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

- IFCB part of FerryBox underway system
- Continuous flow of sea water
- Sampling every 20 minutes
The Imaging FlowCytobot (IFCB) is an automated, submersible microscope

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 rubrum* and an unidentified diatom, bottom row: *Aphanizomenon flos-aquae* and *Nodularia spumigena*. The scale in the images varies.
Quantitative observations of phytoplankton - a challenge

- 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 unpublished)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

- **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

**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

- 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 fluorescence
- 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.
  - pCO₂
  - pH
  - Phytoplankton - IFCB
- Water sampling device
R/V Svea Ferrybox

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Preliminary results from cruise in July 2022

R/V Svea IFCB July 2022
Sampling locations (n = 293)

* Toxin producers
Acquisition of plankton images during field work

Images of identified organisms used to produce classifiers

Manual identification of organisms in training set

Images of identified organisms used to produce classifiers

Classifiers used to analyse the whole set of images

Results

Species composition and cell abundance

Step 1
In situ instrument (IFCB) used

Step 2
Phytoplankton identification specialist needed

Step 3
Analysis of the whole data set – final results produced

> 1,000,000 images of plankton

Training set of images

Quality estimate

Number of correct identifications
False positives
False negatives
Cyanobacteria from satellite 12-18 July 2022

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
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