TA PROJECT REPORT

1. Project Information

Proposal reference number	JS3_CALL_2_4039_AQUA-Action-2
Project Acronym (ID)	AQUA-Action-2
Title of the project	AQUACOSM-JERICO Pilot Supersite Action @ Utö
Host Research Infrastructure	Utö marine station
Starting date - End date	3.9.2022 - 5.9.2022 (hosts continued the observations until 7.9.2022 – Remote Access for two days)
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2. Project objectives

Our project AQUA-ACTION-2 aimed at relating two overarching objectives:

1. Scientifically, the tests conducted at Utö marine station should assist our experimental work at SYKE MRC-lab, by providing a comparison of different imaging instruments (IFCBs, CytoSense, other sensors) installed in an AQUABOX system monitoring the Baltic Sea plankton at Utö, and especially improving our understanding of sensor and instrument specific uncertainties in abundance and biomass estimation of natural plankton communities.

2. Our second objective was to utilize this opportunity for knowledge transfer and harmonisation of competence between European Research infrastructures, by collaboratively developing best practices and technology solutions for plankton imaging. We specifically aimed to initiate RI-RI collaboration in using Artficial Intelligence (AI) for high-throughput analysis of plankton image on several instrument platforms. Our aim during the Utö visit was to exchange experiences and discuss best approaches for integration of IFCB instruments in operational multi-sampling platforms. This includes automated and remote maintenance practices, physical, fluidical and electronic integration, and practices in data transfer.

We expect that this knowledge exchange and harmonisation approach will benefit the wider plankton community by supporting the development of platform-agnostic and open-source approaches for plankton analyses.

3. Main achievements and difficulties encountered

The scientific teams involved in this activity from IGB (TA users), FMI (operator) and SYKE (operator) successfully shared their expertise and knowledge, harmonised competences for plankton imaging technologies and during the stay at Utö marine station located (UMS) on Utö Island (59º 46'50N, 21º 22'23E) at the outer edge of the Archipelago Sea (Baltic Sea) https://en.ilmatieteenlaitos.fi/uto. Long-term measurements of physico-chemical parameters are in line with image-based data acquisition of Baltic Sea phytoplankton by using the permanently running IFCB installed in the AQUABOX (Kraft et al. 2021, 2022).
During the visit of the IGB team at Utö, the SYKE-team helped the IGB-team to set up the IGB-Imaging Flow CytoBot (IFCB) and connected it to the AQUABOX installed at Utö marine station. The IFCB at Utö got maintenance and parallel measurements of algae cultures were performed to confirm that they measured similar values (which they did, within ca 5-10% from each other each run), before attaching both to the water intake for the AQUABOX. Parallel measurements of phyto- and microplankton species with both IFCB imaging flow cytometers (from IGB and SYKE) were then used to compare measurements between both instruments including different properties. We achieved benchmark comparison of taxa identification, abundance measurements and biomass estimations with both IFCB instruments supported by the convolutional neural network (CNN) analyses developed by SYKE (Kraft et al. 2022). These measurements will also be compared with pigment group-dependent abundance analyses in the CytoSense performed by Lumi Haraguchi (SYKE).
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. The 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 Bochinski et al. 2019), and strengthen our open-source program initiatives at both involved institutions, e.g. to develop approaches more independent of instrument platforms preferably across the involved institutions, and beyond. This collaboration will also support planned collaboration in the new HORIZON Europe project Aqua-INFRA, where both IGB and SYKE are partners with focus on plankton imaging.
This project has a strong relevance at the European level, through the collaboration between key persons and institutions in both JERICO-S3 and AQUACOSM-plus RIs forming a direct RI-RI collaboration to enable transfer of knowledge, harmonisation of competence, develop best practices and technology solutions between European Research infrastructures.

4. Dissemination of the results

- Raw data obtained by the instrument IGB-IFCB during the access to Utö, will be available on open source after quality control and will be part of an open access paper comparing the IFCB instruments used for analysing the Baltic Sea 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 short-term comparison of IFCB instrumental set-up and data acquisition of the Baltic Sea phytoplankton monitoring data will be published in scientific peer-reviewed journals in collaboration with SYKE and FMI/UMS teams on location.

- Results from the measurements and method developments achieved at the Utö station and the mesocosm experiment will be presented in scientific meetings in collaboration with SYKE, FMI/UMS and the other teams on location.
- Publication of next version of Label Checker (GitHub) or similar is under planning; AI tools developed at IGB were shared with SYKE to collaborate on developments.
- Results and benefits of the RI-RI collaboration will be disseminated through the channels of the EU-networks - JERICO-S3, AQUACOSM-plus and the Aqua-INFRA projects.

5. Technical and Scientific preliminary Outcomes

1) Monitoring infrastructure and plankton analyses at Utö

The scientific teams involved in this activity from IGB, FMI and SYKE successfully shared their expertise and knowledge, harmonised competences for plankton imaging technologies and during the stay at Utö Marine Research Station (Laakso et al. 2018). The Utö Atmospheric and Marine Research Station of Finnish Meteorological Institute is located on Utö Island (59º 46'50N, 21º 22'23E) at the outer edge of the Archipelago Sea, Baltic Sea (Figure 1). Marine observations are lead by the Head of Group, Lauri Laakso (FMI) and biological observations are lead by the Head of laboratory, Jukka Seppälä (SYKE). The Finnish Meteorological Institute started meteorological observations on the island in 1881, marine observations in 1900 and atmospheric trace gas and aerosol measurements in 1980. Utö Atmospheric and Marine Research Station is part of the HELCOM marine monitoring network, European Monitoring and Evaluation Programme EMEP, Integrated Carbon Observing System ICOS, The Aerosol, Clouds and Trace Gases Research Infrastructure ACTRIS and Coastal research infrastructure network Jerico-RI. Currently, it is developed to become a national ACTRIS site. Real-time observations from the station available at https://en.ilmatieteenlaitos.fi/uto.

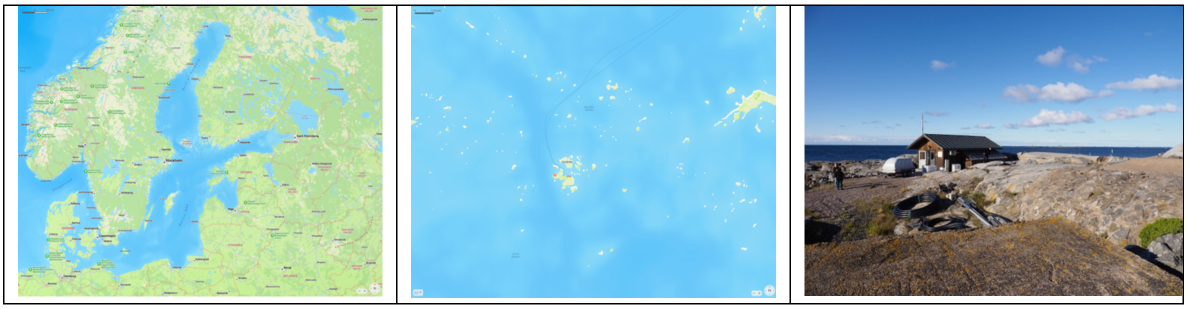

Figure 1. Location and photo of Utö Marine Station on the island Utö, Baltic Sea (Photo: Jens Nejstgaard).

The TNA activity by the IGB team enabled a collaboration between FMI, SYKE and AQUACOSM-plus, including the set-up and field-testing of the IGB-IFCB in parallel with the SYKE-IFCB at Utö. Long-term measurements of physico-chemical parameters of the Baltic Sea at Utö are combined with image-based data acquisition of phytoplankton by using the permanently running IFCB installed in the AQUABOX (Kraft et al. 2021, 2022). We compared the results of both IFCB instruments measuring the Baltic Sea phytoplankton community by a continuous flow-through setup and collected image data with both IFCBs in September 2022. As only a stay between 3-5 September was possible for some of the participants, including the IGB team, the rest of the running of the IFCB had to be conducted as Remote Access until the local team took the instrument back from Utö to Helsinki, after the mission was completed on 7 September 2022. The IFCBs capture images of planktonic cells and processed a 5-mL sample every 20 minutes. Both instruments were operated with chlorophyll *a* trigger to capture images of chlorophyll-containing cells and to prevent detritus and other non-living material to be imaged. The size of the imaged particles ranged from 5 µm nanoplankton to large colonies or filaments such as cyanobacteria with a length of ~300 µm. A 150 µm mesh was used at the instrument inlet to prevent the instrument from clogging.

2) Set-up and installation of the IGB-IFCB at Utö

The research teams from IGB, SYKE and IMF successfully shared their knowledge and expertise to harmonise competences for plankton imaging technologies. In particular, IGB strongly benefitted from this TNA, as IGB's IFCB has been set up (mounted, aligned, filled with fluids) with the critical 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. The team effort was successful and we could finally install the IGB-IFCB in the AQUABOX system at Utö which is connected to the Baltic Sea water inlet and to run plankton samples in parallel with the SYKE-IFCB (Figure 2).

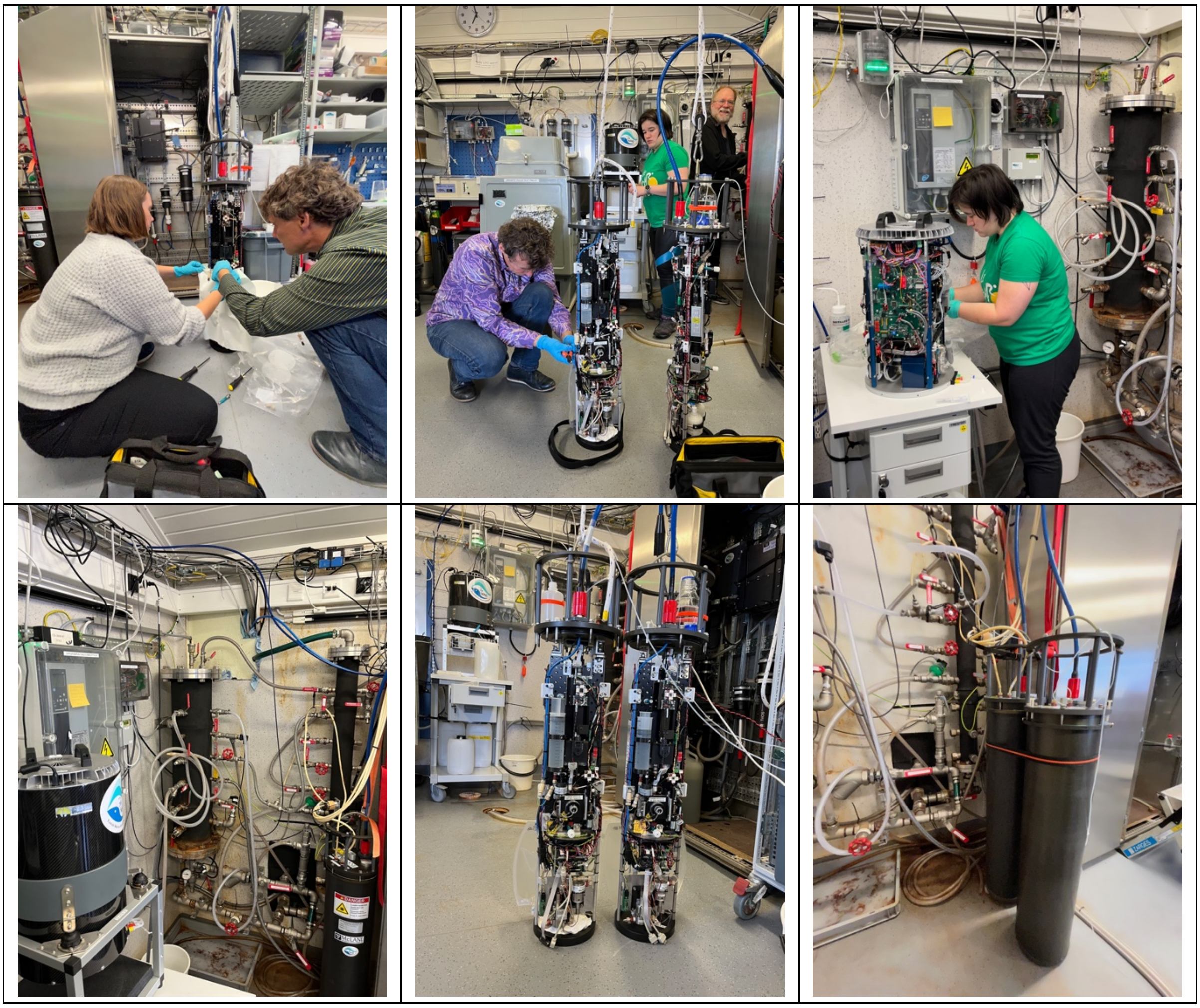

Figure 1. Top row, from left to right: Kaisa Kraft and Jens Nejstgaard, setting up the IGB-IFCB attaching the fluid pouches to prepare for operation; Jens Nejstgaard working on the IGB-IFCB with Lumi Haraguchi working on the CytoSense and Jukka Seppälä working on the AQUABOX in the background; Lumi Haraguchi setting up the CytoSense; Lower row, from left to right: The water intake tubing for the AQUABOX; comparing the IGB and SYKE-IFCBs before closing the instruments; both IFCBs connected to the AQUABOX water intake system (Photos: Stella A. Berger, IGB).

3) Comparison of measurements between both IFCBs

In both IFCBs, the total number of images per sample and image numbers of IFCB-specific estimates at four different equivalent spherical diameter (ESD) ranges were compared.

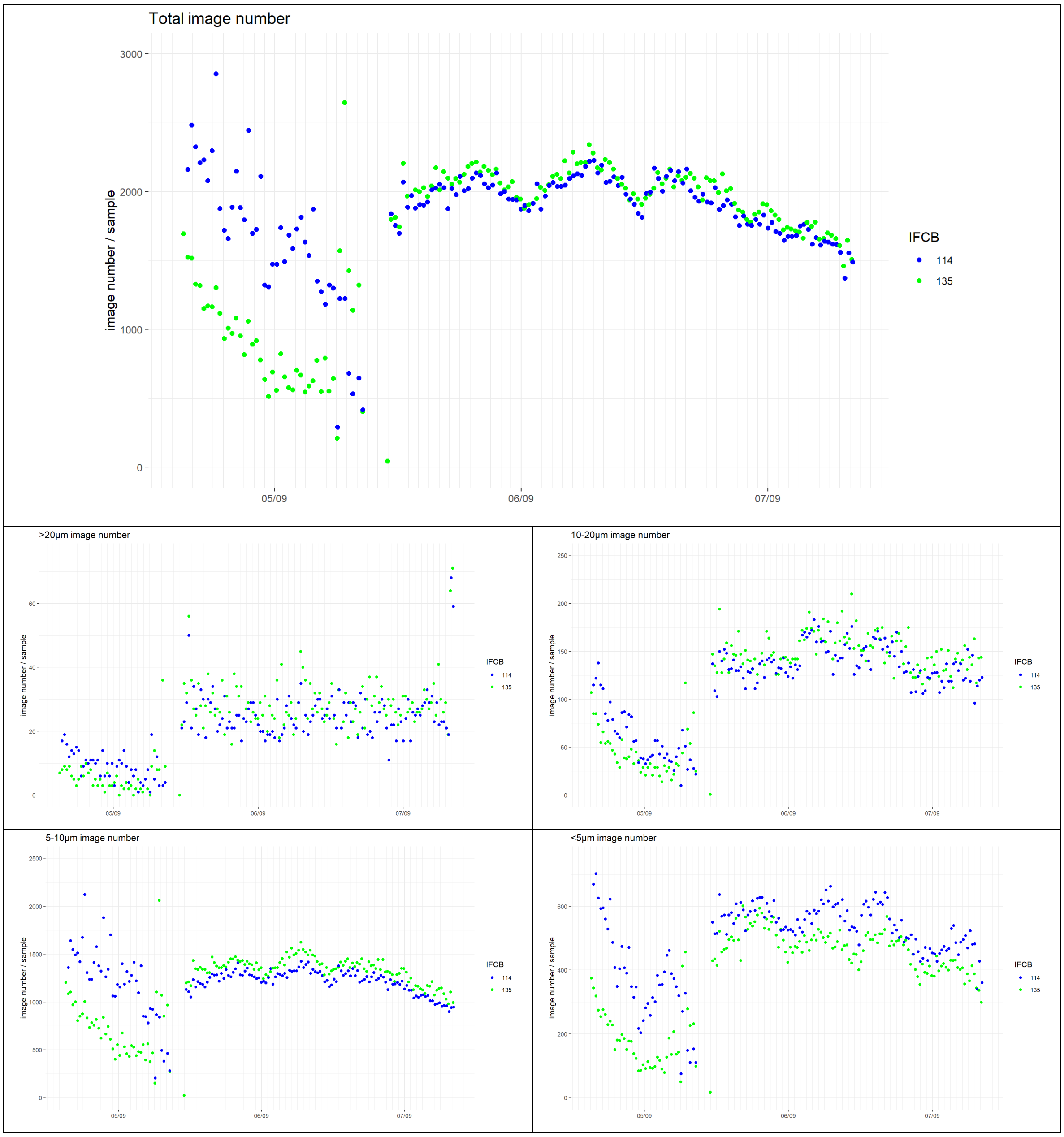

Figure 3. Comparison of IFCB instruments (SYKE-IFCB 114 in blue and IGB-IFCB 135 in green) measuring Baltic Sea water samples including natural phytoplankton. Total image numbers (upper large graph) and different equivalent spherical diameter (ESD) ranges > 20 µm, 10-20 µm, 5-10 µm, < 5 µm (lower graphs). Preliminary data kindly provided by Kaisa Kraft, SYKE.

Despite that the biomass estimates have been very similar between the two IFCBs tested at SYKE in the previous project AQUA-ACTION-1, the total numbers per sample and the size-specific numbers at first appeared to be very different between the two IFCBs compared at Utö (Figure 3). This can be seen in the left part of all panels of Figure 3 where systematically higher numbers were detected in the SYKE-IFCB. We discovered that this was related to which of the water intakes that was used for the respective IFCB instrument. The error could be found in the intake system i.e. the intake micro tube was pressed against the side of the intake tube in one of the two hose connections (Figure 4). The problem could be solved during the period of Remote Access, and after this, both instruments gave very similar results as indicated in the right part of all panels in Figure 3. Total image numbers were very similar between both IFCBs as well as image numbers in the size groups 10-20 µm ESD and >20 µm ESD. Image numbers slightly differed between both IFCBs in the size groups <5µm ESD and 5-10 µm ESD (Figure 3). Overall, differences in image numbers between both flow cytometers decreased as particle size increased because the IFCB provides better quantitative observations at a ESD ranging between 10 and 80 µm. Despite our effort, minor differences in the instrument settings in recognizing small-sized cells still remained. For instance, the pixel to µm calibration has not been done to IFCB 135 (IGB), instead we used the conversion factor from IFCB 114 (SYKE), which may affect in which size class the images go (i.e. data from IFCB 135 showed less images in 5-10 µm and more in < 5µm size class).

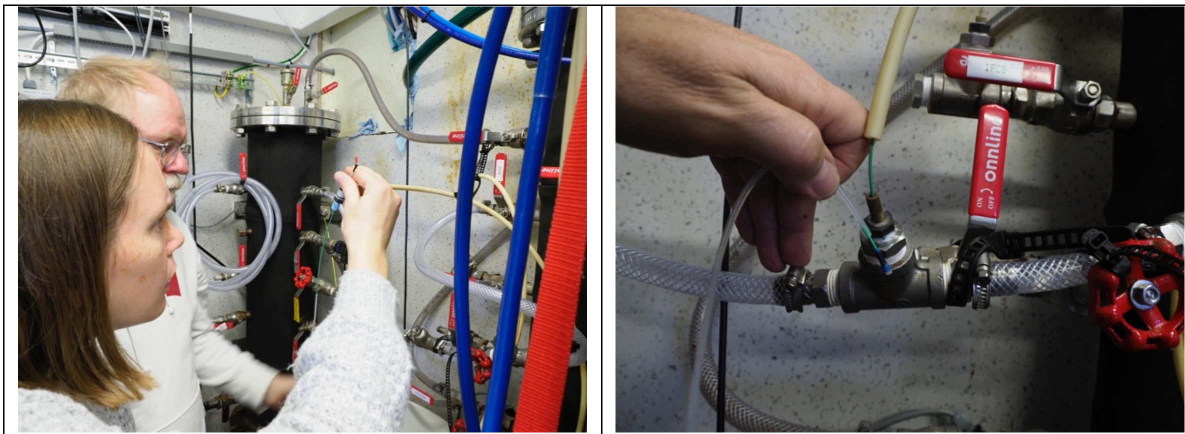

Figure 4. Critical control, and reinstallation of the hose connection to the AQUABOX system.

4) Use of IFCB image data to assess plankton dynamics in pelagic aquatic ecosystems

The visit to Utö measurement station and the SYKE 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" (Boschinki et al. 2019). The primary ambition for the IFCB was to be installed as a long-term monitoring instrument *in situ.* After thorough interpretation, our results should enable us to infer to some extent the effects seasonal developments in plankton communities from imaging data. The exercise at Utö helped us to define i) how to best install IFCB for continuous operations on location and when getting back to IGB to install the IFCB at Lake Stechlin and ii) estimate instrument specific uncertainties for imaging by comparing the the images quired in both IFCBs and technical connections to the AQUABOX.

The collaborative study between FMI, SYKE and AQUACOSM-plus, including the set-up and field-testing of the IGB-IFCB in parallel with the SYKE-IFCB supported by long-term observational physical and chemical data at Utö and the experimental data at SYKE, will contribute to better understanding of how phytoplankton communities are affected by extreme climatic forcing. Several aspects of interests for the research community studying pelagic plankton system can be achieved i.e. to enhance spatio-temporal analyses of plankton samples and support time-consuming microscopic analyses. Thus, this activity contributed to the tools needed to cope with Grand Challenges for aquatic ecosystems connected with global climate change. The knowledge-transfer during this activity enabled future phytoplankton data collection also at the freshwater LakeLab platform in Lake Stechlin, North Germany, upon return of the IGB-team.

	Thanks to the great team at Utö marine station and specifically to Lauri, Kaisa, Jukka, Otso, and Lumi for the collaborative experience, super teamwork and wonderful impressions of the island and engaged and informative guidance by Lauri on the Utö island, Jens and Stella

5) References

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Stechlin, 28/11/2022 ___________________	St. Bofer (signature) ______________________________
Location and date	Signature of principal investigator