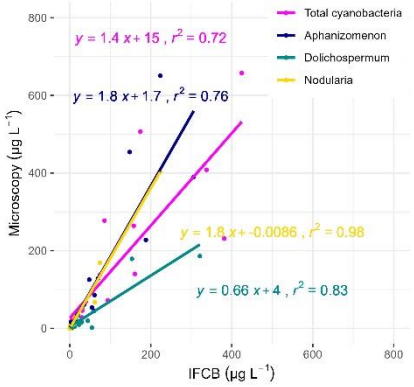
Cyanobacteria bloom development in 2018, 2020, 2021 & 2022

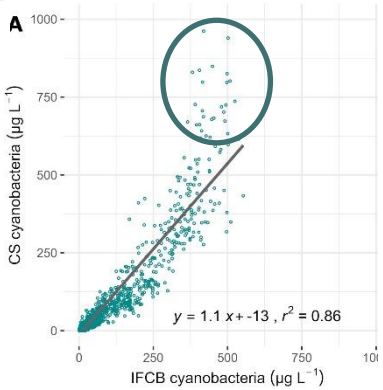
- Initiation with water temperature of approx. 15°C with max. peak at approx. or close to 20°C
- Variation in regard of whole community biomass
- Peaks coincide with satellite based FCA



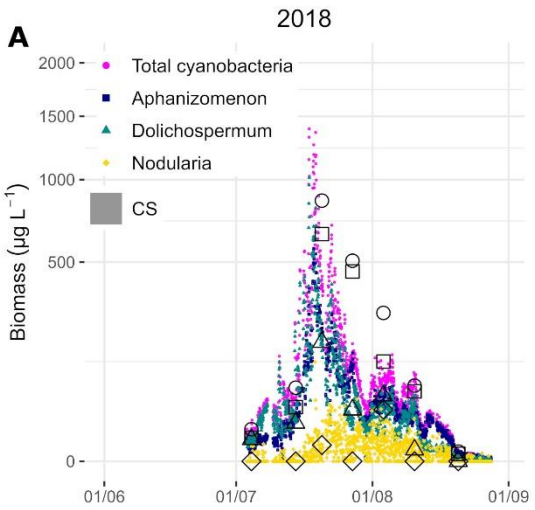
Kraft et al. manuscript

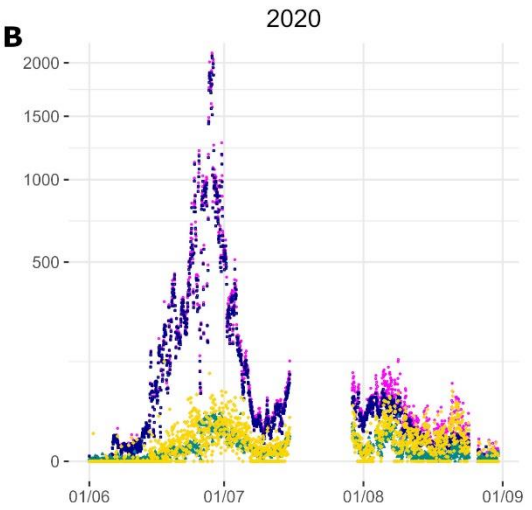
IFCB cyanobacteria, CytoSense & light microscopy

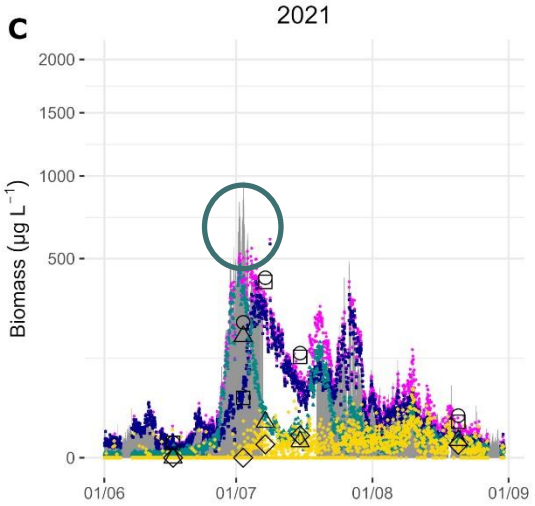


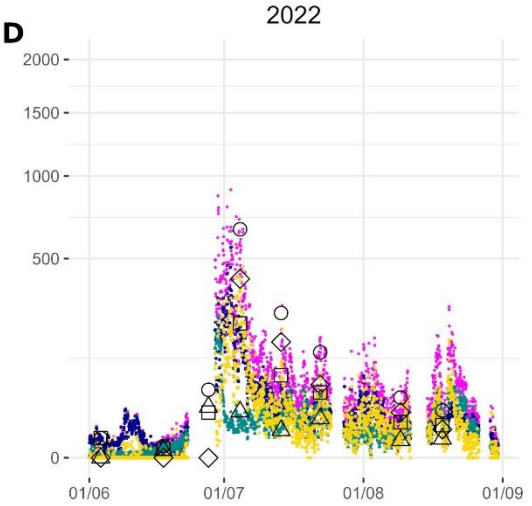


- Same overall bloom development
- Good agreement between IFCB & CS
- Some differences in exact biomass between IFCB & light microscopy









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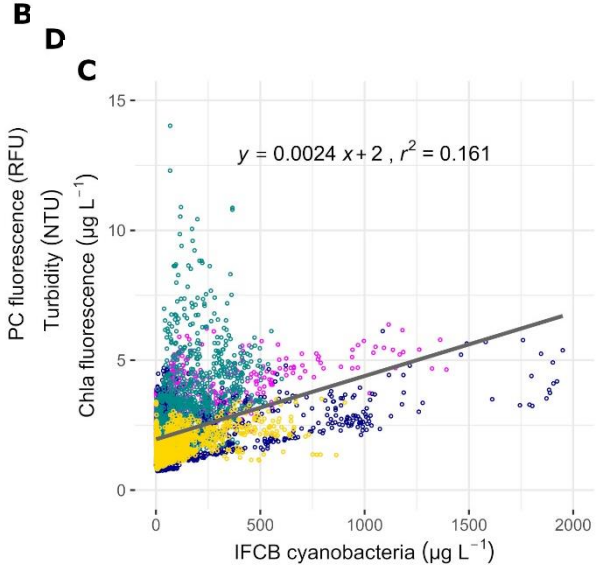
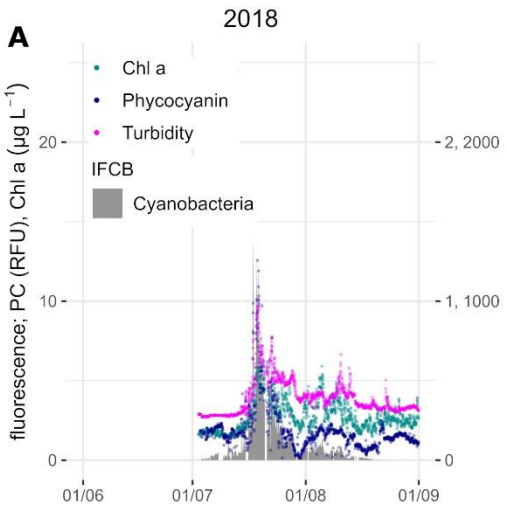
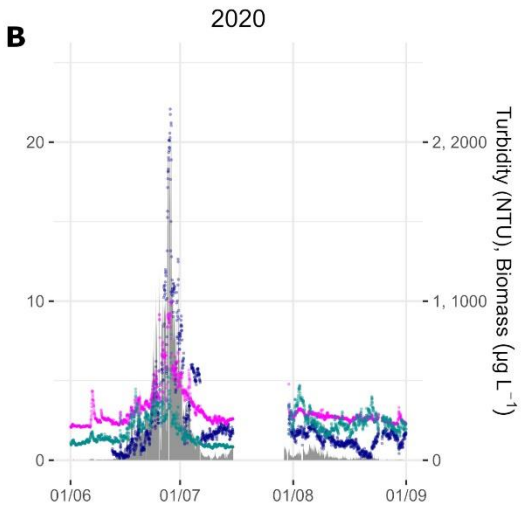
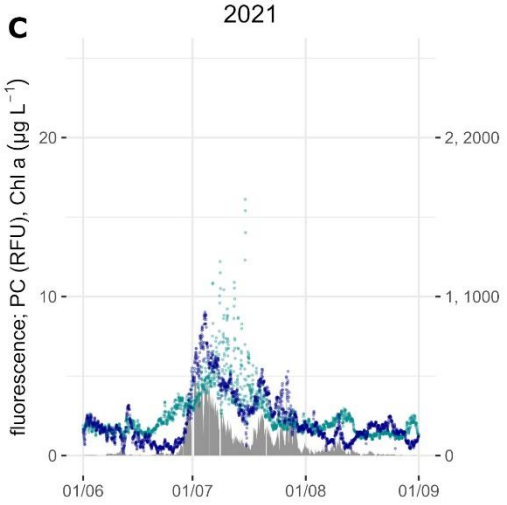
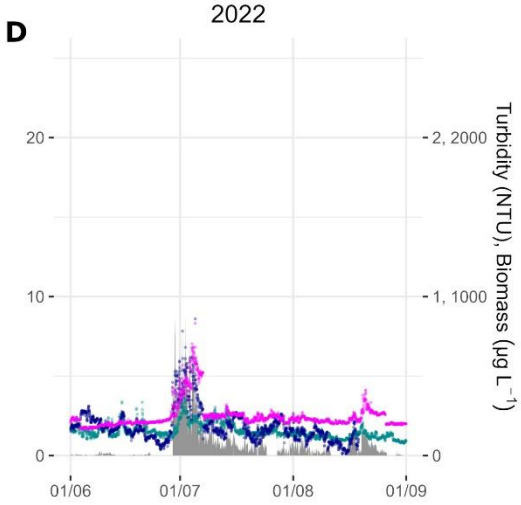
IFCB cyanobacteria, Chl *a* & PC fluorescence & turbidity











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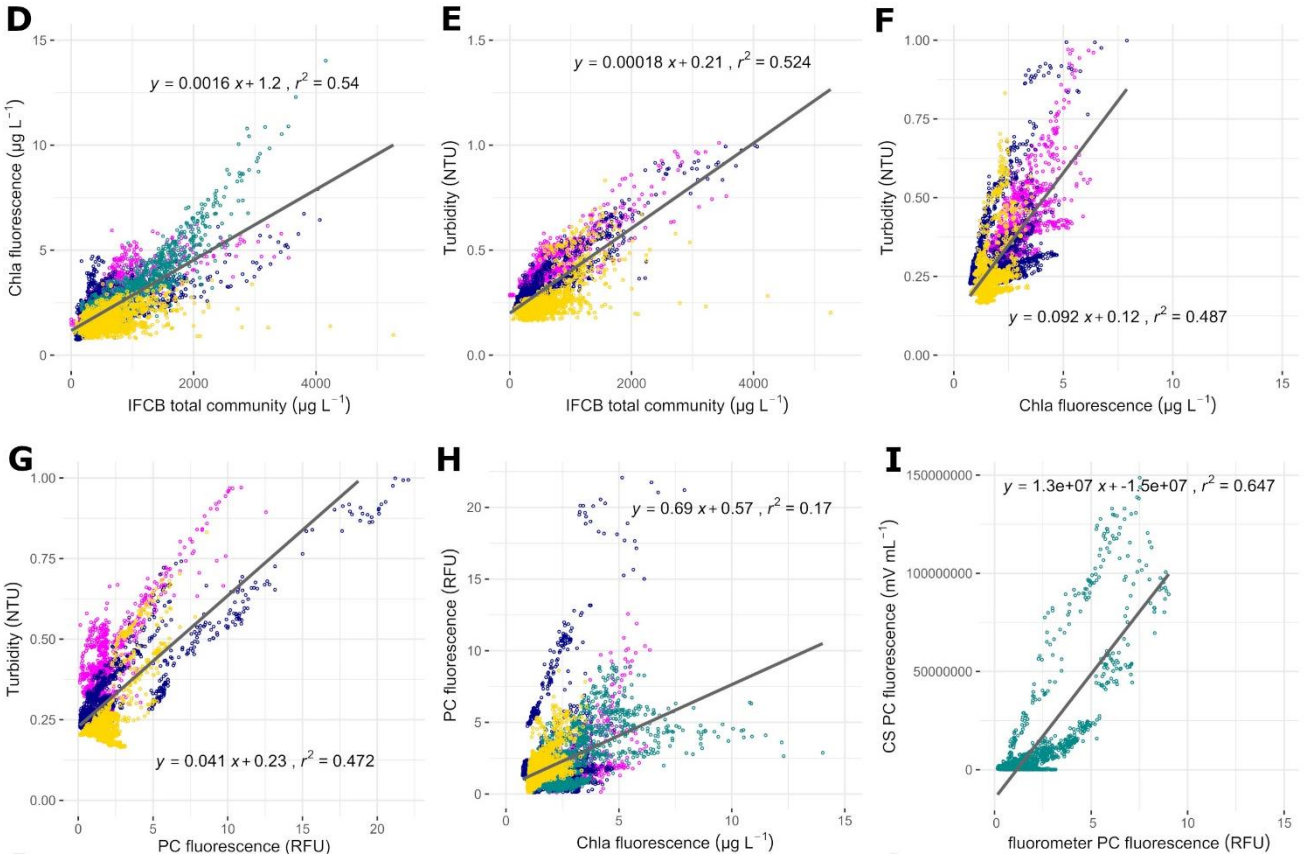
IFCB total community, Chl *a* & PC fluorescence & turbidity



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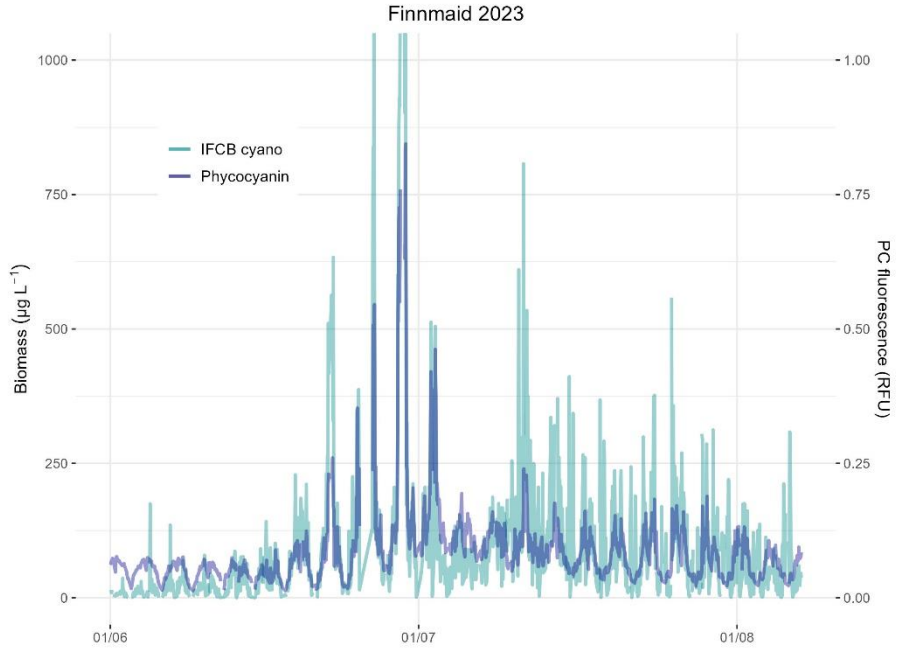
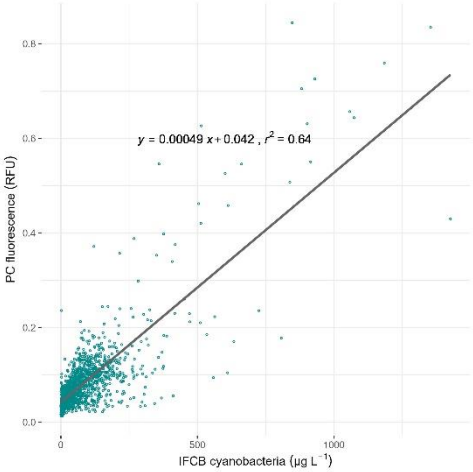
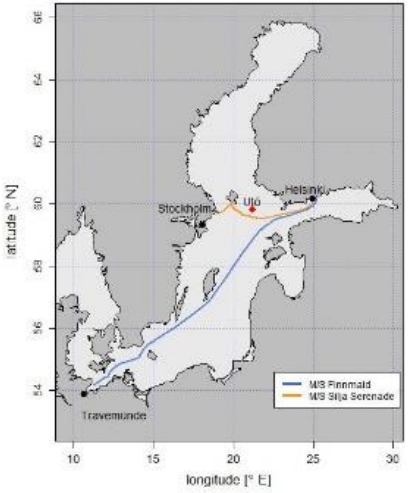
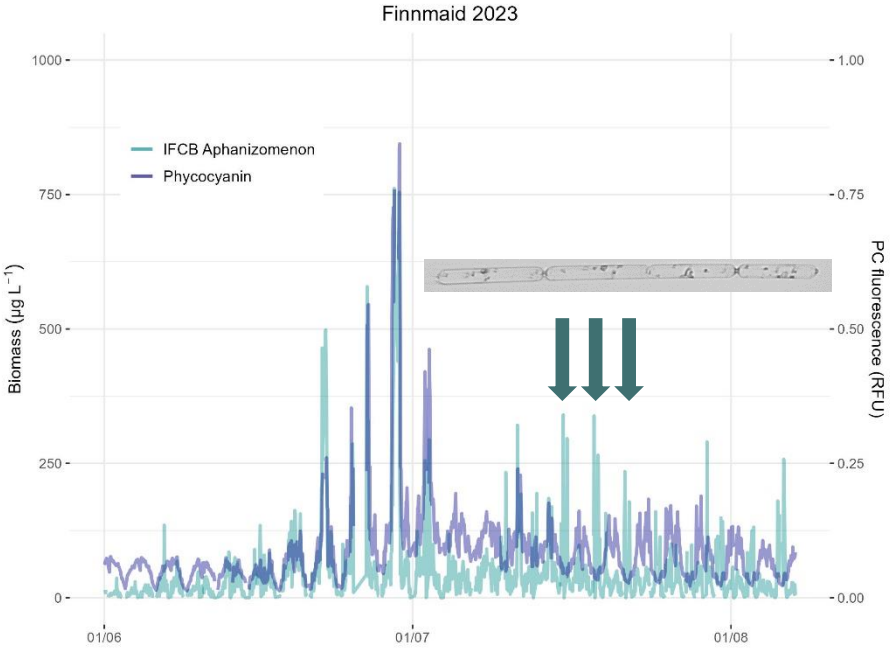
IFCB cyanobacteria & PC fluorescence on Finnmaid

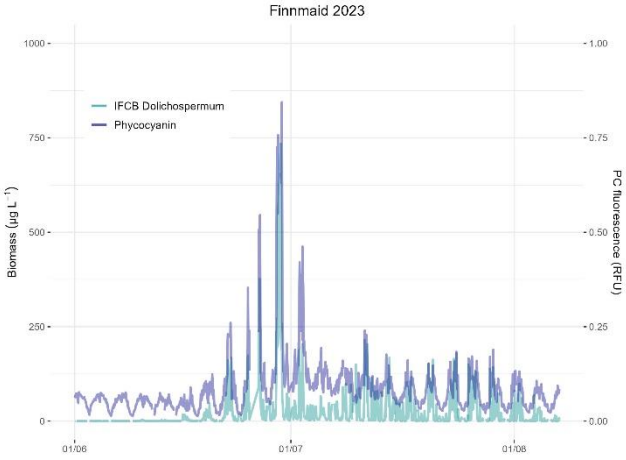


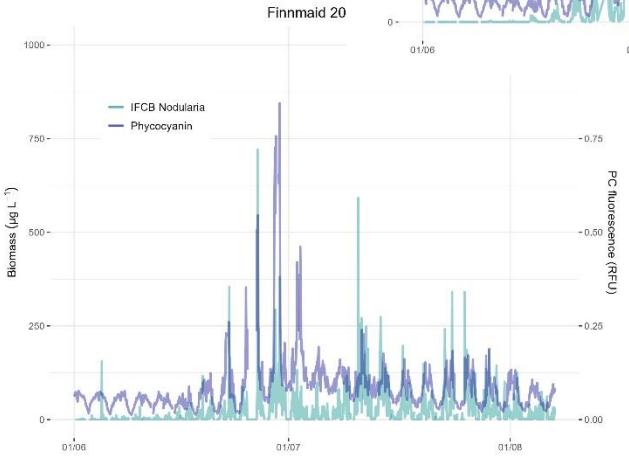




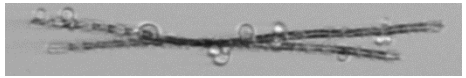
IFCB cyanobacteria & PC fluorescence on Finnmaid

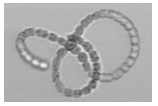
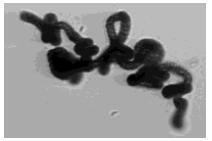


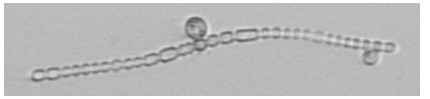




Conclusions





- IFCB is a great instrument for following the blooms
  - ➢ species-specific dynamics in high-frequency

- IFCB corresponds to CS and agrees with light microscopy on the higher biomass periods, however, some discrepancies in the magnitude of the total cyanobacteria and the species-specific biomasses – IFCB also agrees with other commonly used methods

- Strong relationship between IFCB cyanobacteria biomass and PC fluorescence in both high and low-biomass situations
  - ➢ IFCB cyanobacteria biomass seems to go together with PC fluorescence also when looking at the preliminary data from Finnmaid

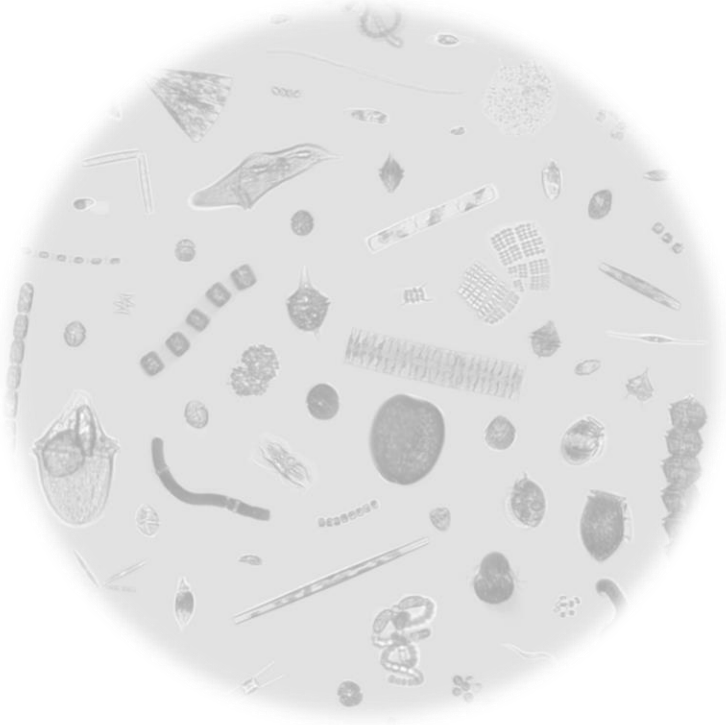
- High-frequency information about the community opens interesting possibilities to investigate species-specific dynamics in relation to environmental conditions, also potential for novel insights into changes in pigmentation through bloom development that can be linked to the overall state of the cells

Thank you!

Team:

- Jukka Seppälä
- Lumi Haraguchi
- Pasi Ylöstalo
- Sami Kielosto
- Annaliina Skyttä

- Lauri Laakso
- Martti Honkanen



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