JERICO and FerryBox workshop
Geestacht, Germany 26-29 September 2022
Presentation made on 28 September

AUTOMATED OBSERVATIONS OF PHYTOPLANKTON IN THE KATTEGAT-SKAGERRAK AND THE BALTIC SEA USING THE IMAGING FLOWCYTOBOT AND OTHER SENSORS ON R/V SVEA

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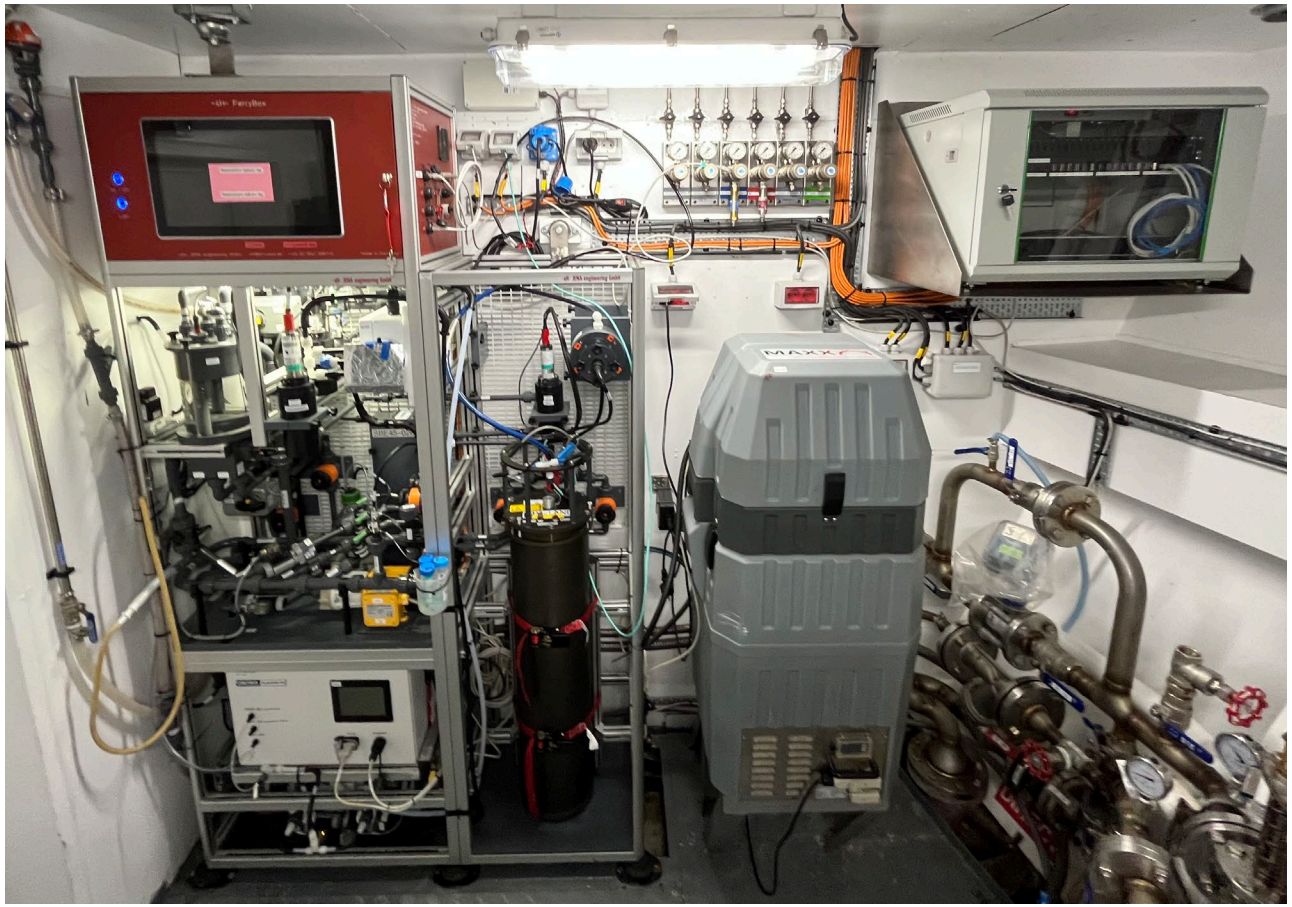
Imaging FlowCytobot, IFCB on R/V Svea

SMHI

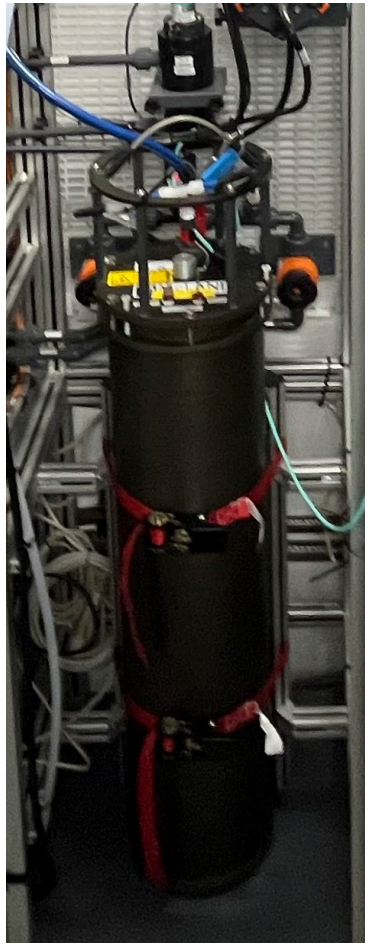
- IFCB part of FerryBox underway system
- Continuous flow of sea water
- Sampling every 20 minutes

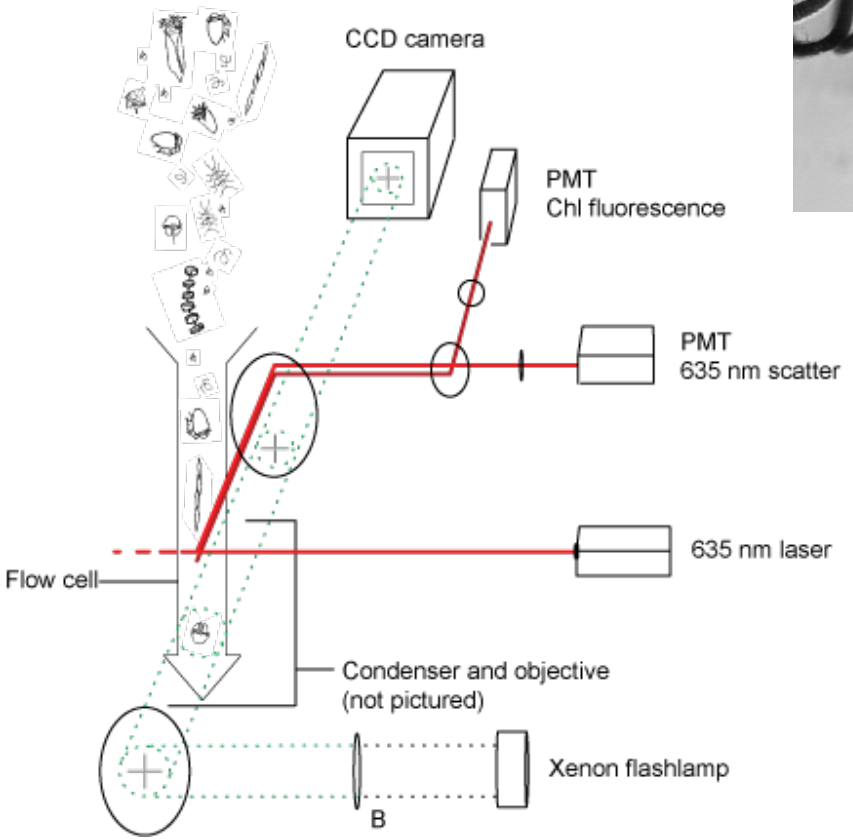


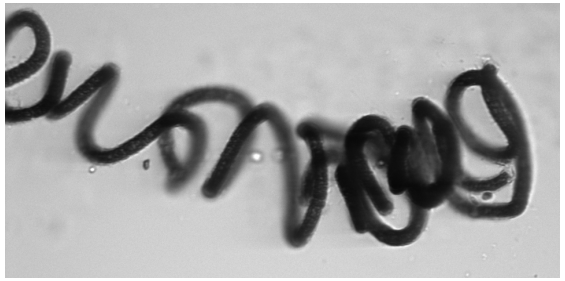
IFCB part of FerryBox underway system

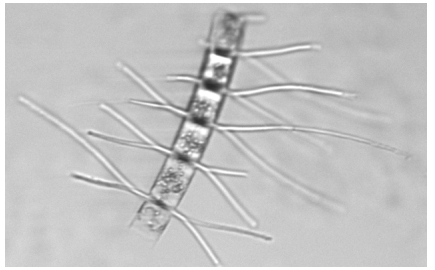
The Imaging FlowCytobot (IFCB) is an automated, submersible microscope

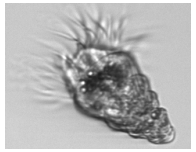
SMHI

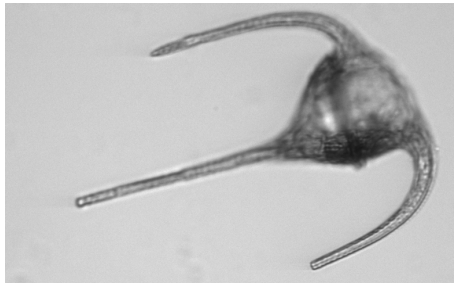


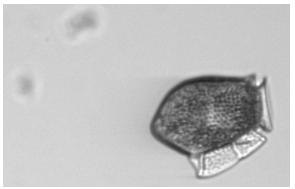






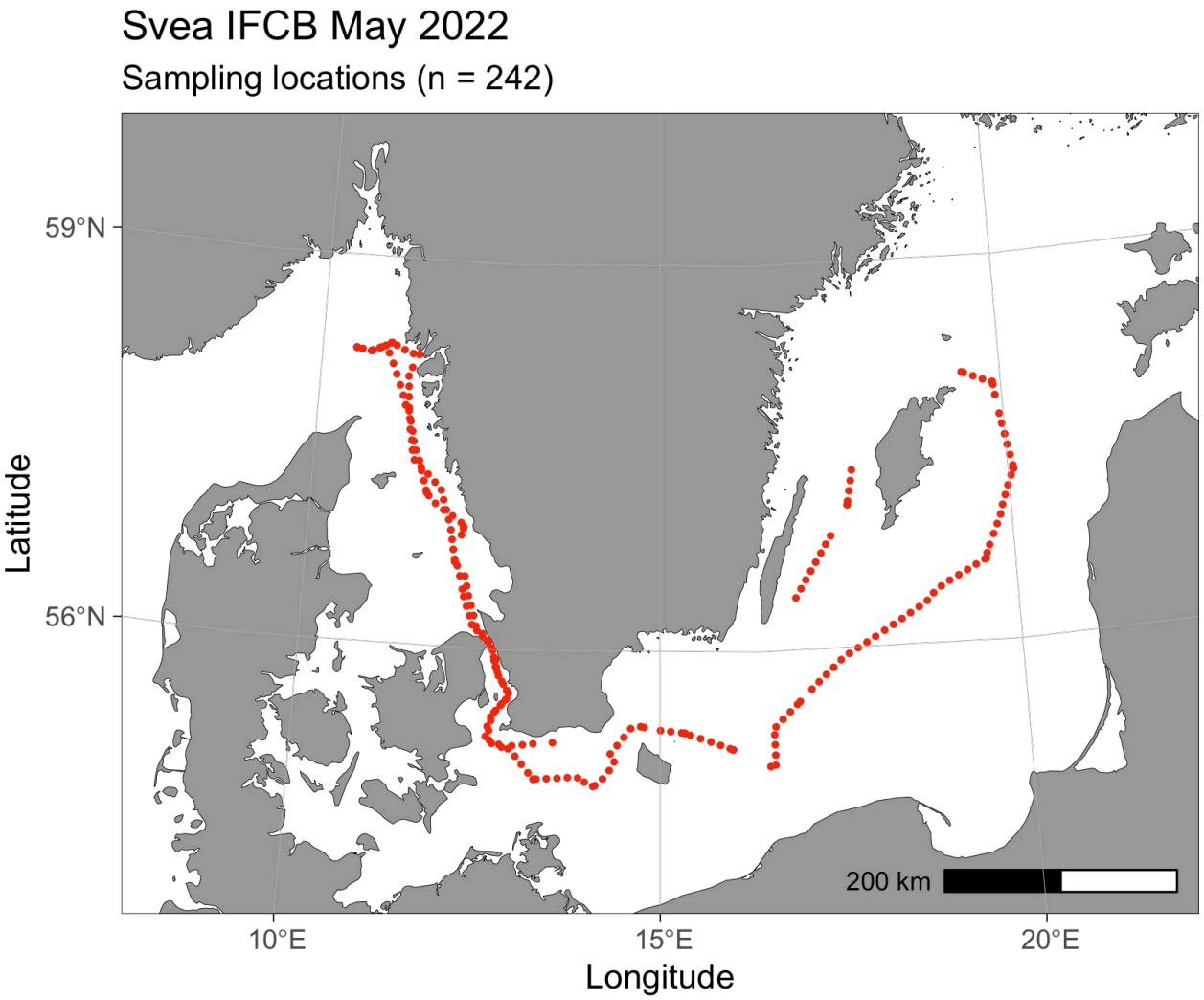


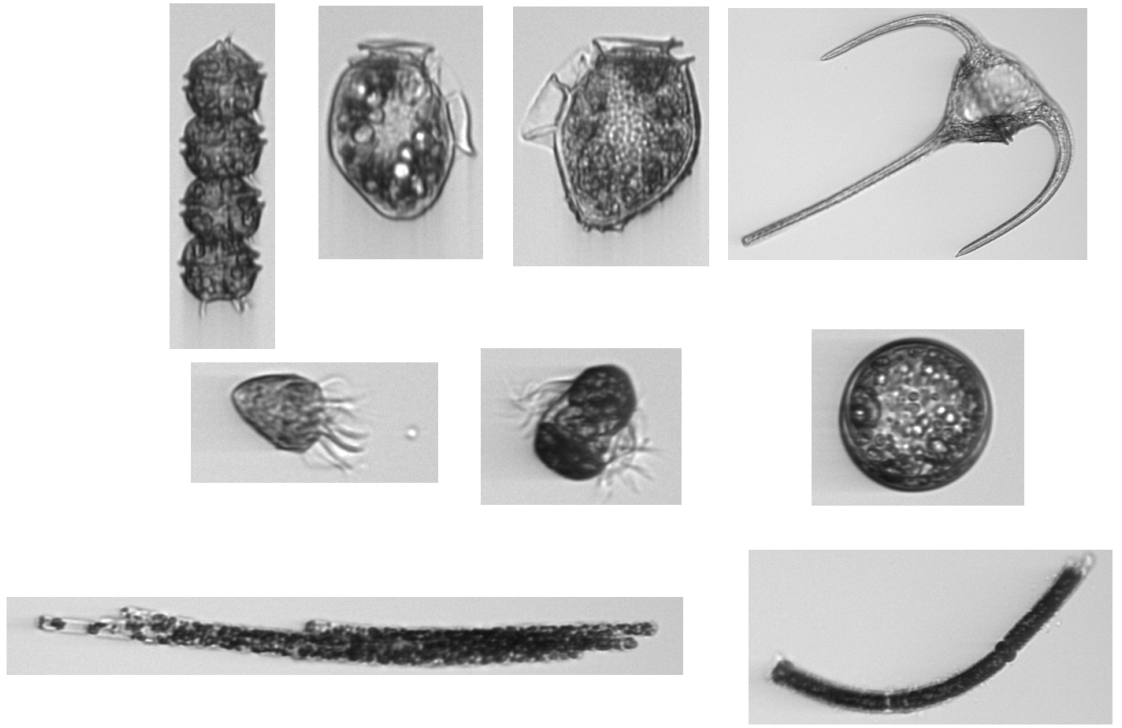




Plankton documented with the Imaging FlowCytobot on RIV Svea 12-18 July 2022

Some results from cruise in May 2022





Photos of plankton from the Imaging FlowCytobot. Left to right:

Top row: *Pedinella catenata*, *Dinophysis acuminata*, *Dinophysis norvegica* and *Tripos muelleri*, middle row: *Strombidium* sp., *Mesodinium rubru*m and an unidentified diatom, bottom row: *Aphanizomenon flos-aquae* and *Nodularia spumigena*. The scale in the images varies.

Quantitative observations of phytoplankton - a challenge

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- Size range from 0.7 µm to ~1 mm (pico 0.2-2, nano 2-20, micro 20-200 µm)
  - Cell numbers do not reflect biomass
  - Calculation of biomass from observations of cells requires information on abundance and size

- Large diversity
  - Morphological species ~700 in the Baltic Sea area (HELCOM-PEG/NOMP list)
  - Genetic species/strains (ASV = Amplicon Sequence Variants) > 7000 (Karlson et al. unpublished)

- Pigment composition does not reflect functional groups or taxonomic groups - there are exceptions (e.g. some cyanobacteria)

Some methods for phytoplankton observations

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- **Morphology**
  - Water sampling and microscopy
  - Automated imaging in flow
- **Genes**
  - Water sampling and analyses of rDNA - metabarcoding
  - qPCR/ddPCR
- **Pigments**
  - Water sampling and analysis of chlorophyll a
  - In situ chlorophyll fluorescence (+phycocyanin and phycoerythrin)
  - In situ absorption spectra
  - Satellite remote sensing of ocean colour - estimates of chlorophyll a
  - Flow Cytometry (scattering and fluorescence)

Some approaches for automation

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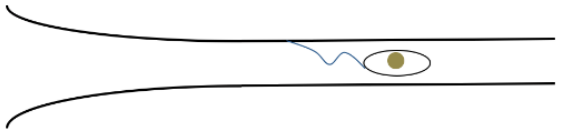
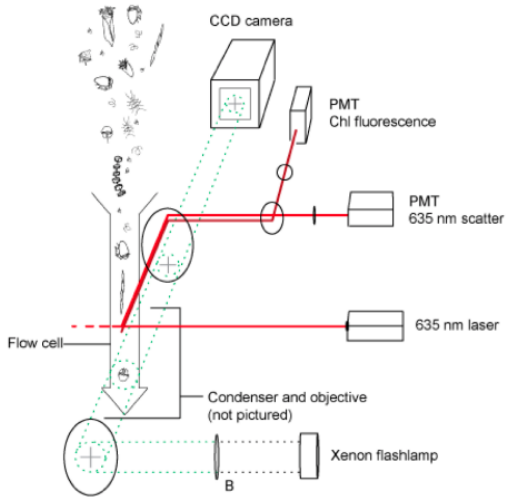
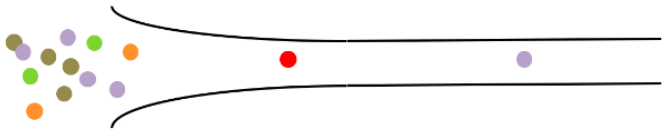
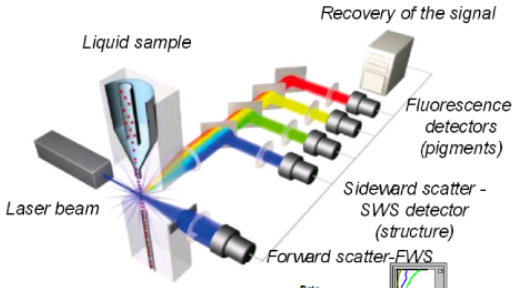
Imaging/in flow
Single cells – size and morphology of organisms



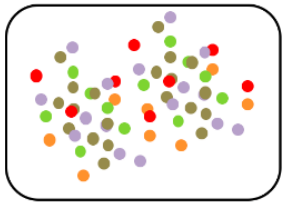


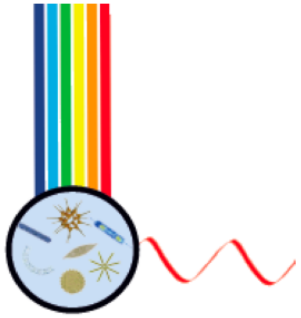
Automated flow cytometry (pulse shape-recording)
Single cells – fluorescence –pigment content and scattering (size, shape)

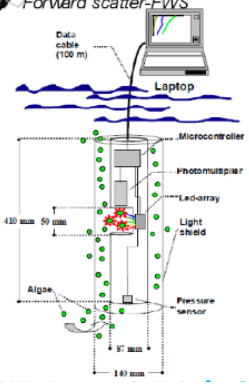




Fluorescence and absorption (multi-spectral)
Pigment based methods – bulk properties +
Variable fluorescence
(photosynthetic parameters)

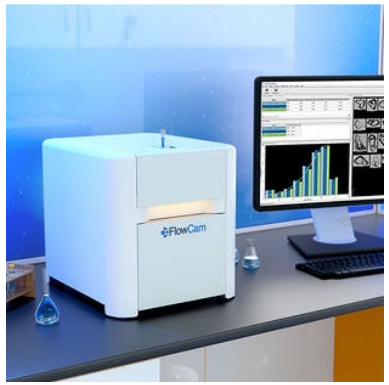






Instruments for imaging in flow

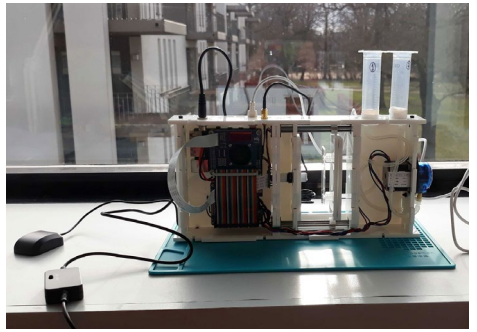
SMHI



FlowCam

Imaging FlowCytobot

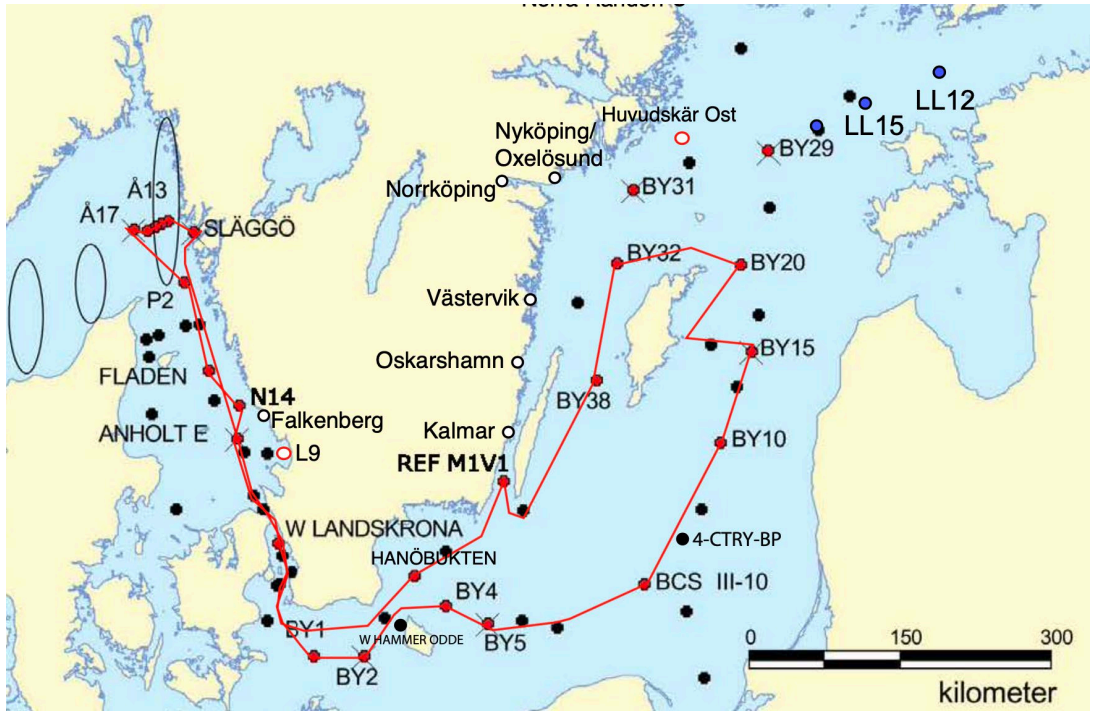
CytoSense



PlanktoScope (IOW)

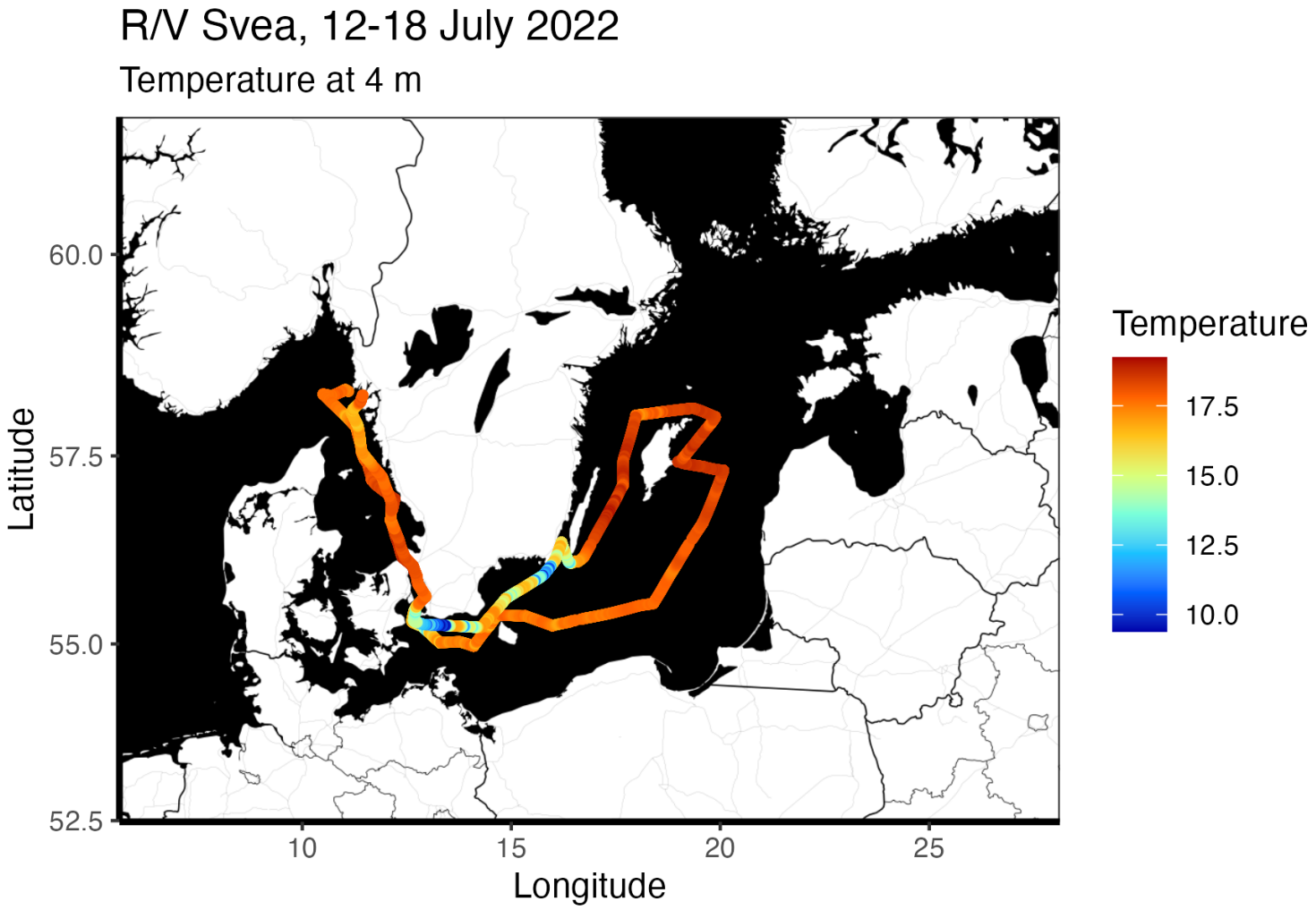
Phytoplankton observations 12-18 July 2022

- Monitoring cruise with R/V Svea
  - Tube sampling 0-10 m
    - Microscopy
  - FerryBox
    - Imaging FlowCytobot
    - Chlorophyll fluorescence
    - Phycocyanin fluorescence
    - Phycoerythrin fluoresence
  - CTD
    - Chlorophyll fluorescence
    - Phycocyanin fluorescence
  - MVP
    - Chlorophyll fluorescence



R/V Svea Ferrybox

- Continuous flow of sea water
- Data collected every minute
  - Salinity
  - Temperature
  - Chl. fluor
  - Phycocyanin fluor.
  - Phycoerythrin fluor.
  - CDOM fluor.
  - pH
- Data collected every 10-20 min.
  - pCO_2
  - pH
  - Phytoplankton - IFCB
- Water sampling device



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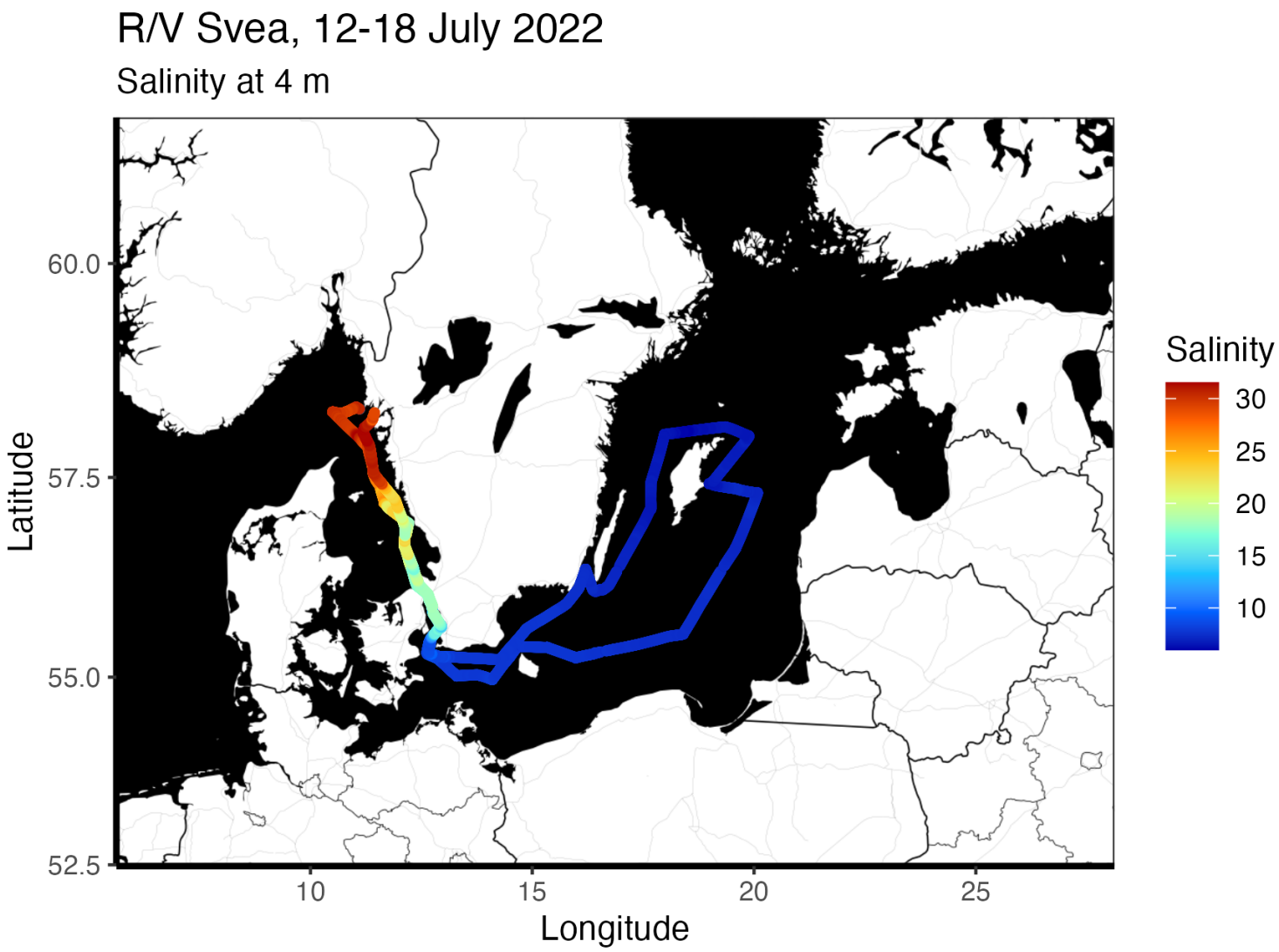

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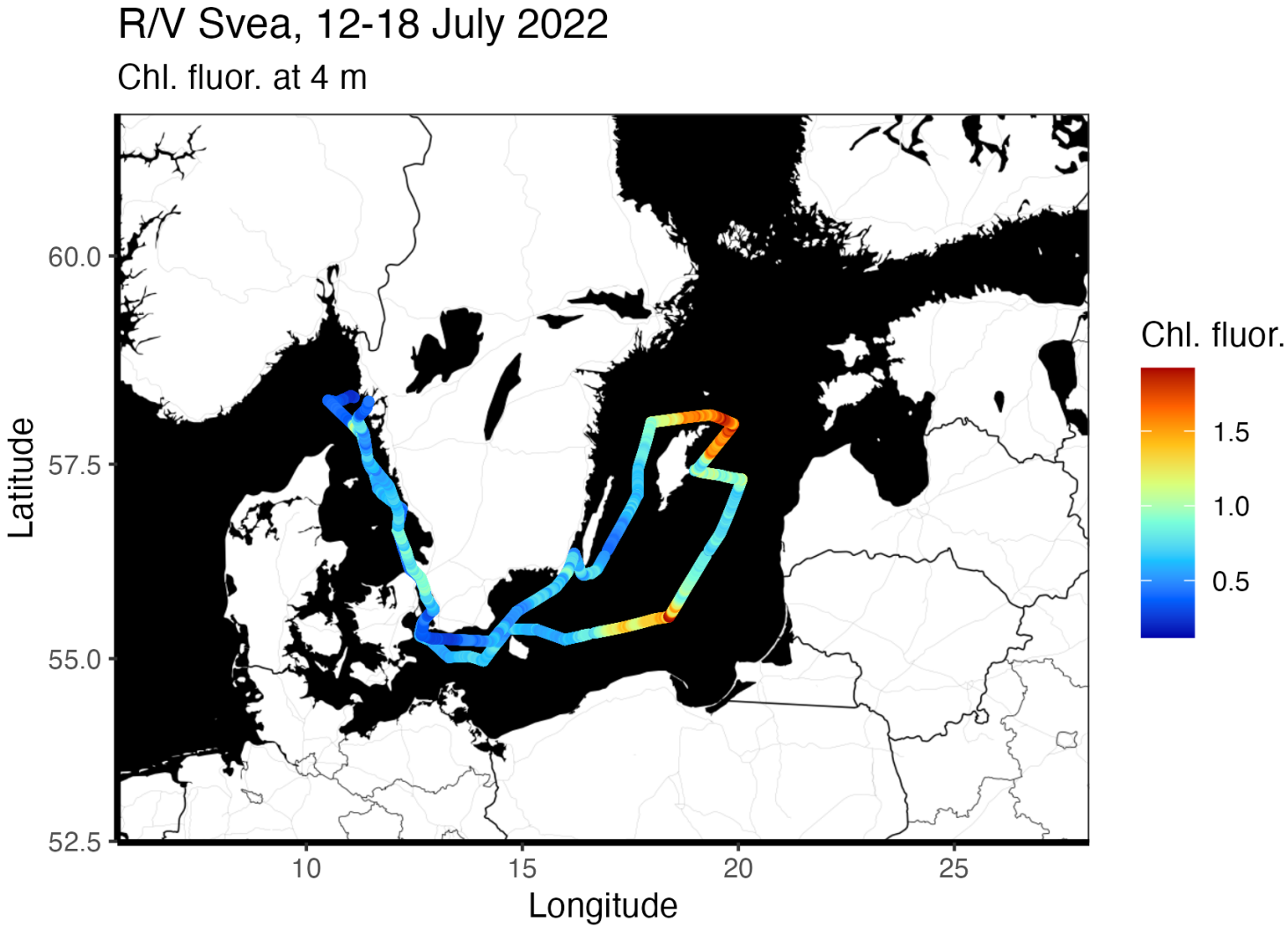

R/V Svea Ferrybox

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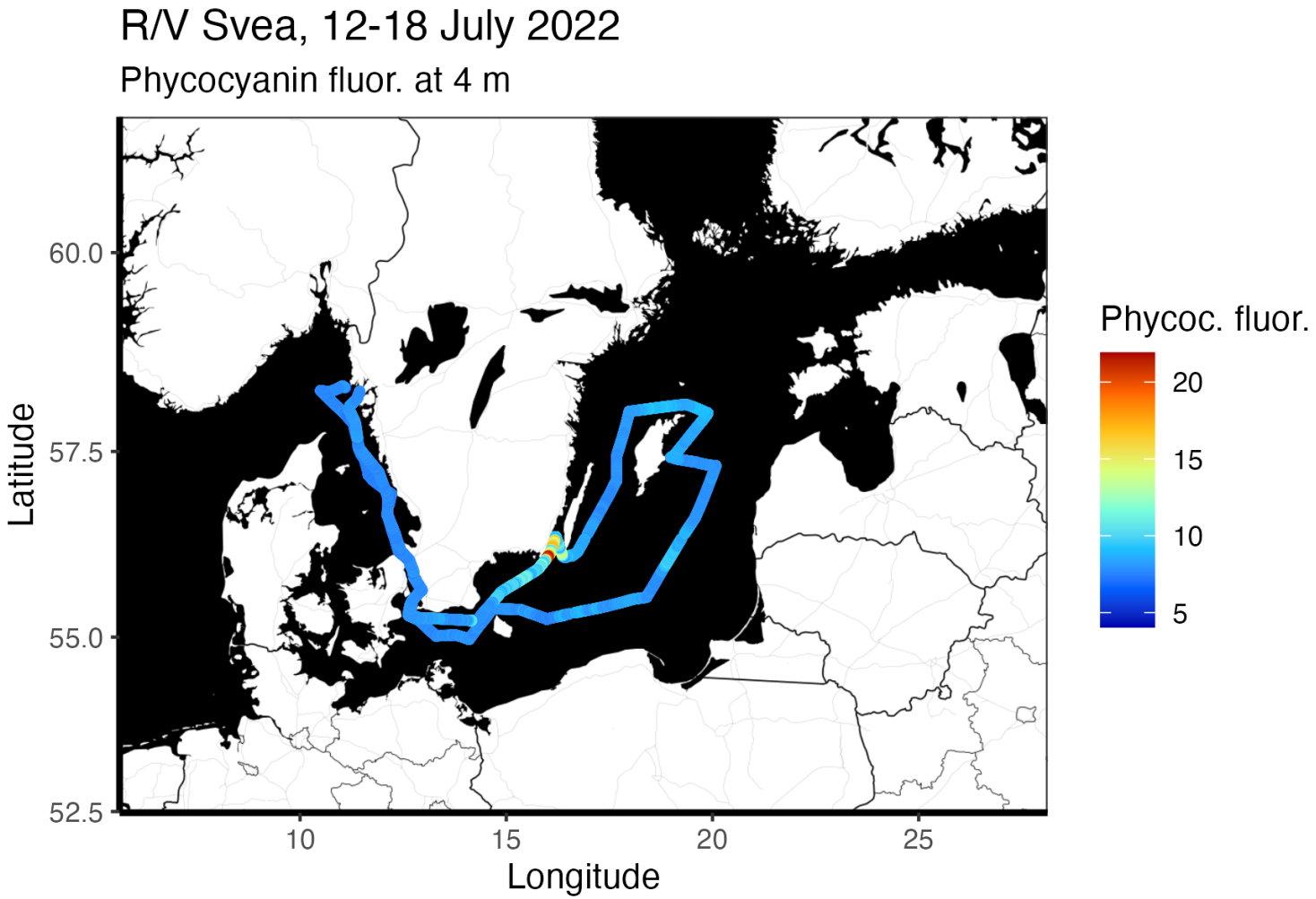
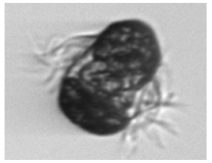
  - Salinity
  - Temperature
  - Chl. fluor
  - Phycocyanin fluor.
  - Phycoerythrin fluor.
  - CDOM fluor.
  - pH
- Data collected every 10-20 min.
  - pCO_2
  - pH
  - Phytoplankton - IFCB
- Water sampling device

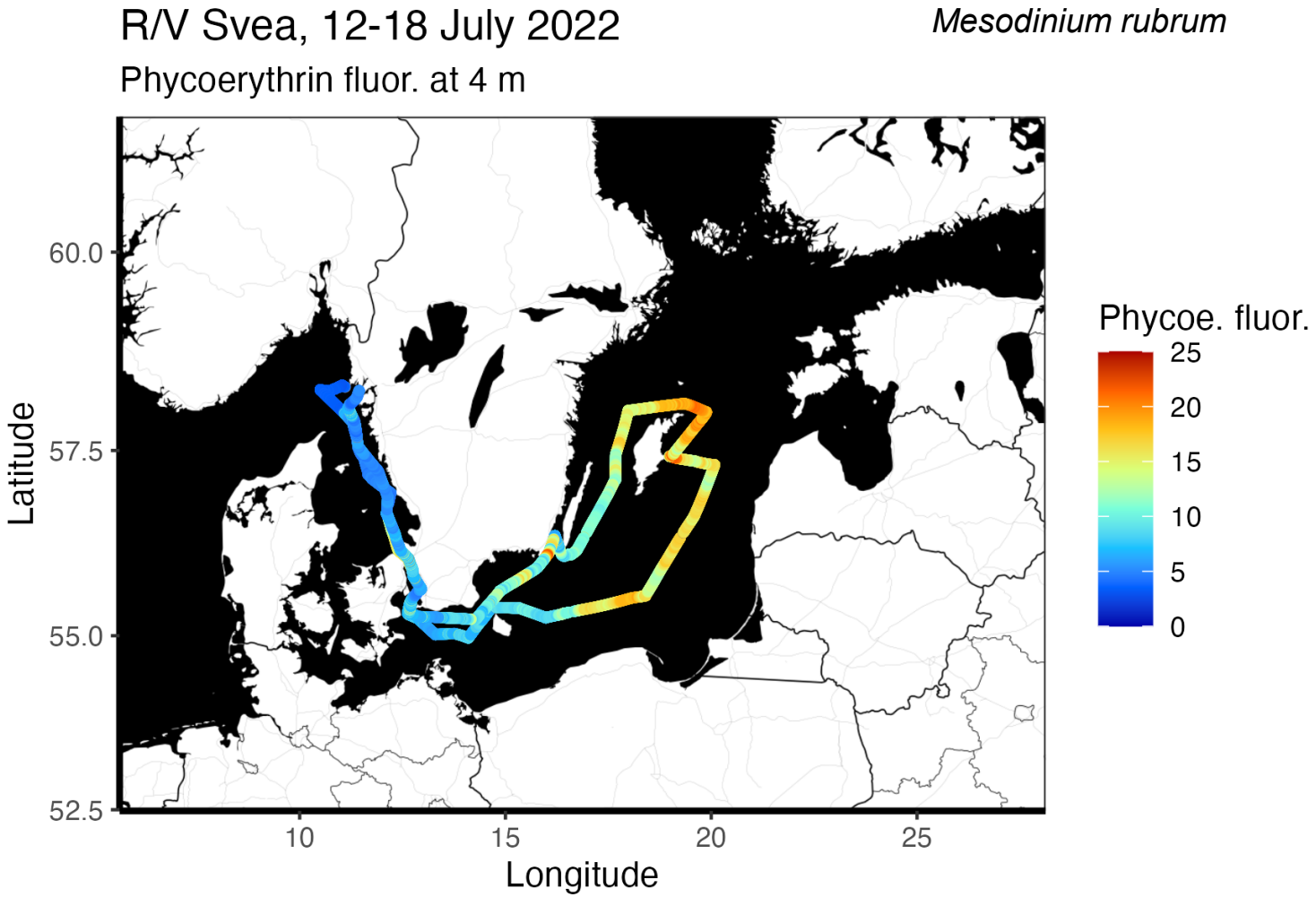


R/V Svea Ferrybox



Mesodinium rubrum

- Continuous flow of sea water
- Data collected every minute
  - Salinity
  - Temperature
  - Chl. fluor
  - Phycocyanin fluor.
  - Phycoerythrin fluor.
  - CDOM fluor.
  - pH
- Data collected every 10-20 min.
  - pCO_2
  - pH
  - Phytoplankton - IFCB
- Water sampling device



Preliminary results from cruise in July 2022

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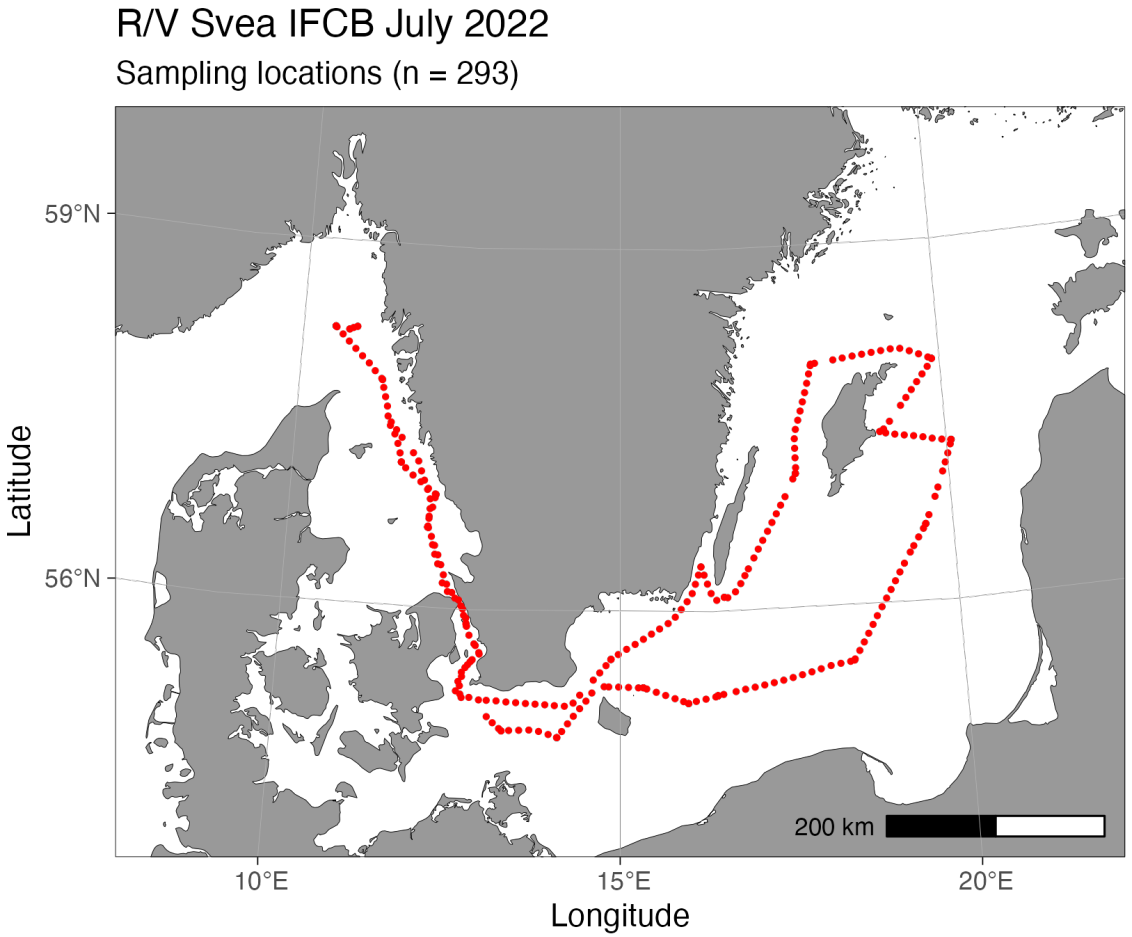

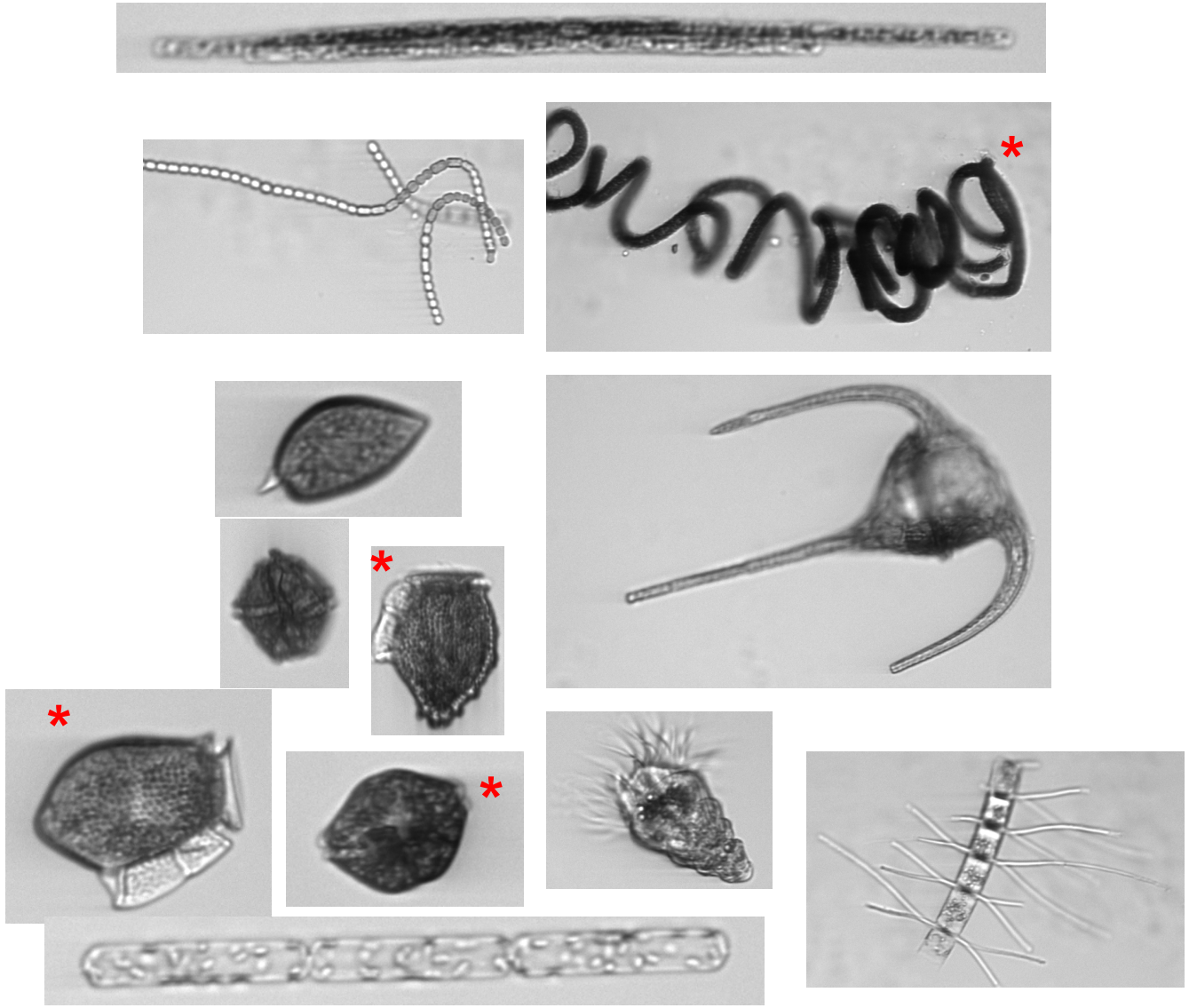

*Toxin producers

IFCB data flow and production of classifiers

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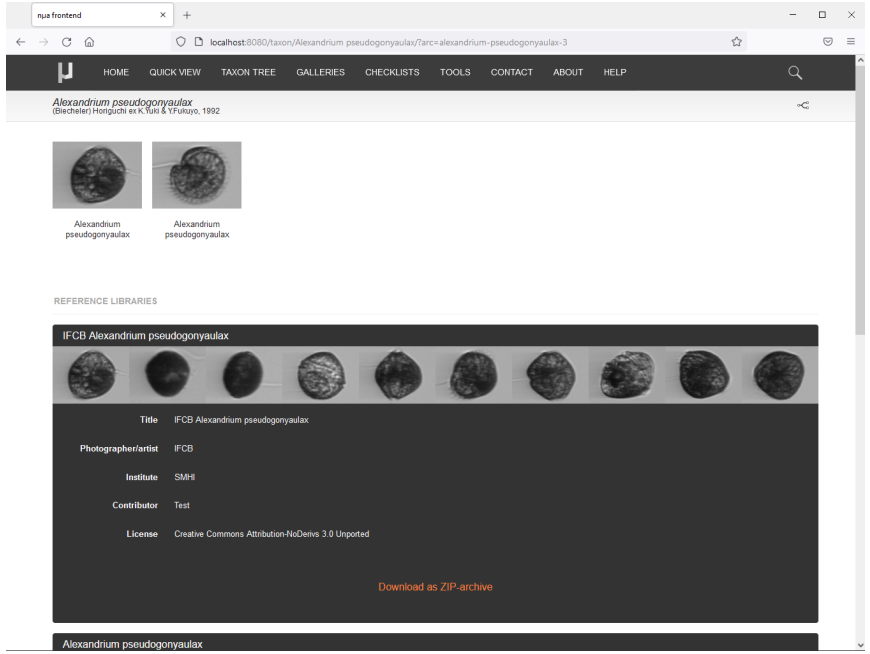
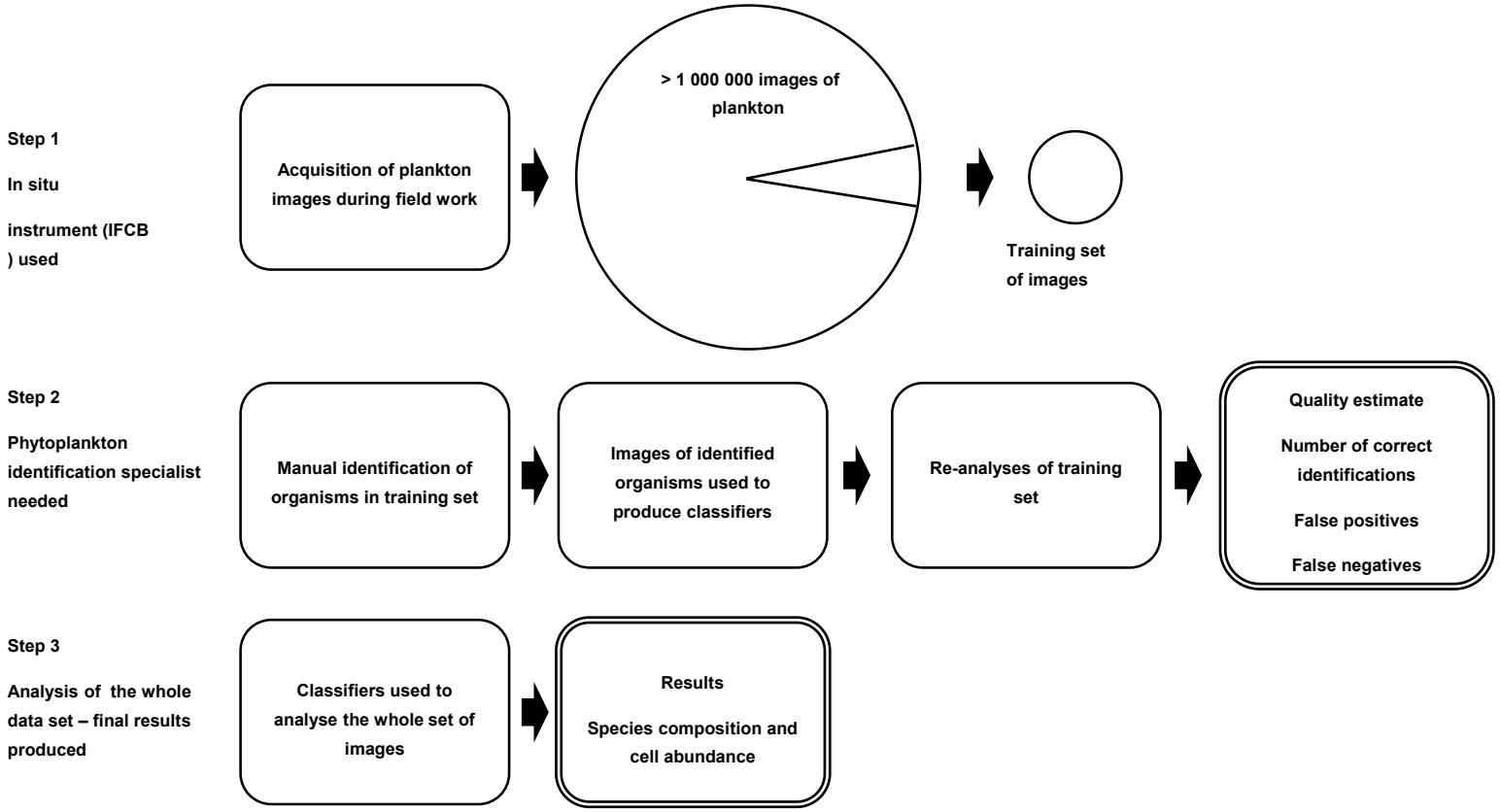
ANNOTATED IMAGES

LIBRARY OF REFERENCE IMAGES



NORDIC MICROALGAE

https://nordicmicroalgae.org



Cyanobacteria from satellite 12-18 July 2022

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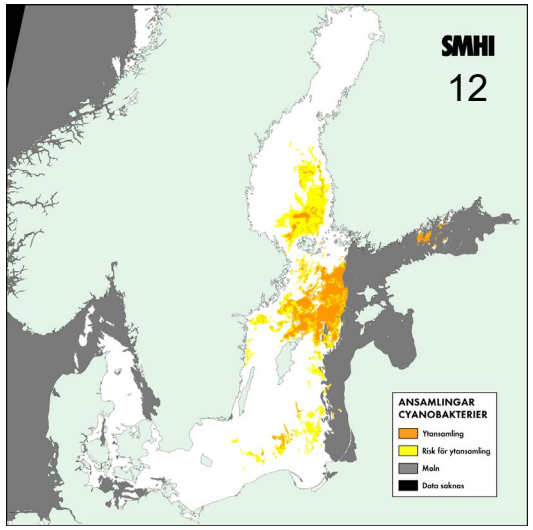
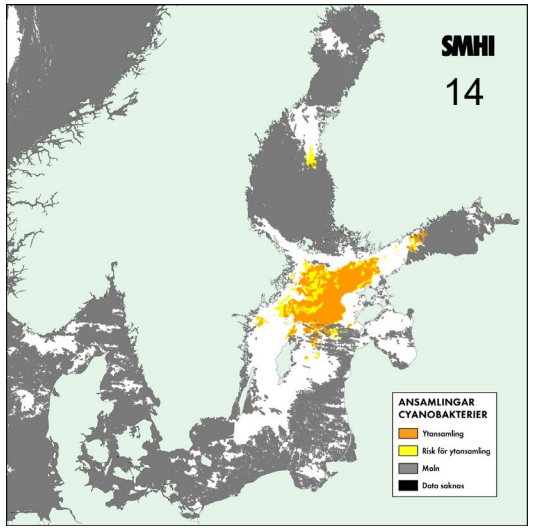
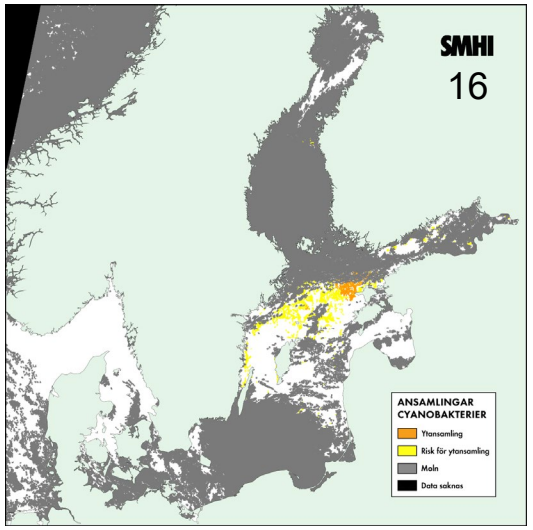
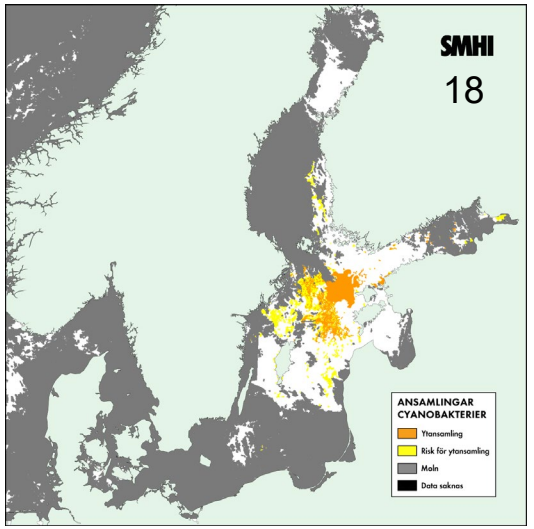
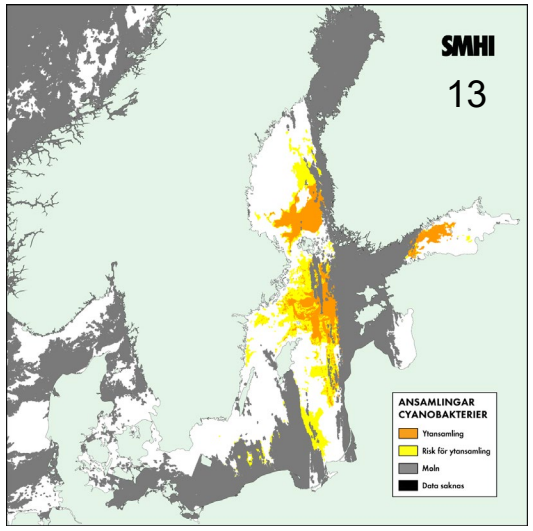
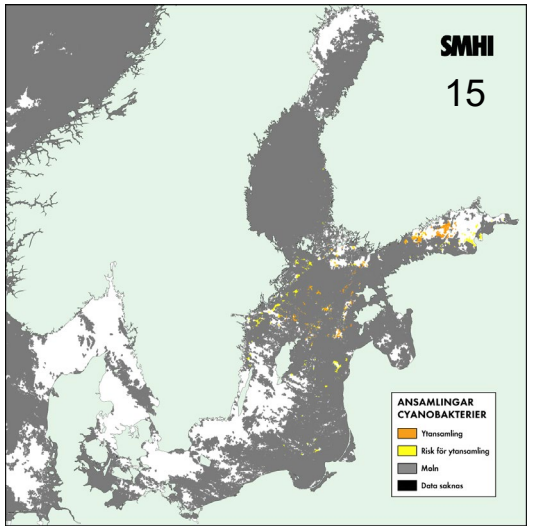


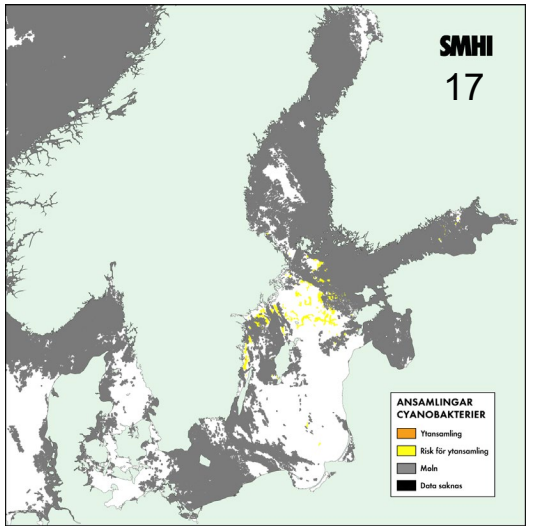










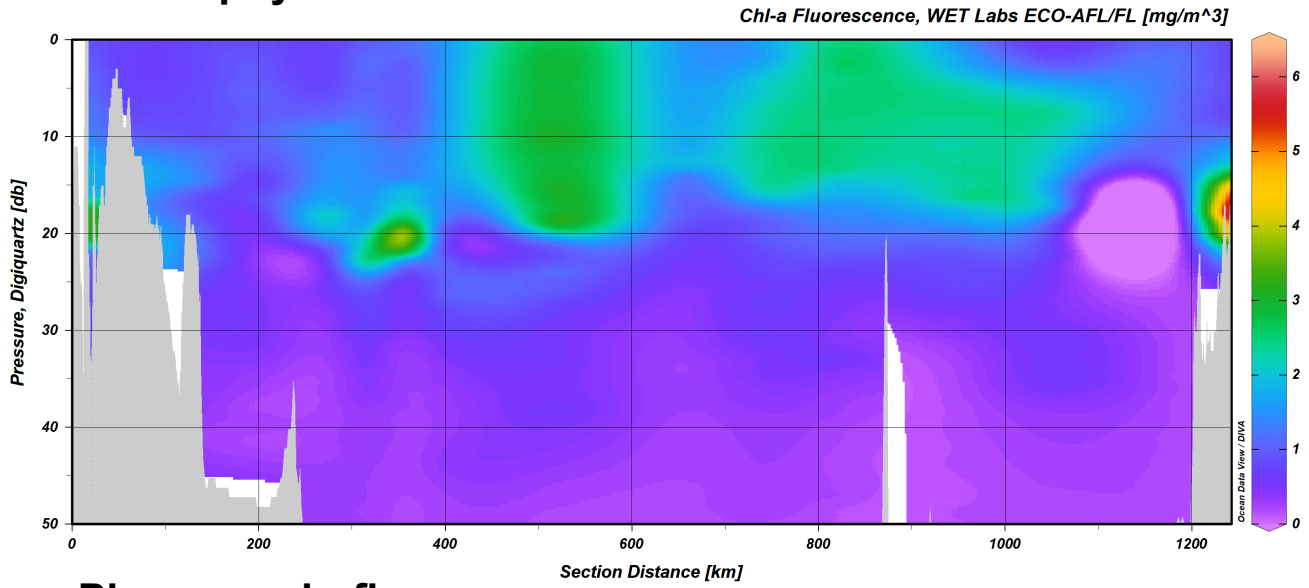


BALTIC ALGAE WATCH SYSTEMS

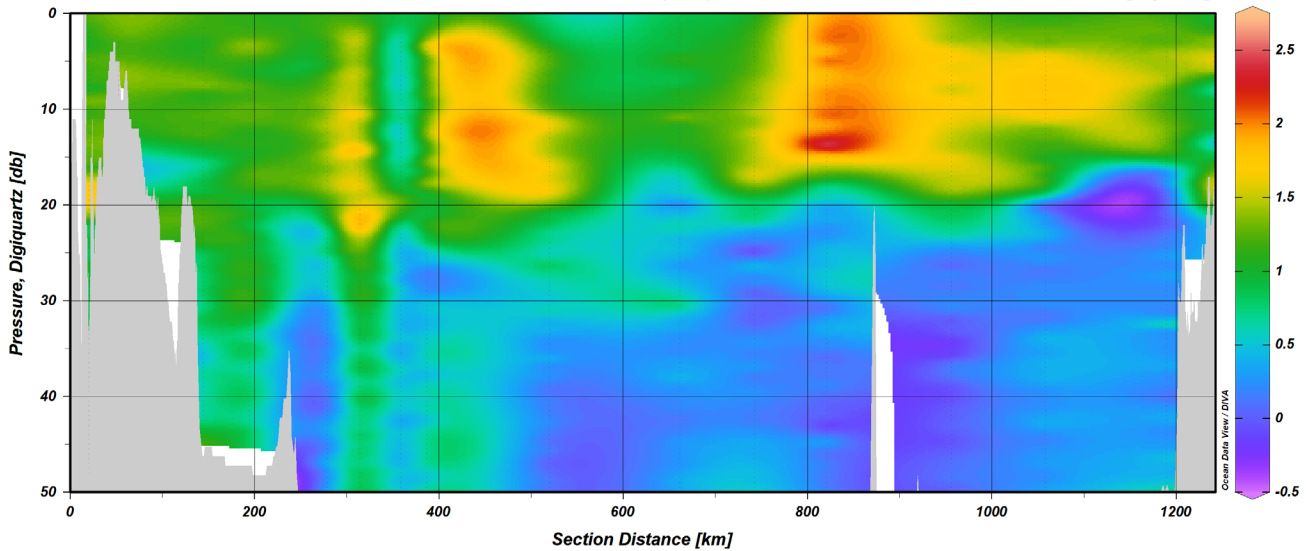
https://www.smhi.se/data/oceanografi/algsituationen

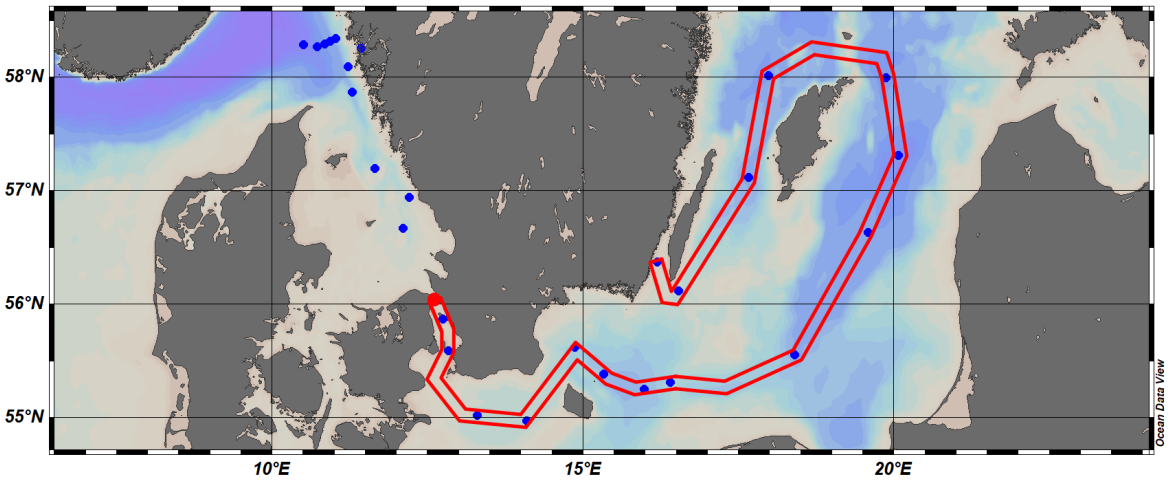
Optical sensors on CTD

Chlorophyll fluorescence



Phycocyanin fluorescence





Summary

- Imaging FlowCytobot
  - Provides detailed information at species or genus level
  - Cell abundance and biovolume and biomass in carbon
  - New IFCB deployed on R/V Svea
    - Cruises carried out in May and July 2022, next one in October
    - Observations of cyanobacteria successful - large colonies overlooked?
    - Fully operational in 2023?
- Satellite remote sensing revealed near surface distribution of some cyanobacteria
- Bio-optical sensors in FerryBox systems, on CTD showed distribution of chlorophyll fluorescence and phycocyanin fluorescence, proxies for biomass

Acknowledgements

- JERICO-S3
- Swedish Biodiversity Data Infrastructure (SBDI)
- Swedish National Marine Monitoring Program
- Colleagues at SMHI and crew involved in sampling on R/V Svea

Swedish Biodiversity Data Infrastructure