Title: An Autonomous Flow through Salinity and Temperature Perturbation Mesocosm System for Multi-stressor Experiments

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Abstract

The rapid environmental changes in aquatic systems as a result of anthropogenic forcings are creating a multitude of challenging conditions for organisms and communities. The need to better understand the interaction of environmental stressors now, and in the future, is fundamental to determining the response of ecosystems to these perturbations. This work describes an automated *ex-situ* mesocosm perturbation system that can manipulate several variables of aquatic media in a controlled setting. This perturbation system was deployed in Kongsfjorden (Svalbard) where ambient water from the fjord was heated and mixed with freshwater in a multifactorial design to investigate the response of mixed kelp communities in mesocosms to projected future Arctic conditions. The system employed an automated dynamic offset scenario where a nominal temperature increase was programmed as a set value above real-time ambient conditions in order to simulate future warming. The freshening component was applied in a similar manner where the decrease in salinity was coupled to track the temperature offset based on a temperature-salinity relationship in the fjord. The system functioned as an automated mixing manifold that adjusted flow rates of warmed and chilled ambient seawater, with unmanipulated ambient seawater and freshwater delivered as a single source of mixed media to individual mesocosms. These conditions were maintained via continuously measured temperature and salinity in all 12 mesocosms (1 ambient-control and 3 treatments, all in triplicates) for 54 days. System regulation was robust as median deviations from setpoint conditions were < 0.15 for both temperature (°C) and salinity across the 3 replicates per treatment. Regulation further improved during a second deployment that mimicked three marine heatwave scenarios where a dynamic temperature regulation held median deviations to < 0.036°C from the setpoint for all treatment conditions and replicates. This perturbation system

has the potential to be implemented across a wide range of conditions to test single or multi-stressor drivers (e.g., increased temperature, freshening, high CO_2) while maintaining natural variability. The automated and independent control for each experimental unit (if desired) provides a large breadth of versatility with respect to experimental design.

1 Introduction

The persistent burning of fossil fuels since the industrial revolution has radically increased atmospheric CO_2. This has led to an enhanced greenhouse effect resulting in a multitude of changing climatic elements such as increasing sea surface temperature (Bindoff et al., 2019). In fjord systems, the confluence of increased fluvial inputs, glacier and permafrost meltwater, stratification and water mass intrusion, as well as increased sea surface temperatures can create periods of extreme physicochemical conditions for nearshore benthic and pelagic marine communities (Bhatia et al., 2013; Poloczanska et al., 2016; Divya and Krishnan, 2017; Bindoff et al., 2019). As ocean changes progress, the need to better understand the effects of combined stressors (e.g., increased temperature and freshening) on marine communities is essential to understand how community function and species richness will be affected as assemblages adjust to new environmental conditions (Kroeker et al., 2017; Wake, 2019; Orr et al., 2020). Methodological approaches to assessing and characterizing the response of organisms and community assemblages to future ocean changes is often pursued by conducting *ex-situ* experiments, utilizing natural analogues (e.g., CO_2 vents), or performing space-for-time substitution (when spatial phenomena are used to model temporal changes); however, this can limit the ability to test the range and dynamics of present and future environmental conditions (Blois et al., 2013; Rastrick et al., 2018; Bass et al., 2021). The use of *ex-situ* experimental systems that manipulate multiple environmental conditions such as temperature and salinity can, thus, be a valuable tool to assess the response to multi-stressors in a future ocean.

The recognition of conducting multi-stressor experiments has become increasingly relevant due to the interaction of environmental drivers in dynamic systems under a changing climate (Kroeker et al., 2020). Nearshore regions such as fjord systems and estuaries can experience an amplified modulation of temperature and salinity on short timescales (Evans et al., 2015; Hales et al., 2016; Fairchild and Hales, 2021). Such instances have been observed in sub-Arctic estuaries where water temperature at a depth of 10 m decreased by 1.5°C in < 10 h, and in temperate systems where the magnitude of salinity change driven by high precipitation displayed a decrease of 4 units in < 24 h (Miller and Kelley, 2021; Poppeschi et al., 2021). Changes of this magnitude are particularly pertinent for Arctic fjords where recent evidence found that changes in salinity from glacial meltwater were capable of directing whether a system is net heterotrophic or autotrophic (Sejr et al., 2022). In this system, the salinity dynamics were observed as a temporal decrease from 31 to 28 over a few days, or as a spatial modification where values changed by 5 units over a 5 – 10 km distance.

Recent advances in the ability to modulate several environmental parameters at once using *ex-situ* mesocosms has been presented via the use of a modular programmable system (Wahl et al., 2015; Pansch and Hiebenthal, 2019). Such systems have demonstrated an ability to apply programmable environmental scenarios as a multifactorial design, or as a delta-change (offset) from ambient conditions that mimic the natural variability of an environment. The advantages of these types of automated systems lie in their ability to overcome the need for capturing and measuring abundant discrete measurements used to regulate experimental conditions, and transcend the logistical difficulties of implementing natural variability to experimental designs. In addition, these systems can reduce the need for constant human observation which may be required to program new regulatory operations or make rapid adjustments to experimentally manipulated conditions.

Here, we describe an autonomous salinity and temperature experimental perturbation mesocosm system (SalTExPreS) that has the ability to modify, and then regulate, salinity and temperature in real-time. The SalTExPreS can perform similar functions as the *ex-situ* mesocosm systems discussed previously (i.e., Kiel-outdoor and -indoor benthocosms), such as applying programmable static or dynamic changes to temperature and salinity, or by replicating natural variability as on offset in real-time, but has the added capability of autonomous control for each experimental unit (e.g., chamber or mesocosm). In this initial deployment of the SalTExPreS, we applied a delta offset (i.e., offset from a measured control) to temperature and salinity as a fractional-factorial treatment design for a two-month long experiment in KongsFjorden, Svalbard, that exposed mixed kelp communities to future temperature, salinity, and irradiance conditions in the fjord. This study demonstrates the stability and flexibility of the SalTExPreS as an experimental tool to be utilized under extreme and dynamic conditions to test the effects of physicochemical multi-stressors on marine organisms and communities in the context of a multi-month experiment.

2 Methods

2.1 Operational Concept of the Experimental System:

The SalTExPreS simulates the drivers in a marine or freshwater system such as temperature, freshening, acidification, or hypoxia as either static or as temporally-variable modifications to ambient seawater. This is accomplished by mixing manipulated source water, whether it is freshwater or warmed water, with ambient water through automatic flow valves that control the volume and rate of water delivered. This is regulated by the constant monitoring of the mixed water conditions in each mesocosm or chamber via a programmable feedback loop that transmits the opening or closing of the automatic flow valves. The automated ability of the SalTExPreS is configured to respond to near instantaneous measurements (several reads per second) to achieve high frequency regulation of the manipulated drivers based on a measured *in-situ* or control reference. The programmable setpoint conditions in each mesocosm are easily controllable through an intuitive computer interface application.

2.2 Site Description and Experimental Design

Kongsfjorden is a fjord system on the west coast of Svalbard (Norway) where the West Spitsbergen Current exchanges warm Atlantic water through sill channels based on differences in density gradients at the fjord mouth. Over the past two decades, a persistent influx of Atlantic water has resulted in the reduction of land-fast ice and the melting of sea terminating glaciers causing enhanced freshwater and fluvial input (Luckman et al., 2015; Tverberg et al., 2019). The influx of freshwater is highest in summer and is accompanied by an important sediment loading with the potential to shoal the euphotic zone from 30 to 0.3 m depth (Svendsen et al., 2002). These advancing climatic changes in Kongsfjorden set a relevant context for the inaugural deployment of the SalTExPreS on a concrete platform situated ~ 12 m from the shoreline in Ny-Ålesund, which is located on southwestern shore of Kongsfjorden ~ 11 km from the fjord mouth.

The SalTExPreS was utilized to implement three treatment scenarios in a fractional-factorial design to represent expected future conditions in Kongsfjorden for an experiment that examined the productivity, survival, and growth response of mixed kelp communities found at 7 m for 54 d. The response of these kelp community assemblages was determined in part by conducting weekly closed system incubations and assessing the growth and metabolism of the kelp in each mesocosm—the details and results of this experiment are discussed elsewhere (Miller et al., 2023). The treatments were realized by multi-driver combinations of temperature, freshening, and irradiance, where treatments 1 and 2 differed in the magnitude of temperature increase, salinity decrease, and irradiance decrease (Table 1). Only temperature was manipulated for treatment 3. The chosen treatment and salinity perturbations were applied as offset values from measured *in-situ* fjord conditions at 11 m which captured the natural variability of the fjord system. The applied temperature offsets used for this experiment reflected the projected SSP2-4.5 and SSP5-8.5 scenarios (Meredith et al., 2019; Overland et al., 2019; Table 1). The chosen decreases in salinity were based on correlations between *in-situ* temperature and salinity during summer 2020 in Kongsfjorden (Gattuso et al., 2023), weeks 22 to 35 (Appendix A1 and Fig. A1). These calculated delta salinity values were applied as offsets in treatments 1 and 2 (Table 1). The third treatment scenario applied a temperature change of + 5.3°C as a way to decouple the multi-stressor system and evaluate a temperature only stress. The effect of turbidity for treatments 1 and 2 were simulated as a decrease in surface irradiance (i.e., ~ 25% and ~ 40% reduction from ambient irradiance at 7 m) by applying a combination of neutral light and spectral filters (LeeⒸ Filters) placed as static fixtures over the top of the mesocosms.

2.3 Experimental System

Water was pumped from Kongsfjorden at a 10 m depth (300 m offshore) using a submersible pump (NPSⒸ Albatros F13T) that was tapped into an underwater intake pipe that fed a header tank in the Kings Bay Marine Laboratory in Ny-Ålesund, Svalbard. Pumped ambient seawater from the header tank was then split into three sub-header tanks within the marine lab where ambient water was (1) left unchanged, (2) chilled to 0 °C, or (3) warmed to 15 °C. Each sub-header tank was plumbed to supply a maximum flow of 6 m^3 h^{-1} for the ambient, 1 m^3 h^{-1} for chilled, and 2 m^3 h^{-1} of warmed water which required a pressure of 0.3 bars for each line to ensure consistent flow rates (Fig 1). The three control mesocosms received a mix of chilled and ambient seawater in order to properly simulate *in-situ* temperatures. The three experimental treatments (nine mesocosms in total) received a mix of ambient, warmed, and freshwater for treatments 1 and 2, whereas treatment 3 received a mix of just ambient and warmed water (Fig 1). Freshwater was sourced from the tap which was fed by the Tvillingvann reservoir close to Ny-Ålesund. The total flow-through rate of each mesocosm was 0.5 m^3 h^{-1} (i.e., each mesocosm turned over every 2 h) of post-mixed media delivered in an open cycle flow-through system, where flow rates of 7 – 8 L min^{-1} were maintained as target setpoints. Continuous flow was maintained throughout the experiment with the exception of weekly 3 h incubations (to perform experiments on the community) where the flow to each mesocosm was shut off. In total, there were twelve circular mesocosms (3 treatments and 1 control, 3x replicates) with a mean diameter of 1.1 m and a volume of 1 m^3, each equipped with a 12 W wave pump (SunsunⒸ JVP-132), a temperature-conductivity probe (Aqualabo, PC4E), an optical oxygen sensor (Aqualabo, PODOC), and an OdysseyⒸ light logger. Fiberglass insulation was secured around the outside of each mesocosm in order to reduce any unintended changes in temperature.

Delivery of ambient, chilled, warmed, and freshwater first ran through an automated mixing manifold that regulated the flow of each media type assuring that proper volumetric proportions passed through the regulator valves to achieve target conditions (Fig. 1). Each source-water flow-line was regulated by an automated 3-way mixing valve (2-way mixing valve for incoming freshwater) which then passed through another mixing valve (12 in total) that was assigned to each mesocosm (Fig. 1) This style of regulation ensured that the proper proportions of manipulated media and ambient water were mixed to achieve setpoint conditions. Any temperature variation induced by mixing freshwater was immediately compensated for by regulating the flow of the warm water line. Details regarding the programmed regulation are discussed further in the appendix (Section A2). The mixed media then passed through a flow meter to measure the flow rate to each mesocosm which was regulated by a manual hand-crank valve used to make minor adjustments to the valve opening position. This regulates the flow to each mesocosm. Measurements by all the pressure sensors, the status of open position for the regulator valves, and flow rates were logged every minute and projected to the user interface via a computer application (Fig. A3).

2.4 Setpoint Regulation

Setpoint temperature conditions of + 3.3, 5.3, and 5.3°C applied to treatments 1, 2, and 3, respectively, were offsets from the control temperature setpoint. The temperature setpoint of the control condition was updated hourly and programmed to replicate the measured i*n-situ* conditions in the fjord logged by the AWIPEV (Alfred Wegener Institute and Institute Paul Emile Victor) FerryBox part of the COSYNA underwater observatory (https://dashboard.awi.de/) situated at a depth of 11 m. Each treatment condition (temperature and salinity offset) was set by manually programming the setpoint value of temperature in the software interface (see appendix A3). The salinity offset was coupled to the temperature setpoint via the correlation described in appendix A1. The measured temperature and salinity observations from inside each mesocosm were recorded multiple times per minute and used to continuously monitor the regulation of the conditions inside each mesocosm. This data transmission was used to program the software controller that performed the automated regulation of mixed media (for details see appendix A2).

2.5 Software

The software application used for the control of the SalTExPreS was developed using Visual Studio Community (2019 edition) with the vMicro extension and Arduino 1.8.13. The program application has a user-friendly interface designed to allow real-time monitoring and parameterization of regulation processes (Fig. A3). The main window displays each mesocosm condition (sensor measured parameters), their piping connections, a connection status for each PLC informing proper communication, date and time of the last received communication packet from the Head PLC, and the status of the experiment (e.g., started or stopped). The interface also displays the valve-open percentage along with the pressure setpoints and the actual measured value for each main source-water inlet. In addition, the *in-situ* data (temperature and salinity) received from the FerryBox is displayed with the time and date of the last logged value utilized to program the real-time setpoint of the control condition. Sensor readings of flow rate (L min^{-1}), O_2 concentration (% saturation), salinity, and temperature (°C) are shown for each mesocosm in conjunction with the treatment setpoints (i.e., temperature, and salinity when relevant). All measured data is stored through the server connection to the cloud, however, there is a backup microSD card on the Head PLC that logs data from all mesocosms every 5 sec. If communication fails between the Head PLC and the interfaced computer, data will not be retrieved by the PC during the communication break, but will be retained by the microSD card.

3 Results

3.1 Regulation of the control condition

The control condition was able to simulate the ambient fjord temperature well over the experimental period deviating < 0.3°C on average across the 3 replicates (Table 2, Fig. 2). The overall quality of the regulation was based on the ability of the system to read the measured data from the FerryBox, or to follow a manually programmed setpoint when communication with the FerryBox was interrupted. During the experiment, the FerryBox went intermittently offline 24% of the time, ceasing transmission of real-time data that resulted in a break of communication to the PLCs. This somewhat frequent break in communication resulted in an average setpoint deviation that was nearly double for the control compared to the treatment conditions (Table 2). The ability to manually program a new setpoint when communication breaks occurred ensured that the control condition was still robustly regulated. Over the entire period of the SalTExPreS deployment, the mean temperature of the control condition increased from ~ 4 to 6.5°C from early July to the end of August (Fig. 3a). The coldest mean temperature of the control condition occurred when a backup pump situated at 90 m was used from 2021-07-14 ~21:00 UTC until 2021-07-26 13:49 UTC while the original pump at 10 m was repaired due to a motor malfunction. During this period, the control condition was ~ 1.0 – 1.5°C cooler than the temperature measured by the FerryBox (Figs. 2, 3). Since a warmed seawater inlet was not supplied to the control condition, the temperature of the control condition remained cooler than the measured ambient conditions at the FerryBox. Despite the cooler temperature for the control, regulation of flow rates, mesocosm turnover time, and variability across the control condition replicates was well maintained by the system.

2.6 Temperature and salinity regulation

The regulation of temperature and salinity in the different treatment conditions (Trts. 1 – 3) was maintained for 54 days (2021-07-03 – 2021-08-26) by the SalTExPreS: the entirety of the planned experiment. For the first 6 days of the SalTExPreS deployment, the treatment conditions were held at control conditions (i.e., no applied offset from the control) before the stepwise increase in temperature began. On 2021-07-10 12:00 UTC a temperature offset of 0.55°C d^{-1} was programmed for treatment 1 while treatment 2 and 3 were programmed to increase by 0.88°C d^{-1} (Figs. 2, 3). The final temperature setpoint above the control condition was reached on 2021-07-15 21:00 UTC. The system needed 4 h to achieve the new temperature conditions (i.e., homogenize the mesocosm to a 0.88°C increase) after the final incremental increase was programmed. To avoid potential discrepancies with the regulation of salinity for treatments 1 and 2, a manual override was applied resulting in the system to realize the final salinity offset value upon the initial temperature increase (Fig. 3b, 4). It took the system 4 h to achieve the salinity offset for treatment 2 adjusting the value from ~34 to 29.8 (Fig. 3b, 4).

The precision of the temperature and salinity regulation across all treatment conditions was well maintained as the mean difference between the measured value and the setpoint was < 0.2°C and < 0.36 for salinity across the entire deployment (Table 2). The mean deviations observed across treatments did not appear to correlate to the degree of offset. Thus, treatment 3 showed the highest precision for temperature regulation, while salinity regulation was the most robust for treatment 2 compared to treatment 1 (Table 2). During the several instances where communication broke with the FerryBox and the Head PLC, the SalTExPreS retained the last measured value at the FerryBox as a contingency protocol. This aided in the ability of the system to maintain a high degree of regulation throughout the entire deployment. The largest deviation from the setpoint value for all treatment conditions occurred during the single instance in which the last read value from the FerryBox was not retained: this occurred on 2021-08-24 04:47 UTC (Fig. 4). Communication was quickly restored after this incident by cycling the program code, and the average deviation of temperature and salinity for treatment 1 for the remainder of the deployment was < 0.16 and < 0.25 for treatment 2.

When adequate flow rates were maintained, the SalTExPreS was able to simultaneously regulate 12 mesocosms at 4 different conditions to deviations in temperature and salinity that were < 0.5°C or 0.5 in salinity from the setpoint value ≥ 80% and ≥ 70% of the time, respectively (Fig. 5). Due to an erroneous setpoint for the control condition during the 90 m pump usage, these times were excluded. When considering the % of time deviations from a temperature setpoint were held to < 1°C, all mesocosms were regulated accordingly 89% of the time and 80% for the salinity treatments (i.e., < 1 unit deviation), with the exception of the 1st replicate for treatment 2.

Discussion

The inaugural application of the fully autonomous SalTExPreS demonstrated the capacity of the system to successfully manipulate temperature and salinity as an offset value from control conditions, thus maintaining, natural, *in-situ* variability for 4 different conditions simultaneously. We utilized this deployment to test the effects of climate change drivers on Arctic kelp communities recognizing the feasibility of the system to perform *ex-situ* experiments on organisms or whole communities (Miller et al., 2023). The versatility of the system not only permits the manipulation of temperature and salinity, but could incorporate other factors such as CO_2 or hypoxia (Gazeau et al., *in prep*). While this experiment used a control offset approach to produce treatment conditions, programmable parametrization of various treatment combinations can be applied depending on the question and design of the experiment. The automated component of the system reduced the logistical hurdles that can arise when performing high precision replication and regulation of experimental conditions that track real-time system variability. While the use of such a system can reduce user oversight and limitations, there is still a need for diligent operation.

Since this initial deployment, we have implemented several changes which have improved the performance of the system that have been realized during a second deployment in the summer of 2022 (Fig. 6). In this experiment, the SalTExPreS was integrated to function with a deployable heat pump to simulate multiple scenarios of heatwave patterns over a nearly month-long experiment. In this instance, temperature regulation was vastly improved as a result of the programmable modifications made since the initial deployment. During this deployment the SalTExPreS mimicked 3 marine heatwave scenarios where a dynamic temperature regulation held deviations to < 0.5°C for 94% of the time in 9 different mesocosms. This was an improvement to the % time of temperature regulation by ~ 15% compared to the first deployment. In the first deployment, inconsistent flow rates and communication errors between the FerryBox and the Head PLC were the primary causes of large deviations (> 2.0 salinity or °C) from setpoint values. For example, flow rates < 2 L min^{-1} accounted for ~ 20% of the large deviations in temperature and salinity regulation. Simple modifications such as 'pop-up' alert windows that warned when a lapse in communication with the FerryBox occurred (FerryBox stopped logging), and the addition of contingency coding instructions (fail-safe instructions) ensuring that the last received *in-situ* data were maintained are the types of modifications that resolved most of the issues. Communication errors were easily remedied by cycling the power on a PLC which is why pop-up alerts were an improvement to the operation. Other extraneous circumstances that could impact flow rates such as pump failure and clogging from the use of raw seawater are conditions that would need to be assessed whenever the SalTExPreS is used. However, these are very manageable situations which can be easily mitigated by an operator.

The novelty of the SalTExPreS lies in its ability to independently regulate an experimental condition in a single experimental chamber (e.g., mesocosm). The operational data produced from this deployment are reliable, easily quantifiable, and provide the highest degree of monitoring frequency for every applied experimental condition. This study has demonstrated its ability to replicate dynamic nearshore systems where temperature and salinity can vary at high frequency (e.g., tidally), but further enhances these conditions to mimic a future scenario by applying an amplitude offset to the natural dynamics of *in-situ* conditions. Wahl et al. (2015) described a system with a similar capability, but regulated treatment conditions by monitoring source water and adjusting that media before it was delivered to each experimental chamber. The SalTExPreS differs here as it measures the conditions inside each experimental chamber (i.e., mesocosm) and regulates based on per second measurements made inside each chamber. This provides the flexibility to individually modulate each experimental chamber providing a broad range of versatility. The lack of infrastructure needed to set up the SalTExPreS makes it easy to deploy and transport. As long as there is a sufficient supply of ambient water and manipulated media, there is little limit to the versatility of automated control for each mesocosm. Many research endeavors and future implementations by the SalTExPreS have the potential to conduct a large range of experimental settings that pertain to environmental perturbations associated with climate change or other anthropogenic forcings. The operation of such a system in extreme environmental conditions has shown the durability of the manifold to endure an adverse Arctic summer and still respond without mechanical failures. With proper operation and user proficiency, this proves to be a highly sophisticated and powerful tool to be utilized for marine and aquatic perturbation experiments.

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Author contributions

C.M. and F.G. conceptualized the frame of the paper while F.G, S.C, and P.U. designed the experimental system. P.U. programmed the software. C.M. wrote the manuscript, performed the data analysis, and constructed the figures and tables while P.U. designed the diagram and schematic figures. All authors participated in the operation of the system and have, thus, commented, and edited during writing.

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Competing interest

The authors declare no competing interests exist.

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Tables

Table 1. Experimental treatment conditions with corresponding positive temperature offsets and the calculated negative salinity offsets. See section A1 and figure A1 for a full description of the temperature-salinity relationship used to calculate salinity offsets. Photosynthetically active radiation (PAR) offsets are the approximate mean value across replicates. Cntrl. is the control and Trt. is treatment.

Treatment	*Temperature*	*Salinity*	*PAR*
Cntrl.	Ambient	Ambient	Ambient
Trt. 1	+ 3.3 °C	Δ 2.5 – 3.0 Δ S = 0.546*T + 0.490	~ 25% decrease from ambient
Trt. 2	+ 5.3 °C	Δ 5.0 – 5.5 Δ S = 0.877*T + 0.089	~ 40% decrease from ambient
Trt. 3	+ 5.3 °C	Ambient	Ambient

Table 2. Absolute mean difference between measured temperature and salinity values against setpoints plus or minus the standard deviation. A weighted average was used for treatments 1 – 3 to account for the 5-day incremental increase. Cntrl. is the control and Trt. is treatment 1 – 3 with replicates a – c.

Treatment	*Mean diff* $Abs(T_{meas.} - T_{set})$	*Mean diff* $Abs(S_{meas.} - S_{set})$	*Manipulated water*			
			Cold	*Ambient*	*Warm*	*Fresh*
Cntrl. a	0.275 ± 0.39	–	x	x		
Cntrl. b	0.291 ± 0.36	–	x	x		
Cntrl. c	0.223 ± 0.36	–	x	x		
Trt. 1a	0.126 ± 0.31	0.116 ± 0.31		x	x	x
Trt. 1b	0.142 ± 0.29	0.148 ± 0.22		x	x	x
Trt. 1c	0.145 ± 0.33	0.171 ± 0.33		x	x	x
Trt. 2a	0.111 ± 0.29	0.357 ± 0.74		x	x	x
Trt. 2b	0.133 ± 0.29	0.149 ± 0.26		x	x	x
Trt. 2c	0.196 ± 0.38	0.128 ± 0.25		x	x	x
Trt. 3a	0.109 ± 0.27	–		x	x	
Trt. 3b	0.112 ± 0.27	–		x	x	
Trt. 3c	0.106 ± 0.28	–		x	x	

Figures

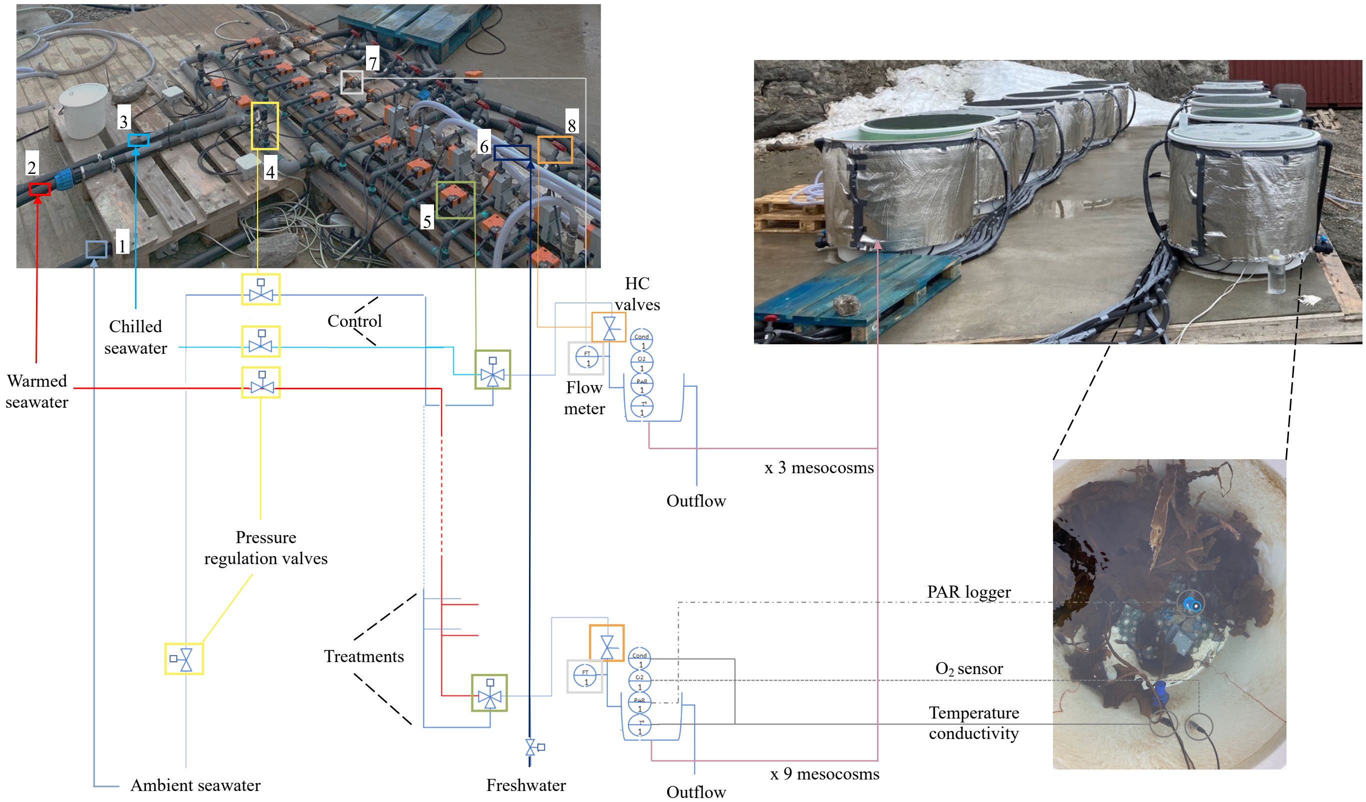


Figure 1. Piping schematic of the SalTExPreS which includes the mixing and regulation manifold. Items 1 – 3 depict the main seawater inlets from the ambient, warmed, and chilled sub-header tanks located in the Kings Bay Marine Laboratory. Seawater from each sub-header tank moves through a 2-way regulator valve followed by a pressure sensor (4) before splitting into individual lines that lead to all 12 regulator valves (5), each assigned to a single mesocosm. For treatments 2 and 3, the freshwater inlet (clear tube; item 6) passes through a 2-way regulator valve before mixing with the ambient and warmed seawater lines. Flow rates are then measured (7) post-mixing, and final flow rates are set using a hand-crank red valve (8). All 12 mesocosms are displayed (upper right photo) with the inside of one mesocosm showing the oxygen and

conductivity sensors along with the Photosynthetically active radiation (PAR) logger (bottom right photo). Table A2 provides the parts list for the items shown in this figure.

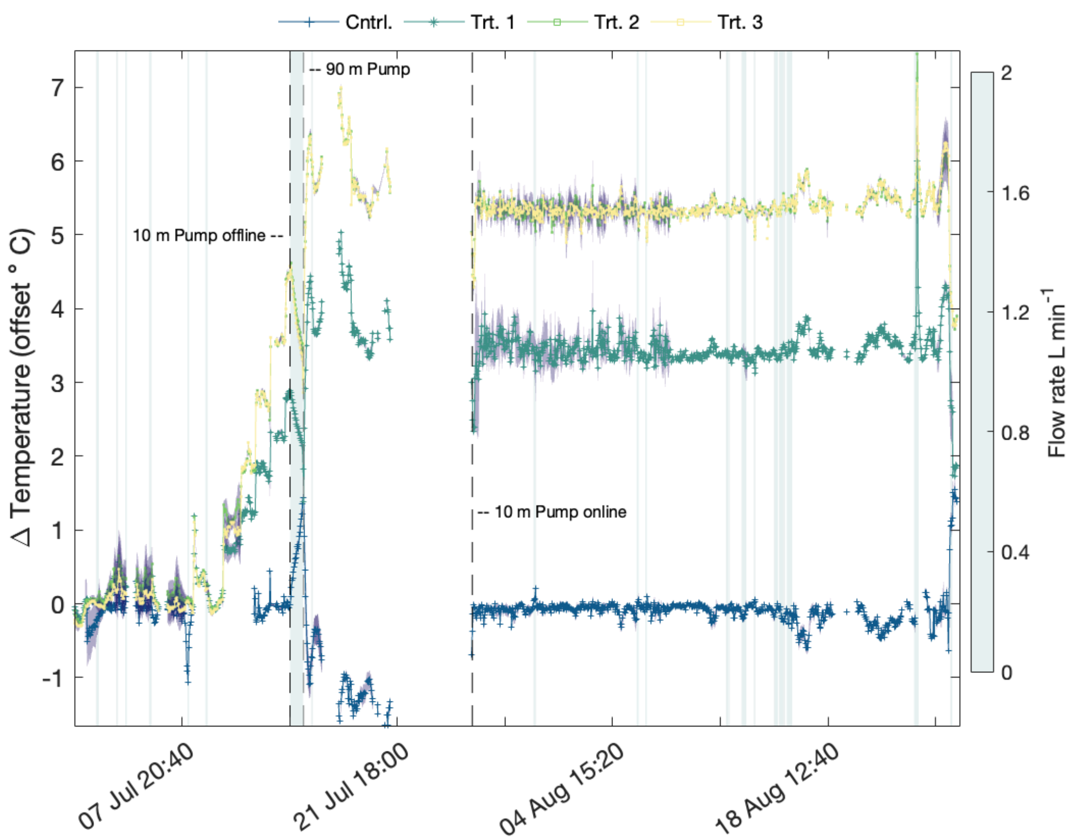


Figure 2. Regulation of the mean temperature offset for all conditions, including the control offset from the FerryBox, and the three treatment offset values from the control condition. The purple shaded region around the mean is the standard deviation and the heatmap isoclines (blue-grey shaded regions) are instances when flow rates were ≤ 2 L min^{-1} (threshold to avoid large deviations > 2.0 salinity or °C). Dashed black lines indicate periods when the pump at 10 m depth and 90 m depth were used to feed the sub-header tanks. The time period presented is the duration of the experimental deployment.

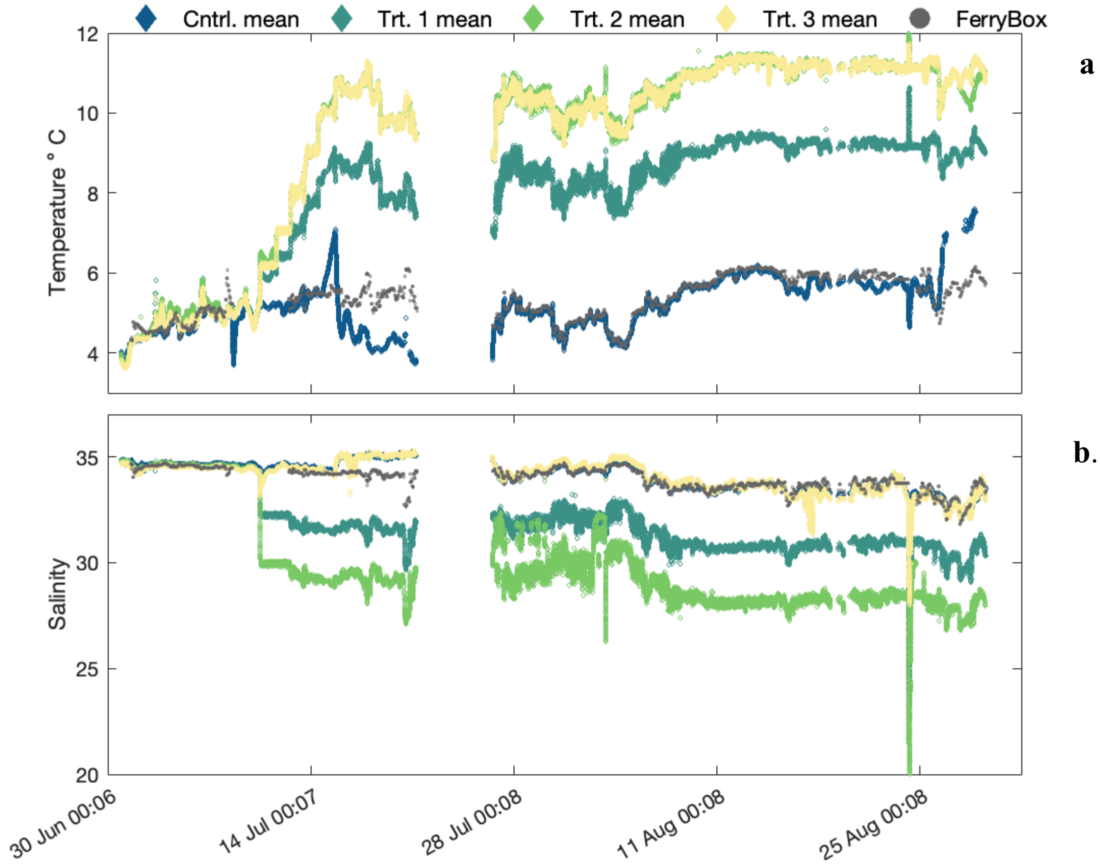


Figure 3. Mean temperature (**a**) and salinity (**b**) for all conditions including those from the FerryBox (dark grey circles) for the entire deployment.

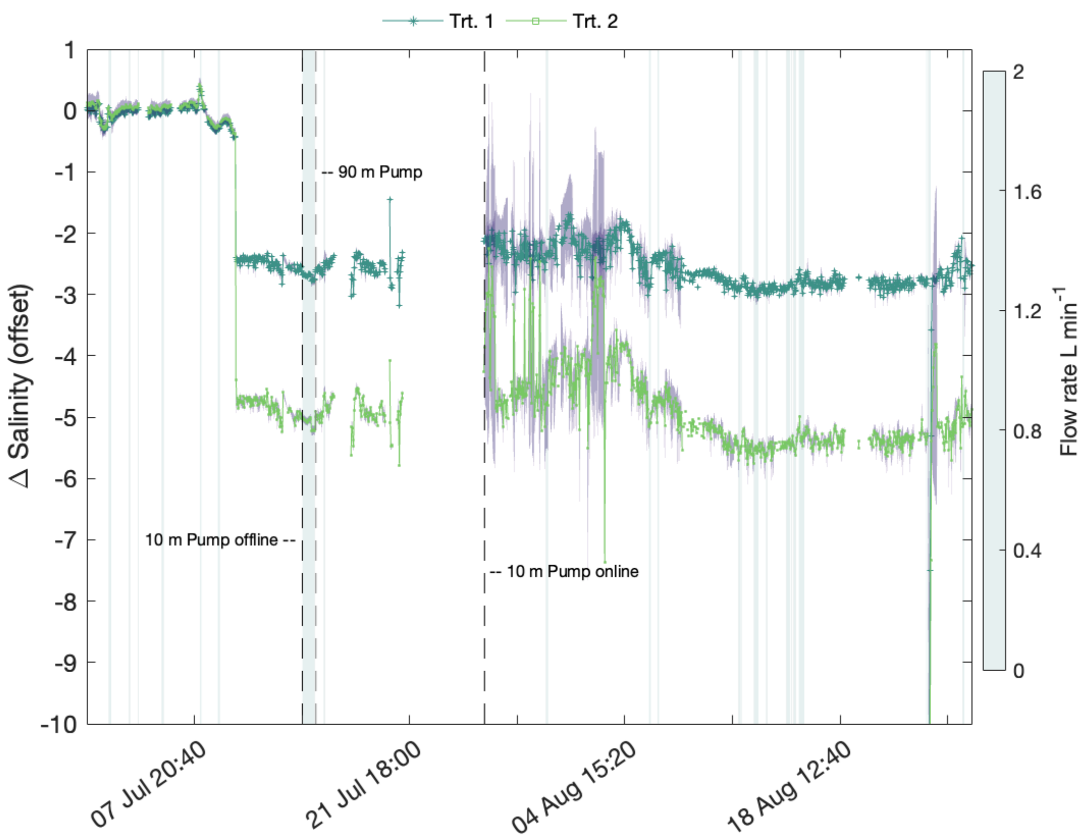


Figure 4. Regulation of the mean salinity offset (Δ salinity) during the experimental period for treatments 1 and 2. The purple shaded region around the mean is the standard deviation and the heatmap isoclines (blue shaded regions) are the instances when flow rates ≤ 2 L min^{-1}. Dashed black lines indicate periods when the pump at 10 m depth and 90 m depth were used to feed the sub-header tanks.

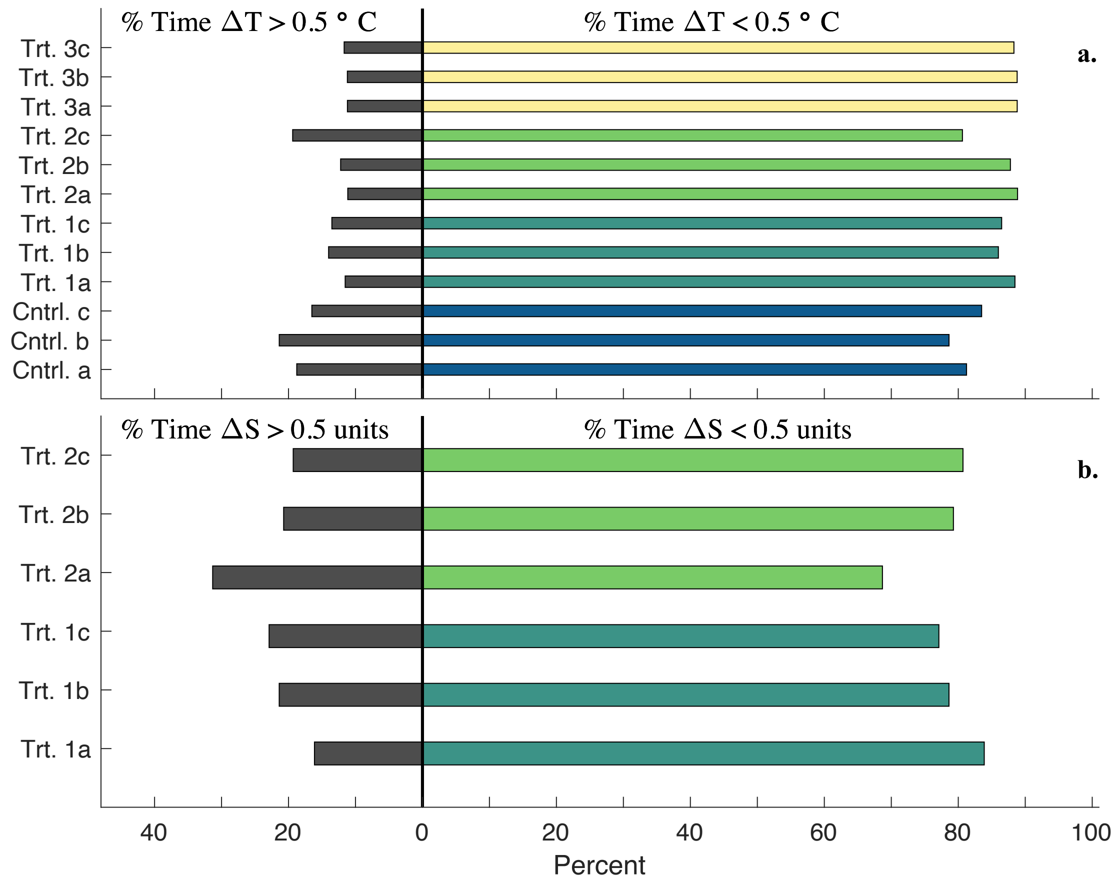


Figure 5. Percent time each mesocosm experienced a deviation > (black bars) or < (colored) 0.5°C (ΔT; **a**) or 0.5 in salinity (ΔS; **b**) when flow rates were above 2 L min^{-1}. This excludes the period when using the 90 m pump (12 d) but accounts for 42 days out of the 54 day experiment. Bar color indicates different treatment groups, as shown on the y-axes.

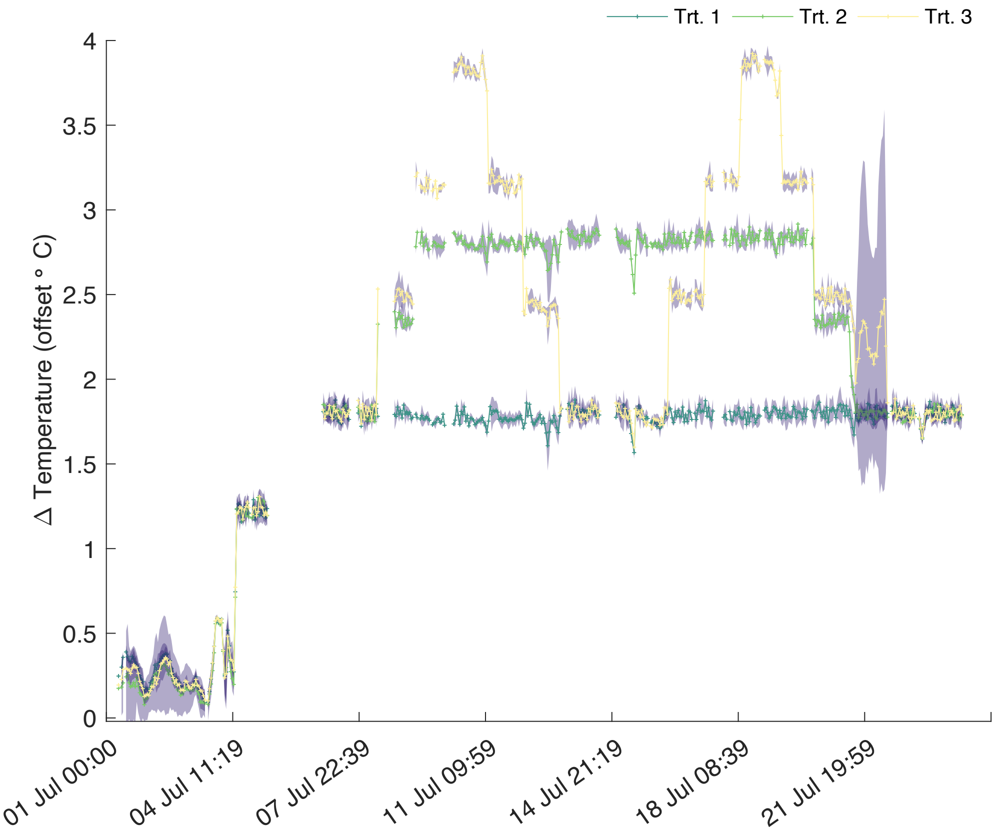
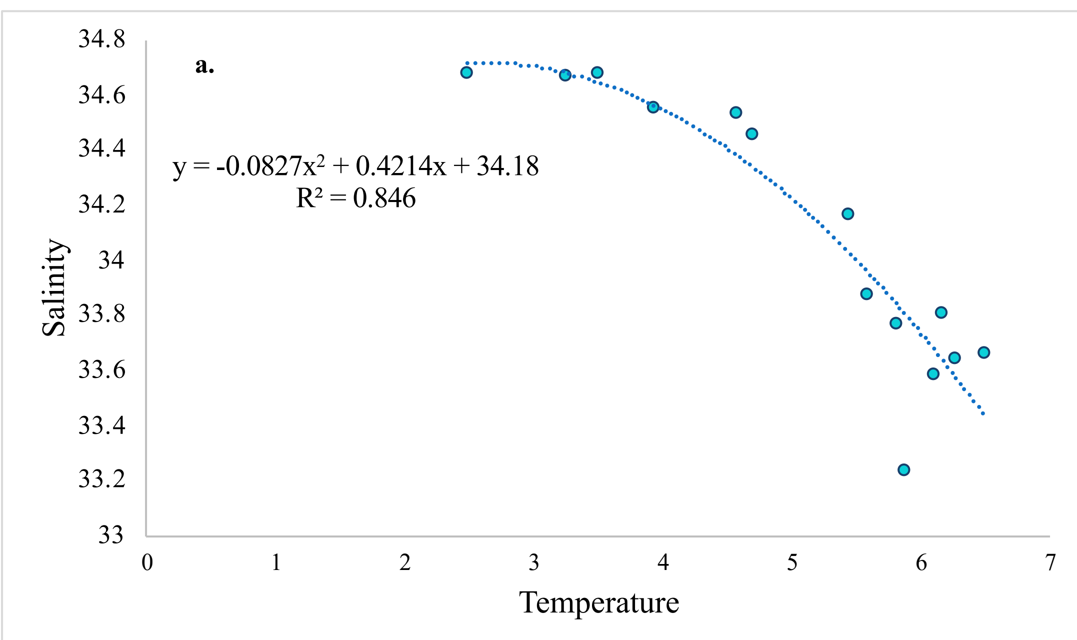


Figure 6. Regulation of the mean temperature offset (Δ Temperature) during the 2nd deployment of SalTExPreS in the summer of 2022 in Tromsø (Norway) performing a variation of heatwave scenarios with three experimental treatments 1 – 3. The purple shaded region around the mean is the standard deviation.

Appendix

A1. Calculation of Salinity Offset

In the summer of 2020—weeks 22 to 35— the mean temperature at 11 m displayed a range from 2.48 – 6.28, with salinity values ranging from 34.67 measured at the minimum 2.48°C and 33.63 measured at 6.28°C (Fig. A1a). The correlation was best fit with a 2nd order polynomial. To project the salinity offset at a future temperature based on this 2nd order polynomial fit, temperatures of + 3.3 and 5.3°C (SSP2-4.5 and SSP5-8.5, respectively) were added to *in-situ* fjord temperatures and salinity was calculated based on the 2nd order polynomial. These estimated salinity values were then subtracted from the mean salinity values observed (y-axis, Fig. A1a) in summer 2020 in order to calculate a delta salinity value for the SSP2-4.5 and SSP5-8.5 scenarios. The relationship between these estimated delta salinity values and the mean *in-situ* temperature (y-axis, Fig. A1a) displayed a robust linear relationship (Fig A1b).



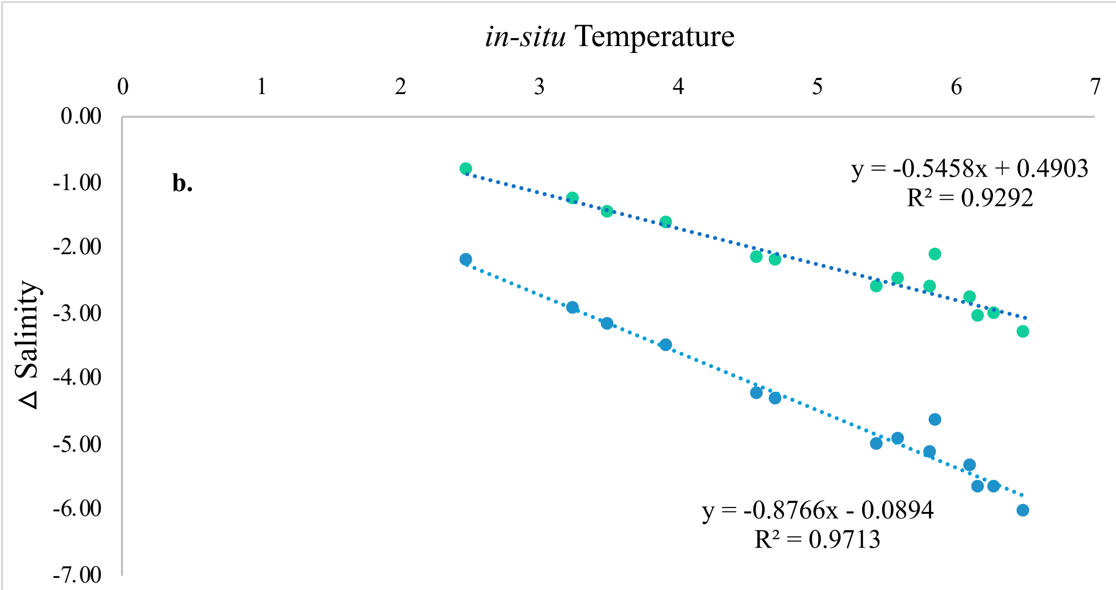


Figure A1. Relationship between temperature and salinity in summer 2020 weeks 22 – 35 in Ny-Ålesund, Svalbard (**a**). Relationship between estimated delta salinity and *in-situ* temperature, where delta salinity was calculated as the difference between the current mean salinity and the salinity estimated at the temperature increase projected for SSP2-4.5 (blue dots) and SSP5-8.5 (green dots) scenarios (**b**).

A2. Temperature and Salinity Regulation

Accurate temperature and salinity regulation was managed using the software PID (proportional integral derivative) controller on the corresponding Programmable Logic Controller (PLC). The PLC operated in PoE mode (power over ethernet) which builds a local area network (LAN) enabling use of Ethernet data cables to carry electrical power. The PID controller measures the difference between the measured value and the nominal value (i.e., the error). This calculates the position and adjustment of the valve opening by multiplying the error, the integral of this error, and the derivative of the error over time, by previously determined coefficients K_p (proportional gain), K_i (integral gain) and K_d (derivative gain), respectively. These coefficients were obtained experimentally using the empirical method of Ziegler & Nichols (1943). These coefficient values may differ from one condition to another.

A2.1. Pressure and Flow Regulation

Each sub-header tank inlet line of ambient, chilled and warmed seawater had its own pressure regulation system enabling equivalent pressure levels to be maintained. This regulation process aided in the ability to adjust flow rates for all mesocosms by using the hand-crank valves (Fig. 1). The system consisted of an analog pressure sensor (SiemensⒸ 7MF1567-3BE00-1AA1) and a two-way analog valve (BELIMOⒸ R2025-10-S2 with LR24A-SR motor). The pressure sensors were placed in-line directly after water from each sub-header tank passed through a regulator valve. The sensor ensured that pressure for each line was maintained at 0.3 bars by transmitting data to the system which then regulated the valve opening position of the incoming flow. A pressure setpoint for all three sensors was predetermined during flow rate test trials. This process took place during the setup of the system where the valve opening was adjusted using a PID regulator (see A2) to maintain the defined pressure setpoint.

A2.2 Automation

The automation was performed using 4 Industrial Arduino-based PLCs (Industrial shieldsⒸ Mduino-42+), with an individual PLC regulating the control condition and each treatment 1 – 3, respectively. Each PLC was responsible for logging data and regulating a specific experimental condition. The PLC regulating the control condition—identified as the Head PLC—was the primary device responsible for communication with the branched PLCs and the monitoring computer (Fig. A2). All monitoring was performed on a PC Windows application (Section A3) and responsible for: (1) reading data received from the PLCs, (2) reading *in-situ* data received from the internet, (3) displaying live data, (4) logging data and sending it to an FTP server, and (5) sending settings and commands to the PLCs. Communication between the PLCs and the PC was ensured using http WebSocket protocol on RJ45 ethernet cables. The communication between the PLCs and the conductivity-temperature and oxygen sensors, flow rate sensors, and regulation valves was executed using a half duplex RS485 (2 wires) protocol, with an analog 4-20mA and an analog 0-10V signal, respectively. All PLCs and wired communication lines were housed in an electrical box installed to an IP68 Fibox enclosure with a 400 V (3P+N+E) 32 A security switch (Fig. A6). All the automation elements use low tension (12 Vdc or 24 Vdc) through circuit breakers and fuses. The electrical box was protected with a 220 V socket.

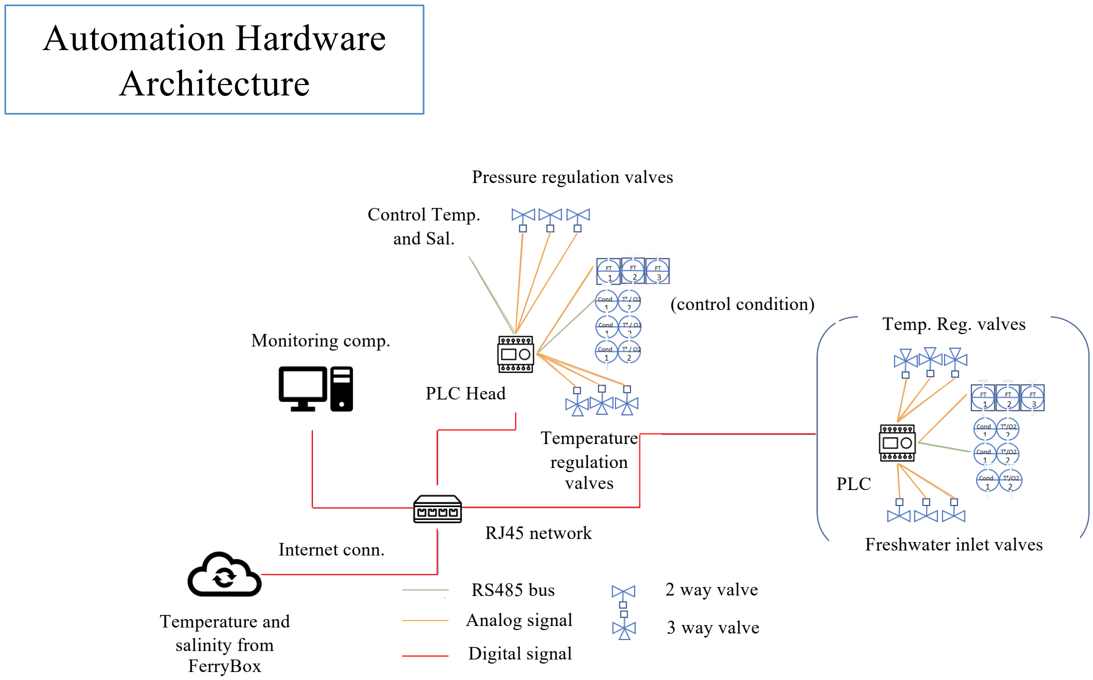


Figure A2. Diagram and flow-chart of the automation system.

A3. Software Development

The code for the application was written in C/C++. The code uses publicly available Arduino libraries (https://www.arduino.cc/reference/en/libraries/) as well as originally designed libraries. All code is available on Github (https://github.com/purrutti/FACEIT). The code is divided into two pathways: 'Master.ino' for the Head PLC, and 'Regul_condition.ino' for the Branched PLCs. A description of the main functions applied in the code for programming the system regulation and features are listed in Table A3.

Table A3. Functions used for programming of software.

Function	Operation	*Ancillary field* Sender ID	*Ancillary field* Command #
RTC.read()	The PLCs are equipped with a RTC chip and battery to keep track of the date. Once set on commissioning, RTC.read() returns the current date and time.		
readMBSensors()	This functions loops through each sensor connected on the RS485 bus. Each Mesocosm has two sensors (O2 and Conductivity/Salinity), so each PLC has 6 sensors connected on its bus. - O2 sensors have addresses ranging from 10 to 12, for mesocosms 0 to 2 of the scenario, respectively. - PC4E sensors have addresses ranging from 30 to 32, for mesocosms 0 to 2 of the scenario, respectively. - Sensors are requested individually and in sequence. A request is made every 200 ms.		
webSocket.loop()	This is a callback function responsible for dealing with the WebSocket communication. The master PLC is the WebSocket server. It listens to slave PLCs requests and to the monitoring PC requests. Requests are JSON formatted. They always contain *anciallry fields:* senderID (ID of the entity sending the request), condID (ID of the requested entity), command (command type of the request). They optionally can also contain a « time » field: Unix-like timestamp (number of seconds since 01-01-1970)	Head PLC (ID = 0) Branched PLCs (ID = 1–3) Monitoring PC (ID = 4)	Request params: setpoints, PID settings (# = 0) Request data: measurement values, regulation outputs (# = 1) Send Params: response to a « request params » request (# = 2) Send Data: response to a « request data » request (# = 3) Calibrate sensor: request for calibrating sensor to specified value (# = 4) Request Head data: specific data measured by Head PLC (pressure & flowrates) (# = 5) Send Head data: a response to a « request Head data » request (# = 6)
regulationTemperature()	This function is responsible for the temperature regulation of the mesocosm. It sets the corresponding three-way valve position using a 0-10V analog signal. The function first checks if the regulation is in « manual override » mode. If so, it applies the override setpoint. If not, it reads the temperature measure in the mesocosm, compares it with the setpoint, and uses the PID settings to set the valve position.		
checkMesocosmes()	This functions loops through every mesocosm every 200 ms and reads analog signals (i.e., flowrates and pressure readings).		
regulationPression() *Only for HEAD PLC*	This function is responsible for the pressure regulation of the mesocosm. It sets the corresponding three-way valve position using a 0-10V analog signal. The function first checks if the regulation is in « manual override » mode. If so, it applies the override setpoint. If not, it reads the pressure measure in the mesocosm, compares it with the setpoint, and uses the PID settings to set the valve position.		
printToSD() *Only for HEAD PLC*	Master PLC is equipped with a microSD card, on which data from all mesocosms is logged every 5 seconds, in one csv file per day. This is for security only, as the microSD card is not easy to remove from the PLC casing. It should not be removed before the end of the experiment.		
regulationSalinite() *Only for Branched PLCs*	This function is responsible for the salinity regulation of the mesocosm. It sets the corresponding three-way valve position using a 0-10V analog signal. The function first checks if the regulation is in « manual override » mode. If so, it applies the override setpoint. If not, it reads the salinity measure in the mesocosm, compares it with the setpoint, and uses the PID settings to set the valve position.		

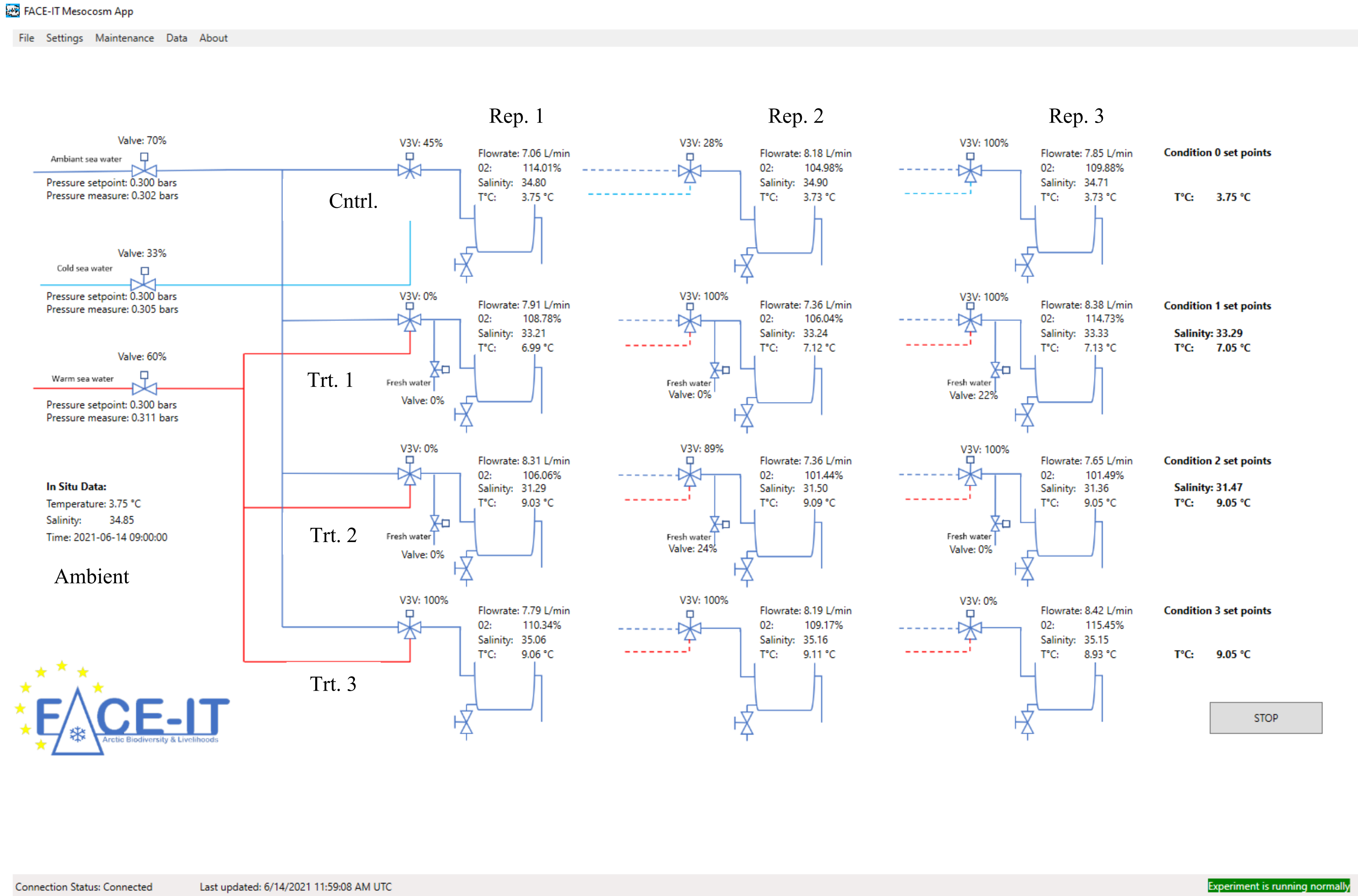

FACE-IT Mesocosm App
File Settings Maintenance Data About
Rep. 1
Rep. 2
Rep. 3
Valve: 70%
Ambiant sea water
Pressure setpoint: 0.300 bars
Pressure measure: 0.302 bars
Valve: 33%
Cold sea water
Pressure setpoint: 0.300 bars
Pressure measure: 0.305 bars
Valve: 60%
Warm sea water
Pressure setpoint: 0.300 bars
Pressure measure: 0.311 bars
In Situ Data:
Temperature: 3.75 °C
Salinity: 34.85
Time: 2021-06-14 09:00:00
Ambient
Cntrl.
Trt. 1
Trt. 2
Trt. 3
V3V: 45%
Flowrate: 7.06 L/min
02: 114.01%
Salinity: 34.80
T°C: 3.75 °C
V3V: 28%
Flowrate: 8.18 L/min
02: 104.98%
Salinity: 34.90
T°C: 3.73 °C
V3V: 100%
Flowrate: 7.85 L/min
02: 109.88%
Salinity: 34.71
T°C: 3.73 °C
Condition 0 set points
T°C: 3.75 °C
V3V: 0%
Flowrate: 7.91 L/min
02: 108.78%
Salinity: 33.21
T°C: 6.99 °C
Fresh water
Valve: 0%
V3V: 100%
Flowrate: 7.36 L/min
02: 106.04%
Salinity: 33.24
T°C: 7.12 °C
Fresh water
Valve: 0%
V3V: 100%
Flowrate: 8.38 L/min
02: 114.73%
Salinity: 33.33
T°C: 7.13 °C
Fresh water
Valve: 22%
Condition 1 set points
Salinity: 33.29
T°C: 7.05 °C
V3V: 0%
Flowrate: 8.31 L/min
02: 106.06%
Salinity: 31.29
T°C: 9.03 °C
Fresh water
Valve: 0%
V3V: 89%
Flowrate: 7.36 L/min
02: 101.44%
Salinity: 31.50
T°C: 9.09 °C
Fresh water
Valve: 24%
V3V: 100%
Flowrate: 7.65 L/min
02: 101.49%
Salinity: 31.36
T°C: 9.05 °C
Fresh water
Valve: 0%
Condition 2 set points
Salinity: 31.47
T°C: 9.05 °C
V3V: 100%
Flowrate: 7.79 L/min
02: 110.34%
Salinity: 35.06
T°C: 9.06 °C
V3V: 100%
Flowrate: 8.19 L/min
02: 109.17%
Salinity: 35.16
T°C: 9.11 °C
V3V: 0%
Flowrate: 8.42 L/min
02: 115.45%
Salinity: 35.15
T°C: 8.93 °C
Condition 3 set points
T°C: 9.05 °C
STOP
FACE-IT
Arctic Biodiversity & Livelihoods
Connection Status: Connected
Last updated: 6/14/2021 11:59:08 AM UTC
Experiment is running normally

Figure A3. Application interface displaying real-time monitoring of ambient conditions as well and control (Cntrl.), and treatment (Trt.) conditions for each replicate (Rep.) in each mesocosm.

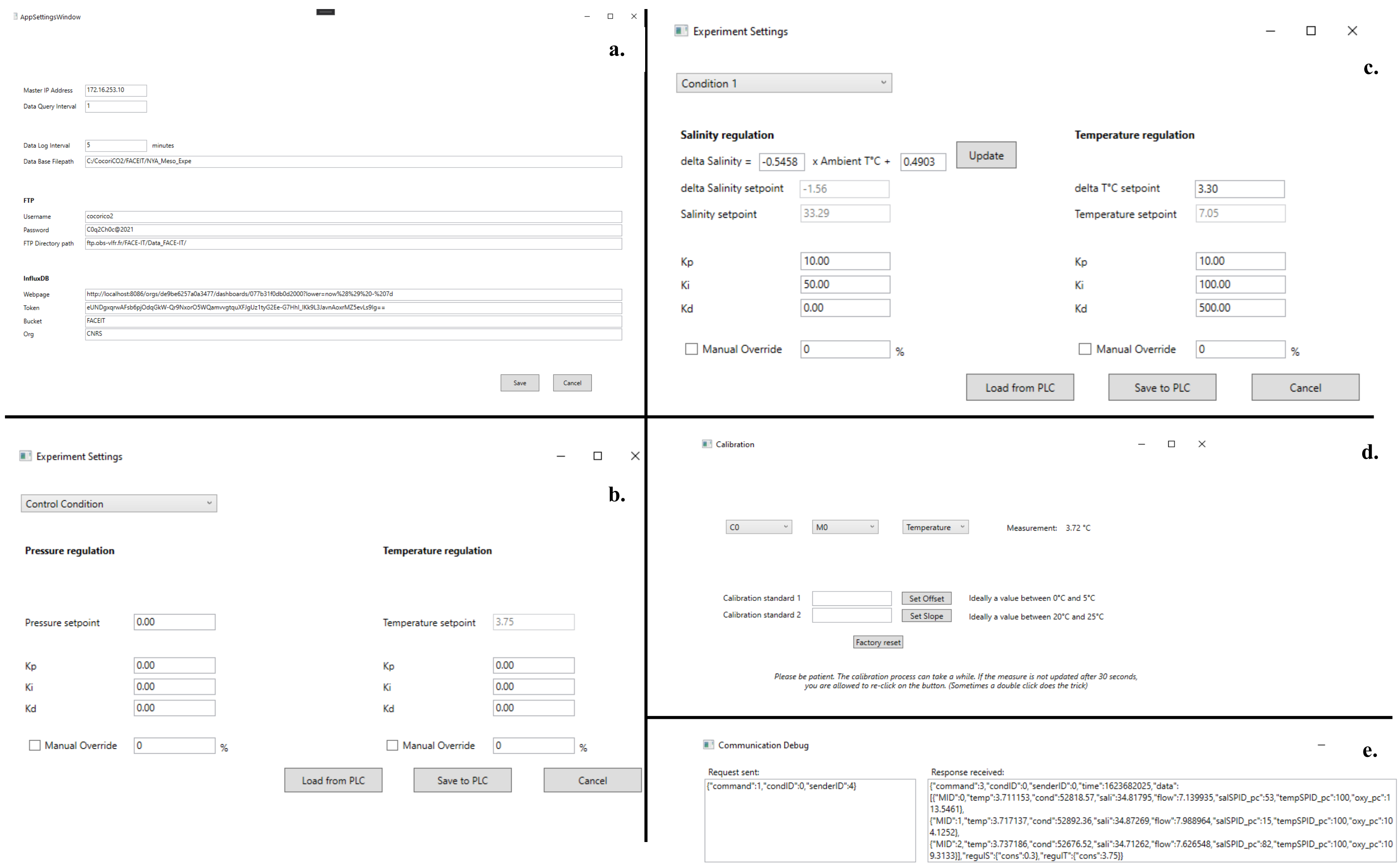
A3.1. Menu bar of PC application

From the interface, the user sets the temperature condition and associated salinity offset, IP address and logging parameters, sensor calibration settings, and pressure setpoints (Fig. A4). Within the menu bar several tabs permit the setup of the project: file, settings, maintenance, and data. Under 'file' the system can be manually connected to, or disconnected from, the PLCs. Connection is usually maintained automatically. The 'settings' tab displays the application and experimental setting options (Fig. A4 a – c). All the settings of the project are stored on the computer (found in 'application settings') that is running the application, which include:

i. *Master IP address*: The IP Address of the Master PLC (centralizing all the data).
ii. *Data Query Interval*: Frequency of queries from the application to the master PLC.
iii. *Data Log Interval:* Number of minutes between logs to file.
iv. *Data Base File Path*: Directory and base filename of the csv data files.
v. *FTP Username, Password, Path*: FTP settings for sending the data file every hour.
vi. *InfluxDB Settings*: For Live Monitoring and local storage of the data.

Under 'experimental settings', the programmed specificities and regulation of the treatment conditions can be adjusted. This includes programming the setpoints for pressure (all main inflow lines), temperature and the salinity-temperature relational equation (on a different tab selected from dropdown), as well as adjusting the K_p, K_i & K_d coefficients for the regulation (see section 2.3.1). The temperature setpoint is provided by the data received from the ferry-box, however this can be overridden if needed. The « Save to PLC » button sends the values to the corresponding PLC and saves the data, while the « Load from PLC » button loads the settings from the PLC. For the purposes of this experiment, the salinity setpoint was calculated based on a delta salinity for treatments 1 and 2 which were derived from the linear relationship with temperature (see section 2.3.1). This can also be overridden if needed by selecting the manual override box.

The ‘maintenance’ tab is where sensor calibration and communication ‘Debug’ operations can be executed (Fig. A4 d, e). Calibration can be performed for each sensor deployed in each mesocosm, and uses a 2-point calibration for temperature and % oxygen. The salinity calibration is done by setting the conductivity value corresponding to a temperature of 25°C rather than the *in situ* measured temperature. The conductivity value is programmed as $\mu S\ cm^{-1}$. The communication process for sensor calibration is between 5 to 10 seconds. The final option in the menu is the ‘data’ tab which displays the historical and live data. The historical data can be interfaced to an html site if desired.



AppSettingsWindow
a.
Master IP Address 172.16.253.10
Data Query Interval 1
Data Log Interval 5 minutes
Data Base Filepath C:/CocoriCO2/FACEIT/NYA_Meso_Expe
FTP
Username cocorico2
Password C0q2Ch0c@2021
FTP Directory path ftp.obs-vlfr.fr/FACE-IT/Data_FACE-IT/
InfluxDB
Webpage
Token
Bucket FACEIT
Org CNRS
Save
Cancel
Experiment Settings
c.
Condition 1
Salinity regulation
delta Salinity = -0.5458 x Ambient T°C + 0.4903
Update
delta Salinity setpoint -1.56
Salinity setpoint 33.29
Kp 10.00
Ki 50.00
Kd 0.00
Manual Override 0 %
Temperature regulation
delta T°C setpoint 3.30
Temperature setpoint 7.05
Kp 10.00
Ki 100.00
Kd 500.00
Manual Override 0 %
Load from PLC
Save to PLC
Cancel
Experiment Settings
b.
Control Condition
Pressure regulation
Pressure setpoint 0.00
Kp 0.00
Ki 0.00
Kd 0.00
Manual Override 0 %
Temperature regulation
Temperature setpoint 3.75
Kp 0.00
Ki 0.00
Kd 0.00
Manual Override 0 %
Load from PLC
Save to PLC
Cancel
Calibration
d.
C0
M0
Temperature
Measurement: 3.72 °C
Calibration standard 1
Set Offset
Ideally a value between 0°C and 5°C
Calibration standard 2
Set Slope
Ideally a value between 20°C and 25°C
Factory reset
Please be patient. The calibration process can take a while. If the measure is not updated after 30 seconds, you are allowed to re-click on the button. (Sometimes a double click does the trick)
Communication Debug
e.
Request sent:
{"command":1,"condID":0,"senderID":4}
Response received:
{"command":3,"condID":0,"senderID":0,"time":1623682025,"data":
[{"MID":0,"temp":3.711153,"cond":52818.57,"sali":34.81795,"flow":7.139935,"salSPID_pc":53,"tempSPID_pc":100,"oxy_pc":113.5461},
{"MID":1,"temp":3.717137,"cond":52892.36,"sali":34.87269,"flow":7.988964,"salSPID_pc":15,"tempSPID_pc":100,"oxy_pc":104.1252},
{"MID":2,"temp":3.737186,"cond":52676.52,"sali":34.71262,"flow":7.626548,"salSPID_pc":82,"tempSPID_pc":100,"oxy_pc":109.3133}],"regulS":{"cons":0.3},"regulT":{"cons":3.75}}

Figure A4. Operation windows for the application and experimental settings (**a**-**c**). These windows are found under the 'settings' tab. Operation windows for sensor calibration and debugging (**d, e**). These are found under the 'maintenance' tab.

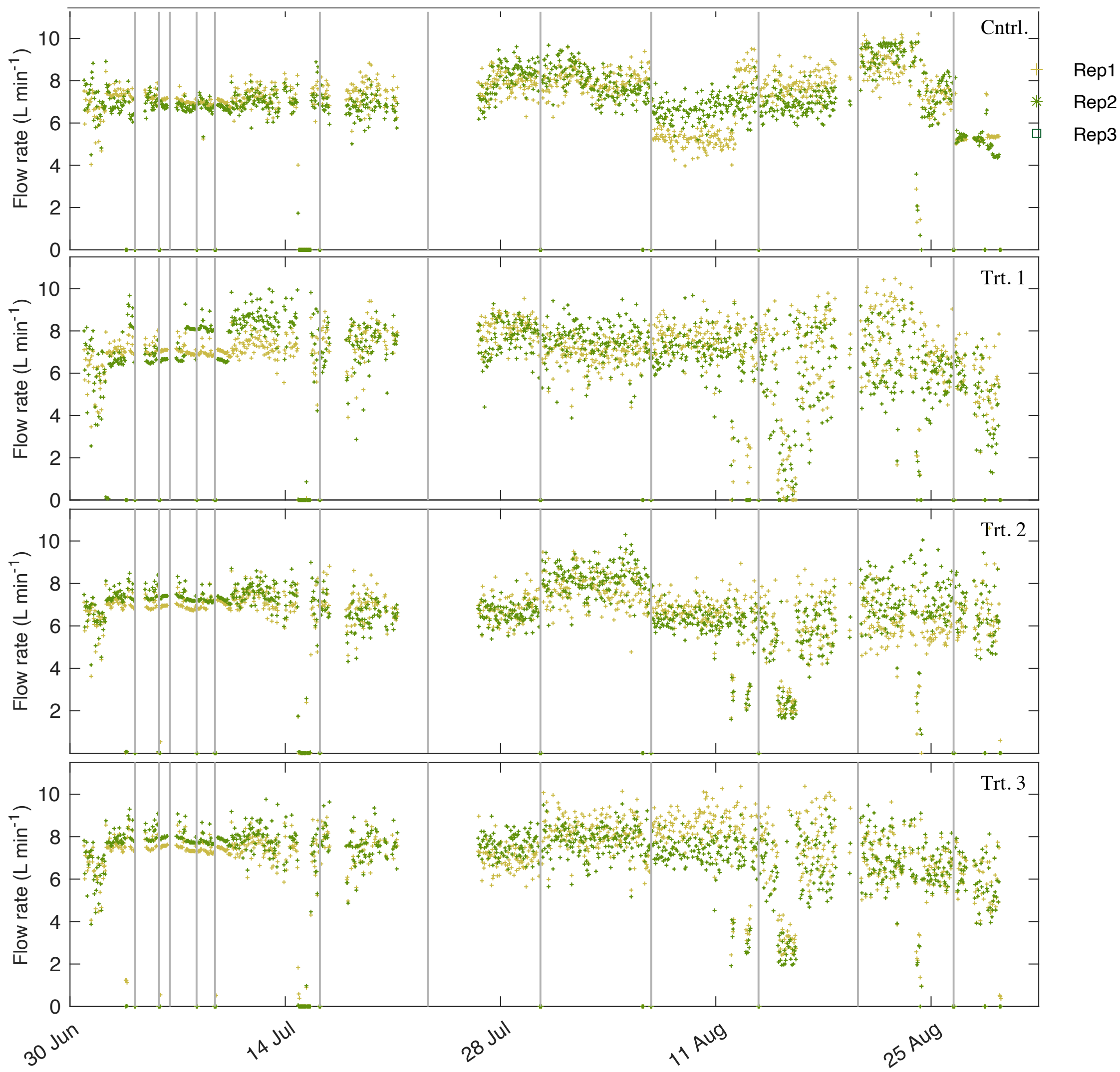


Figure A5. Flow rates for control condition and treatments 1-3 for the entirety of the system deployment. Black vertical lines are when incubations were performed and the system shut-off for a period of 3 h. Flow rates went to zero at these times.

Figure A6. Electrical cabinet used for SalTExPreS

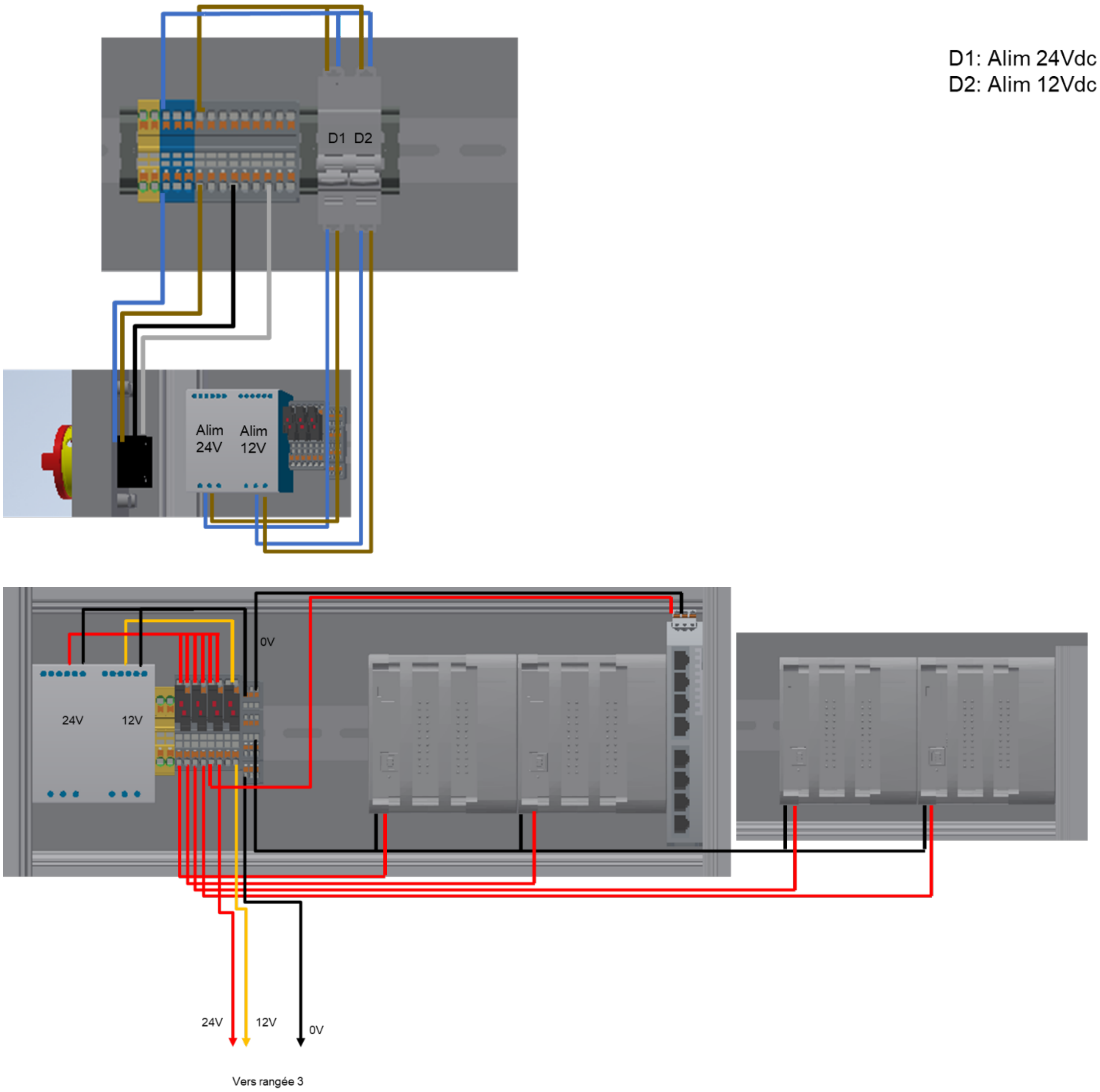


Figure A7. Electrical schematic for wiring within the electrical box.

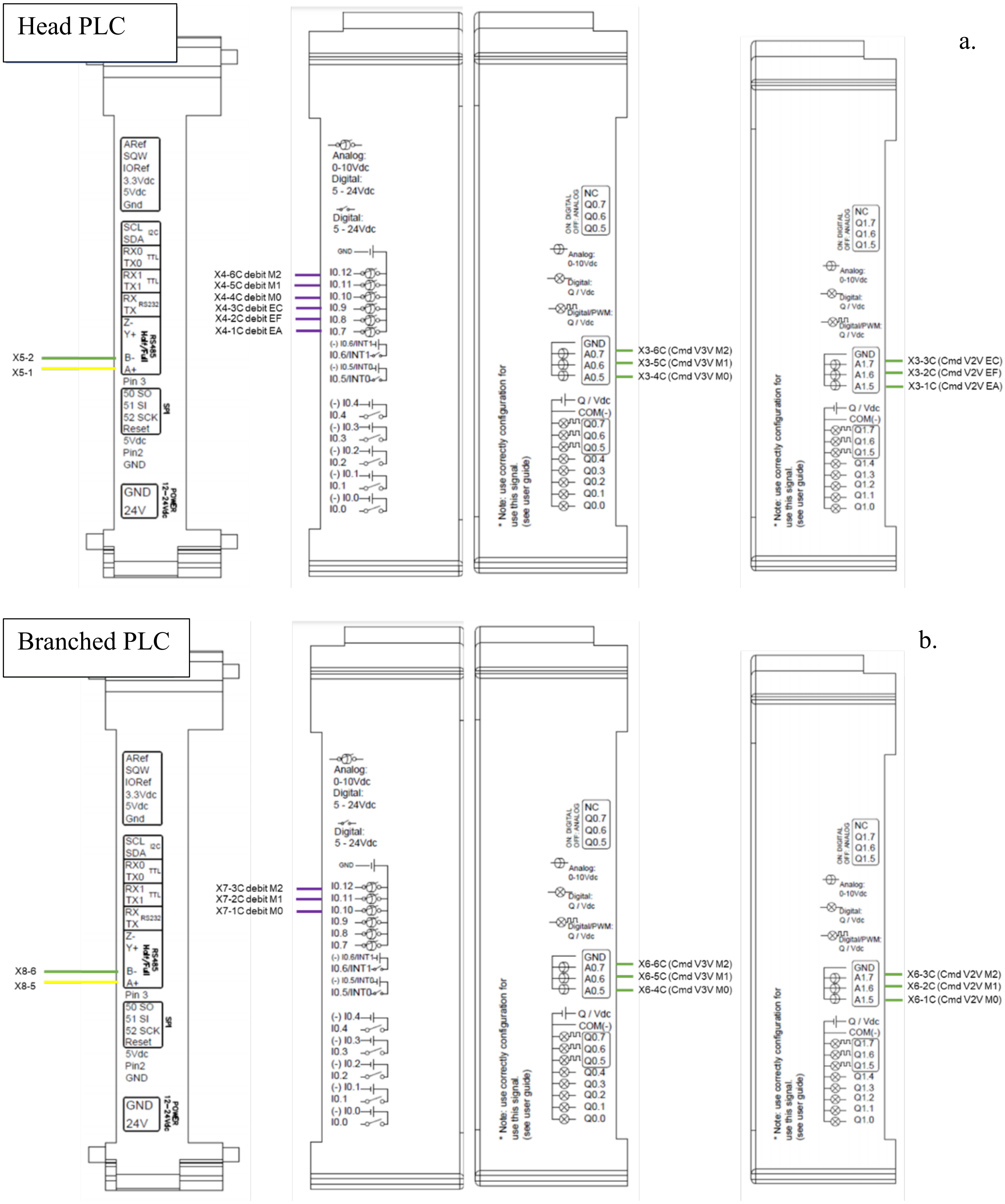


Figure A8. PLC controller diagram for Head (a) and Branched (b) operations.

Table A2. Parts list with manufacturer model numbers.

Group	Item	Supplier/manufacturer	Model / details	Quantity
Hydraulic system				
	Mesocosms	home made	1000 L fiber glass	12
	Seawater pump	NPS, BradFord, UK	Albatros F13T	1
	PVC-U tubing and fittings		20mm, 32mm & 50mm diameter	–
	Insulated flexible hose		19mm diameter	100 m
Sensors				
	Conductivity / temperature	Aqualabo, Champigny sur Marne, France	PC4E	12
	Oxygen	Aqualabo, Champigny sur Marne, France	PODOC	12
	Pressure	Siemens, Munich, Germany	7MF1567-3BE00-1AA1	3
	Flow rate	IFM, Essen, Germany	SV3150	12
Actuators				
	Pressure regulation valves	BELIMO, Hinwil, Switzerland	R2025-10-S2 with LR24A-SR motor	3
	Temperature regulation valves	BELIMO, Hinwil, Switzerland	R3015-10-S2 with LR24A-SR motor	12
	Salinity regulation valves	BELIMO, Hinwil, Switzerland	R2015-10-S2 with LR24A-SR motor	6
Automation cabinet				
	Cabinet	Fibox, Espoo, Finland	FIB8120017N	1
	Security switch	KRAUS-NAIMER, Karlsruhe, germany	KNA002245	1
	12 vdc power supply	TDK Lambda, New York, USA	LAMDRL30-12-1	1
	24vdc power supply	TDK Lambda, New York, USA	LAMDRB240-24-1	1
	PLC	Industrial Shields, Barcelona, Spain	Mduino-42+	4
	Ethernet switch	HIRSCHMANN-INET, Neckartenzlingen, Germany	HIR942132002	1