TA PROJECT REPORT

1. Project Information

Proposal reference number	JS3_CALL_2_4038_AQUA-Action-1
Project Acronym (ID)	AQUA-Action-1
Title of the project	AQUACOSM-JERICO Pilot Supersite Action @ SYKE
Host Research Infrastructure	SYKE-MRC (Marine Research Centre) Mesocosm Facility
Starting date - End date	18.8.2022 - 3.9.2022
Name of Principal Investigator **Home Laboratory** **Address** **E-mail address** **Telephone**	Dr. Stella A. Berger Department of Plankton and Microbial Ecology, Leibniz Institute of Freshwater Ecology and Inland Fisheries (IGB), Zur alten Fischerhuette 2, 16775 Stechlin stella.berger@igb-berlin.de +49 151 40092826 (mobile)

2. Project objectives

Our project AQUA-ACTION-1 aimed at relating two overarching objectives:
1. Scientifically, we took the opportunity to study how the Baltic Sea plankton community is responding to extreme events such as sudden shifts in temperature, by mimicking a heat wave in the SYKE-MRC Indoor Mesocosm Facility. The experimental set-up was based on observations by continuous long time series recorded by the facility owner. As the IGB-team has recently studied effects of extreme events on lake ecosystems, we specifically wanted to compare our results in freshwater plankton systems with similar pressures on coastal plankton.
2. The second objective was to take this opportunity for knowledge transfer and harmonisation of competences between European Research infrastructures i.e. AQUACOSM-plus partner IGB (hosting the LakeLab facility, Germany) and JERICO-S3 partner SYKE (hosting the SYKE-MRC Indoor Mesocosm Facility, Finland) by collaboratively foster further development and best practices of technology solutions especially for mesocosm research and plankton imaging. We specifically aimed to initiate RI-RI collaboration at using Artificial Intelligence for high-throughput analysis of plankton images on several instrument platforms i.e. two Imaging Flow CytoBots (IFCB), a CytoSense and a FlowCam.
With the support of the scientific team at SYKE, we intended to use complimentary instrument parks, and exchange experience and knowledge in the application of the different imagers. Finally, both IGB and SYKE groups share a strong incentive to develop open-source programs that are independent of instruments.
We expect that this exchange will benefit these overall objectives, and the wider plankton community by supporting the development of platform-agnostic and open-source approaches for plankton analyses.

3. Main achievements and difficulties encountered

Both scientific teams at IGB and SYKE successfully shared knowledge and harmonised competences for plankton imaging technologies during the time course of the indoor mesocosm experiment. In particular, the SYKE-team helped the IGB-team to set up the Imaging Flow CytoBot (IFCB) in the AQUABOX for benchmark comparison of taxa identification and abundance measurements with the convolutional neural network (CNN) developed by SYKE (Kraft et al. 2022). Parallel measurements of phyto- and microplankton species with the FlowCam performed by the IGB-team during the mesocosm experiment allowed to compare measurements between the IFCB imaging flow cytometers and the FlowCam including different properties such as phytoplankton abundance and biomass estimations by FlowCam and IFCB associated with pigment group-dependent abundance measured in the CytoSense.

This knowledge transfer permitted to create a valuable and rich data-set in terms of annotated images from all the imaging platforms that will be further used in the image library for CNN improvements. These annotated images will then be further used to test and compare the results of the different automated approaches developed at the host institution SYKE and at IGB. The IGB-team will use these results to further develop the "LabelChecker" software currently version 2 based on open-source algorithms, and strengthen our open-source program initiative that are independent of instruments (Bochinski et al. 2018). Finally, IGB expects after thorough interpretation of the experimental results to be able to infer the effects of heat waves on plankton systems from bulk phytoplankton measurements and imaging data.

Unfortunately, the S::SCAN spectro::lyser underwater spectrophotometer could not be setup due to instrument failure and limited manpower for repair.

4. Dissemination of the results

- JRA-TA activity was presented in the media on the JERICO-RI webpage: (https://www.jerico-ri.eu/2022/09/06/joint-jerico-s3-and-aquacosm-plus-study-on-baltic-sea-heatwaves/)
- And on the AQUACOSM-plus web page: https://www.aquacosm.eu/news/article/joint-aquacosm-plus-and-jerico-s3-study-on-baltic-sea-heatwaves,

  https://www.aquacosm.eu/news/article/participation-in-a-mesocosm-experiment-at-syke-helsinki-hcmr-team-and-little-maria
- Raw data obtained during the mesocosm experiment by the instruments run by the IGB team , i.e. IGB-IFCB and the FlowCam, will be available on open source after quality checking and will be part of an open access paper describing the effect of a simulated heat-wave on the plankton community and size distribution. QC data described above will be available on open source (by CC) in an open source publisher (for example Pangaea https://pangaea.de) and on the scientific journal site (if available).
- Results from the method developments and the mesocosm experiment will be presented in scientific meetings in collaboration with SYKE and the other teams on location.
- Results from the method developments and the mesocosm experiment will be published in scientific peer-reviewed journals in collaboration with SYKE and the other teams on location.
- Publication of next version of the Label Checker software on open source (e.g. GitHub) is planned. So far, AI tools developed at IGB have been shared with SYKE to collaborate on future software developments for AI-supported automatised plankton classification.

5. Technical and Scientific preliminary Outcomes

5.1) Set-up and installation of the IGB-IFCB

Both teams IGB and SYKE successfully shared their knowledge and expertise to harmonise competences for plankton imaging technologies. In particular, IGB'S IFCB has been set up (mounted, aligned, filled with fluids) with the support of Kaisa Kraft (Figure 1), a PhD student at SYKE specialized in automated identification and detection of plankton by combining IFCB and CNN technologies (Kraft et al. 2021, 2022). IGB's IFCB has been calibrated under the same conditions as SYKE's IFCB. After a row of difficulties, in particular pump dysfunctions and lasers alignment issues, the team effort was successful and we could finally install the IGB-IFCB in the AQUABOX system which is connected to the experimental mesocosm tanks and to run plankton samples in parallel with the SYKE-IFCB (Figure 2).

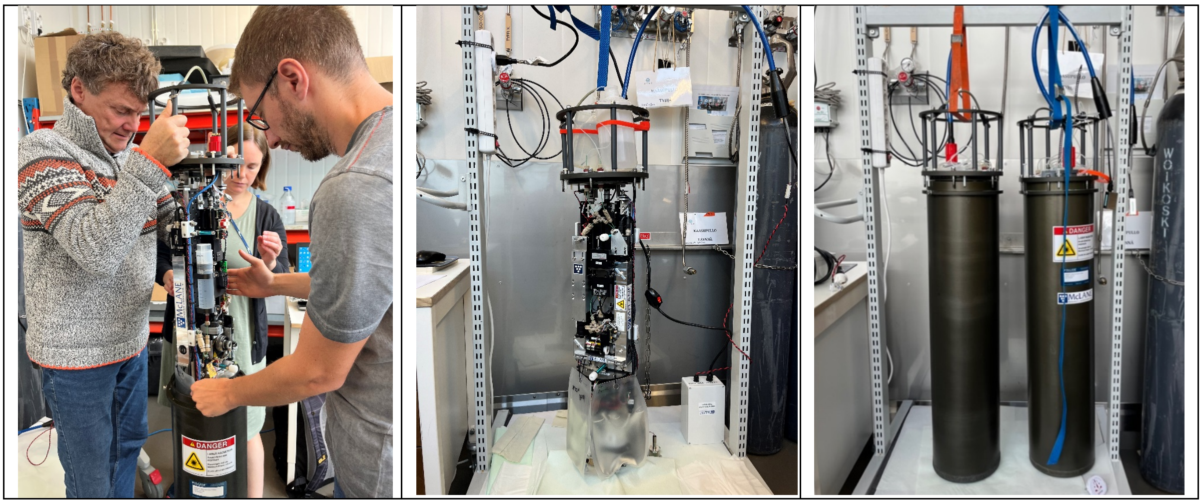

Figure 1. Setting up the IGB-IFCB (left, Jens Nejstgaard, Kaisa Kraft, Christian Dilewski); Naked hanged up IGB-IFCB filled with fluids and ready to measure (mid); IGB-IFCB (135) and SYKE-IFCB (114) ready be installed in the AQUABOX (right); (Photos: Stella A. Berger, IGB).

Figure 2. From left to right: Christian Dilewski trouble shooting the night measurement routine in the IFCB software; Installation of the IGB-IFCB in the AQUABOX system to run plankton samples in parallel with the SYKE-IFCB (Photos: Stella A. Berger, IGB).

5.2) Comparison of measurements between both IFCBs

In both IFCBs, image-specific biovolumes were computed and the biovolumes were converted to biomass (µg L^{-1}) assuming a plasma density of 1 g cm^{-3} (CEN 2015; see Kraft et al. 2022). We compared IFCB-specific phytoplankton biomass estimates at four different equivalent spherical diameter (ESD) ranges (ESD = 6*biovolume/PI^(1/3)).

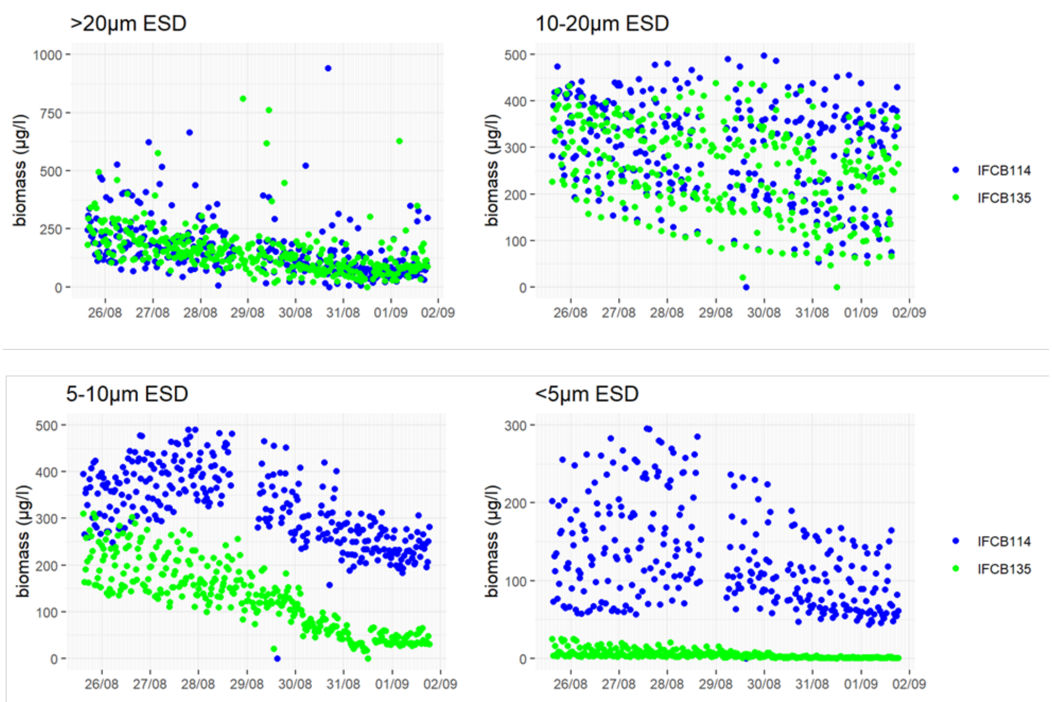

Figure 3. Comparison of IFCB-specific biomass estimates at four different equivalent spherical diameter (ESD) ranges (preliminary results provided by Kaisa Kraft, SYKE).

Preliminary results look very promising and the phytoplankton biomass estimates were very similar between both IFCBs at ESD above 10 µm (size groups 10-20 µm ESD and >20 µm ESD). However, the biomass estimates differed between the IFCBs at ESD below 10 µm (size groups < 5 µm ESD and 5-10 µm ESD). Overall, differences in biomass estimates between both flow cytometers decreased as particle size increased. Currently, the IFCB provides best quantitative observations at a ESD > 10 µm. Despite our effort, some differences in the instrument settings in recognizing small-sized cells still remained and need to be improved in future. For instance, the pixel to µm calibration has not been done for the IFCB 135 (IGB), and we used the conversion of the IFCB 114 (SYKE). Finally, we compared only single small samples volumes (< 5mL). Consequently, larger sample volumes have to be tested to get a better comparison of IFCB-specific results.

5.3) FlowCam measurements during the mesocosm experiment and response of plankton size

Phytoplankton cell size is a key functional trait that often governs resource acquisition, growth, reproduction or interactions with consumers. Temperature changes affect the metabolic rates of phytoplankton that are allometrically scaled with cell size. It is thus expected to observe higher proportion of small species with fast metabolism during heat waves, as compared to larger species with slower metabolism. The IGB team ran samples from each mesocosm every day (or second day) in a FlowCam equipped with a 100 µm flow cell and 10x objective to obtain plankton images in the size range between 3 and 100 µm (Figure 4 and 5).

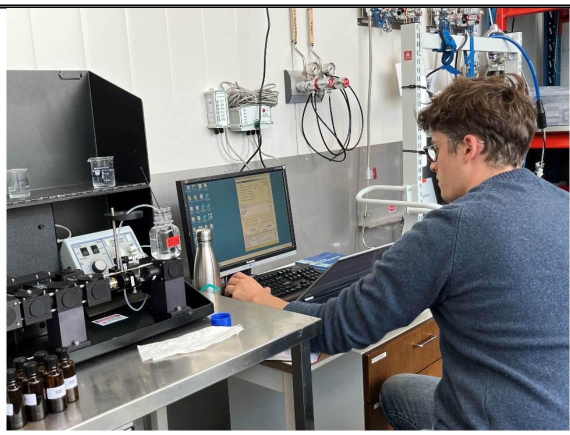

Figure 4. Sampling of the mesocosms (left, IGB-team and Sami) and run of plankton samples in the FlowCam (Alexis Guislain, right) during the SYKE mesocosm experiment at temperature setting of 16, 18, 20, 22°C 4x replicated (Photos: Stella A. Berger, IGB).

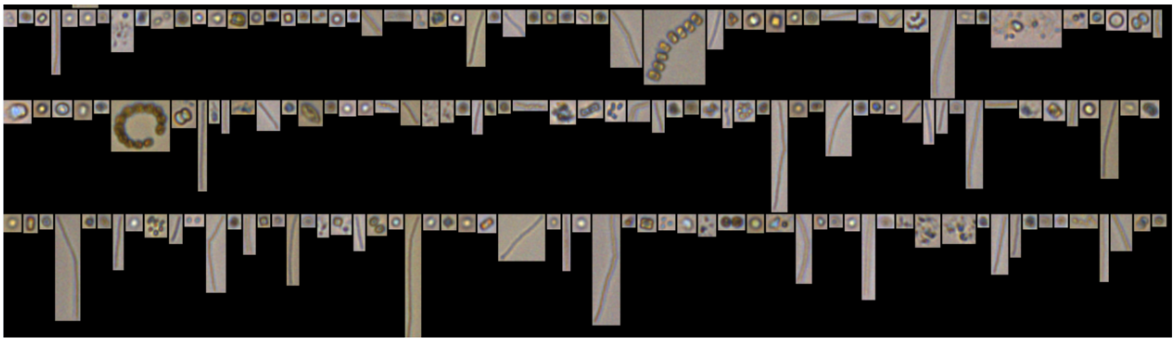

Figure 5. Example of collage images obtained with the FlowCam on August 30th 2022 from the community incubated at 16°C (Photo, IGB).

Preliminary results supported the introductory rationale (Figure 6). Particles (algae plus detritus) below ESD of 20 µm accounted for 90% of the total community biovolume when incubated at 22°C. In contrast, the ESD range had to be twice as large for the community incubated at 16°C to reach 90% of the community biomass. These differences in size distribution during heat waves are expected to have severe consequences on carbon cycling or size-dependent predation for instance.

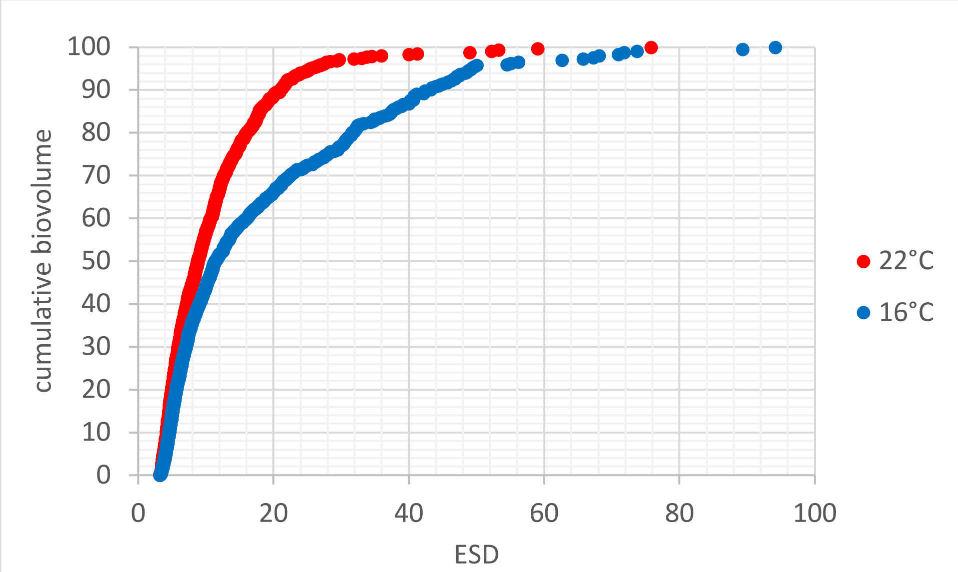

Figure 6. Size distribution (ESD) of the community biomass (expressed as biovolume = ESD*pi^(1/3)* 1/6) in the communities incubated at 22°C and 16°C. The contribution of smaller organisms to the total community biovolume increased as temperature increased (Graphic, Alexis Guislain).

5.4) Use of IFCBs and FlowCam image data to assess the effects of heat wave on plankton

The mesocosm experiment gave us the opportunity to further develop a calibrated suite of flow cytometers with different properties and to create a valuable and rich data-set in terms of annotated images that will be used to further develop the "LabelChecker" (Bochinski et al. 2018). The primary ambition for the IFCB was to be installed as a long-term monitoring instrument in situ. The FlowCam however, was dedicated to laboratory measurements. Both IFCB and FlowCam have now the possibility of being calibrated and complement each another (Figure 3). The FlowCam software has been updated by IGB to Visual Spreadsheet 4.19 to improve volume estimation of plankton particles. FlowCam includes all particles also those without fluorescence signal such as heterotrophic microzooplankton, flagellates and detritus, thus, comparison with IFCB needs classification and sorting of particles, which will be supported by LabelChecker 2.0. After thorough interpretation, our results should enable us to infer to some extent the effects of heat waves on plankton systems from imaging data.

Thanks for the great team at SYKE and specifically Kaisa, Katri, Jukka, Maiju, Pasi, Sami, Timo, Otso, Teresa, Noora, Mari, Joanna, Christian, Anne-Mari and more as well as the opportunity for fruitful collaboration with all other research teams visiting SYKE.

5.5) References

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Stechlin, 18/11/2022 ______________________________ Location and date	____________________ Signature of principal investigator