Using different FlowCams to observe grazers

GlobalHAB symposium on automated in situ observations of plankton

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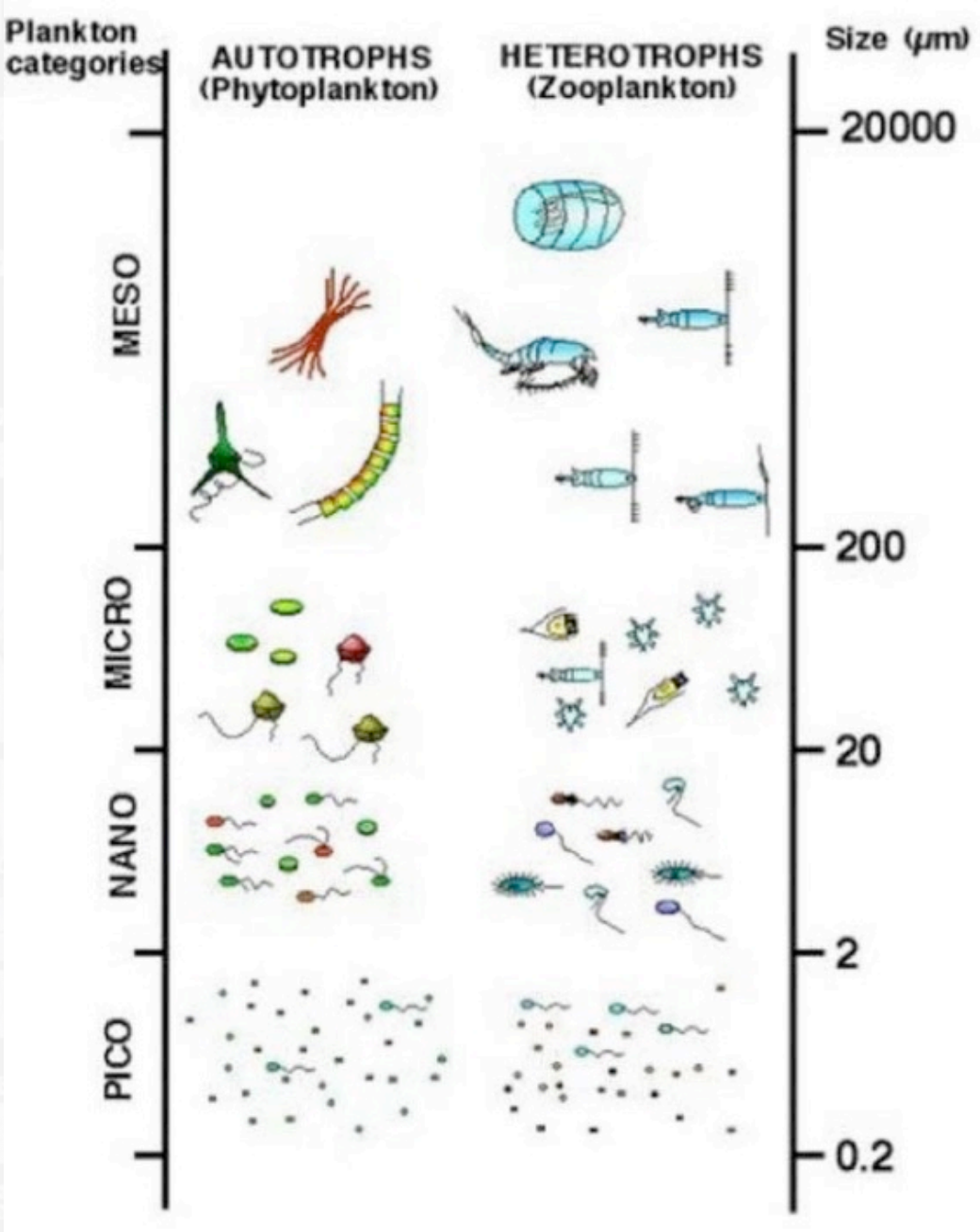
Overview of this presentation

- Objective and challenges
- How we come from FlowCam data to classified data
- Some examples of our use of premature classifiers
- Combining the data from different FlowCams and instruments

Objective

Implement FlowCam in routine monitoring of coastal «grazers» using automatic image recognition at IMR in Norway

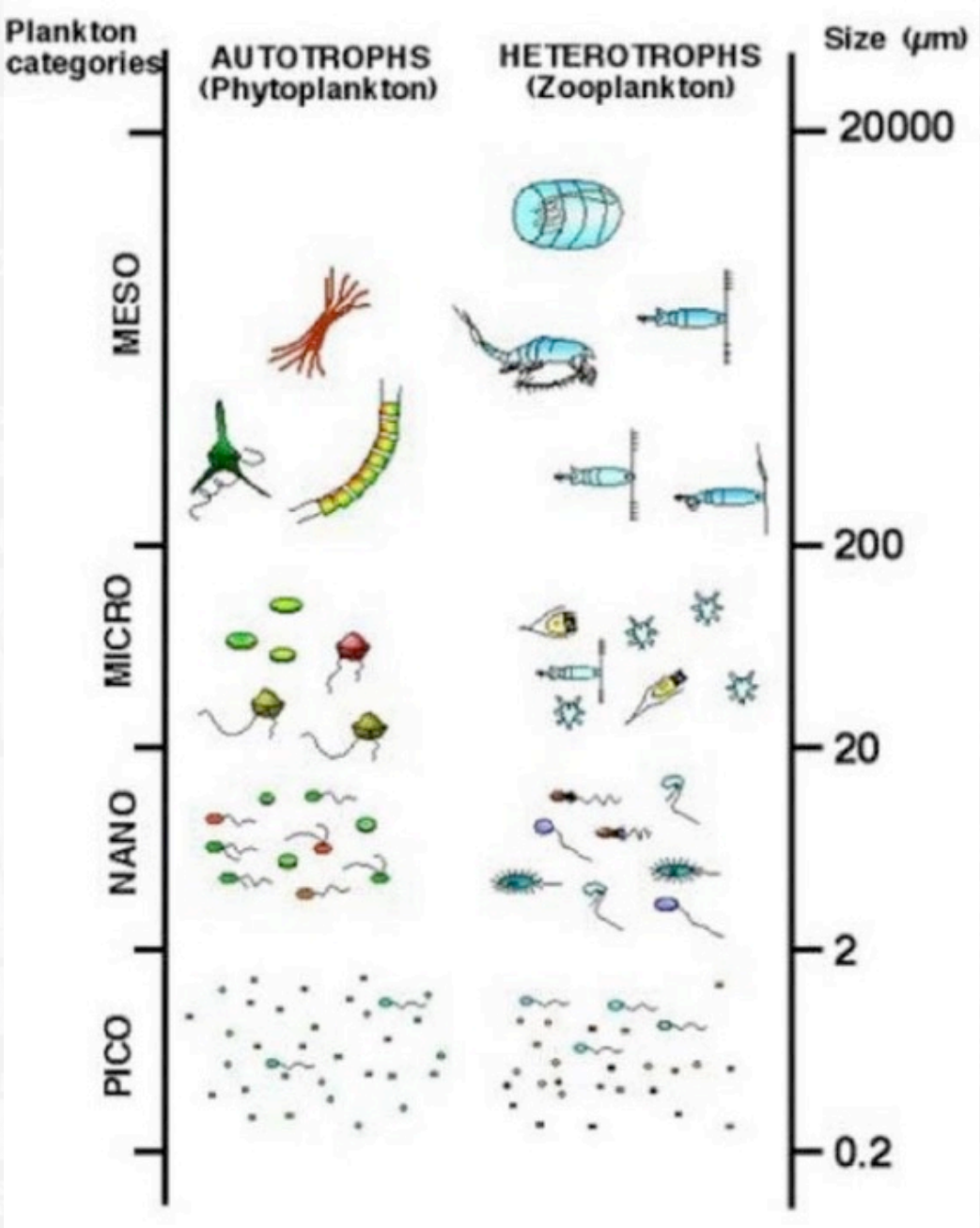


From **Alcaraz** and **Calbet 2003**

Challenges

- Large size range (5- 8000 µm in length)
    - The need to combine different magnification settings and FlowCam instruments and make multiple training sets

- Often low abundances
    - Requires large volume of water to be imaged
- Many without pigments and fluorescence signals
    - The need to use autoimage-mode and classify large numbers of non-living particles (e.g. detritus, bubbles, background images)
    - Including diatoms
    - All image-types has to be classified



From **Alcaraz** and **Calbet 2003**

Different instruments for different plankton size fractions

Flowcam macro, FCM (50-2000µm)

- Mesozooplankton
- WP2 and multinet plankton net trawls
- Formalin fixed

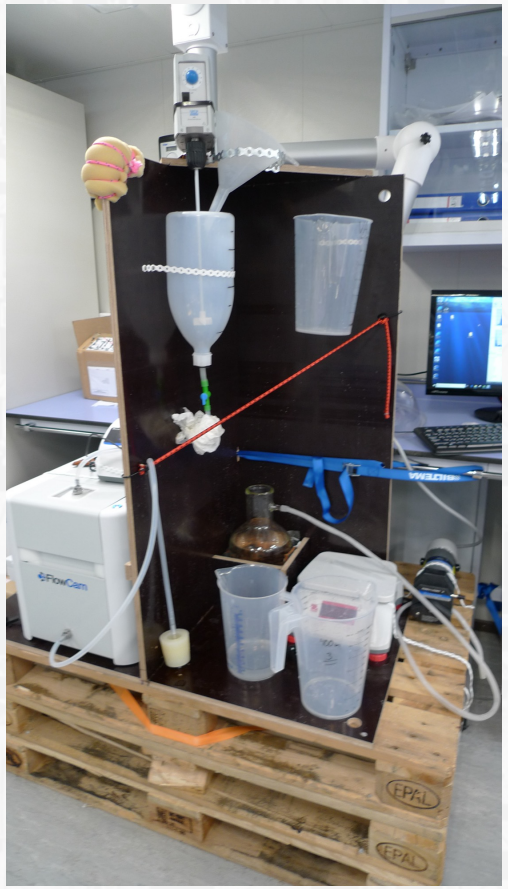
Flowcam VS-1, FCVS (35-500µm)

- Microplankton
- **Unfiltered** seawater samples (500 ml)
- Lugol`s fixed

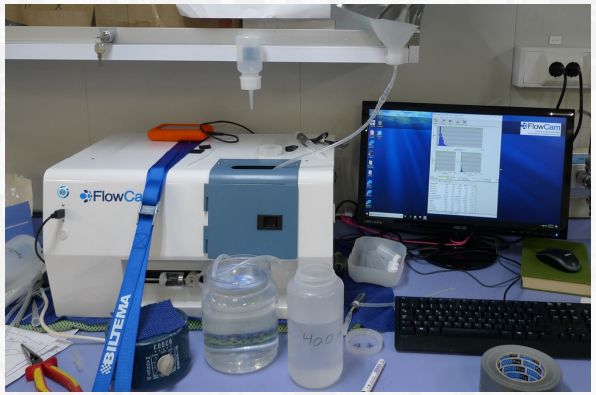
Flowcam 8400, FC8400 (5-300µm)

- Nano/microplankton
- Lugol fixed

Flowcam macro



Flowcam VS



Flowcam 8400

Flowcam principle in short

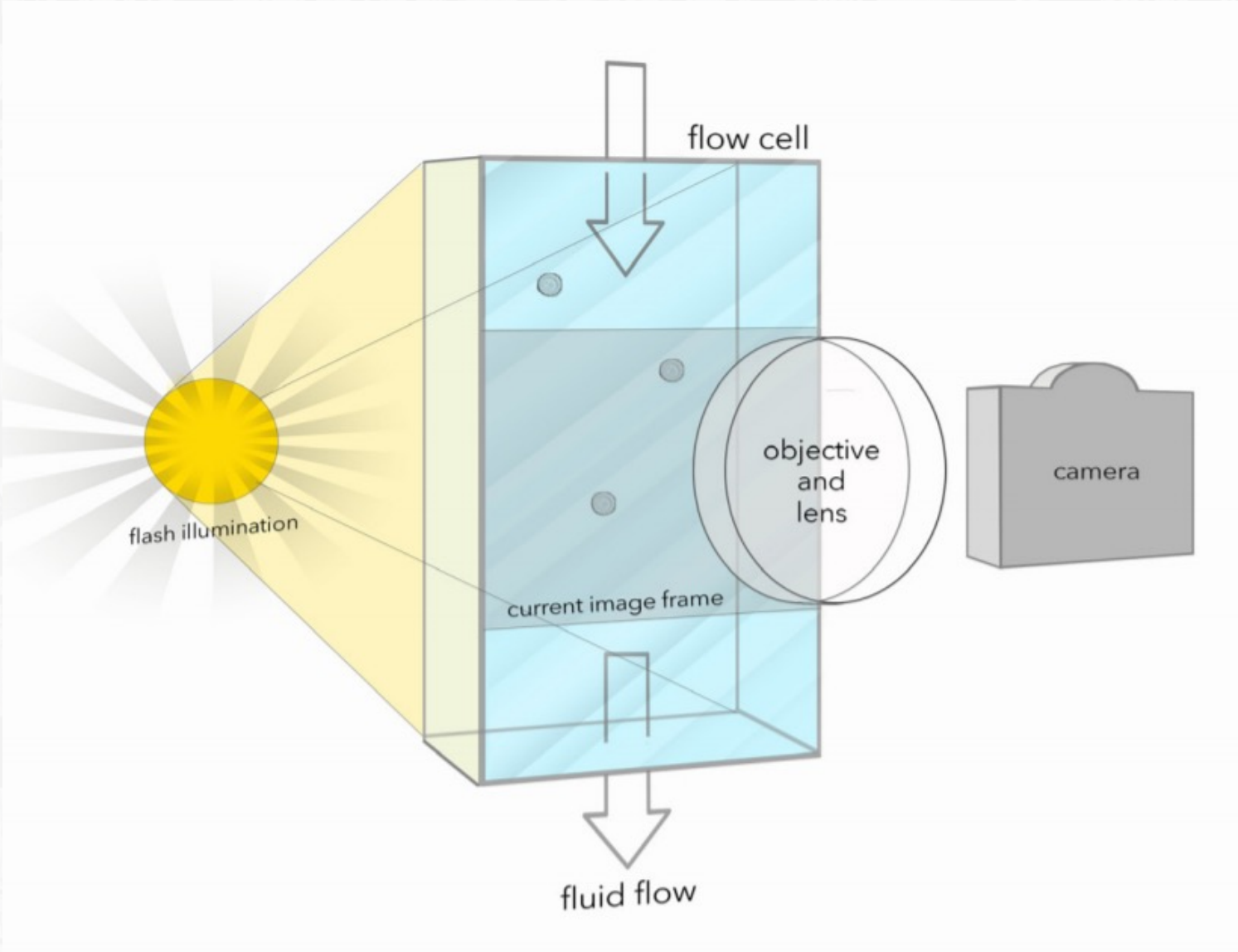
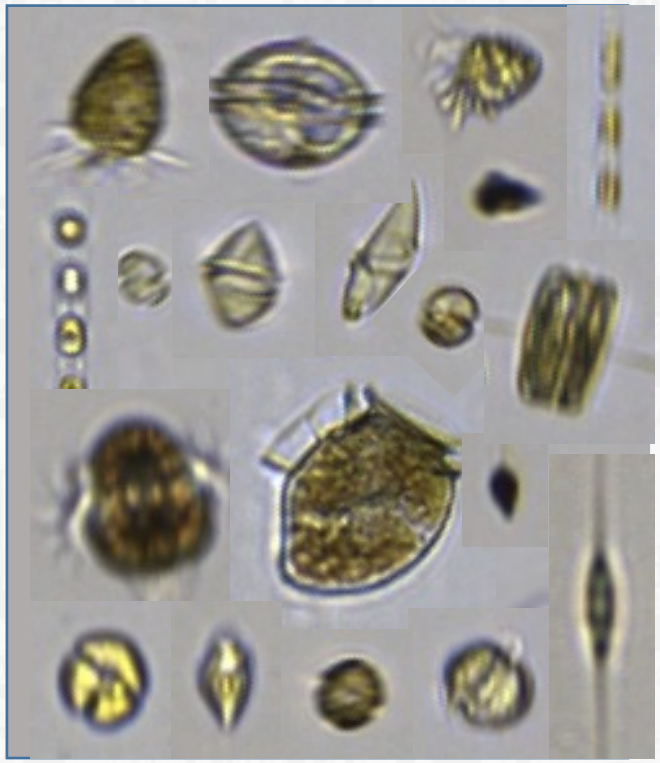


Image examples from FC8400, FCVS and FCM

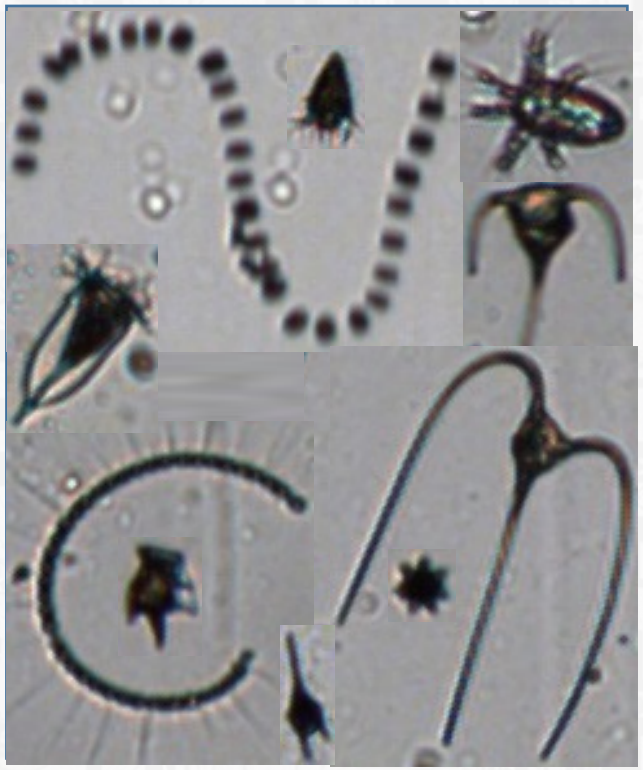
10x objective, 100 µm flowcell



FC8400

Nano- microplankton

2x objective, 800 µm flowcell



FCVS

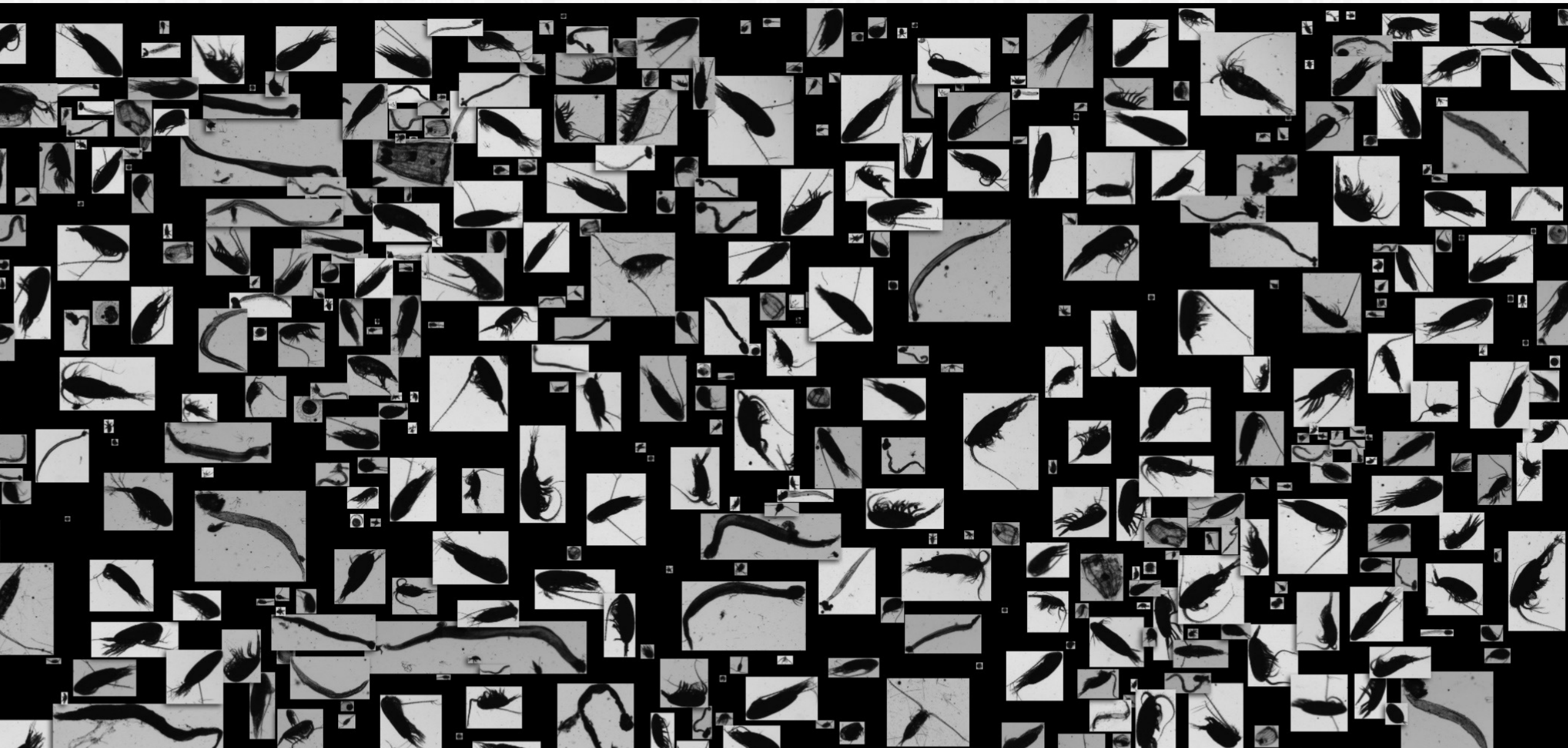
Microplankton

0.5x objective, 800 µm flowcell

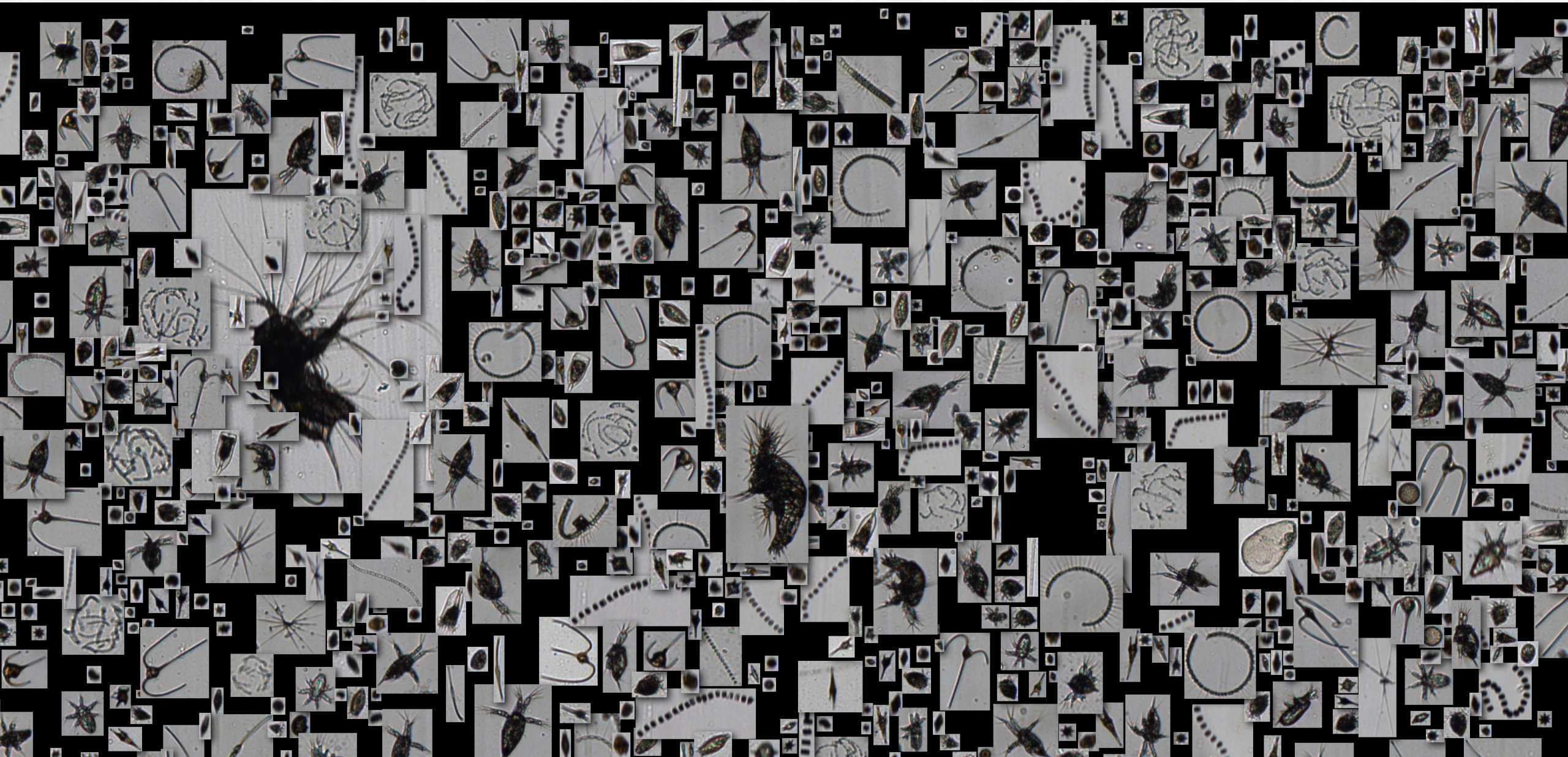
FCM

Mesozooplankton

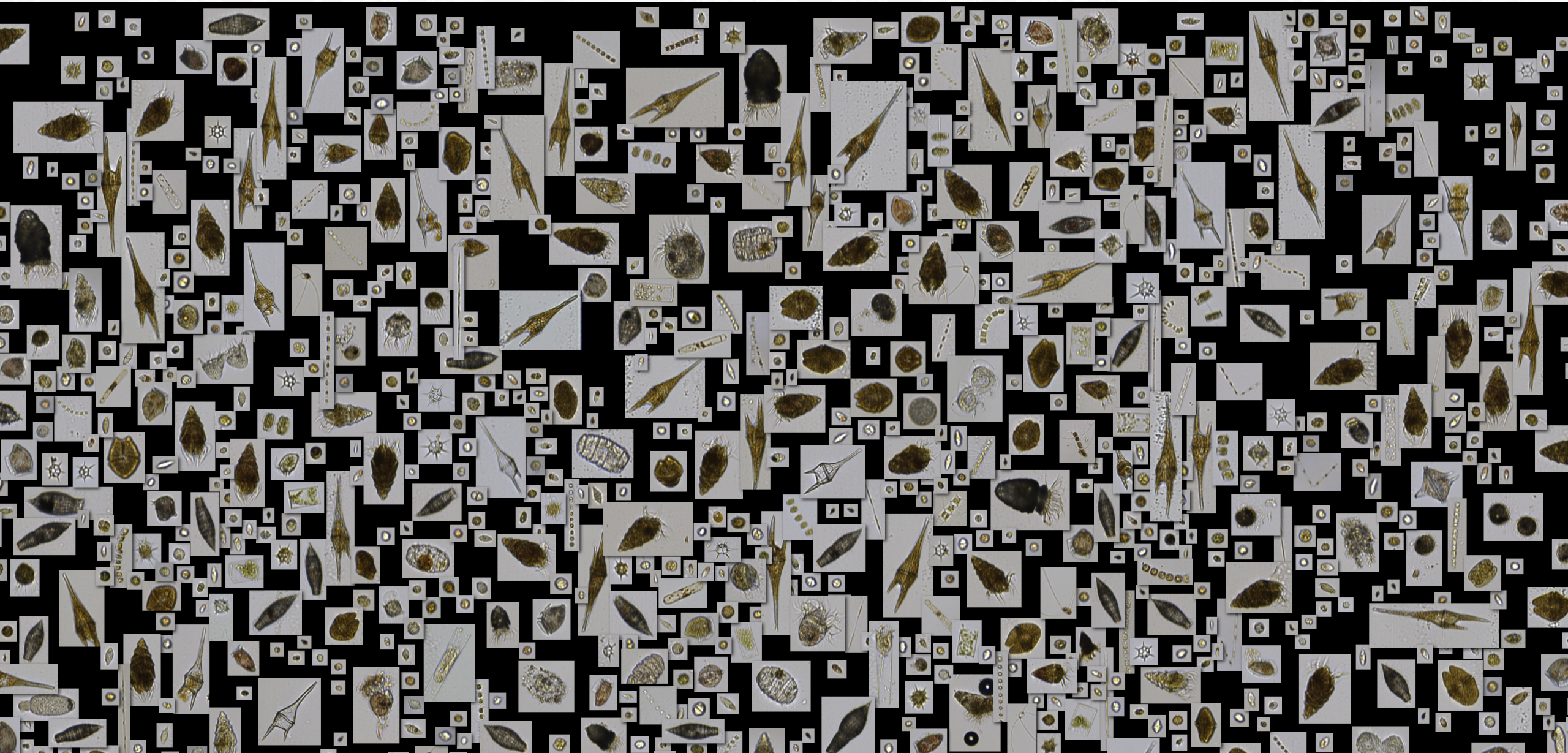
Mesozooplankton – FCM – 12.5 x magnification – 0.5x objective



Micro- and mesozooplankton – FCVS – 20 x magnification – 2x objective



Nano- and mesozooplankton – FCM – 100 x magnification – 10x objective



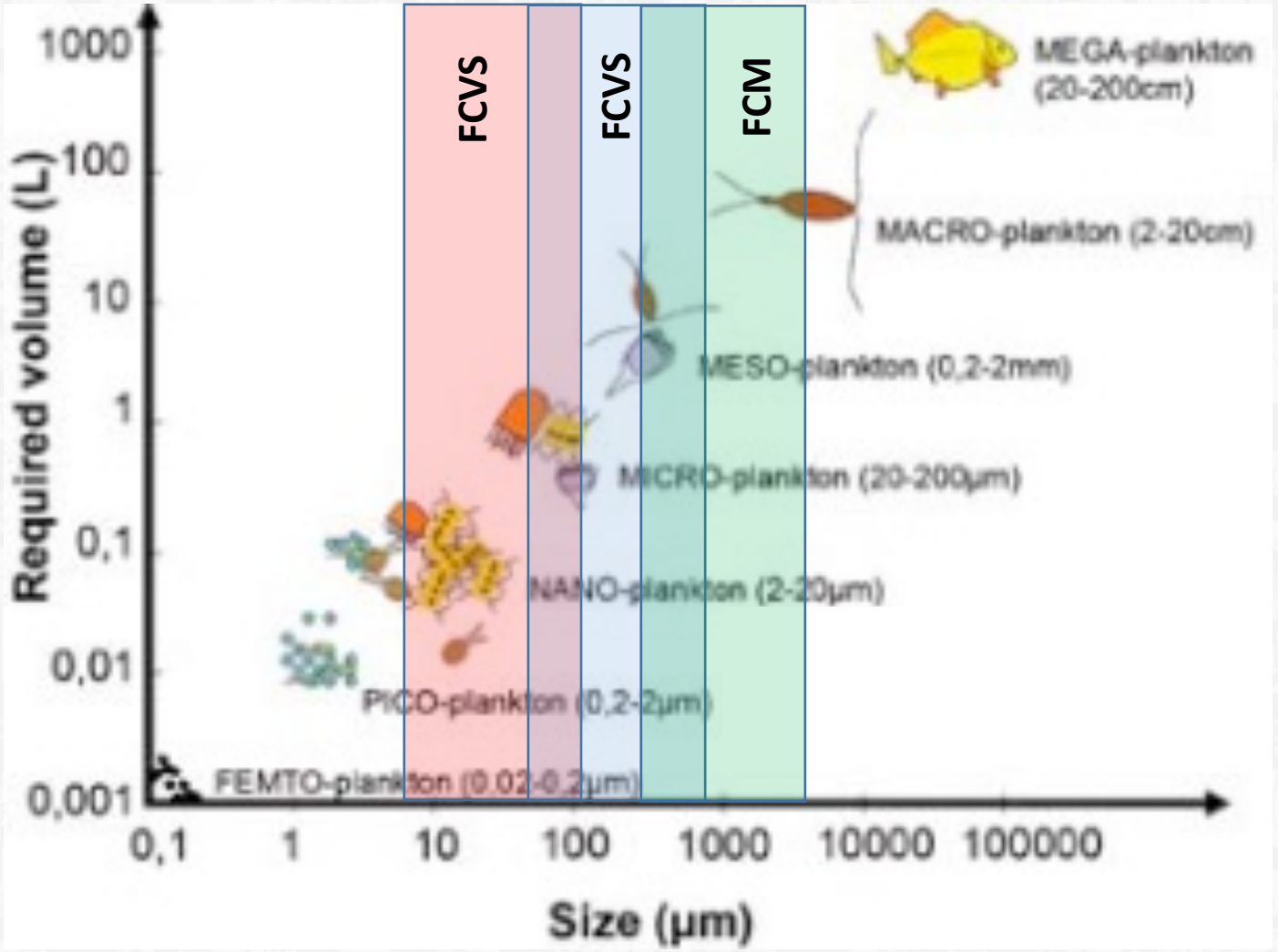
FlowCams and sample volume

- **Mesozooplankton and FlowCam Macro**

Samples are highly concentrated (25-1000m^3) and need splitting and dilution for imaging with FCM

- **Microzooplankton, HNF`s and mixoplankton**

Samples analysed using two flowcams:
1 - FlowCam VS - 2x objective, 800µm flowcell and flowrate of 25 ml min-1. 500ml samples analyses in ca. 20 mins
2 – FlowCam 8400 – 10x objective, 100µm flowcell and a flowrate of 0.6ml min^{-1}. 40 ml^{-1} imaged in 25 mins

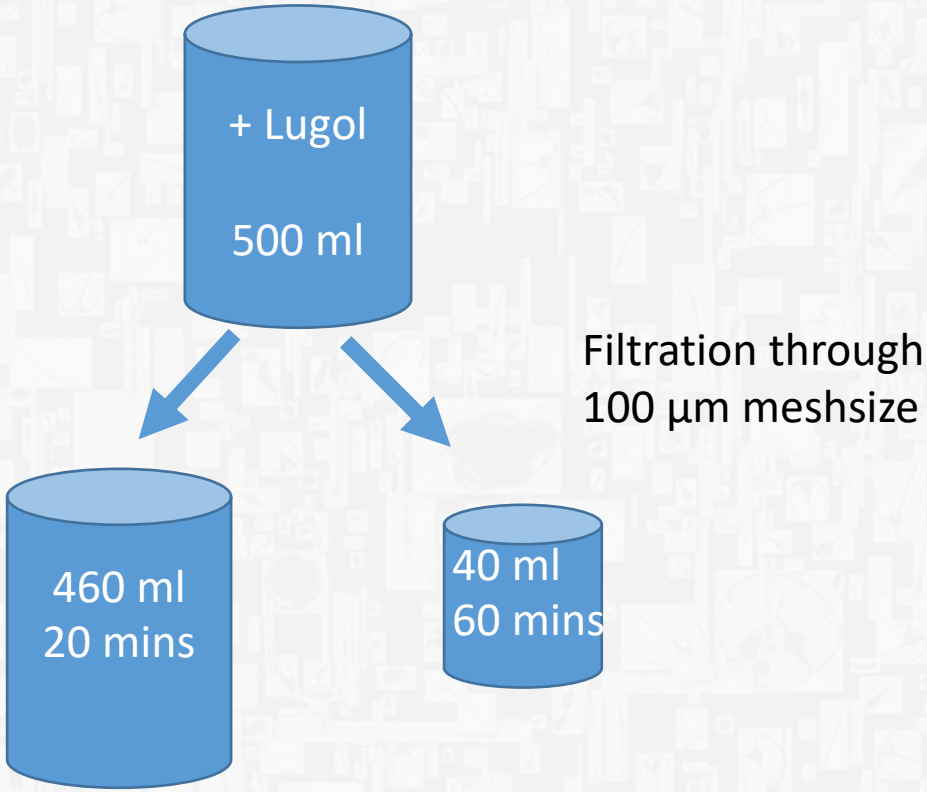


From Mostajir et al. 2012 – Modified by Sieburth 1978

Sampling for coastal microplankton and mesozooplanton

Nano/Microplankton

Whole seawater sample from 5m Niskin bottle



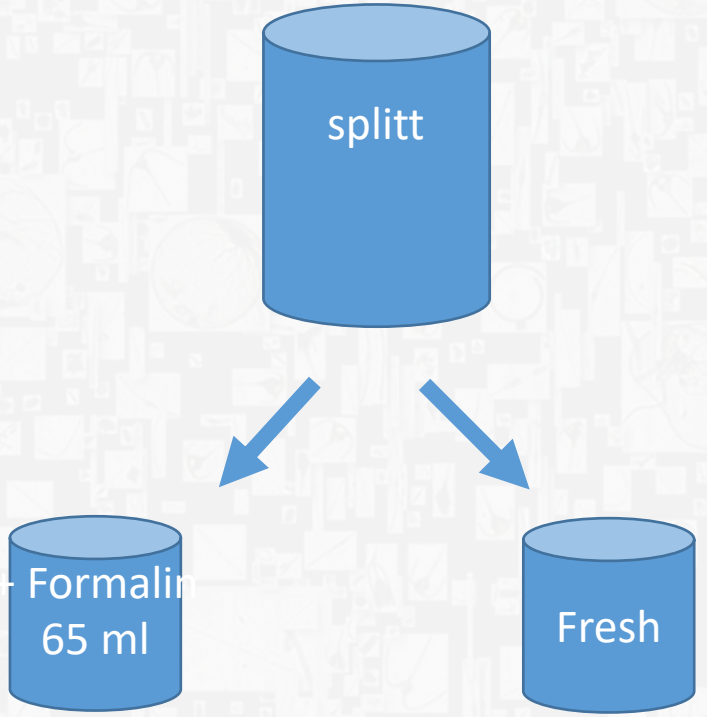
Filtration through 100 µm meshsize

460 ml FCVS with 10x and 100µm flowcell

FC8400 with 10x and 100µm flowcell

Mesozooplankton

180 µm WP2 and Multinet tows – concentrate of up to hundreds of m^3



Mesozooplankton < 2000 µm is worked up namually at home and with the FCM

Macroplankton > 2000 µm is worked up manually at sea

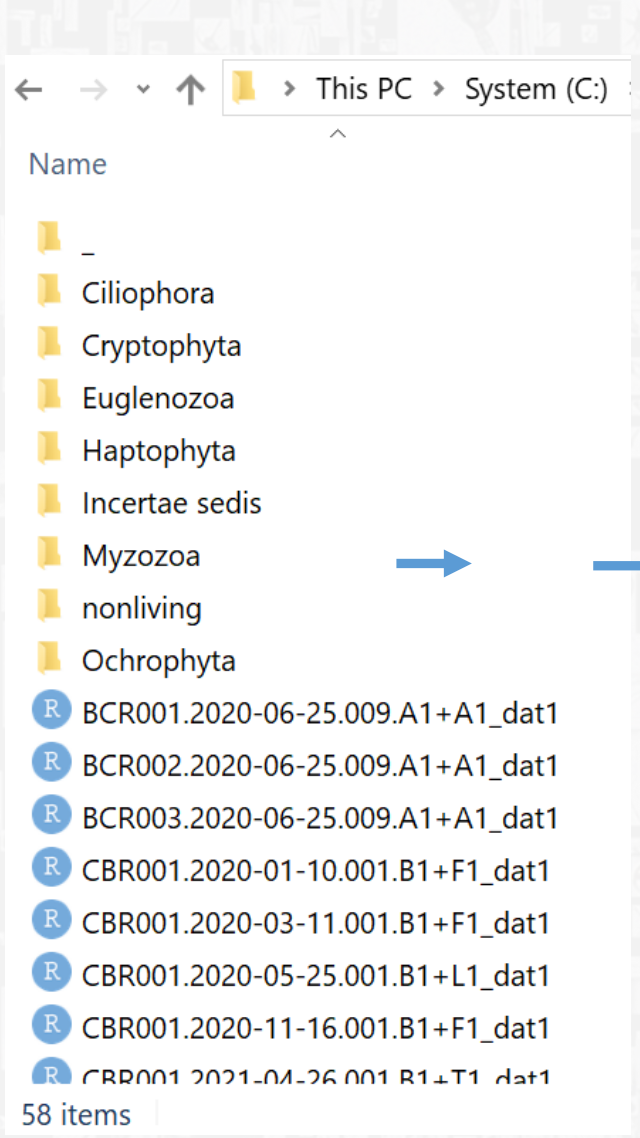
Training sets and classifiers

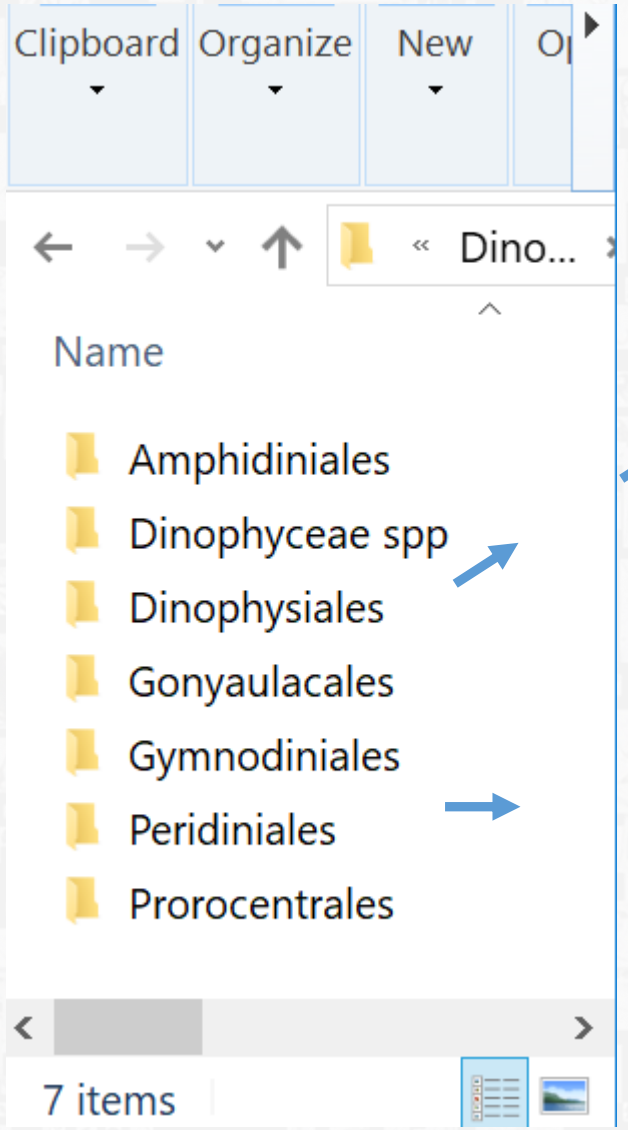
Training sets and classifiers

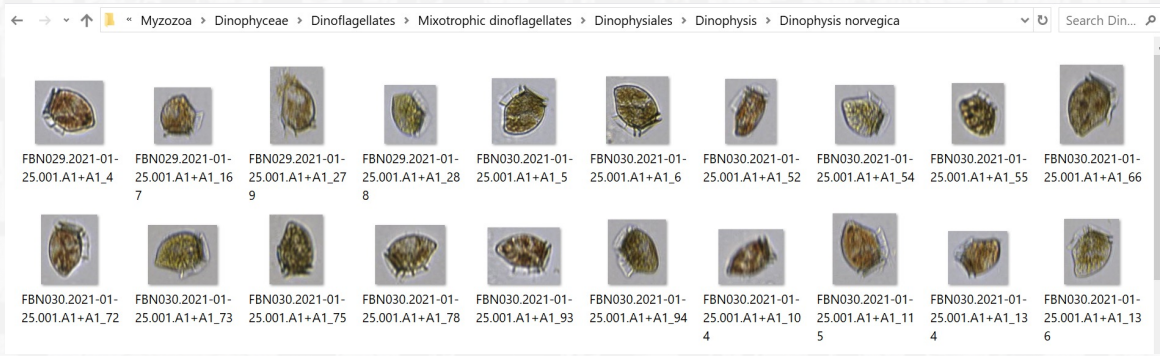
- We use the r-package «**Zooimage**» (Grosjean et al. 2012) to make training sets and classifiers
- We use the r-package «**fc2zi**» by Eva Álvarez (2011, 2012 and 2014) to extract images and additional particle features from the FlowCam raw collage images in batch (up to hundreds of samples)
- In addition to naming the files according to Zooimage requirements, **fc2zi** performs an instrument artifact (e.g. background images, repeated images etc) filtration procedure and prepares the images for «Zooimage» in batch (hundreds of sample)
- The output from the process (individual images and feature table) can be used for training or classified if samples

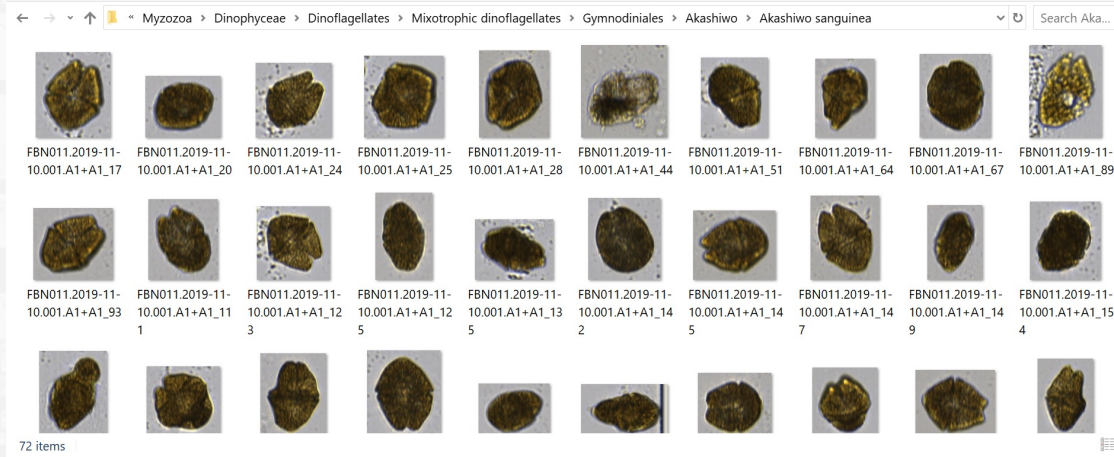
Working with training sets

Very easy to share with specialists for annotation of images in any file or image explorer









Working with training sets

Taxonomical training set – images are sorted and annotated to highest possible taxonomic level from phyla to species. Images put in annotated folder structure according to the phylogeny found in **WoRMS**

Functional training set

- functionally similar taxa can be grouped together before training

E.g. particles may be classified into **autotrophs**, **mixotrophs** and **heterotrophs**

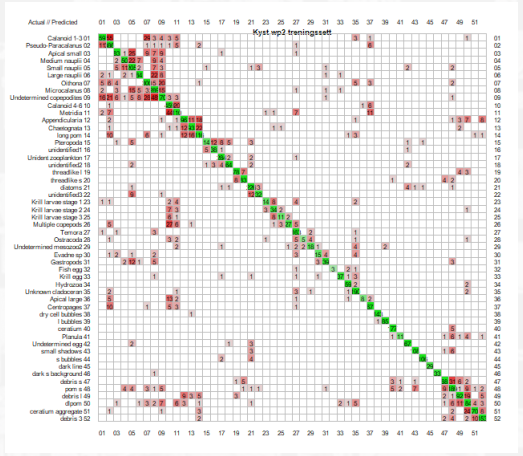
We have been using **Random Forest** classifiers

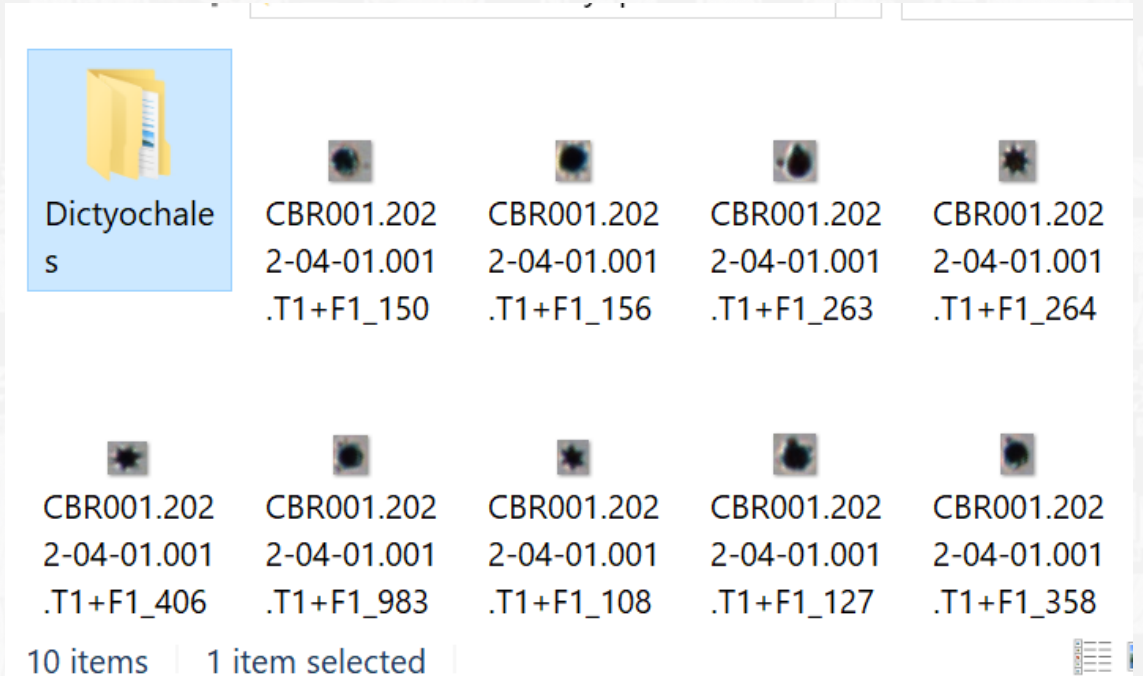
Evaluating our classifiers

We have been using **Random Forest** classifiers

Ten fold cross validation

Visual check – images automatically sorted according to the classifier into the folders of the training data





Improving the training set

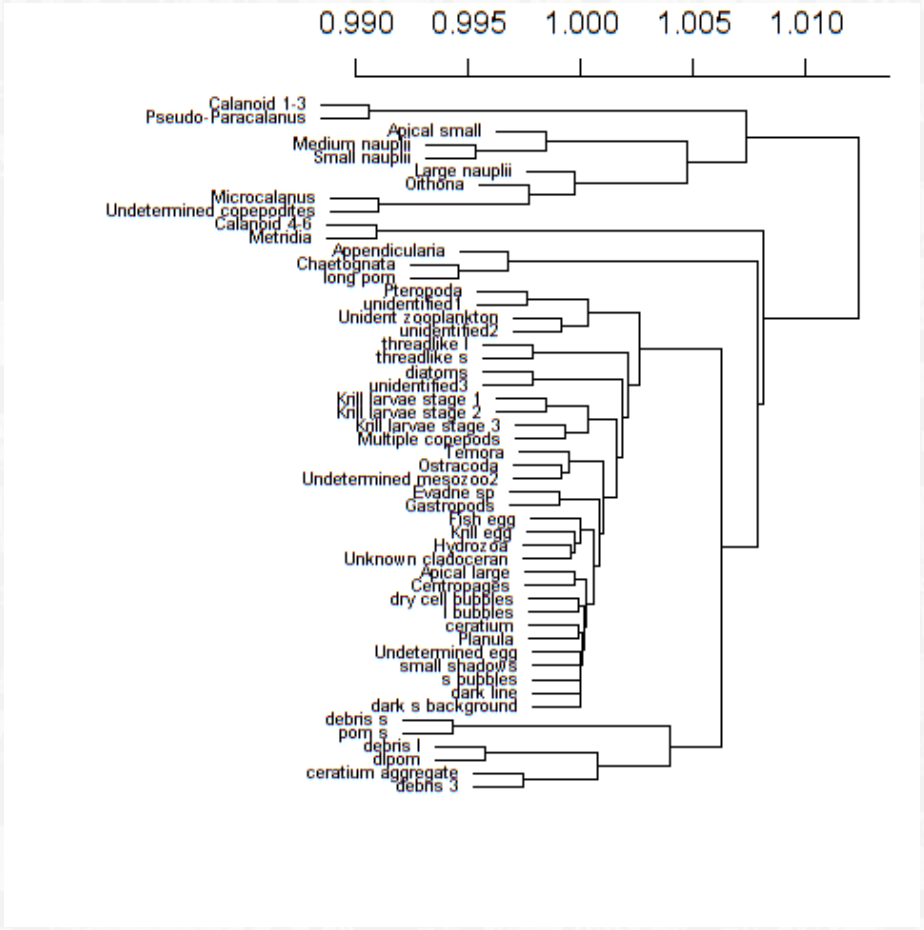
The initial and premature classifiers are used to automatically place unannotated images into the folder structure of the training set and visually checked

Problematic groups are improved by moving representative images to the correct folders, before retraining the classifier. We repeat this MANY times until we find the automatic sorting of images into the groups of the training set folder structure acceptable

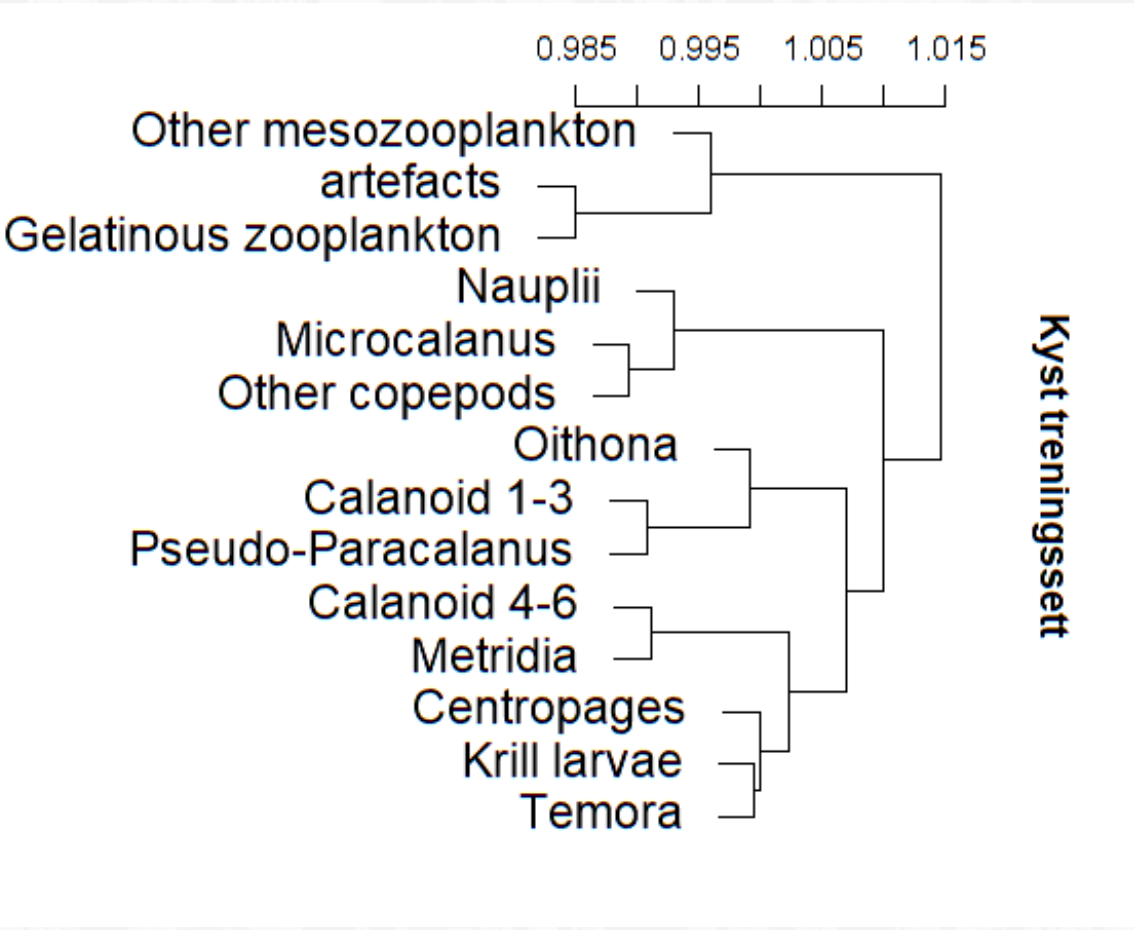
Not for HABs: The classifier will perform better at lower taxonomical detail. If you can´t wait (like me) you can group the classes at a **lower level of taxonomical detail** or in a **functional** way.

Setting the level of taxonomic or functional detail before classifying samples

Classifier trained on all groups in the trainng set



Classifier trained on merged groups in the trainng set



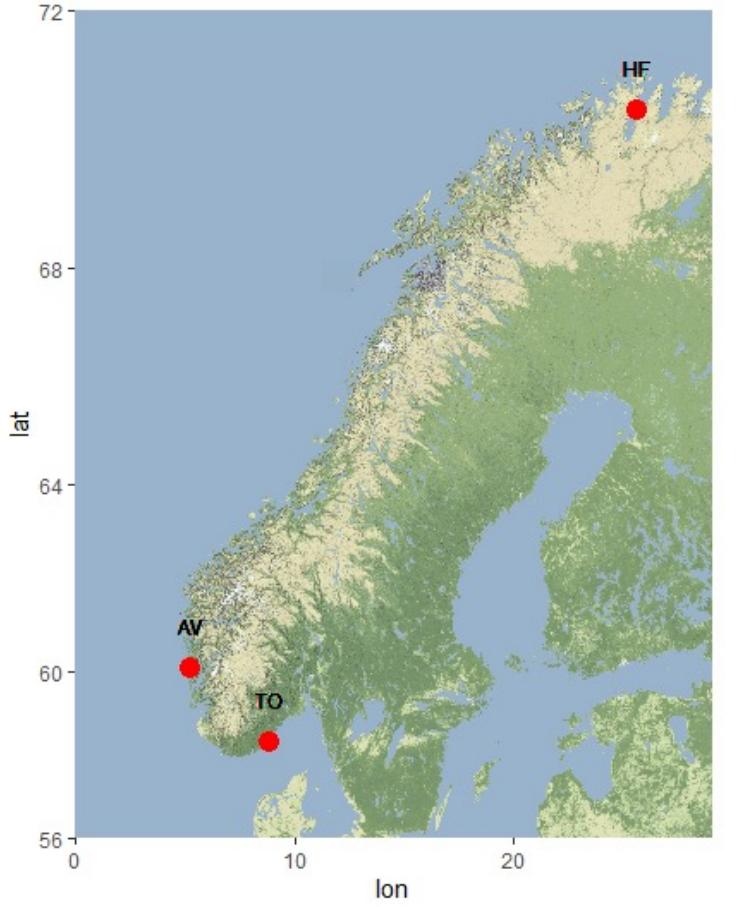
Data output from classification
Taxa

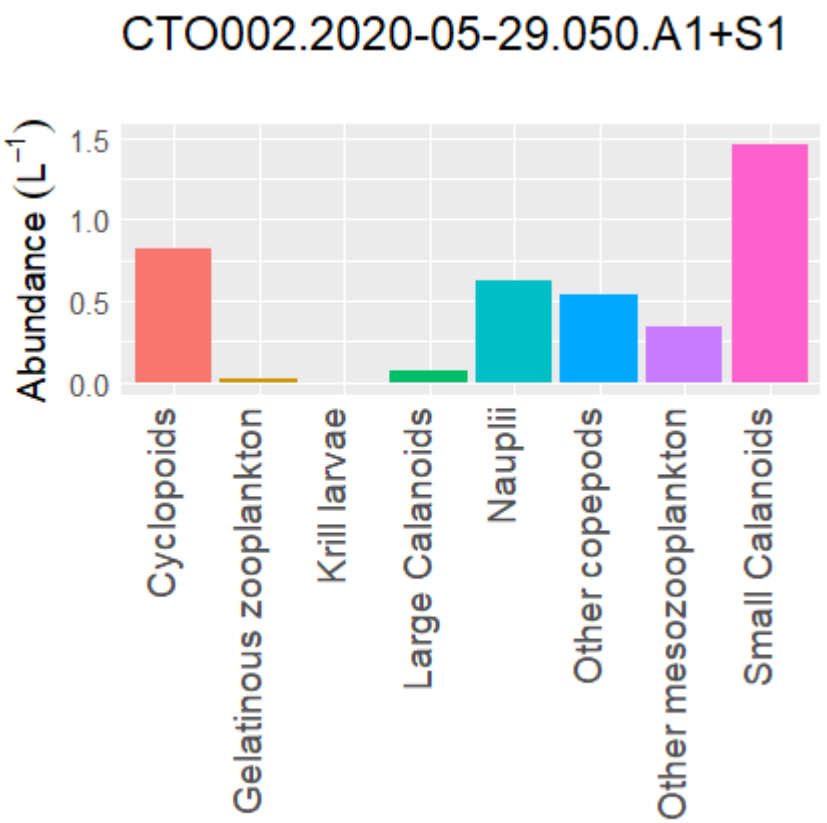
Example of data output from single FlowCam runs

Mesozooplankton sample – WP2 net 180µm

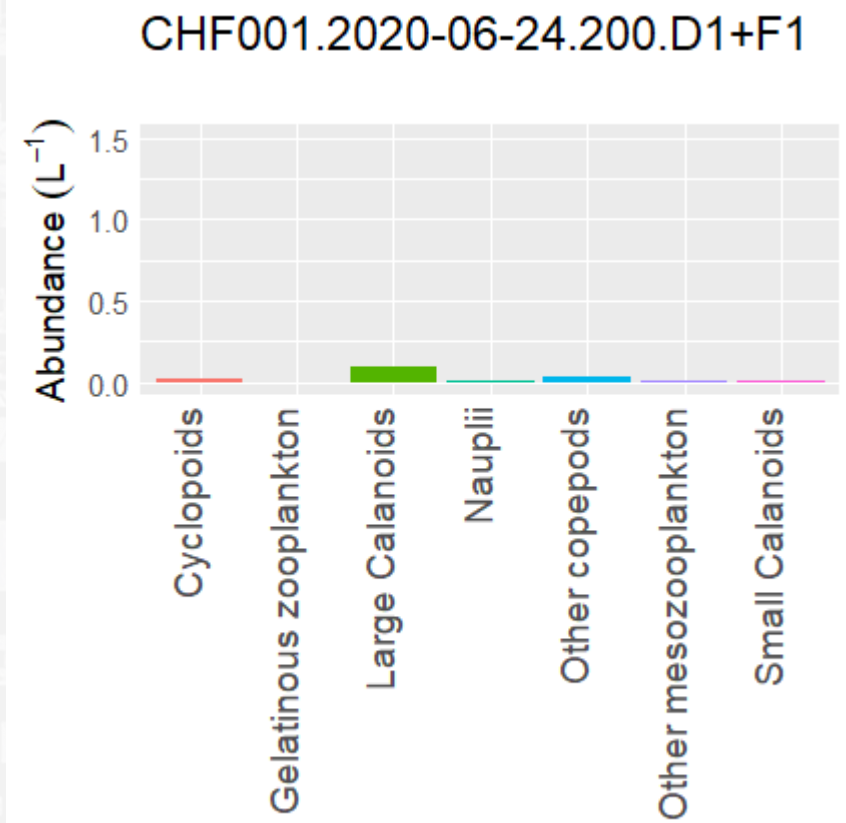
Southern Norway – Torungen St2

Northern Norway - Holmfjord





St2 – 29 -05 -2020 – haul depth = 50m



St1 – 24.06-2020 – haul depth = 200m

Example of data output from single FlowCam runs

Mesozooplankton sample – WP2 net 180µm

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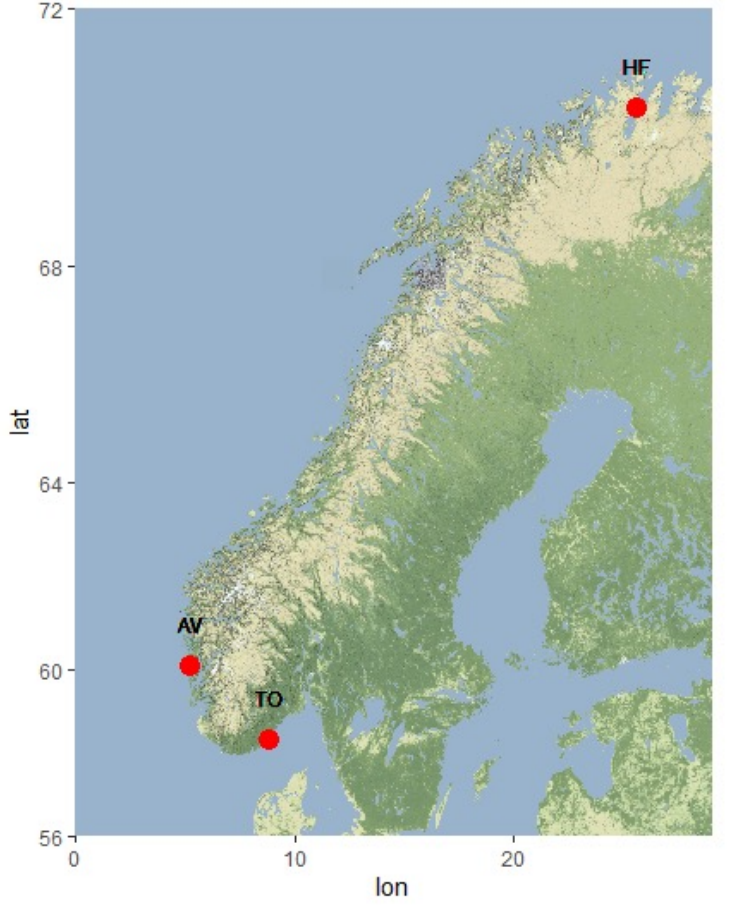
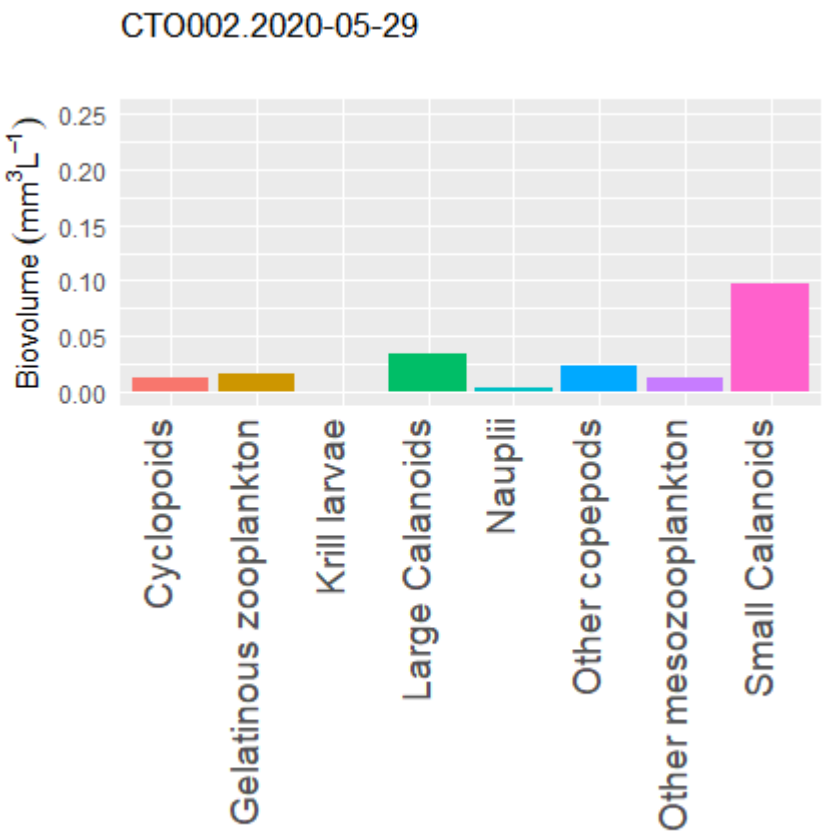
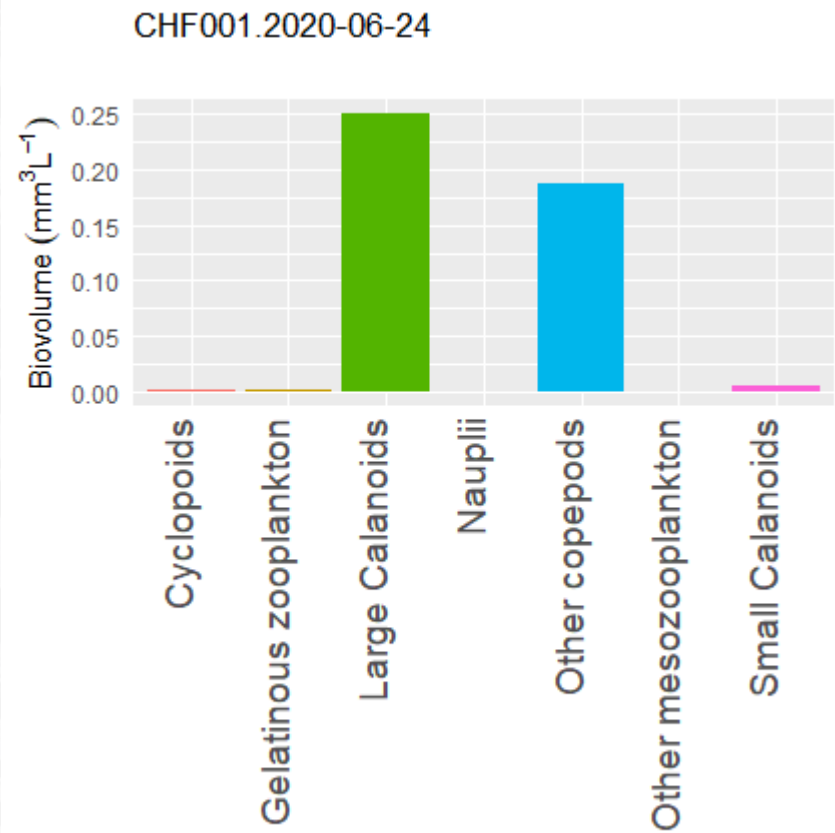




St2 – 29 -05 -2020 – haul depth = 50m



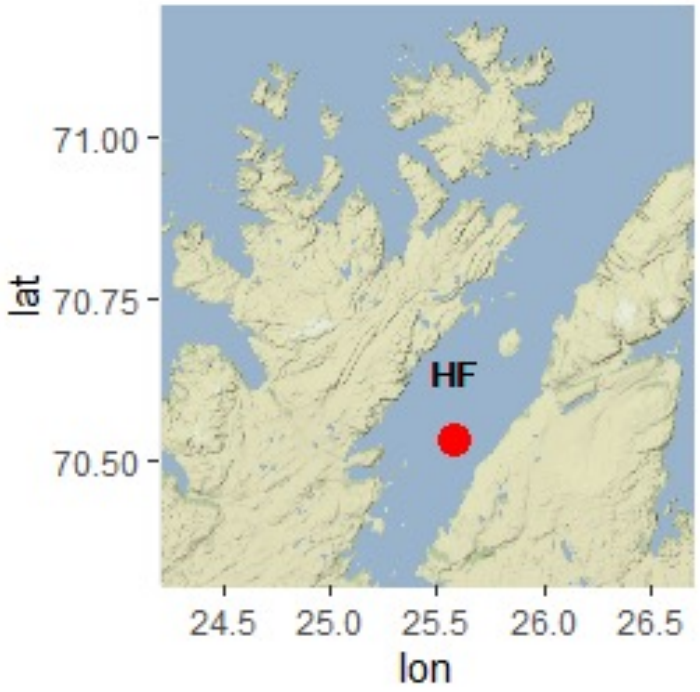
St1 – 24.06-2020 – haul depth = 200m

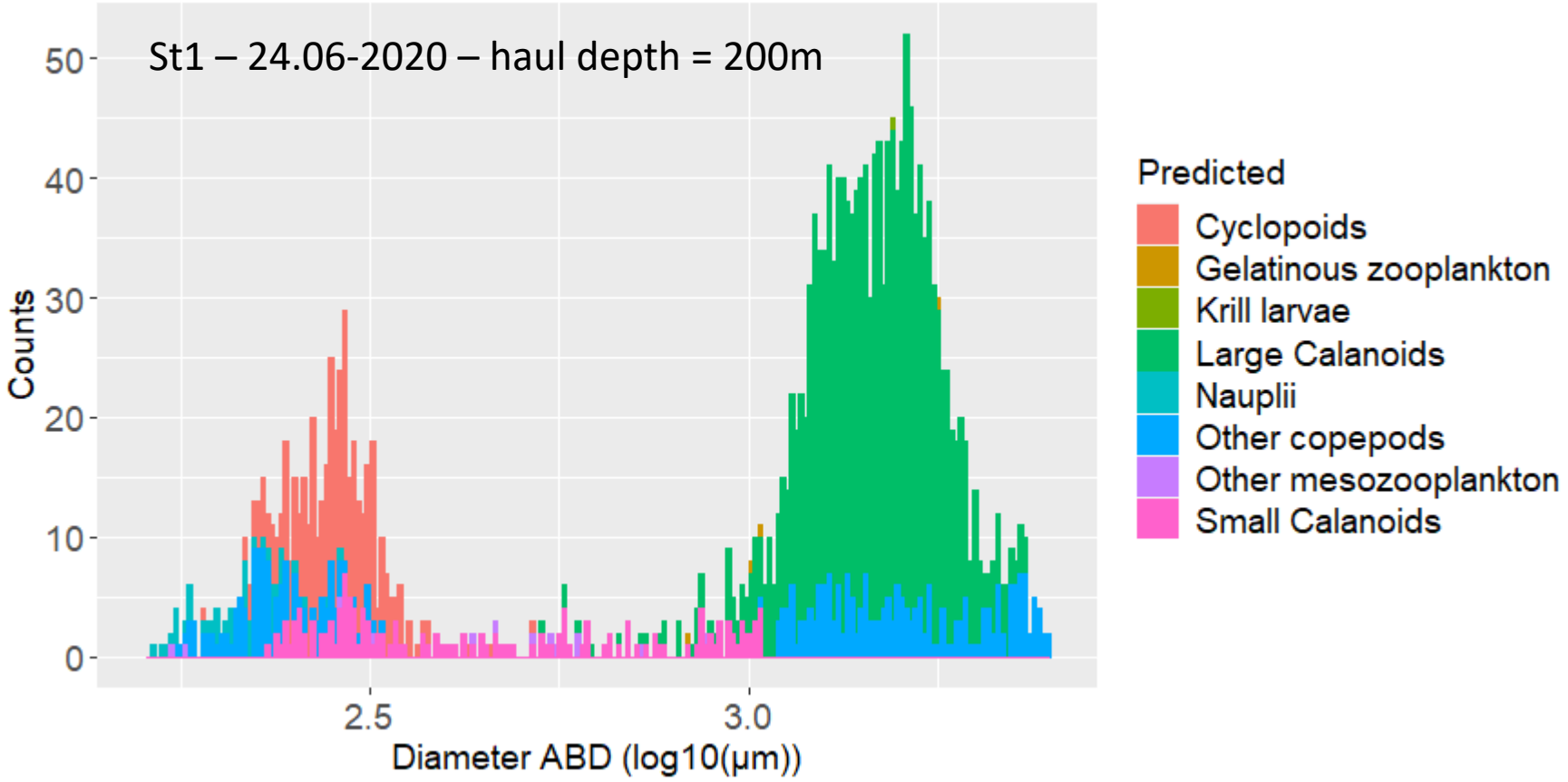
Data output from classification
Size structure

Example of data output from single FlowCam runs

Mesozooplankton sample – WP2 net 180µm

Mesozooplankton - Northern Norway - Holmfjord

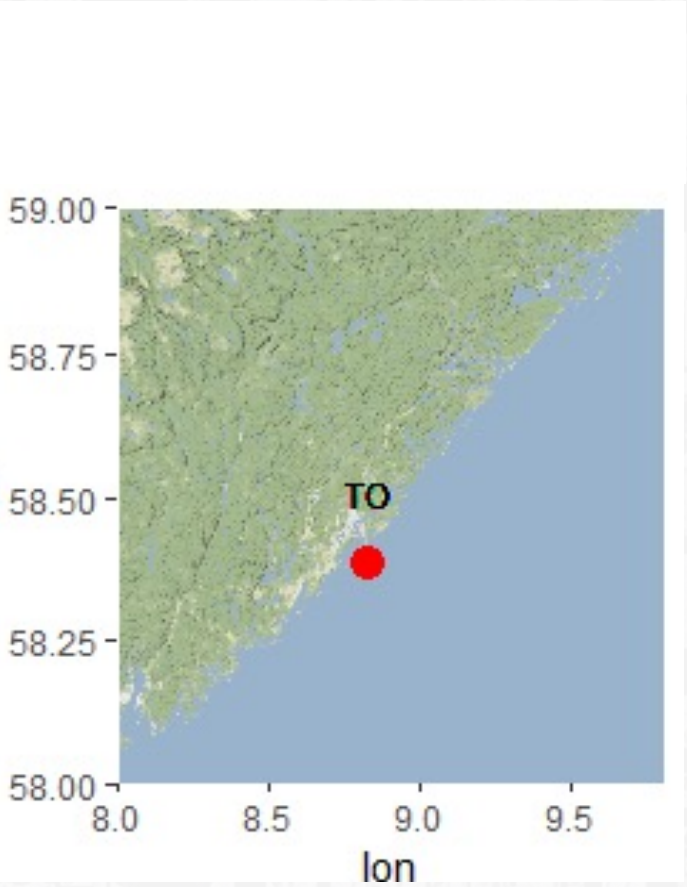


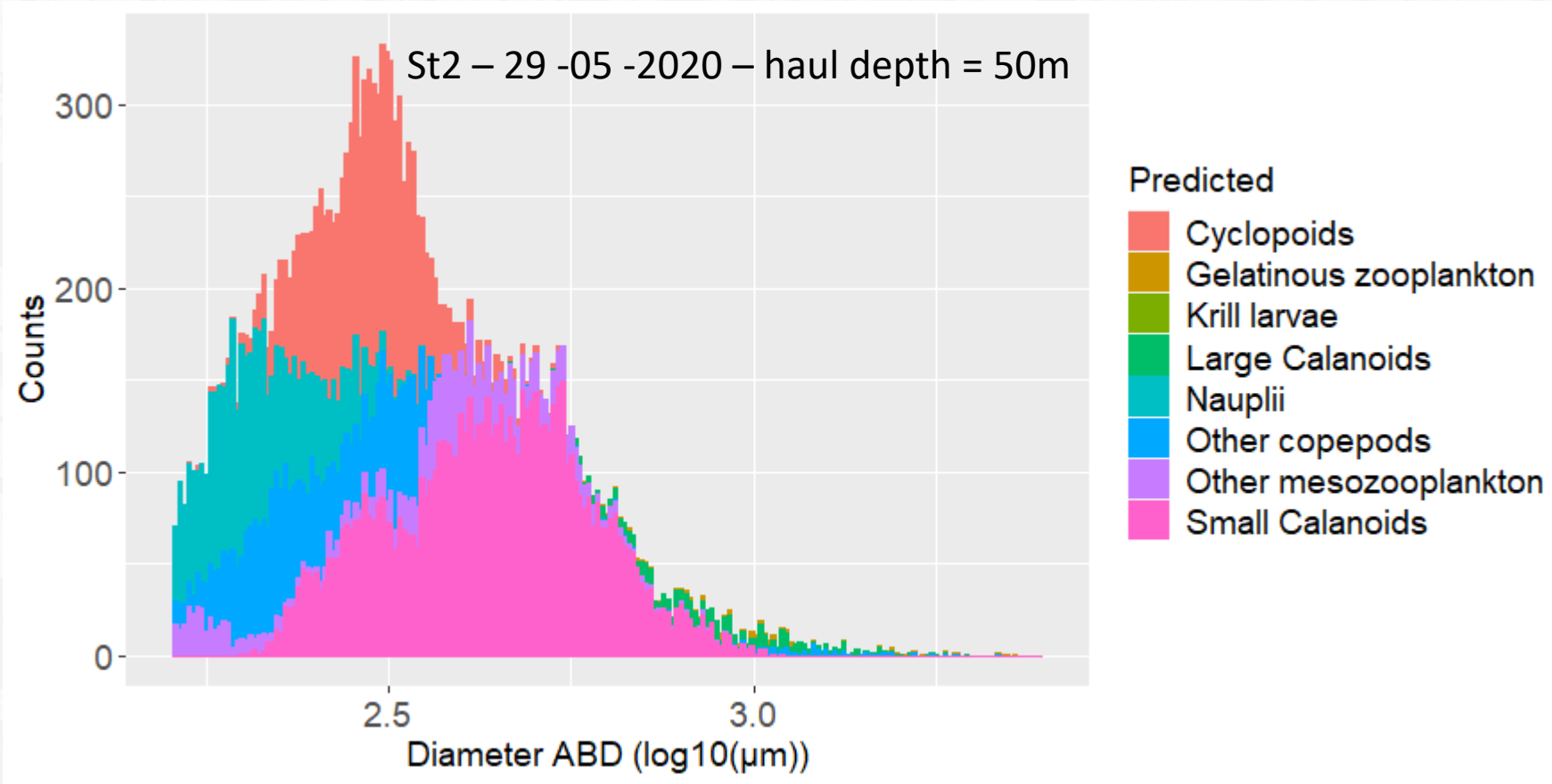


Example of data output from single FlowCam runs

Mesozooplankton sample – WP2 net 180µm

Mesozooplankton - Southern Norway – Torungen St2





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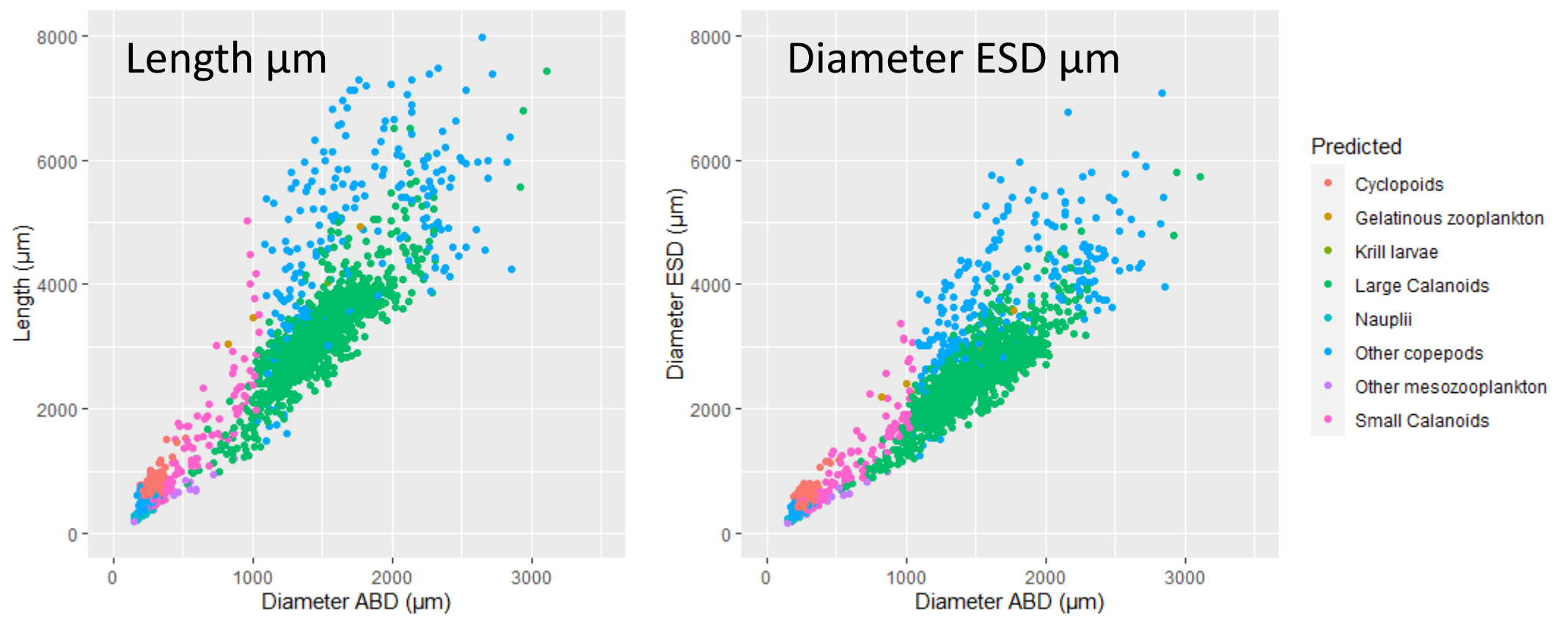
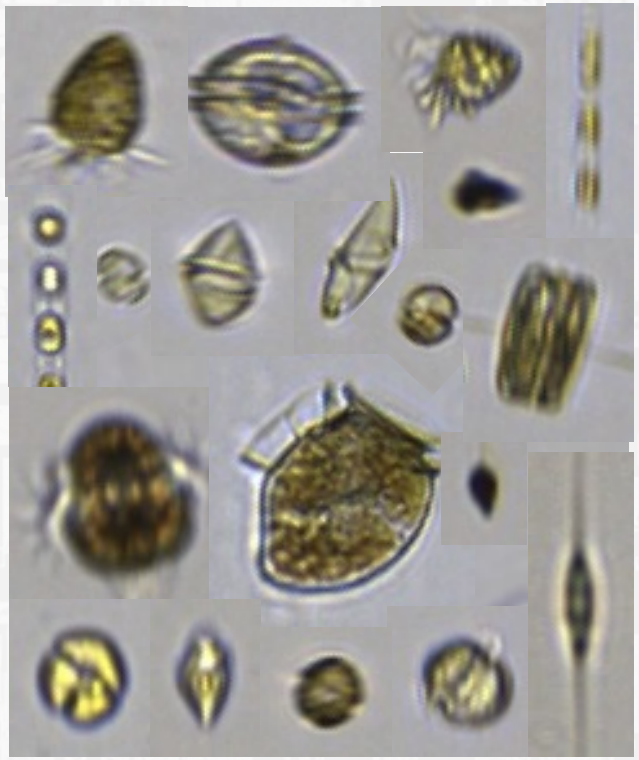
Mesozooplankton sample – WP2 net 180µm

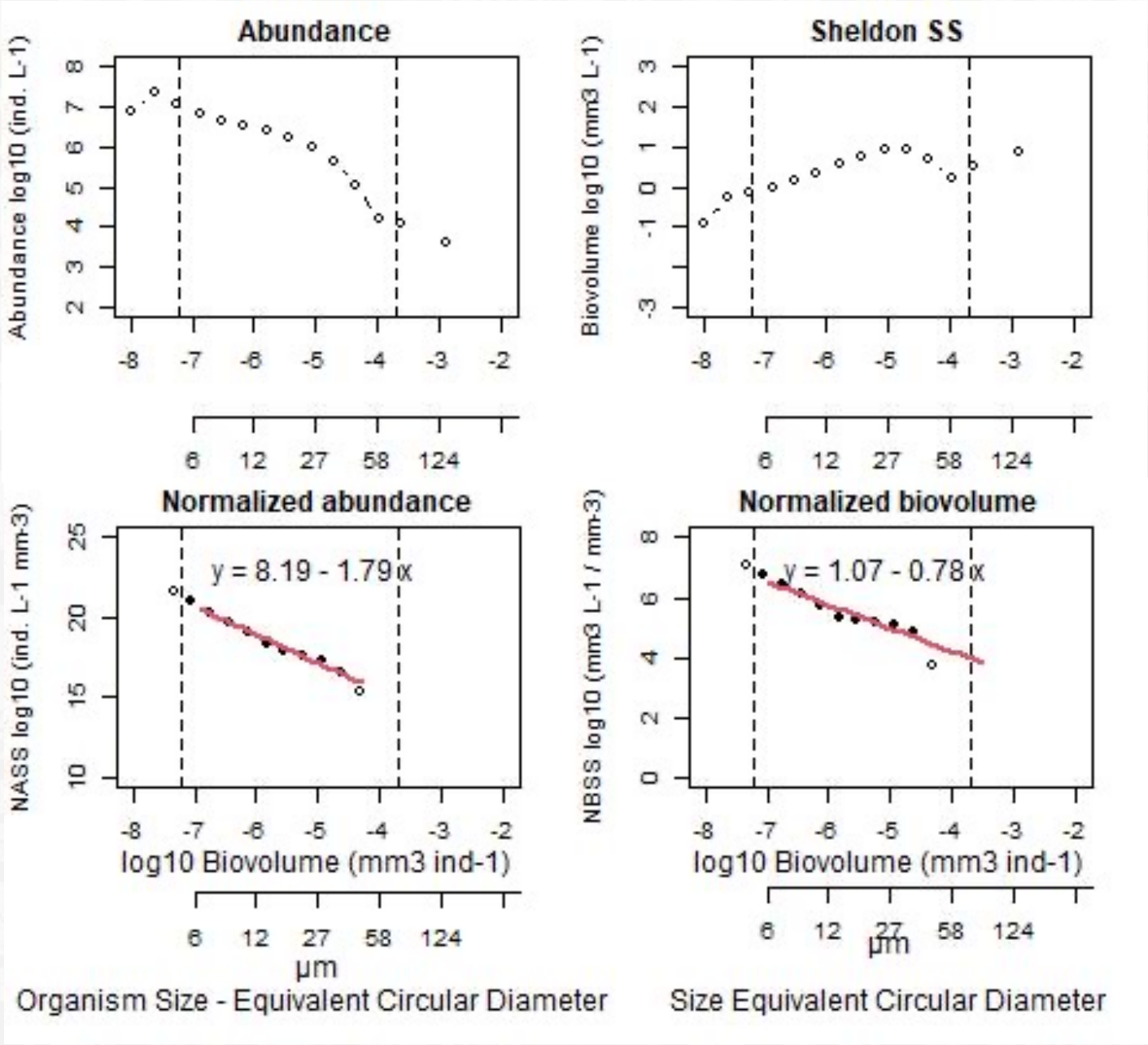
Mesozooplankton - Northern Norway – Holmfjord



Area Based Diameter (ABD)

Size structure

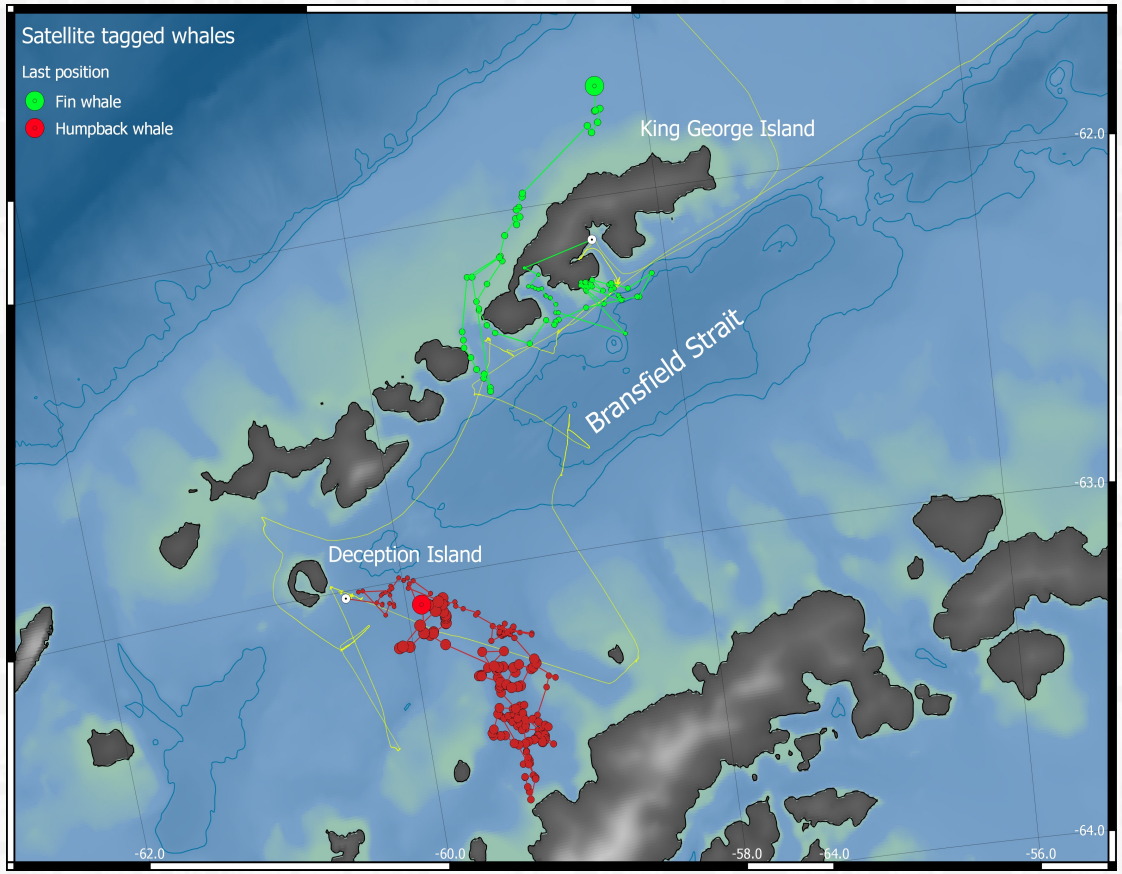


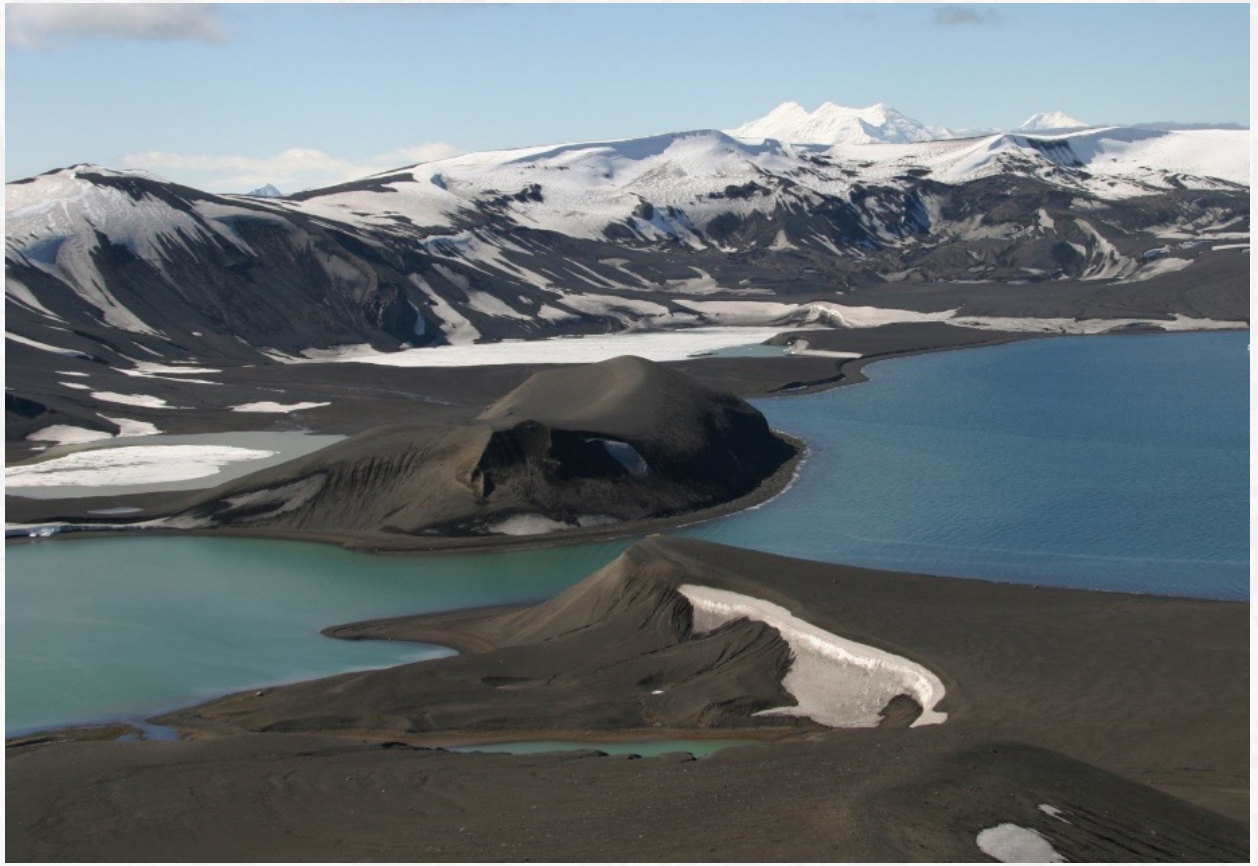


Data valuable even without taxa…

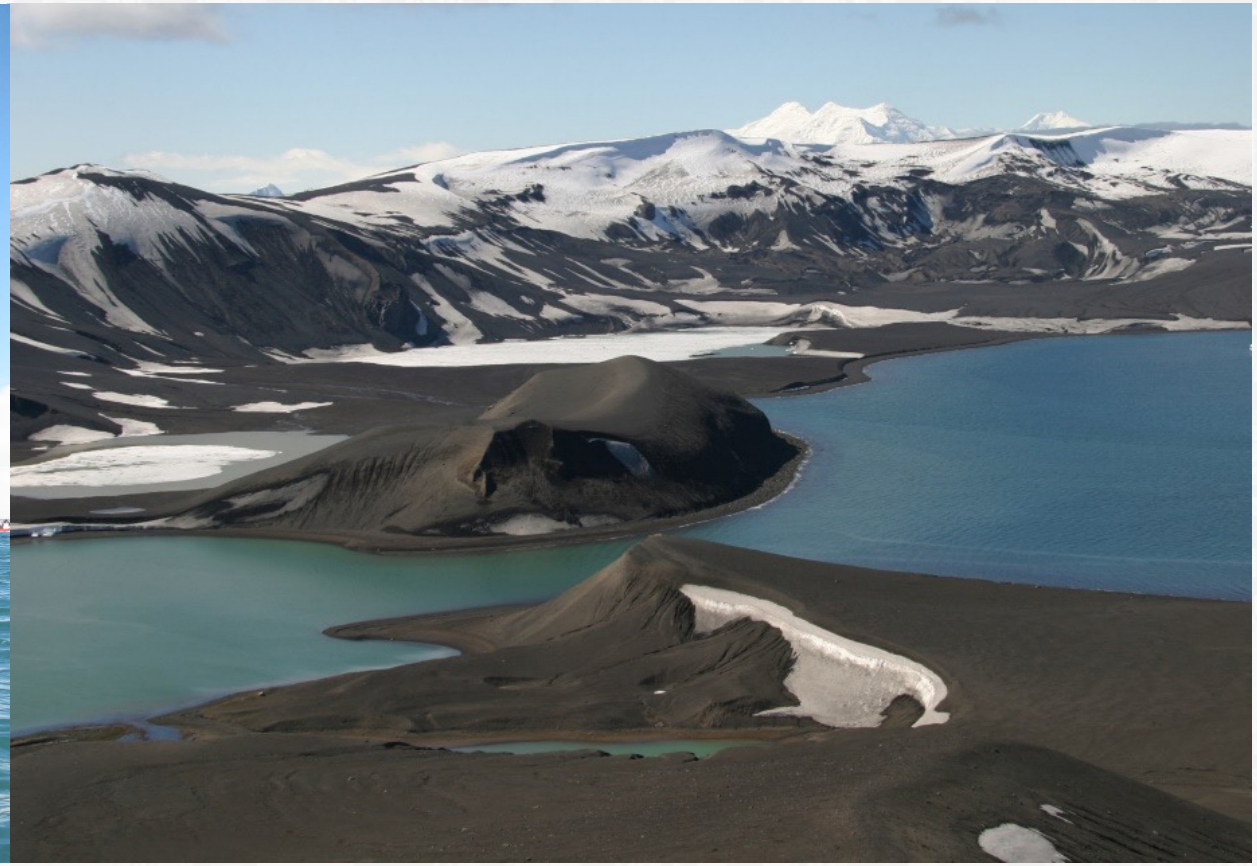
Examples of usage

Example of early use of a premature training set and classifier



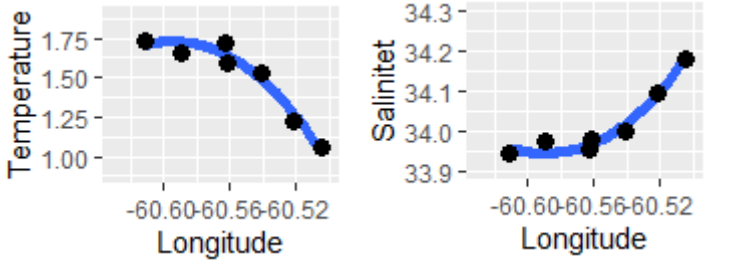


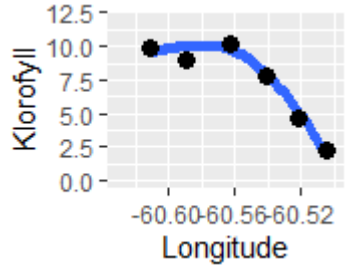
Example of use of a premature training set and classifier

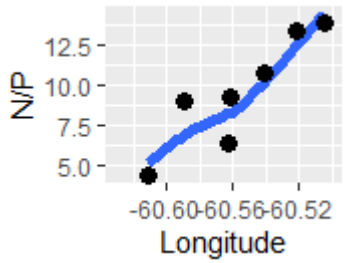


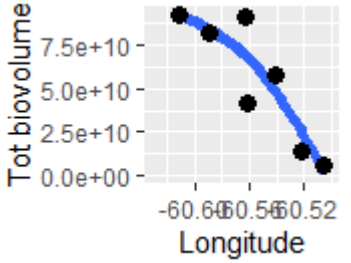
Deception Island – Antarctica

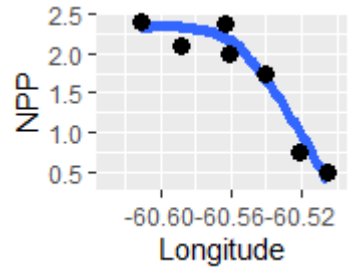
Deception Island

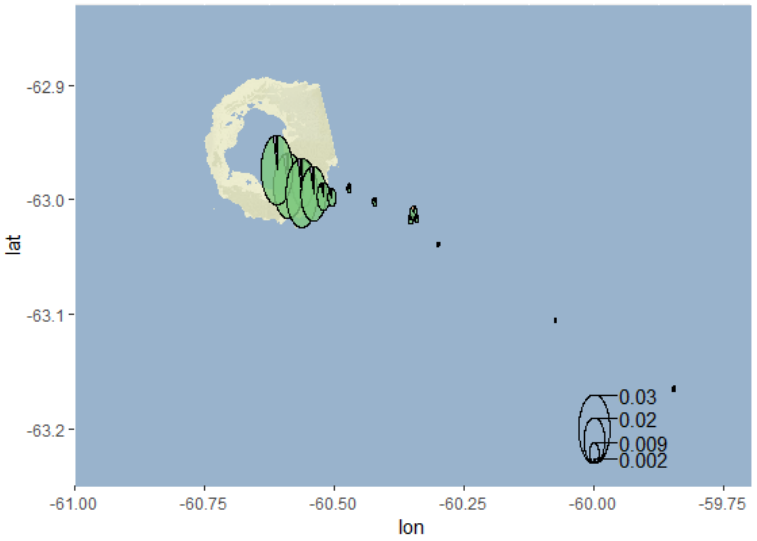




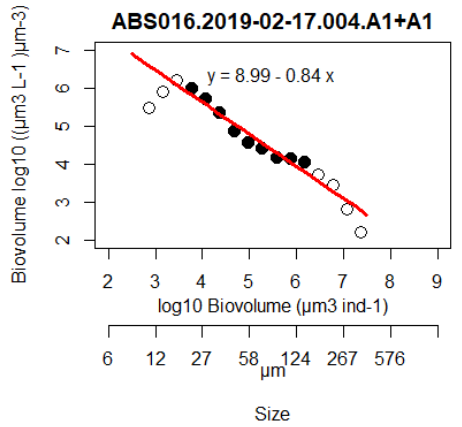


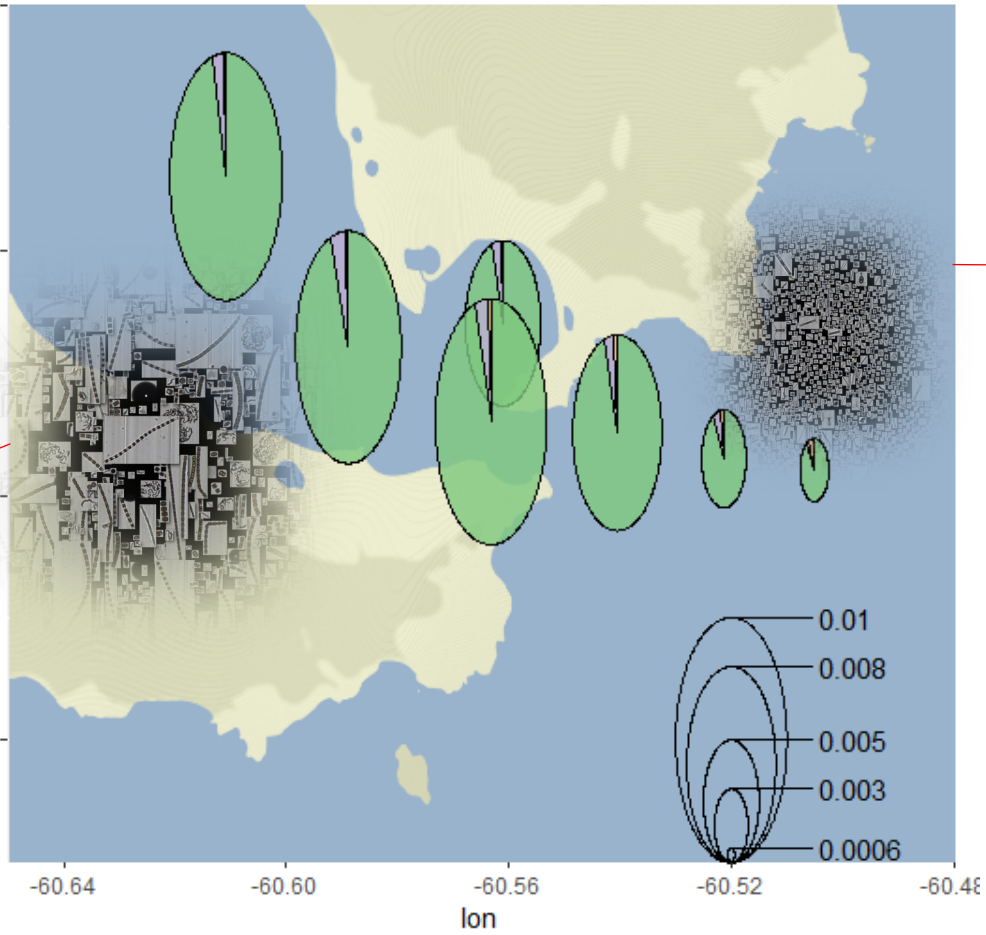


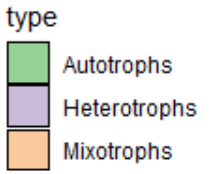




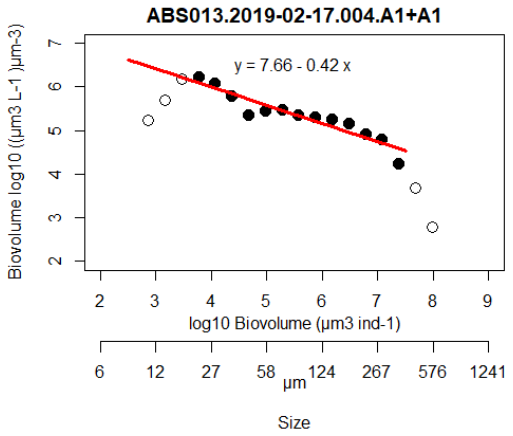
Biomassestørreslsesfordeling ute

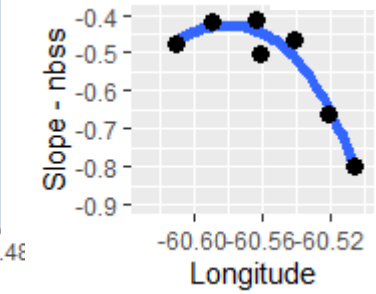


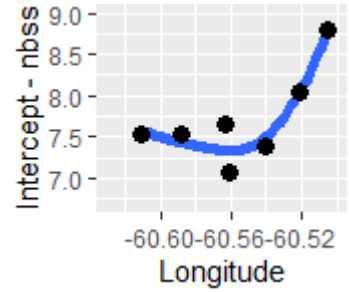




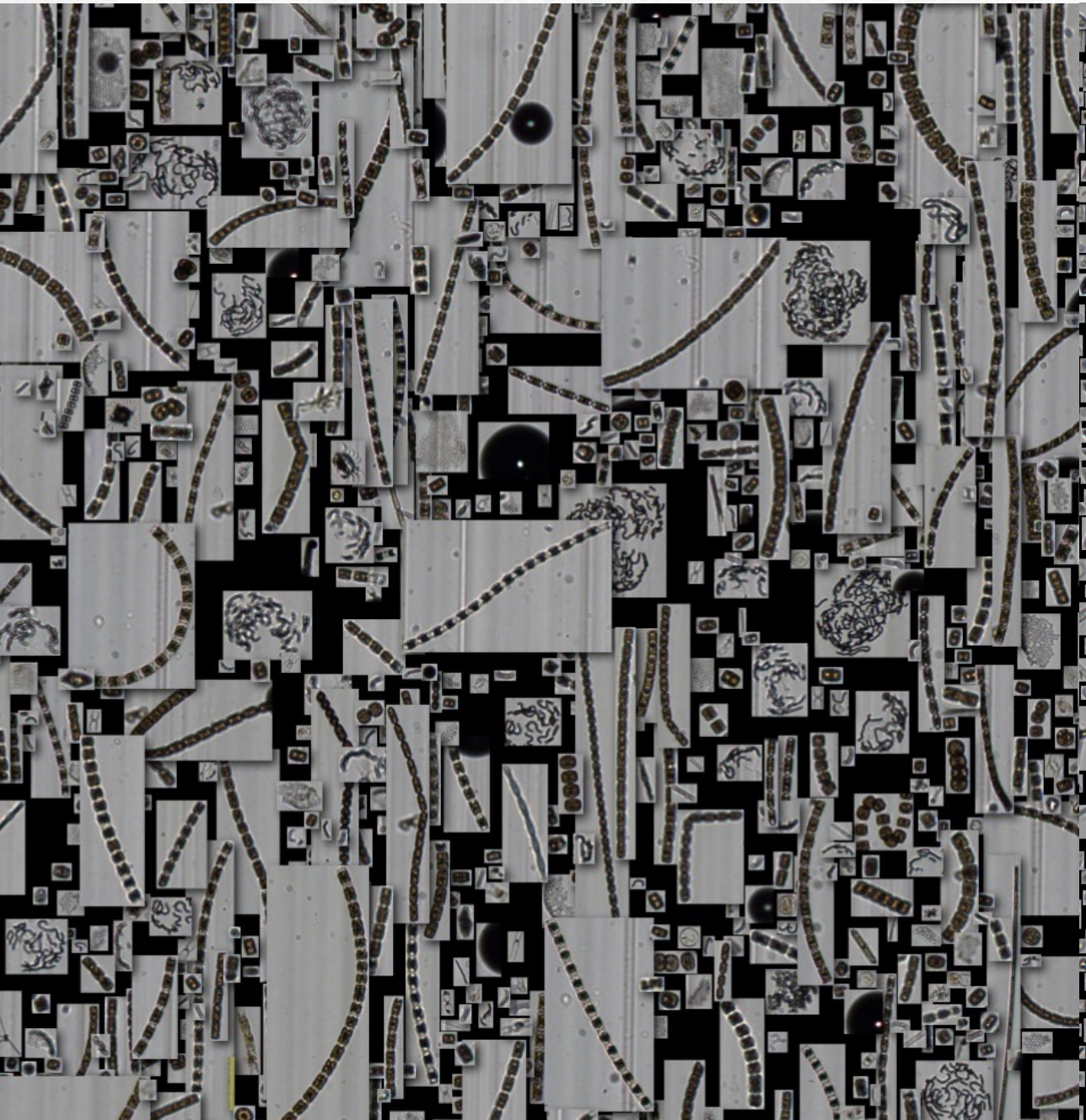
Biomassestørreslsesfordeling inni



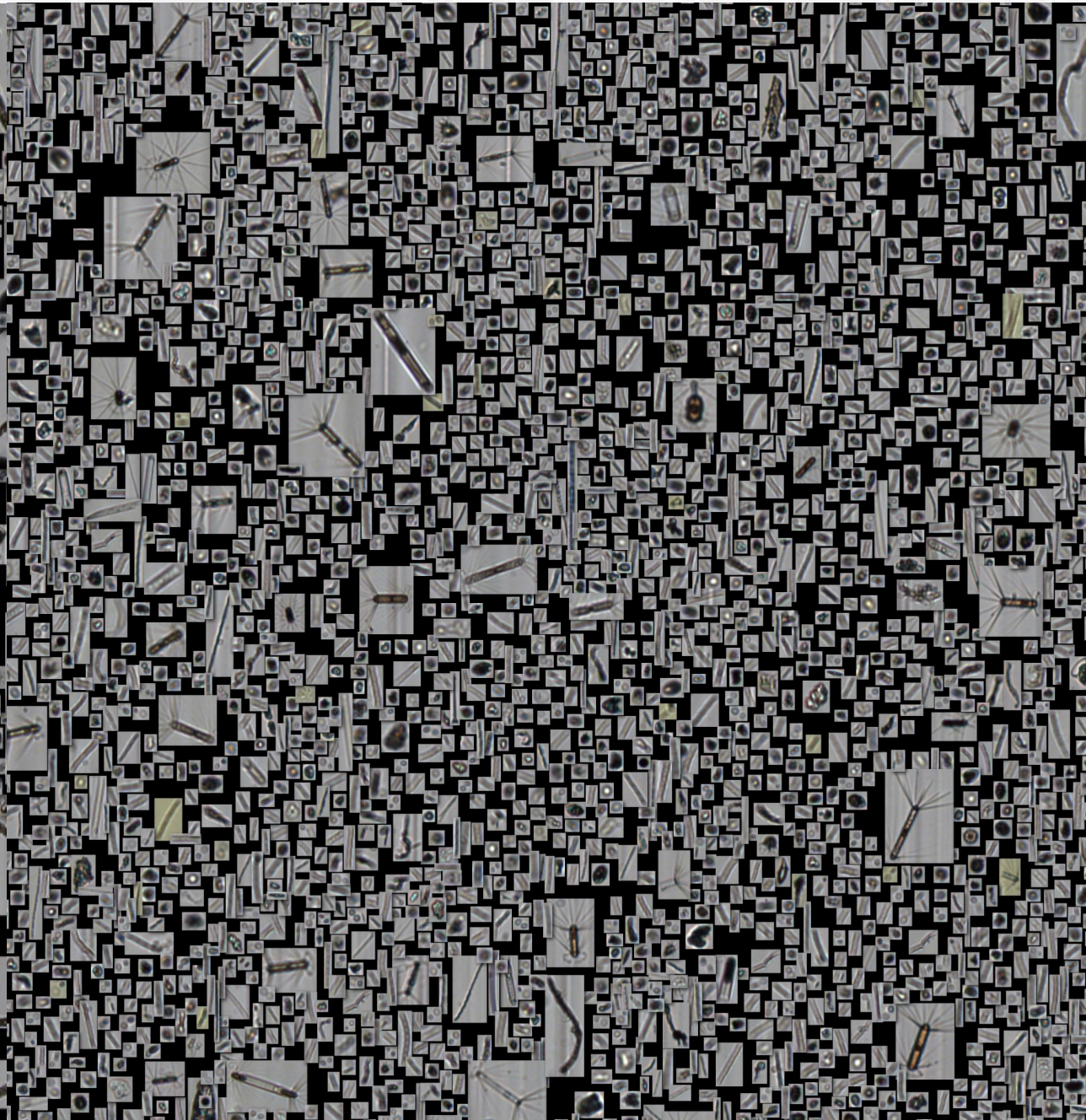




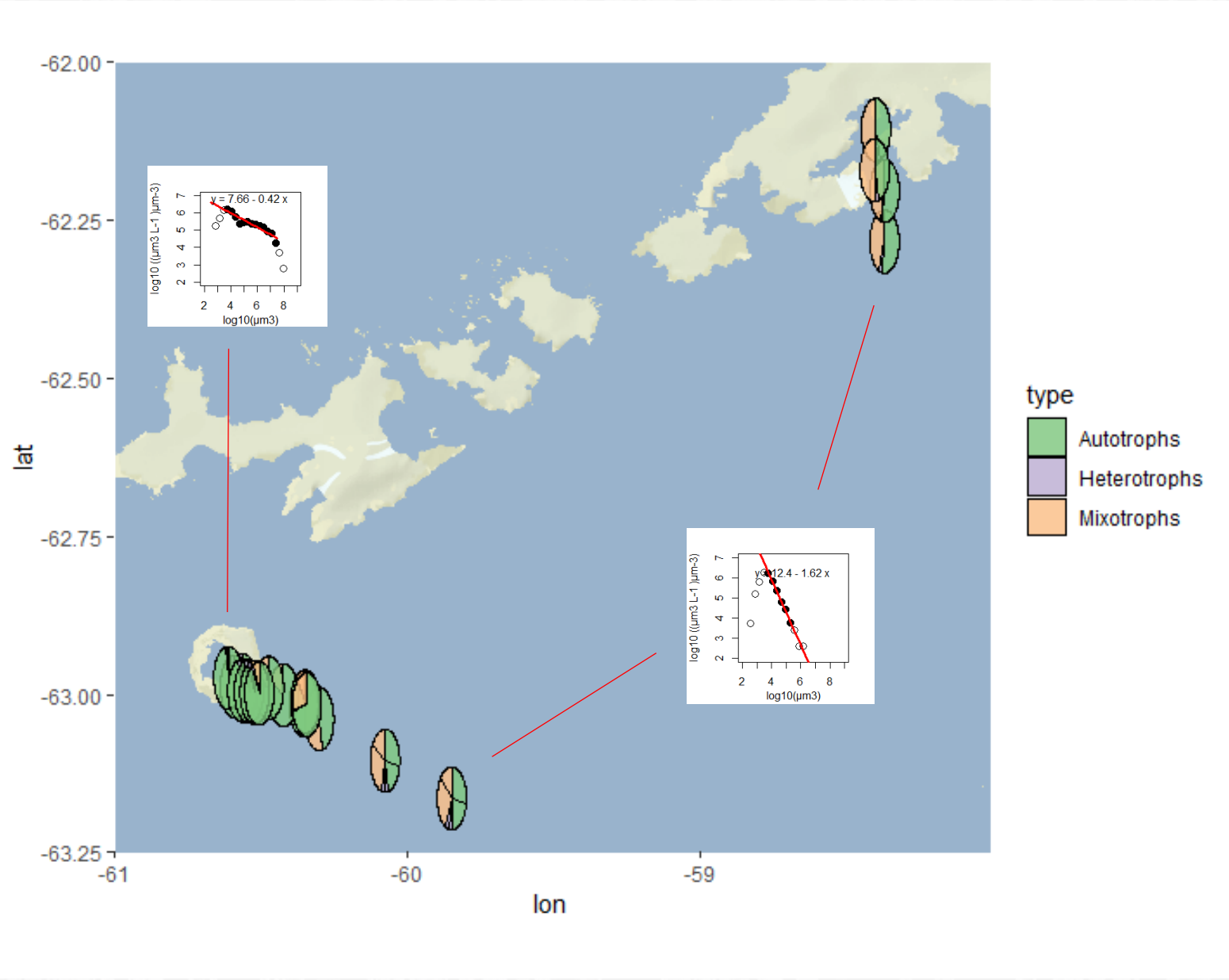
Within the Deception Island



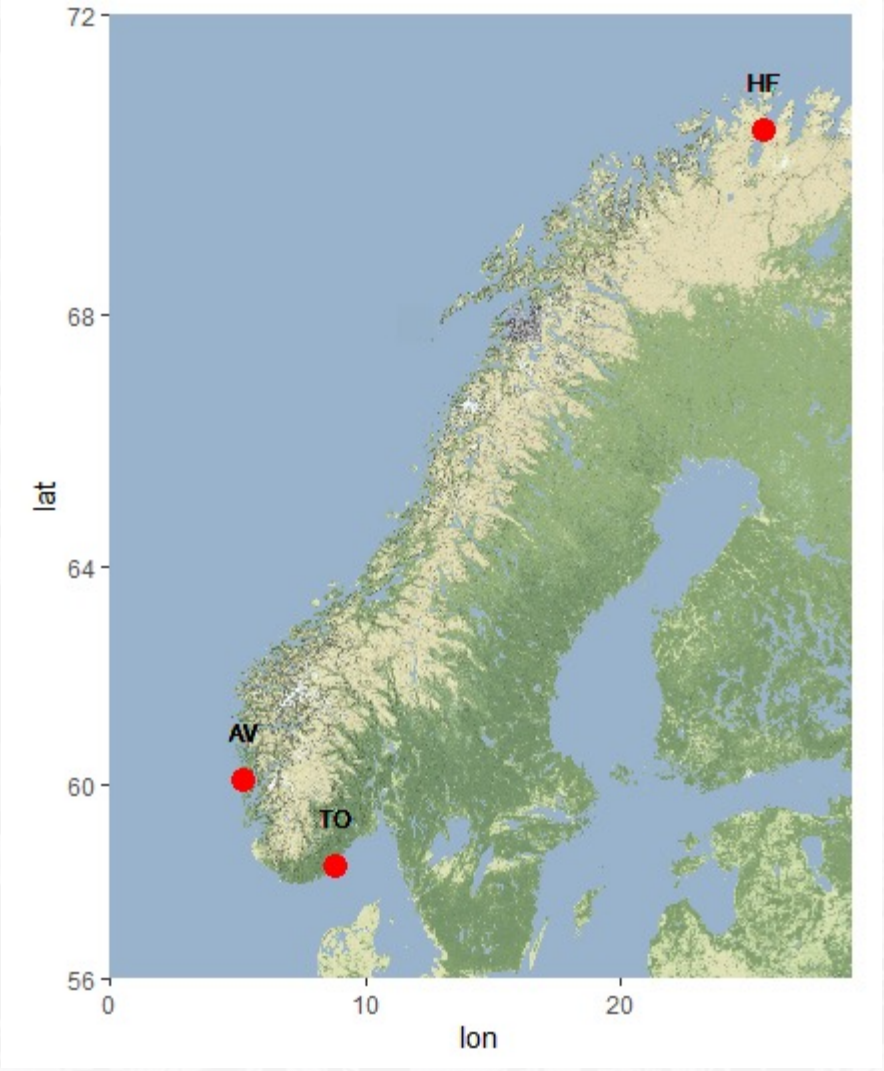
Just outside the opening of the entrance into the caldera



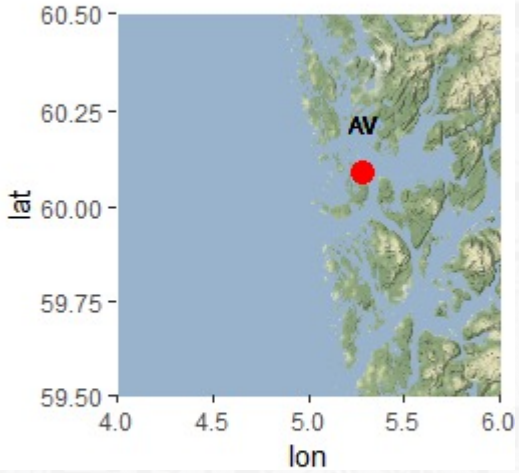
Classification to trophic group



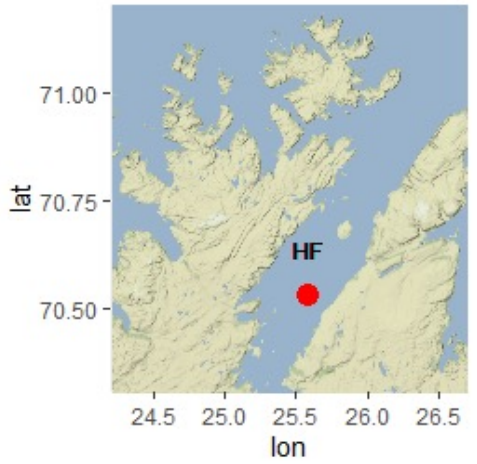
IMR coastal monitoring stations for chemistry, physics and plankton abundance, biovolume and size structure (by FC)



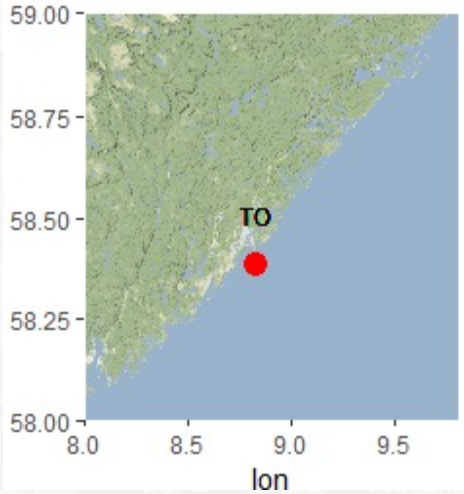
Austevoll



Holmfjord

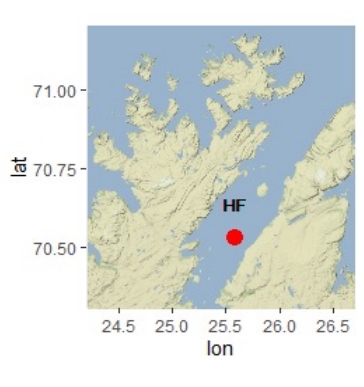


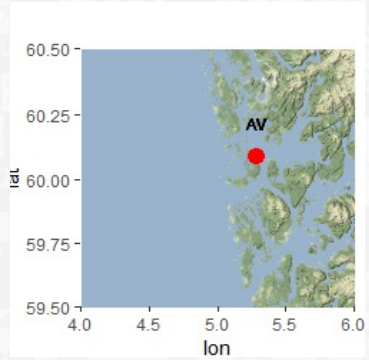
Station 2 - Flødevigen

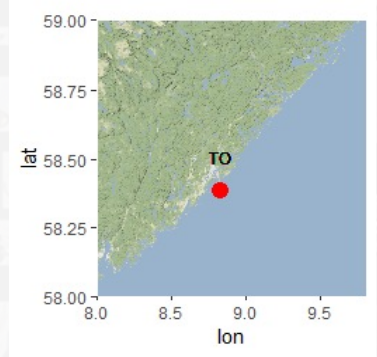


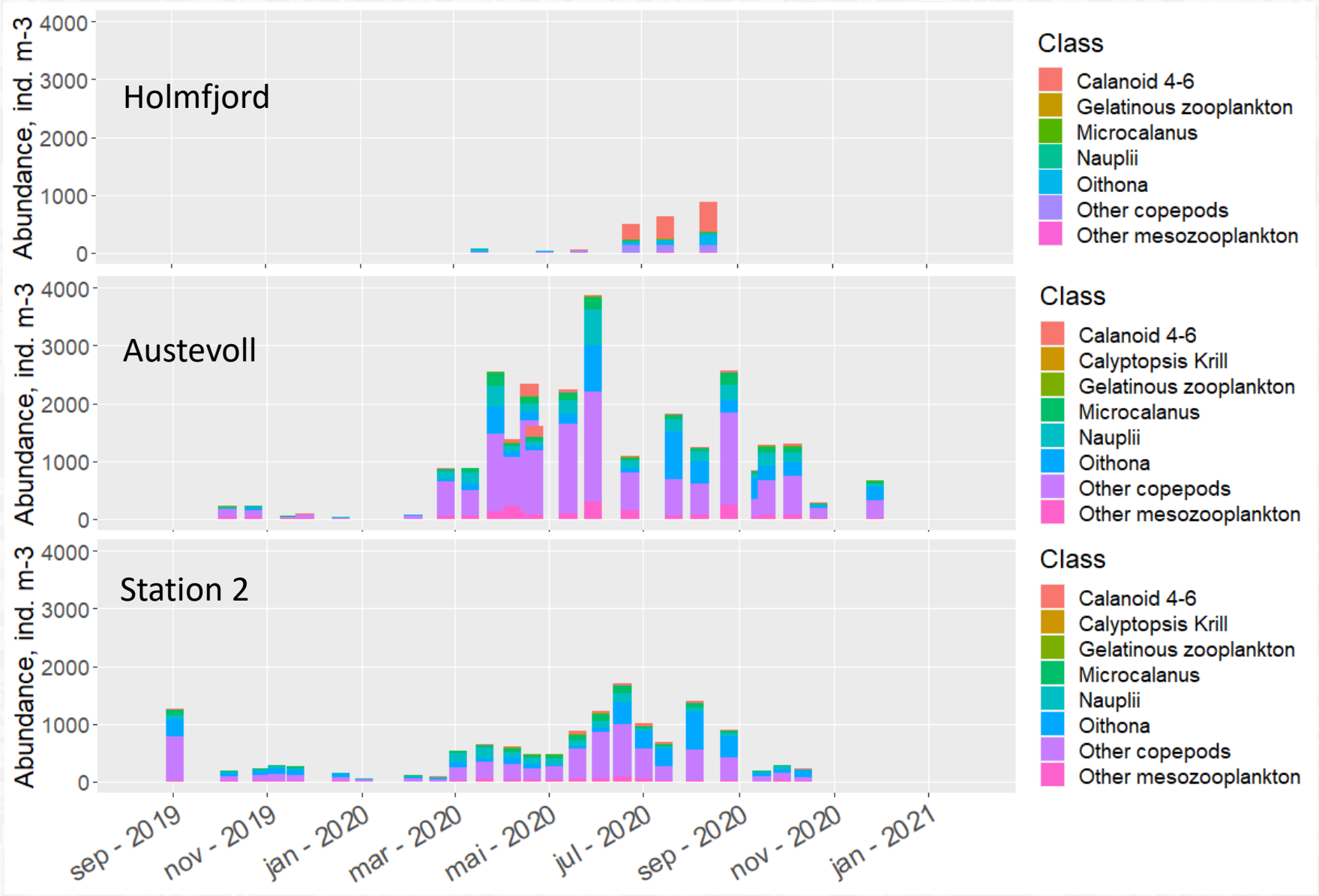
Seawater samples (500 ml) from 5 meter depth were obtained from Niskin bottles every 2-4 weeks at three coastal stations and fixed in acidic Lugol (2% final concentration). The samples were imaged using a FlowCam (20x magnification, 800µm flowcell) and the imaged volume ranged from ~100-130 ml), resulting in an detection limit of ~(1/0.1), or 10 individuals L^{-1}.

Mesozooplankton abundance 180 – 2000µm

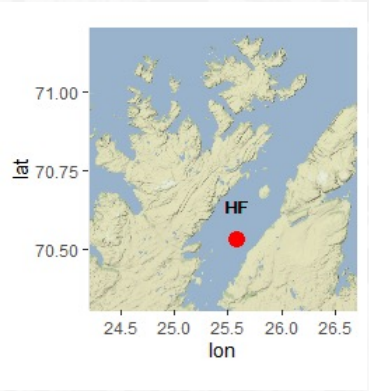


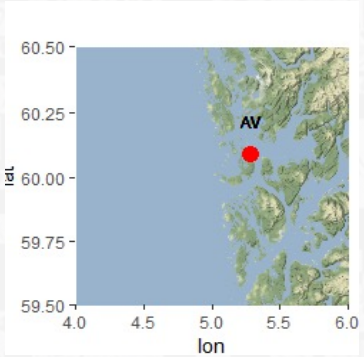


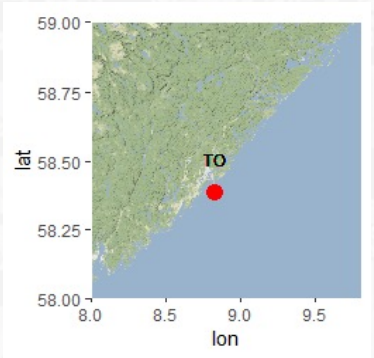


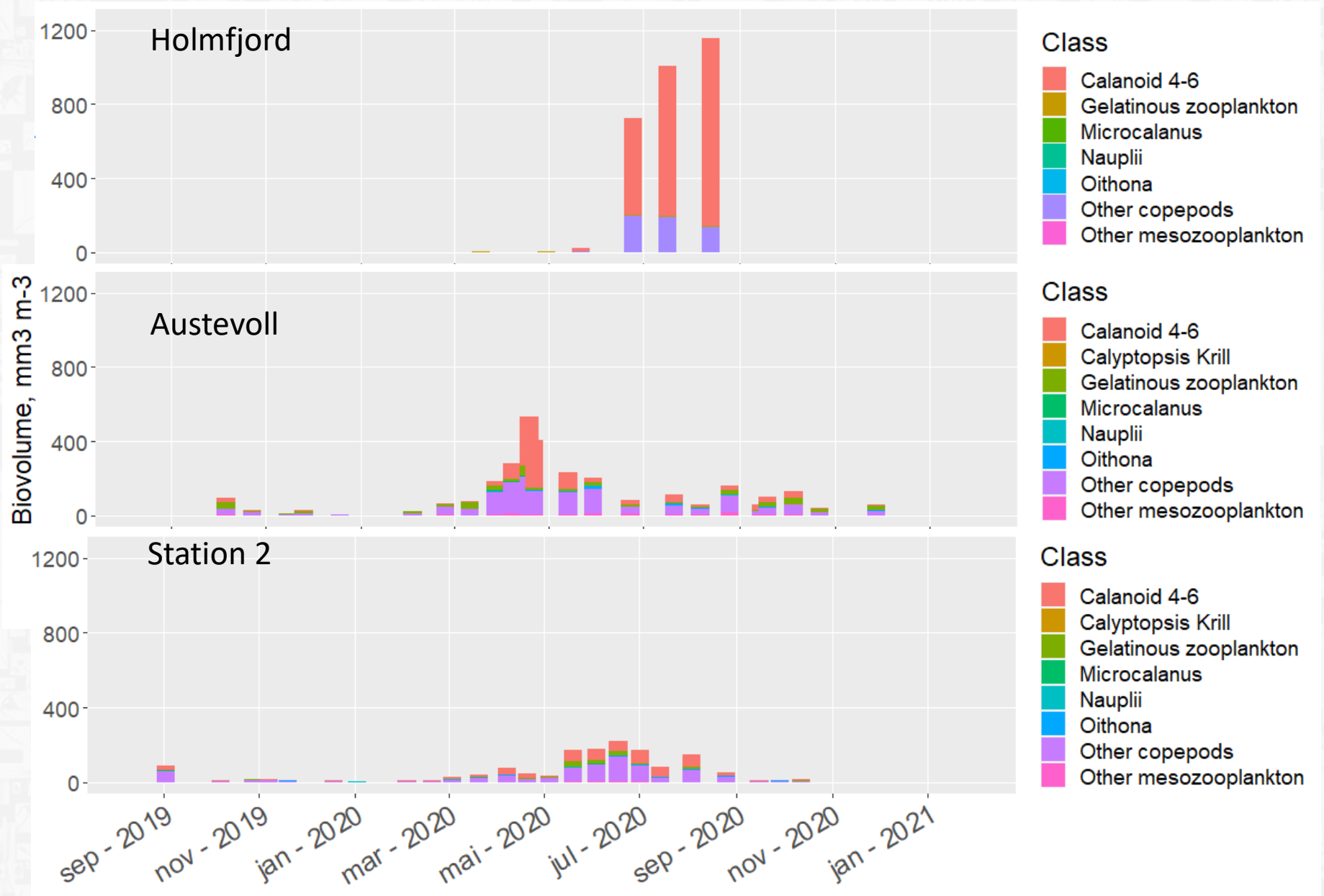


Mesozooplankton biovolume from 180 – 2000µm

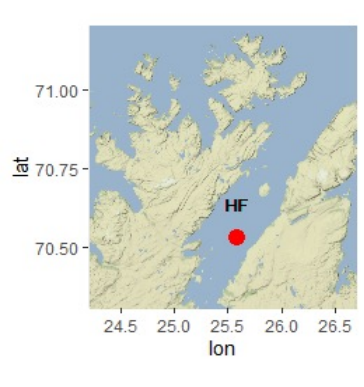


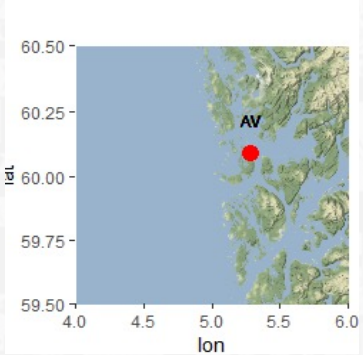


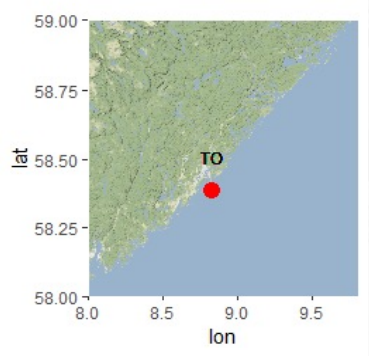


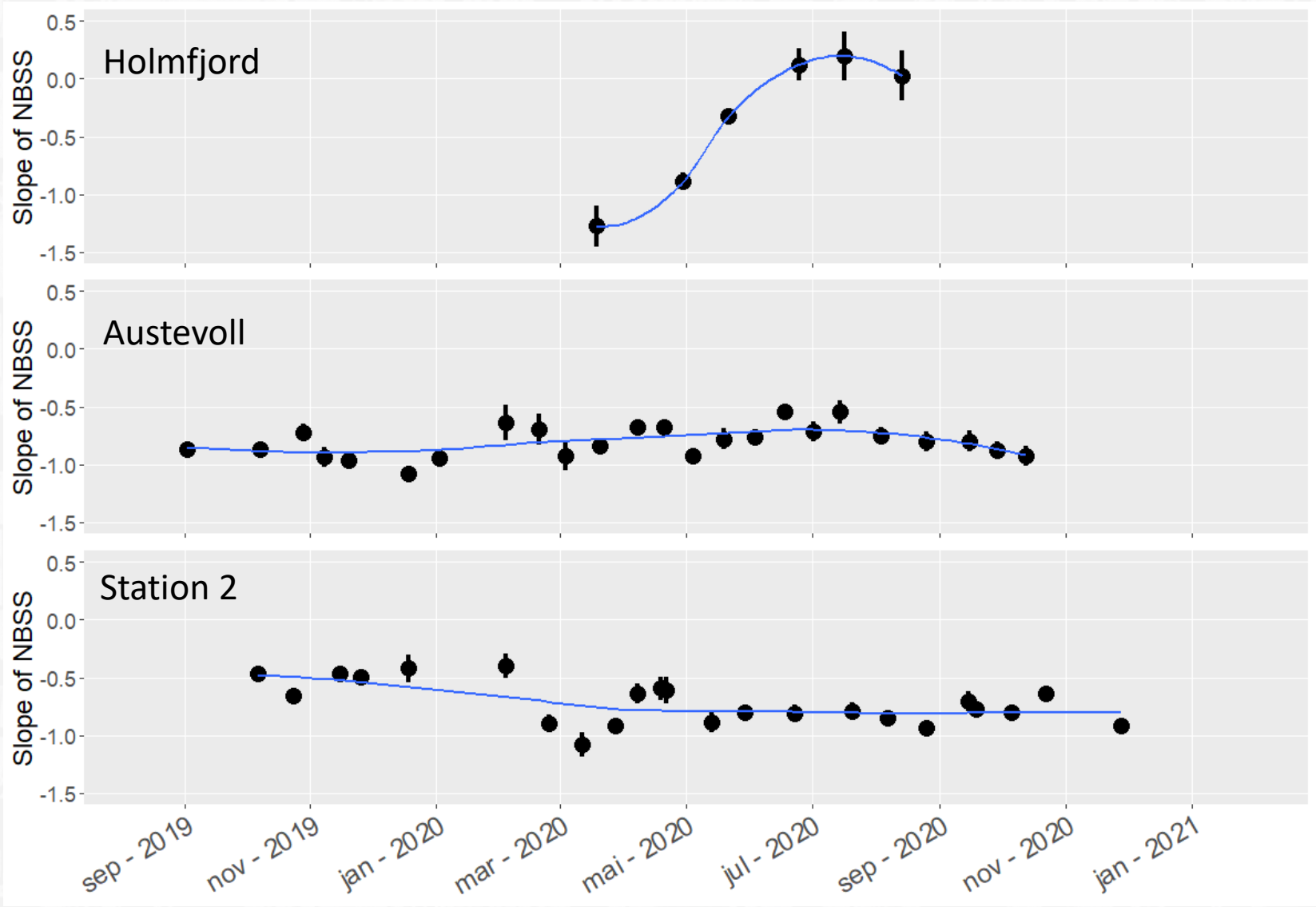


Mesozooplankton size structure 180 – 2000µm - Slope

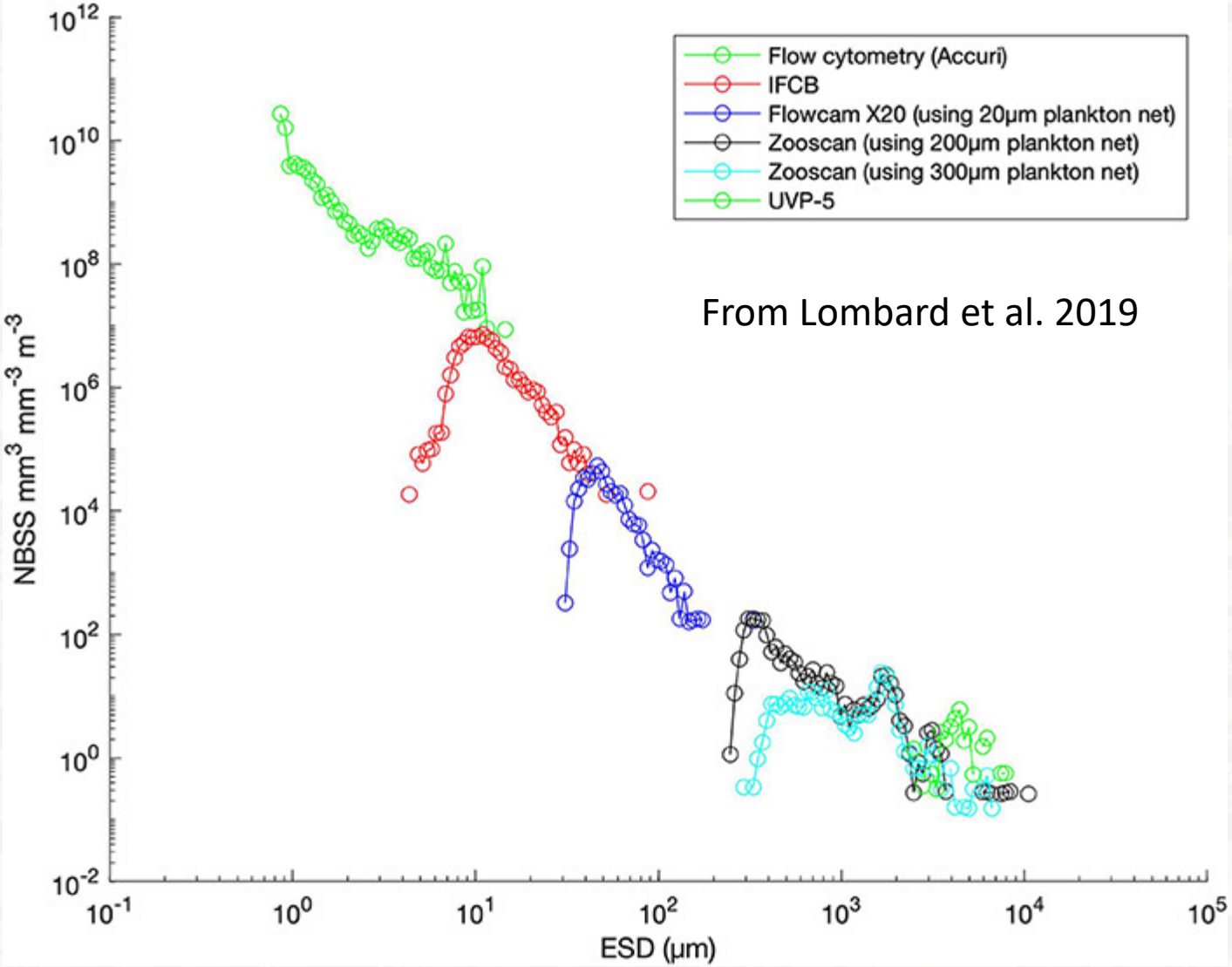






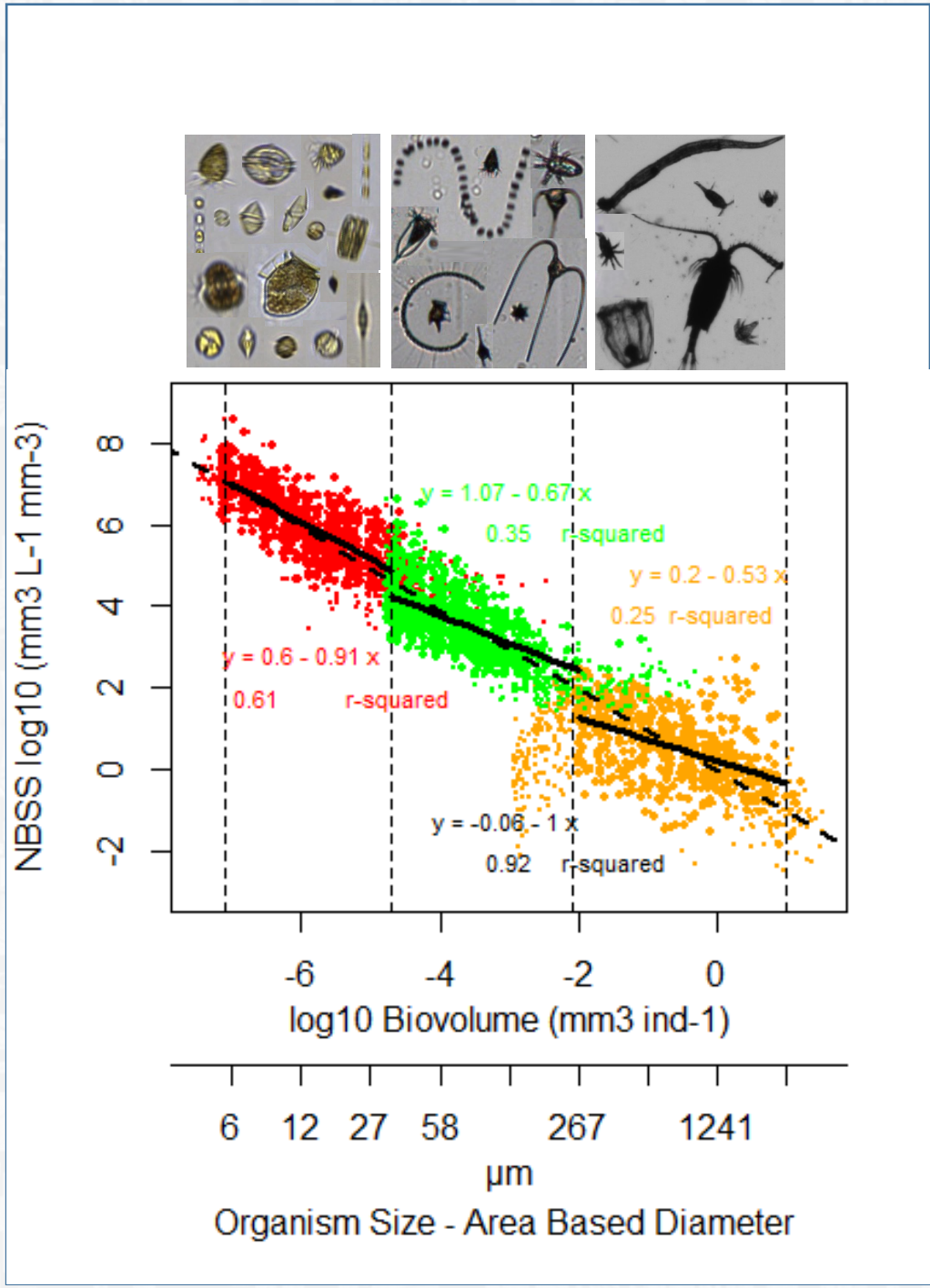


Inspiration to how we may combine results obtained from multiple flowcams



From Lombard et al. 2019

Size structure may guide us in merging the results from different magnification settings and instruments in terms of abundances and bivolumes of taxa across a large range of organism sizes



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