Project Title:	AQUACOSM: Network of Leading European AQUAtic MesoCOSM Facilities Connecting Mountains to Oceans from the Arctic to the Mediterranean
Project number:	732065
Project Acronym:	AQUACOSM
Proposal full title:	Network of Leading European AQUAtic MesoCOSM Facilities Connecting Mountains to Oceans from the Arctic to the Mediterranean
Type:	Research and innovation actions
Work program topics addressed:	H2020-INFRAIA-2016-2017: Integrating and opening research infrastructures of European interest

Deliverable No 8.1:
Report on the current status and aims for AquaBox

Due date of deliverable:	31 August 2017
Actual submission date:	20 December 2017
Version:	v1
Main Authors:	Robert Ptacnik (WCL) , Timo Tamminen (SYKE)

This project has received funding from the *European Union's Horizon 2020 research and innovation programme* under grant agreement No 732065

This project has received funding from the *European Union's Horizon 2020 research and innovation programme* under grant agreement No 732065

Project ref. number	**731065**
Project title	**AQUACOSM: NETWORK OF LEADING EUROPEAN AQUATIC MESOCOSM FACILITIES**

Deliverable title	Report on the current status and aims for AquaBox
Deliverable number	D8.1
Deliverable version	Final
Contractual date of delivery	31 August 2017
Actual date of delivery	20 December 2017
Document status	Public
Document version	Final
Online access	Yes
Diffusion	PUBLIC
Nature of deliverable	
Workpackage	WP8
Partner responsible	WCL
Author(s)	Robert Ptacnik (WCL), Timo Tamminen (SYKE)
Editor	
Approved by	Jens Nejstgaard (IGB)
EC Project Officer	Agnès Robin

Abstract	
Keywords	

This project has received funding from the *European Union's Horizon 2020 research and innovation programme* under grant agreement No 732065

Table of Contents

1. Executive summary

AquaBox is an automated, multichannel and multiparameter sampling and measurement unit to be developed in AQUACOSM for mesocosm experimentation. The aim is to enhance high-frequency data collection, in order to address the dynamic responses and interactions within the microbial food web of planktonic systems. This document describes briefly the aims, background knowledge, current status and R&D plan for AquaBox during the first steps of the AQUACOSM project.

This deliverable official submission date was Month 08 (August 2017) and related to Subtask 8.1.2: Workshop on flow-through technology at SYKE/Helsinki (Lead: SYKE, contributors: WCL, LMU, CNRS, Month 8) and feeds from the results of this workshop. This Workshop was postponed to be held in the Fall 2017 as the lead organiser (SYKE) moved into new buildings over the summer. Due to the relocation of the institute D8.1 had to be postponed. The first request to Agnès Robin was sent by email on July 17 2017, and approved by email reply on July 25 2017.

2. What is "AquaBox"?

2.1 Task description (8.2.2)

Based largely on the Ferrybox technology that has emerged from previous projects (among them FP7 JERICO), and is currently being further developed at SYKE (H2020 project JERICO-NEXT), we will in the AQUACOSM project design a compact and modular flow-through system (**AquaBox**) suitable for high-precision monitoring of mesocosms.

The AquaBox will be designed as a central unit incorporating a modular suite of measurement probes and analysers. This basic concept allows for flexible set-ups in experimentation with different scientific targets, requiring specific sets of measured parameters. It aims at cost-efficiency as measurement probes need not to be replicated for each experimental unit. The basic restraint of the system is the necessity to have the experimental units within reasonable reach from the AquaBox unit, to allow for automated sample retrieval via a stationary tubing array.

The AquaBox thus combines peristaltic pumps and multichannel valves, flow cells and sequential flow technology, performing autonomous measurements successively from several mesocosms installed in a joint rig. By employing flow-through sensors/analysers and open access (OA) software for system control, data retrieval, data quality assurance and quality control (QA/QC), and wireless data transfer, the system can perform multiple measurements on automatically retrieved water samples, allowing for high precision and reproducibility, as well as semi-continuous measurement frequencies hitherto unobtained in mesocosm studies.

A modular, readily expandable system structure and control software for the AquaBox is first developed and tested in lab scale, with a basic set of sensors for key properties most prone to temporal variation in planktonic systems during mesocosm experiments (temperature, oxygen, in vivo phytoplankton pigment fluorescence at several wavelengths).

A key development task in 8.2.2 will be the assessment of feasibility and demonstrations of expanding the basic AquaBox structure to integrate additional functions, and measurements requiring advanced analysers, into its physical and control architecture, such as:

- automated water sample retrieval,
- inorganic nutrient analyses (miniaturized wet chemistry),
- Fast Repetition Rate Fluorometry (FRRF) and Pulse Amplification Modulated (PAM) fluorometry as primary productivity proxies,
- dissolved organic matter (CDOM and FDOM),
- in-water carbonate system chemistry (pCO_2, pH),
- interphase for gas exchange measurements across water surface,
- flow cytometry, and
- image analysis of individual cells of phytoplankton and zooplankton species (e.g. FlowCAM, CytoSense, FlowCytobot).

It is foreseen that a large share of these analysers will be temporarily obtained within the AQUACOSM partnership for basic functional tests and demonstration. The selected advanced features/analysers, chosen on the basis of the development work and laboratory and field tests, will be acquired and incorporated into a high-end AquaBox pilot version by the end of the project.

2.2 Background and synergies in AquaBox R&D

Partner SYKE has extensive experience in developing and running flow-through automated measurements onboard Ferrybox systems in ships-of-opportunity, as well as in developing a complex flow-through measurement system for the new Utö Marine Research Station (Finland). The scheme of the current development version of

Co-funded by the European Union

Ferrybox system is presented in Fig. 1, and main measurements for the Utö flow-through system are depicted in Figs. 2 and 3.

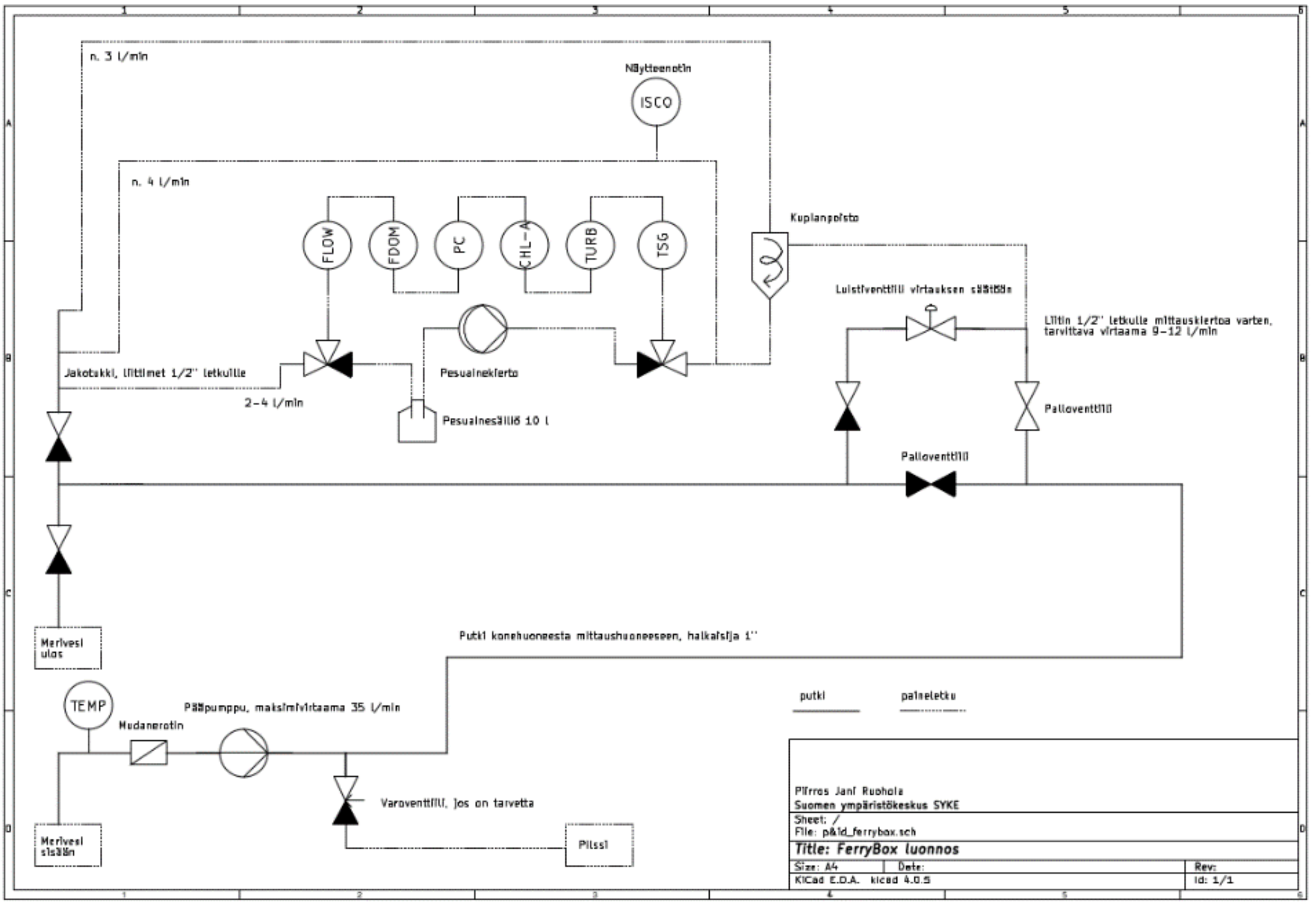

Fig.1. The current version of SYKE Ferrybox R&D ("Algaline 2.0") takes advantage of small computers and open-source electronics; data acquisition and automatic controls done with Raspberry Pi and Arduino. It utilizes simple DAQ software with a GUI, written in Python and Python's Qt5 bindings.

Co-funded by the European Union

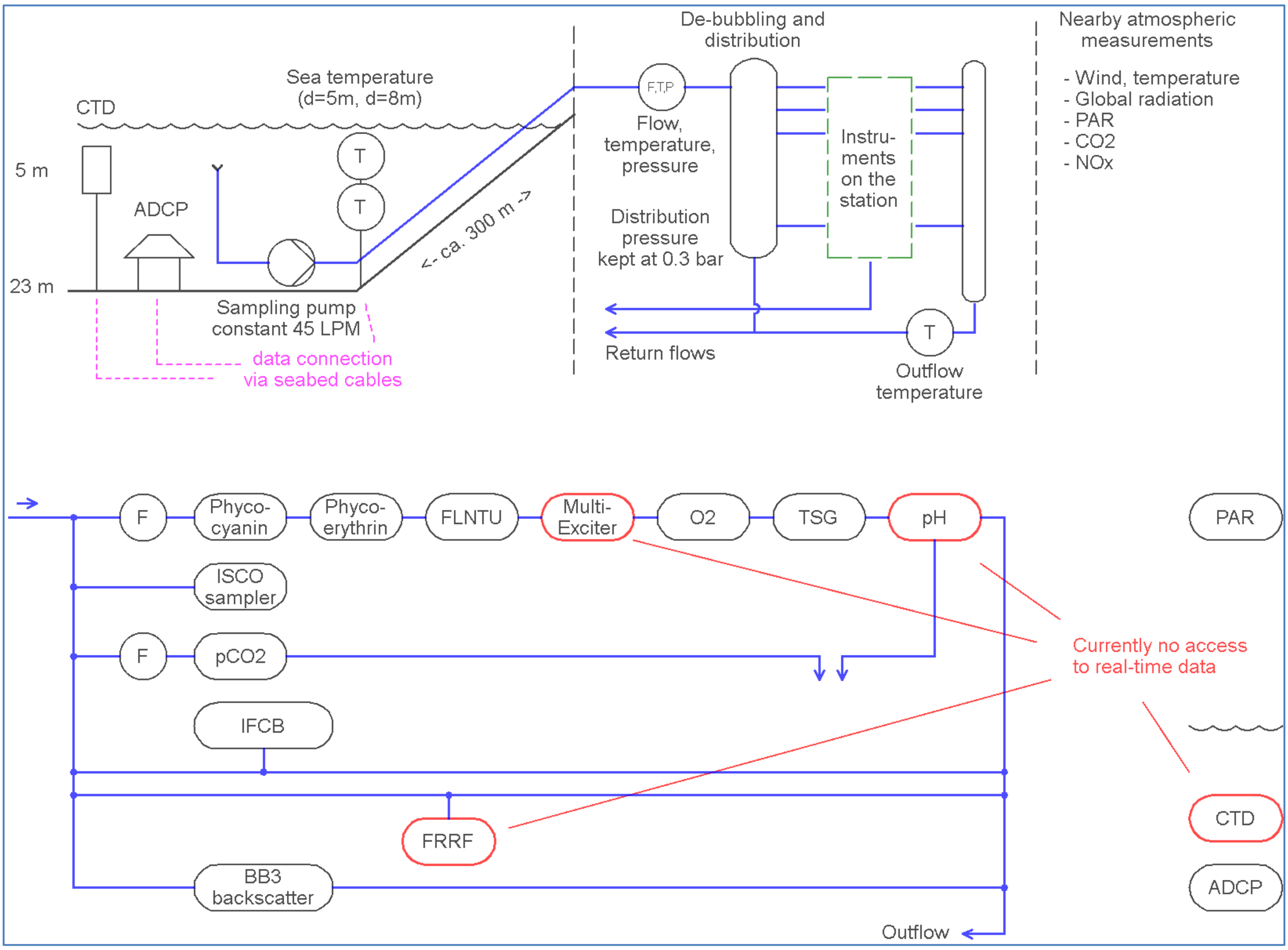

Fig. 2. Scheme of the automated Utö Marine Research Station. Control and data acquisition software are scripted in Python. All data are remotely accessible in real time except for notifications in red.

Co-funded by the European Union

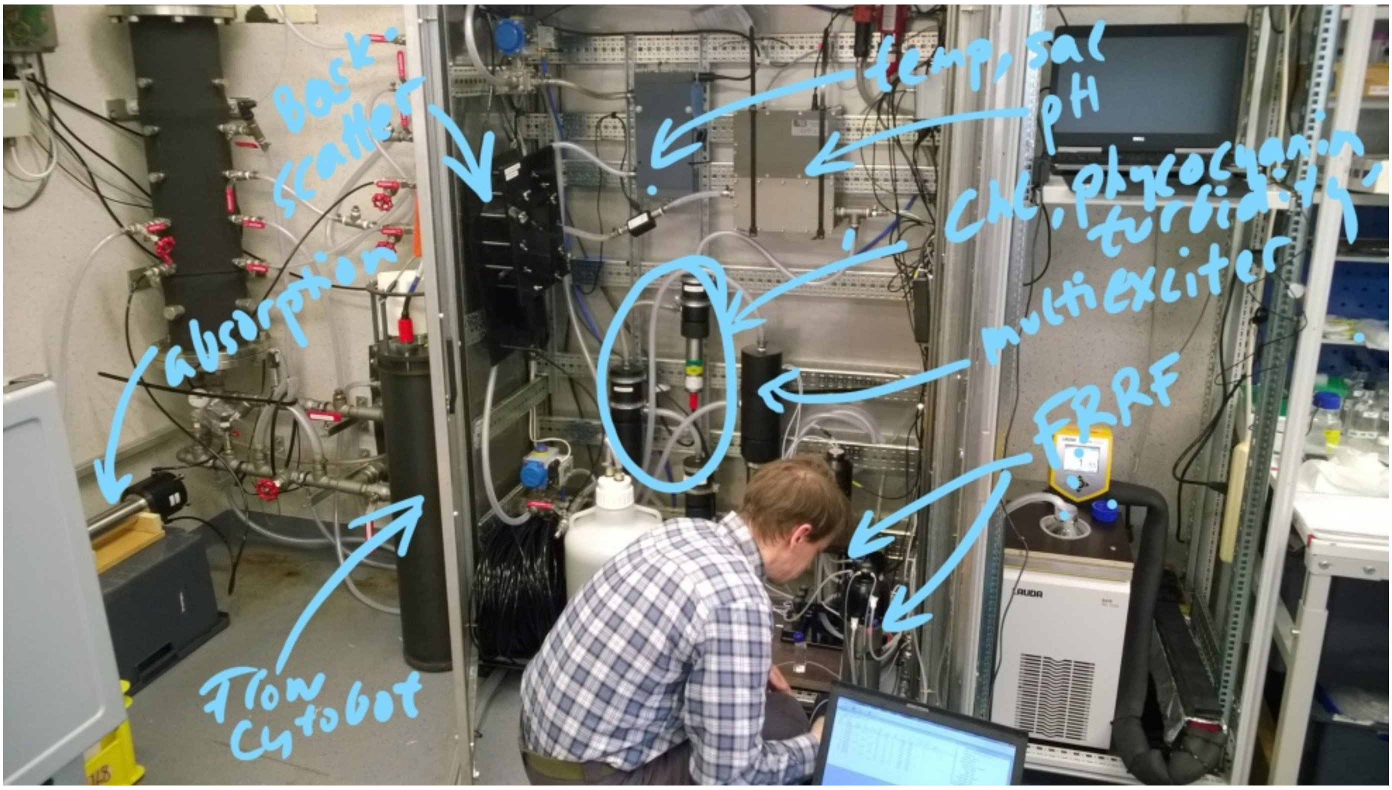

Fig. 3. And in reality, it looks like this (part of the automated Utö Marine Research Station). The AquaBox will require considerable downscaling, but the control and data acquisition software are scale-independent, so synergy with previous systems is essential for the AquaBox R&D work.

These automated measurement systems are developed within the H2020 INFRAIA project JERICO-NEXT by partner SYKE, and the experience gained from these will be directly accessible for AquaBox R&D work in AQUACOSM. The specific challenges in the AquaBox development are partly connected to the arrangement of sample rotation with multiple sample input streams required in mesocosm experimentation (as opposed to a single flow-through stream), and partly to accommodation of measurements requiring different temporal scales (from microseconds up to several minutes) in the same system architecture and control.

Partner WasserCluster Lunz (WCL) has considerable experience in running chemostat experiments (Exponentially Fed Batch Method, Fischer et al. L&O Methods 2014). WCL will implement a downscaled AquaBox to be employed in chemostats (microcosms, volume ~500ml). Here the same computer that controls the through-flow of the chemostat will be used to control automated measurements (peristaltic pumps, multivalve, sensor readings).

3. AquaBox R&D approach and schedule up to Month 24

The AquaBox development plan is based on addressing 3 conceptually different measurement types within a modular, automated sample transfer/measurement framework:

- “***immediate***”: directly from flow-through stream in sequential arrangements (sample streams to waste); response times of measurement probes much less than seconds
- “***delayed***”: collection of sample stream to temporary reservoirs, for parameters requiring much more than a time window of seconds, due to e.g. probe response times, analyzer measurement cycle times, or a need for sample dark acclimation in bio-optical measurements (sample streams to waste)
- “***catch & release***”: sampling loops from exp. units to non-invasive measurement(s) and back to units – for measurements requiring larger volumes and ~minutes of sample stream (potentially pCO_2, imaging of larger particles)

The conceptual version of the automated multiple sample retrieval/measurement system was presented in the AQUACOSM kick-off meeting (Month 1), and the development of system architecture and control scripts (in Python) for pumps, multichannel valves and measuring instruments started immediately. The nucleus version of the scripts was tested in a small lab-scale installment (tissue culture bottle scale – Fig. 4), which was demonstrated for the WP8 partnership in a workshop arranged by SYKE in Helsinki, November 2017. As explained in the executive summary, the workshop, as well as this Deliverable, was originally scheduled for Month 8, but because of relocation of the SYKE laboratory facilities in 2017, both were postponed to the end of the first year, as described above.

The R&D schedule for the next steps is as follows:

Ver. 0.2 (lab scale; demo in Helsinki WS, Nov 2017):

1. basic control software architecture (Python)
2. automated sampling module from X experimental units (10^2 ml scale)
    a. µl-scale peristaltic pump/tubing
    b. multichannel valve for input stream
3. dark acclimation loop
4. “immediate” flow-through measurement module
    a. variable fluorescence parameters (PSI AquaPen)

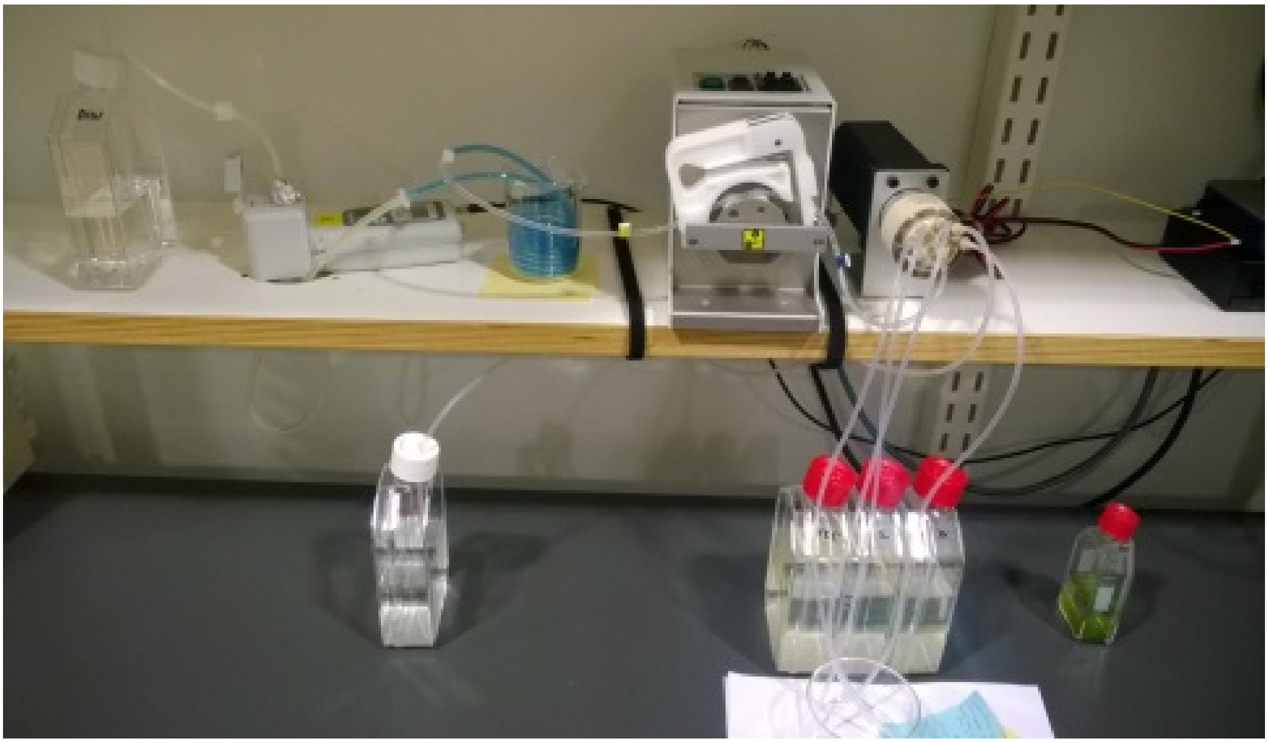

Fig. 4. Lab-scale test and demonstration unit for first R&D steps (Ver. 0.2).

Ver 0.3 (mesocosm scale from here on):

5. upscale to ml-to-liter scale peristaltic pump/tubing for automated sampling
6. upscale to large-bore (ID 2-4 mm) multichannel valve(s) for input stream
7. next “immediate” flow-through measurement modules
    a. chl a fluorescence (Trios Nanoflu)
    b. phycocyanin fluorescence (Trios Nanoflu)
    c. phycoerythrin fluorescence (Chelsea)

Ver 0.4 additions:

8. next “immediate” flow-through measurement modules
    a. pH (Sunburst AFT-pH)
    b. O_2 (Aanderaa)

*******************target: first test runs March 2018***********************

Ver. 0.5 additions:

9. module for “delayed” measurements (#sample reservoirs = #experimental units)
10. module for sampling from reservoirs (small-bore multichannel valve & µl-scale peristaltic pump)
11. “delayed” measurement module:
    a. Fast Repetition Rate Fluorometer (Chelsea)
    b. inorganic nutrients (FIAlyzer-2000)

*******************target: first test runs August 2018*************************

Test runs, troubleshooting and full validation of the Ver. 0.5 system (with immediate and delayed measurement modules in place) is anticipated to take most of the second year of the project (2018).

4. R&D schedule up to Month 45

Further aims of AquaBox development during the 3rd and 4th years of the project (2019-2020) are:

1. Address the “catch & release” sampling principle for non-invasive measurements requiring large volumes/prolonged time, either due to relatively long stabilization time of the measurement (*p*CO2), or e.g. low abundances of target organisms for imaging techniques.
2. Incorporation of automated water sampling module into the AquaBox system.
3. Feasibility tests of incorporation of additional/alternative measurement techniques and instruments in AquaBox, as brought forth by interests of the wider AQUACOSM partnership.
4. Testing of the basic AquaBox system in indoor mesocosm experiments, with parallel analyses of comparable parameters by traditional techniques from discrete water samples.
5. Applying a lab-scale AquaBox in chemostat experiments (WCL Lunz) for semi-automated generation of high frequency data in chemostat experiments.
6. Testing of off-grid power alternatives for field experiments.
7. Field test in a selected joint AQUACOSM experiment.
8. D8.3 delivered in Month 45 (“Final report AquaBox”).