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

Author list: Cale A. Miller[1,2*], Pierre Urrutti[1], Jean-Pierre Gattuso[1,3], Steeve Comeau[1], Anaïs Lebrun[1], Samir Alliouane[1], Robert W. Schlegel[1], and Frédéric Gazeau[1]

1. Sorbonne Université, CNRS, Laboratoire d'Océanographie de Villefranche, 181 chemin du Lazaret, F-06230 Villefranche-sur-Mer, France

2. Present address: Department of Earth Sciences, Geosciences, Utrecht University, Utrecht, The Netherlands

3. Institute for Sustainable Development and International Relations, Sciences Po, 27 rue Saint Guillaume, F-75007 Paris, France

[*]*Correspondence to:* Cale A. Miller (c.a.miller@uu.nl)

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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 *in situ* mesocosm perturbation system that can manipulate aquatic media in a controlled setting on land. The employed system manipulated ambient water from Kongsfjorden, (Svalbard) by increasing temperature and freshening the seawater to investigate the response of mixed kelp communities to projected future Arctic conditions. This system manipulated temperature and salinity in real-time as an offset from incoming ambient seawater to conditions simulating future Arctic fjords. The system adjusted flow rates and mixing regimes of chilled, heated, ambient seawater, and freshwater, based on continuously measured conditions in a total of 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. The implementation of this system has a wide range of versatility and can be deployed in a range of conditions to test single or multi-stressor conditions while maintaining natural variability.

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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 increasing sea surface temperatures (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 nearshore marine communities is essential to understand how community functions and species richness will be affected as assemblages transition to new environmental conditions (Kroeker et al., 2017; Wake, 2019; Orr et al., 2020). Assessing and characterizing the response of organisms and community assemblages to future ocean change is often pursued by conducting *ex situ* experiments, using natural analogues, or 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 physicochemical 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.

A principal challenge of conducting an *ex situ* multi-stressors experiment lies within the ability to consistently modulate, replicate, and regulate the experimental conditions in real-time. To date, the majority of experiments conducted on marine organisms and communities have implemented only static changes to physical stressors with a limited capacity to induce

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variability by either manually changing conditions at set time points or using coarse automation with static setpoints and thresholds (Olariaga et al., 2014; Pansch and Hiebenthal, 2019; Kroeker et al., 2020). Often, this can fail to capture the high frequency variability of *in situ* conditions. When considering the dynamics of physicochemical conditions in nearshore systems that can notably change within tidal cycles (Evans et al., 2015; Hales et al., 2016; Miller and Kelley, 2021; Fairchild and Hales, 2021), replication of these environmental scenarios necessitates the development of an autonomous system in order to properly conduct experiments over various periods of time. The advantages of implementing an automated system are that it can overcome the need for capturing and measuring the abundant discrete measurements used to regulate the experimental conditions. This can also remove the need for constant human observation which may not be feasible in the long-term, but may be required to program new regulatory operations and make rapid adjustments to the experimentally manipulated conditions.

Here, we describe an autonomous salinity and temperature experimental perturbation mesocosm system (SalTExPreS) that can regulate salinity and temperature in real-time. This system was employed outdoors, along with static light filters to mimic the increase in turbidity and associated irradiance attenuation due to glacial melting to perform a two-month long experiment in KongsFjorden, Svalbard, exposing mixed kelp communities to future Arctic conditions. The rapidly changing conditions in Kongsfjorden are partly due to intrusion of Atlantic water that increase sea surface temperature, as well as freshening from retreating sea-terminating glaciers and enhanced terrestrial flow from proglacial streams (Tverberg et al., 2019). Such a dynamic multi-stressor environment was ideal for the SalTExPreS deployment. This study focuses on the stability and flexibility of SalTExPreS as an experimental tool to be

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utilized under extreme and dynamic conditions to test the effects of physicochemical multi-stressors on marine organisms and communities.

2 Methods

2.1 Experimental design

Three experimental treatments representing expected future conditions in Kongsfjorden were considered to examine potential changes in the productivity, survival, and growth of mixed kelp communities present at a 7 m depth in the fjord. This involved manipulating multi-stressor combinations: temperature, freshening, and irradiance (Table 1). The response of communities was determined by conducting weekly assessments of growth and metabolism via closed system incubations (Miller et al., *in prep*). The temperature anomalies used for this experiment represent future Arctic sea surface temperatures projected following scenarios SSP2-4.5 and SSP5-8.5 (Meredith et al., 2019; Overland et al., 2019). The salinity offsets were based on correlations between *in situ* temperature and salinity during the 2020 Kongsfjorden summer (Gattuso et al., *2023*), weeks 22 to 35 (Fig. A1). This correlation was then used to estimate salinity offsets derived from the temperature offset values extrapolated from a linear fit for treatments 1 and 2 (Table 2). In the third treatment, only temperature was manipulated as a way to decouple the multi-stressor system and evaluate a temperature only stress representing coastal areas not affected by glacial melt. The effect of turbidity for treatments 1 and 2 was simulated as a decrease in surface irradiance (i.e., ~ 30 % and ~ 50 % from ambient irradiance at 7 m and corresponding spectra) by the application of a combination of neutral light and spectral filters (Lee© Filters) placed as static fixtures overtop the mesocosms (Fig. S2).

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Exposed kelp communities were reconstructed in each experimental unit (i.e., a single mesocosm) to represent the species composition found at ~ 7 m depth (Hop et al., 2012; Bartsch et al., 2016; Paar et al., 2019). The biomass of the selected species totaled ~ 4 kg (wet weight) and was composed of *Alaria esculanta* (1 kg), *Saccharina latissima* (1.5 kg), and *Laminaria digitata* (1.5 kg). Fauna for each mesocosm was limited to the most abundant organisms found in the field: sea urchins (*Strongylocentrotus pallidus*, *Strongylocentrotus droebachiensis*), snails (*Margarites* spp.), and brittle stars (*Ophiopholis aculeata*) with total biomass weights ~ 400 g for urchins and < 150 g for snails and sea stars at the start of the experiment.

2.2 Experimental system

Twelve circular mesocosms (3 treatments and 1 control, all in 3 replicates) of 1 m^3 (diameter extending from 1.0 at the bottom to 1.2 m at the top, and ~1.2 m high) equipped with a submersible mixing pump, a temperature-conductivity probe, and an optical oxygen sensor (see parts list in Table A1) operated as individual experimental units for the entirety of the 54-day experiment. The determined temperature and salinity offsets applied to treatments 1 – 3 followed a dynamic deviation tracking the control condition. The control condition was designed to represent the actual *in situ* condition in Kongsfjorden at the approximate kelp community depth (7 m). A submersible pump (NPSⒸ Albatros F13T) was deployed at a 10 m depth, 300 m offshore tapped into an underwater intake valve that was plumbed into the header tank of the Kings Bay Marine Laboratory in Ny-Ålesund, Svalbard. Pumped ambient seawater was split into 3 sub-header tanks within the marine lab where temperature was manipulated to obtain: ambient seawater, chilled seawater at 0 °C, and warmed seawater at 15 °C. Each sub-header tank was plumbed to supply a constant flow of manipulated fjord water to each mesocosm placed on an open outdoor platform. The control mesocosms received a mix of chilled and ambient seawater

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to compensate for any potential warming that could occur from transit to the header tank, and finally to the mesocosms. The three experimental treatments received a mix of ambient and warmed water in order to achieve the projected future temperature offsets (Fig 1). Each mesocosm received 0.5 m^3 h^{-1} of seawater in an open cycle which equated to a flow rate of 7 – 8 L min^{-1}. The flow was maintained throughout the experiment with the exception of periods when flow to each mesocosm was suspended for 3 h during weekly whole-system incubations for community metabolism determination.

The main inflow pipes (ambient, chilled, and warmed) were plumbed in combination with a freshwater tap line (Fig. 1) into a control manifold that mixed the manipulated media regulating flow rates using a continuous monitoring system and a series of controlled valves (Fig. 2). Continuous minutely monitoring of the inflow pressure (pre-mixing) and outflow rates to each mesocosm provided high frequency logging and observation of mesocosm condition.

2.3 Parameter regulation

2.3.1 Temperature and salinity

The control from which temperature and salinity regulation were automated was based on hourly *in situ* readings from the AWIPEV (Alfred Wegener Institute and Institute Paul Emile Victor) FerryBox which is part of the COSYNA underwater observatory (https://dashboard.awi.de/). The inlet which is located ~ 400 m from the submersible pump was at 11 m depth. Control conditions were set hourly by the logged COSYNA data, referred to henceforth as the FerryBox. Each treatment condition was retained via manually programmed offsets from the control condition set in the software interface (Section 2.5). The program automatically calculated the salinity offset based on the preset temperature offsets (Table 1). Regulation was maintained via regulation flow

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valves utilizing minutely measurements of temperature and salinity inside each mesocosm making instantaneous adjustments. This was accomplished using an analog three-way mixing valve for temperature and two-way valve for salinity (BELIMOⓒ R3015-10-S2 with LR24A-SR motor). Each valve was plumbed within the manifold and applied to each mesocosm flow line where its open-position was adjusted (using a PID regulator) to reach the temperature and salinity setpoint value.

Accurate temperature and salinity regulation was obtained using a software PID controller on the corresponding Programmable Logic Controller (PLC), in PoE mode (proportional on error). The PID controller measures the difference between the measure and the setpoint (i.e., the error), and calculates how the valve opening should be adjusted by multiplying the error, which is the integral of the error and the derivative of the error, by previously determined coefficients Kp (proportional gain), Ki (integral gain) and Kd (derivative gain), respectively. These coefficients were obtained experimentally using the empiric method of Ziegler & Nichols (1943), and may differ from one condition to another.

2.3.2 Pressure and flow regulation

Each main inflow line of ambient, cold and warmed seawater had its own pressure regulation system established to maintain equivalent pressure levels, which aided in adjusting the flow rates to the mesocosms. 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). A pressure setpoint for all three sensors (Kp, Ki, Kd in software interface) was applied to adjust the valve opening in order to regulate the pressure to the defined setpoint. Each water line with

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post-mixed seawater flowing to each mesocosm was then manually adjusted and controlled with a hand-crank valve. An analog flow rate sensor (IFM© SV3150) was placed in-line with the piping going to each mesocosm located directly before each hand-crank valve. This provided up-to-date logged flow rate values (updating every 30 sec), which could then be monitored to set incoming flow to each mesocosm.

2.4 Automation

The automation was performed using 4 Industrial Arduino-based PLCs (Industrial shields© Mduino-42+), with a single PLC regulating the control and each treatment 1 – 3. Each PLC was responsible for data logging and the regulation of a specific scenario. 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 2.5) 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 to an FTP server, and (5) sending settings and commands to the PLCs. Communication between the PLCs and the PC was made using http WebSocket protocol on RJ45 ethernet cables, while communication between the PLCs and the conductivity-temperature and oxygen sensors, flow rate sensors, and regulation valves was executed using half duplex RS485 (2 wires) protocol, an analog 4-20mA signal, and 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. 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.

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2.5 Software

The code for the application was written in C/C++, and developed using Visual Studio community (2019 edition) with the vMicro extension and Arduino 1.8.13. 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.

The program application has a user-friendly interface designed to allow real-time monitoring and parameterization of regulation processes (Fig. A3). 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). The main window displays each mesocosm condition (temperature, salinity, % O_2, and flow rate), 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 experiment status (e.g., started or stopped). The interface also displays the valve-open percentage along with the pressure setpoints and actual measured value for each main inlet.

In addition, the *in situ* data (temperature and salinity) received from the FerryBox is displayed with the date of the last logged value utilized as the programmed real-time condition for the control. 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). While measured data is stored through the server connection, there is a backup microSD card on the Head PLC that logs data from all mesocosms

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every 5 sec. The microSD should not be removed before the end of the experiment if possible. 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.

2.5.1 Menu bar

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. There is also an exit button that closes the application. 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 Kp, Ki & Kd 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

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corresponding PLC and saves the data, while the « Load from PLC » button loads the settings from the PLC. Loading should be done automatically but this button serves as a way to verify that settings were saved properly. 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.

Results

3.1 Regulation of the control condition

The control condition from which all treatment conditions were offset tracked ambient fjord temperature well over the experimental period deviating on average across replicates < 0.3 °C (Table 2, Fig. 3). The overall quality of the regulation was based on the ability for the system to read the measured data from the FerryBox, or to be overridden when communication was interrupted and acknowledged by the user. During the experiment, the FerryBox went intermittently offline for 24 % of the time. During these periods the real-time dashboard

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populated by the COSYNA underwater observatory ceased updating and resulted in a break of communication to the PLCs. There were two main disruptions in the regulation of the control conditions resulting from the main seawater pump failure and a disconnection from the FerryBox. On 2021-07-14 ~21:00 UTC the pump deployed in the fjord suffered a motor malfunction causing a spike in ambient temperatures due to suspended incoming flow of water to the main header tank (Figs. A5, A6). An alternative deep-water pump (90 m) was used as a replacement until the pump at 10 m depth could be repaired on 2021-07-26 13:49 UTC. During this period, the control condition was colder than the target fjord temperature at 7 to 10 m depth by > 1 °C (Figs. 3, 4) until the proper pump depth was reestablished. The average deviation (Table 2) excluded this period as the control condition was unable to be warmed as only cold and ambient water lines were plumbed to the control condition (see section 2.2). The other instance occurred on 2021-08-24 04:47 UTC when setpoint values did not retain the last FerryBox reading and dropped to 0 °C. This issue was quickly resolved (< 8 h) by resetting the PLC.

3.2 Temperature and salinity regulation

Regulation of the temperature and salinity treatment conditions was maintained for 54 days (i.e., entirety of the experiment) (2021-07-03 – 2021-08-26) by the SalTExPreS: the entirety of the planned experiment. All mesocosm conditions were held at ambient temperature and salinity for the first 6 days dedicated as an acclimation period before proceeding with an incremental rise in temperature over a 5-day period starting on day 7 (2021-07-10 12:00 UTC). Treatment 1 temperature increased by 0.55 °C d^{-1}, while treatment 2 and 3 increased by 0.88 °C d^{-1} (Figs. 3, A5). The temperature offsets achieved final values at ~ 2021-07-15 21:00 UTC, 4 h after the final incremental increase (Fig. 3, A5). Due to technical issues with the incoming FerryBox data, the final salinity offset values for treatments 1 and 2 were reached immediately with the first

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incremental temperature increase on 2021-07-10 12:00 UTC because the programmed setpoint based on the linear relationship with temperature was set as a manual override (Fig. 4).

The regulation of temperature and salinity was well maintained over the duration of the experiment as the difference between the measured and setpoint value was < 0.3 °C and < 0.36 salinity across the three replicates per treatment (Table 2). Stochastic periods of deviations that were greater than the mean value (Table 2) was due to various anomalies. In the event of a communication break between the FerryBox and the Head PLC, the system is programmed to automatically retain the last value read. Thus, regulation would be maintained based on this last *in situ* temperature, unless a manual value was programmed as an override. On 2021-08-24 04:47 UTC, when connection with the FerryBox was interrupted, the last value was not retained and resulted in strong deviations in the regulation until a manual value could be implemented (Fig. A5). This happened on only one occasion and was resolved by rerunning the program code. The disconnection from the FerryBox was due to interrupted logging by the FerryBox on the COSYNA dashboard (https://dashboard.awi.de/), and was not a result of programming miscommunication. The only lasting miscommunication error between the program and PLCs occurred from 2021-07-21 to 2021-07-26 where data logging ceased, however, regulation was maintained. The result was a loss of all measured data during this time period, but resulted in an addition to the programming code which provided a 'disconnection pop-up window' henceforth. Connection could easily be reestablished by resetting the master PLC (i.e., pressing the reset button or cycling the power).

The salinity regulation for treatments 1 and 2 across replicates experienced a sudden freshening on 2021-08-03 07:30 UTC resulting in an upward shift of the mean offset value (Fig. A5). This event was quickly resolved by a system reset and lasted for only 2 h. Deviations in one

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mesocosm (1^{st} replicate of treatment 2) occurred intermittently from 2021-07-26 to 2021-08-03 due to a recurring communication issue with the treatment 2 branched-PLC. A fast reset temporarily fixed the issue, but a reprogramming was required to properly resolve the issue. The mean salinity offset during this time ranged from -7.0 to -2.5 (Fig. 4) compared to the set value of c.a. – 4.5, which was the primary contributor to the final value for the standard deviation (SD).

The main cause of temporary, but frequent, setpoint deviations larger than the mean value was due to low flow rates (Fig. A6). Stochastically throughout the experiment, flow rates averaging 7 to 8 L min^{-1} would vary as a result of partial clogging, concomitant use of header tank water from other parties in the marine lab, or occasional communication errors leading to erroneous temperature setpoints that increased the ambient or heated seawater flow in a mesocosm to outpace the filling of the respective header tanks. These instances were few, however, and were not a result of the programmed control regulation of the SalTExPreS. Several deviations > 2.0 salinity or °C could be explained by flow rates dropping to values < 2 L min^{-1} (Fig. 4, 5). These low flow rates accounted for ~ 20 % of the large deviations in temperature and salinity regulation. Occasions when flow rates were < 2 L min^{-1} appeared to be minor in duration and typically self-resolved (Fig. A6).

When accounting for the various incidences of improper regulation, the SalTExPreS was able to simultaneously regulate 12 mesocosms comprised of 4 different conditions (1 control and 3 treatments) with an accuracy that deviated > 0.5 °C or 0.5 in salinity from the setpoint ≤ 20 % and ≤ 30 % of the time, respectively, when flow rates were above 2 L min^{-1} and excluding the period of 90 m pump use (Fig. 5). Due to an erroneous control value during the 90 m usage, these times were excluded. The % time deviation from a temperature setpoint > 1 °C reduced to < 11 % for all treatments, and ≤ 20 % for all salinity treatments with the exception of the 1^{st}

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replicate for treatment 2. Regulation of temperature resulted in a higher accuracy than regulation over salinity. This higher accuracy was trivial, however, as the average difference between temperature and salinity accuracy as % time > 0.5 was ~ 1.5 % higher for temperature when excluding the 1st replicate for treatment 2.

Discussion

The inaugural application of the fully autonomous SalTExPreS demonstrated the capacity for this system to be successfully utilized for conducting *in situ* experiments on organisms or communities exposed to multiple environmental drivers with a very good stability of treatment conditions and an excellent match of the real-time natural variability. The versatility of the system not only permits the manipulation of temperature and salinity, but could incorporate other factors such as CO_2 or nutrient concentrations (Gazeau et al., *in prep*). The automated component of the system can reduce logistical hurdles needed to perform 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 handling.

Some of the operational challenges encountered during the 2-month experiment in Svalbard were able to be mitigated or resolved henceforth. The use of pop-up alert windows when a lapse in connection occurred, or when data is not logging, along with secondary coding instructions (as fail-safe instructions) ensuring that the last received *in situ* data were maintained are examples how improvements made during the experiment facilitate a more robust deployment for the future. These improvements are now incorporated into the available code for programming of the SalTExPreS. These new editions were implemented during a second

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deployment that occurred in the summer of 2022 under a similar experimental design, and resulted in fewer lapses and frequencies of mis-regulation (Fig. 6). Further, we forewent attempting to regulate the control condition (i.e., mixing chilled seawater with ambient to account for unintended warming during transit from fjord to header tank to mesocosm) during this second deployment as transit time and distance from pumped fjord water was substantially less than this first application. The result of this decision was the complete resolution of connection issues that regulate the control setpoint. As for some of the more common disruptions that can occur during long-term experimental setups using raw seawater such as pump failure and clogging, both of which impacted the performance of the SalTExPreS, were extraneous instances that are not relevant to its direct performance. Other issues such as a sudden glitch in the programming which resulted in a sudden freshening on 2021-08-03 07:30 UTC, or the persistent miscommunication with the treatment 2 branched-PLC from 2021-07-26 to 2021-08-03 UTC, could have been reduced by more fastidious monitoring of the SalTExPreS regulation. We note that some of these issues were easily resolvable by resetting the Head PLC or cycling the power of the system. In short, efficient user operation could further reduce deviations and increase the accuracy of the SalTExPreS regulation.

This first and initial deployment of the SalTExPreS used to conduct a multi-stressor experiment provided robust results for determining mixed kelp community metabolic responses to future Arctic conditions (Miller et al., *in prep*). Many research endeavors and implementations by the SalTExPreS have the potential to conduct a larger range of experimental settings that pertain to environmental perturbation associated with climate change or other anthropogenic forcings. The operational data produced are reliable, easily quantifiable, and provide the highest degree of frequency for the monitoring of experimental conditions. 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 aquatic perturbation experiments.

Data availability

The recorded parameters presented in this paper will be posted to Pangea and are available for access.

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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. C.M. wrote the manuscript and constructed the data analysis figures and tables while P.U. designed the diagram figures. All authors revised, commented, and edited during revision.

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Competing interest

The authors declare no competing interests exist.

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Tables

Table 1. Experimental treatments with given temperature and calculated salinity offsets based on linear correlation with temperature (Fig. A1).

Treatment	*Temperature*	*Salinity*	*PAR*
Control	Ambient	Ambient	Ambient
Trt 1	+ 3.3 °C	Δ 2.5 – 3.0 y = 0.546*T + 0.490	~ 30% decrease from ambient
Trt 2	+ 5.3 °C	Δ 5.0 – 5.5 y = 0.877*T + 0.089	~ 50% decrease from ambient
Trt 3	+ 5.3 °C	Ambient	Ambient

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Table 2. Absolute mean difference between measured temperature and salinity 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	

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Figures

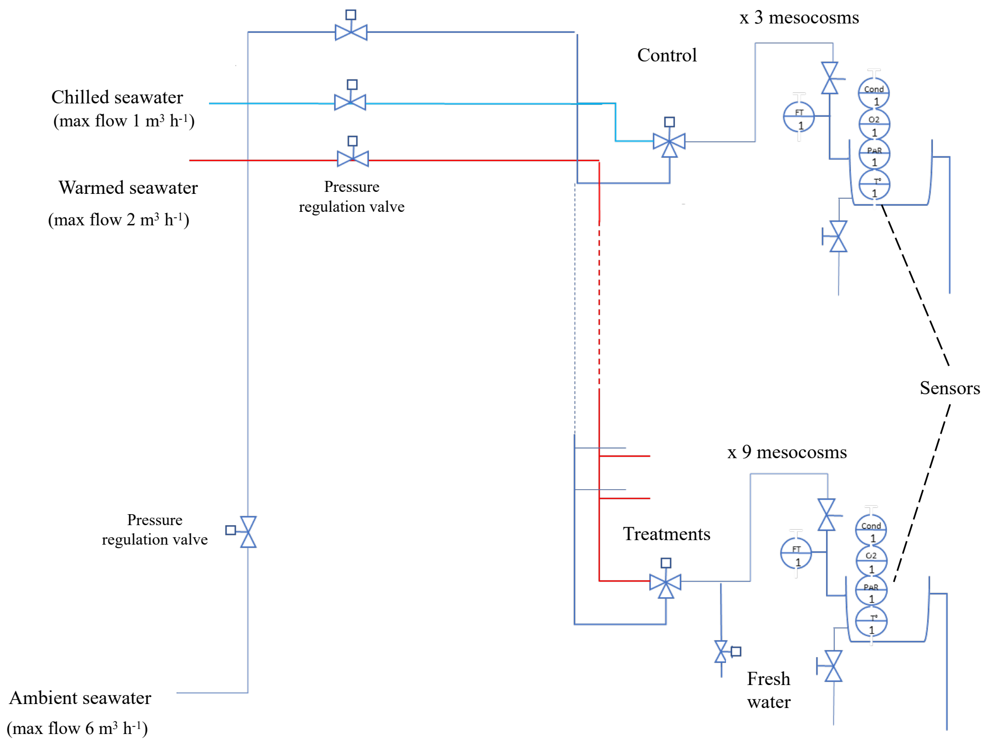

Figure 1. Piping schematic of mixing and regulation manifold for SalTExPreS.

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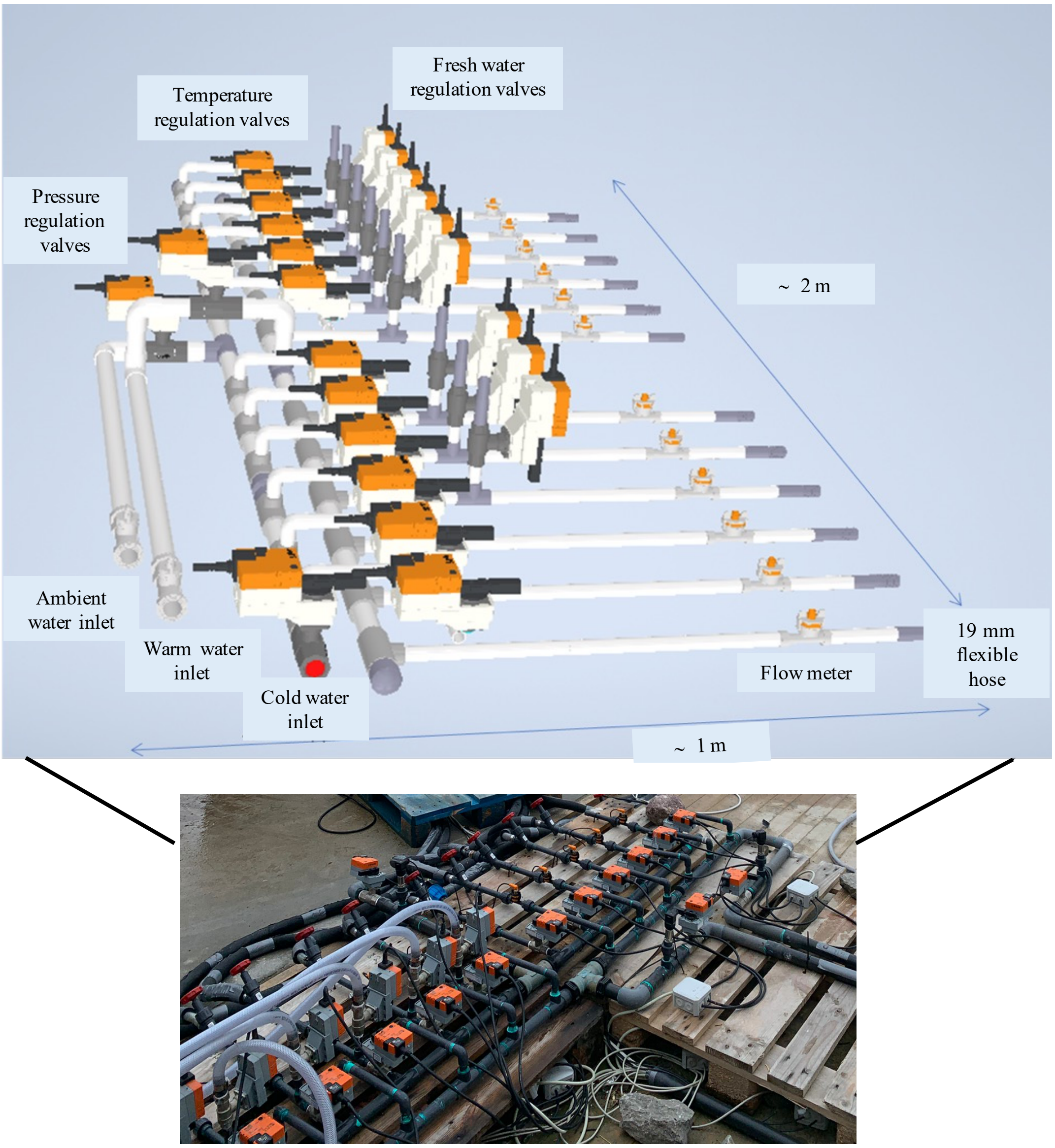

Figure 2. Visual diagram of manifold layout and actual application at the experimental site in Ny-Ålesund, Svalbard.

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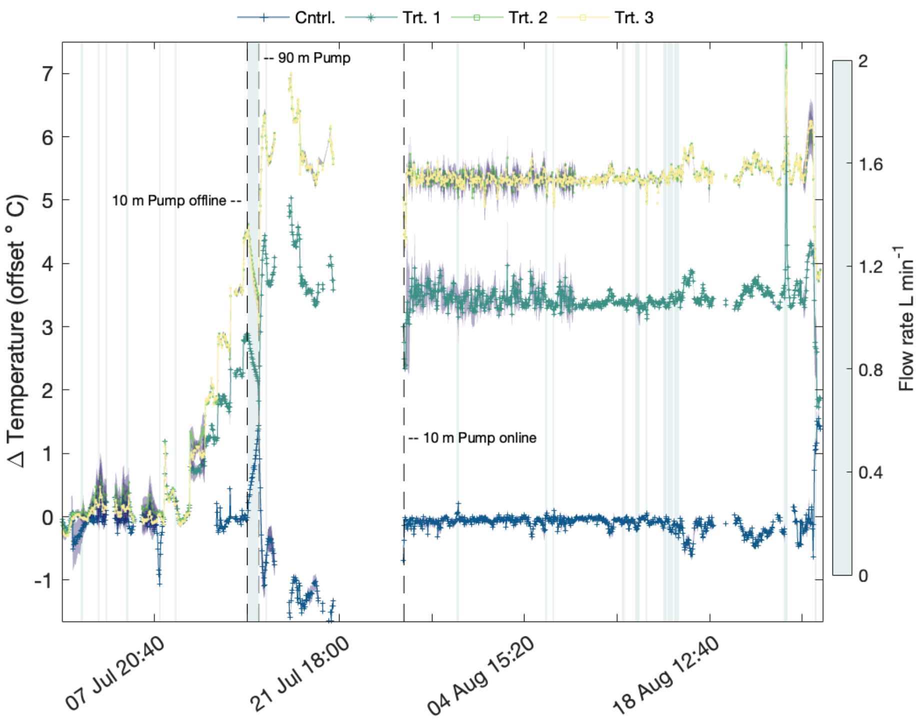

Figure 3. Regulation of the temperature offset during the experimental period for the mean value of all treatment conditions and ambient-control. The purple shaded region around the mean is the standard deviation and the heatmap isoclines are the instances when flow rates ≤ 2 L min^{-1}. Dashed black lines indicate periods when the pump at 10 m depth and 90 m depth were used to feed header tanks.

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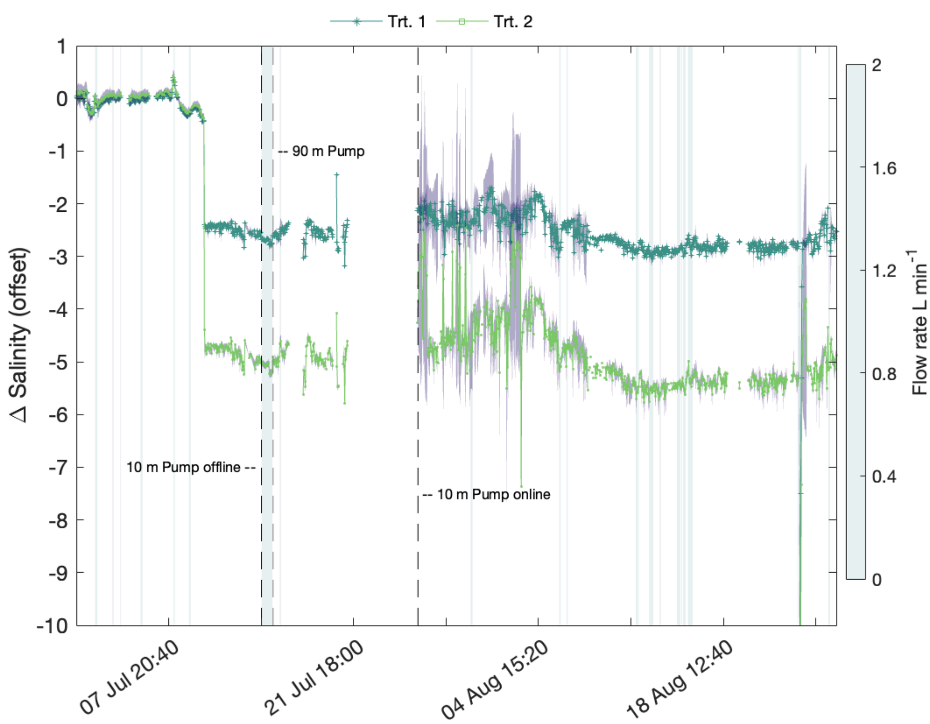

Figure 4. Regulation of the mean salinity offset (Δ salinity) during the experimental period for treatments 1 and 2. The purple shaded region around the mean is the standard deviation and the heatmap isoclines are the instances when flow rates ≤ 2 L min^{-1}. Dashed black lines indicate periods when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

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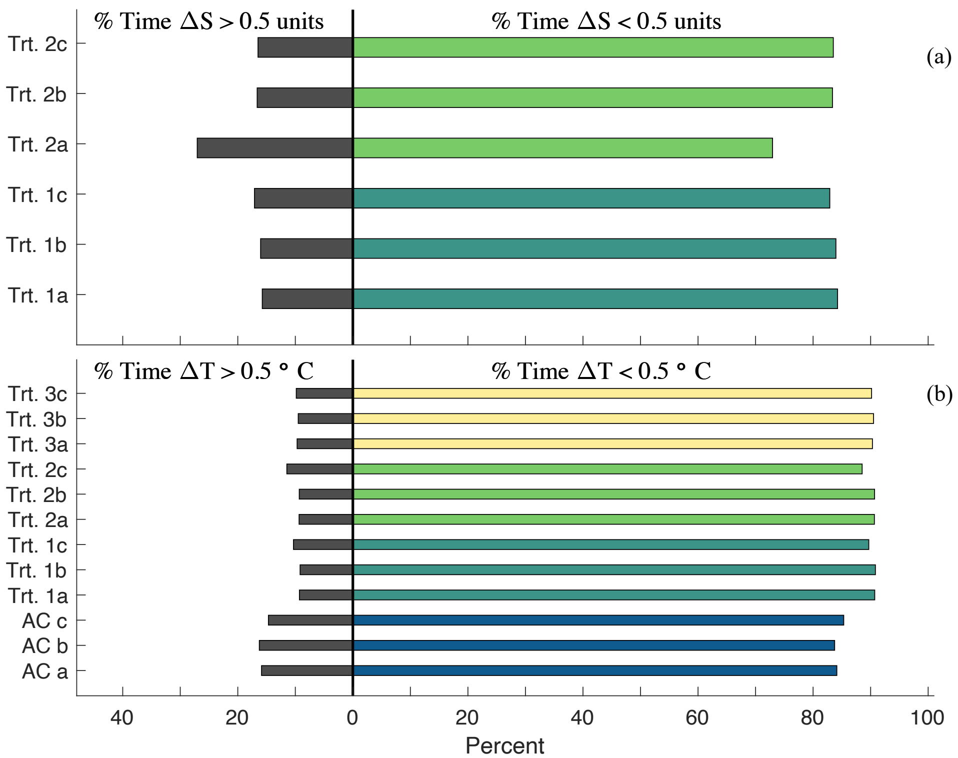

Figure 5. Percent time each mesocosm had a deviation > (black bars) or < (colored) 0.5 (ΔS; **a**) or 0.5 °C (ΔT; **b**) when flow rates were above 2 L min^{-1} spanning 42 days out of the 54 days of the experiment: excluding the time when using the 90 m pump as regulation setpoints were invalid due to an erroneous control.

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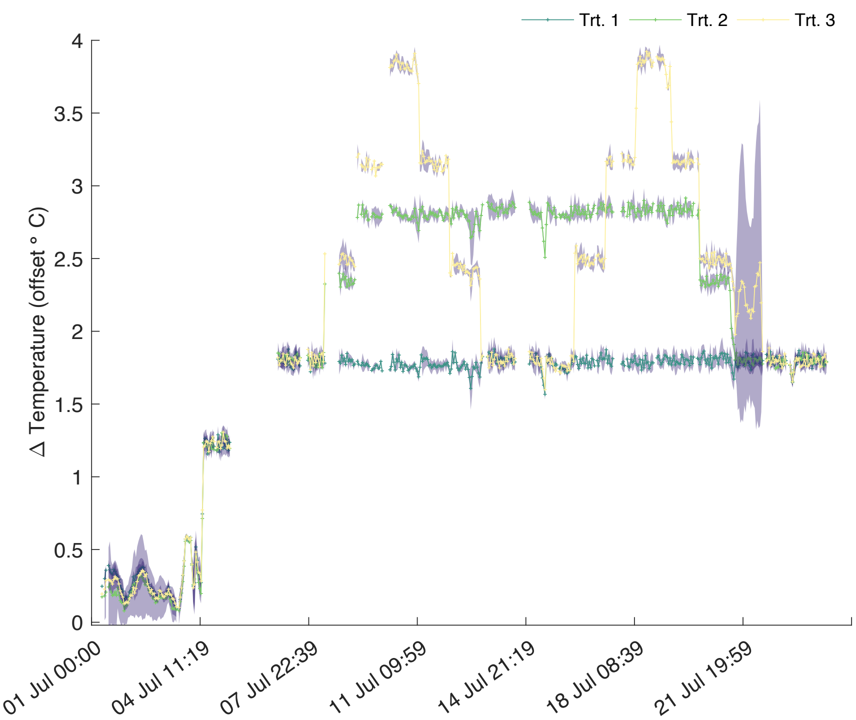

Figure 6. Regulation of the mean temperature offset (Δ temperature) during the 2^{nd} deployment of SalTExPreS in the summer of 2022 in Tromsø (Norway) performing a variation of heatwave scenarios with three experimental treatments 1 – 3. The purple shaded region around the mean is the standard deviation.

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Appendix A

Table A1. 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 *ancialry 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.		

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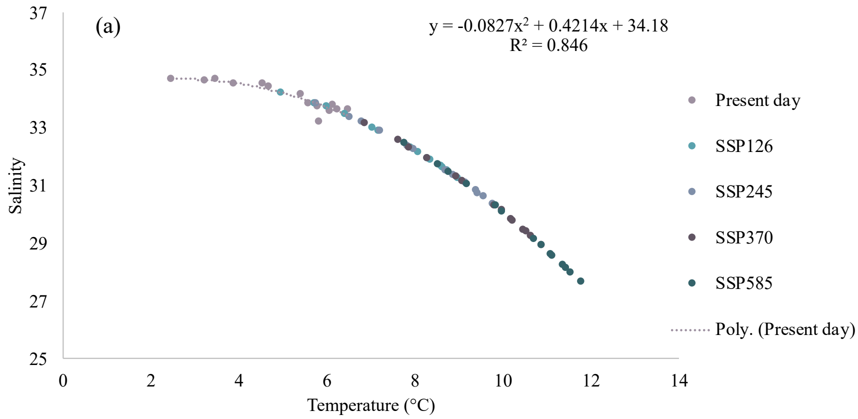

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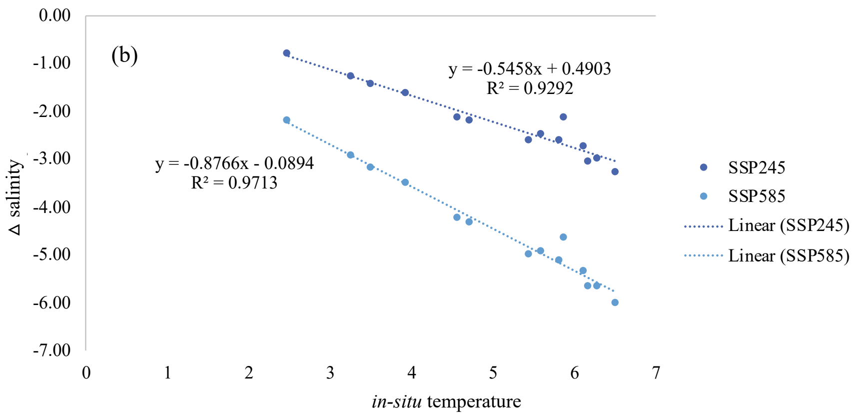

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644 **Figure A1.** Relationship between temperature and salinity in summer 2020 weeks 22 – 35 in Ny-

645 Ålesund, Svalbard. (**a**) Relationship extrapolated using temperature offsets for projected 2100

646 SSP scenarios. (**b**) Applied salinity offsets based on relationship with temperature used for

647 treatments 1 and 2 (Table 1).

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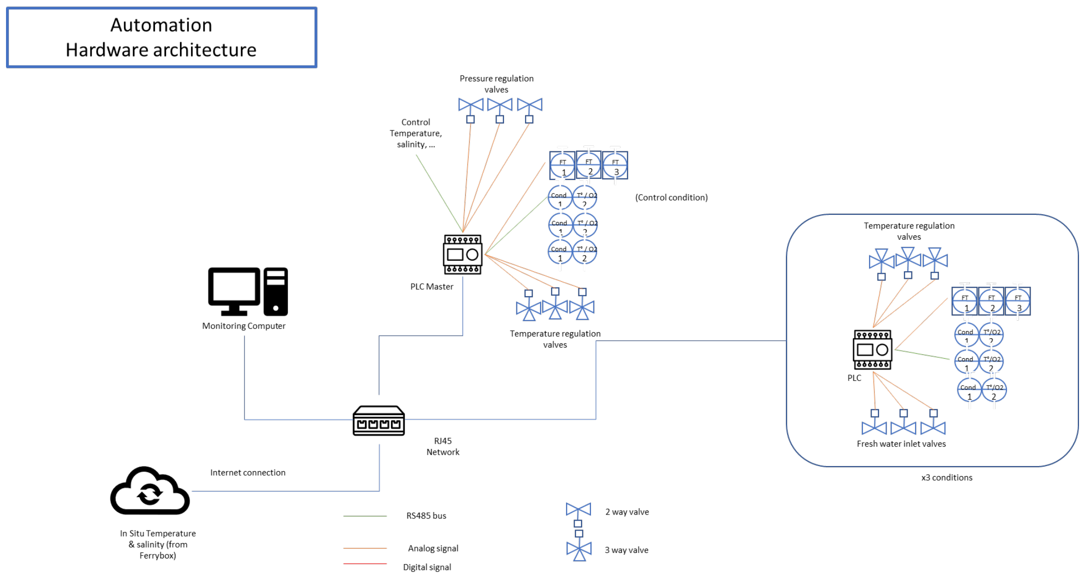

Figure A2. Diagram and flow-chart of the automation system.

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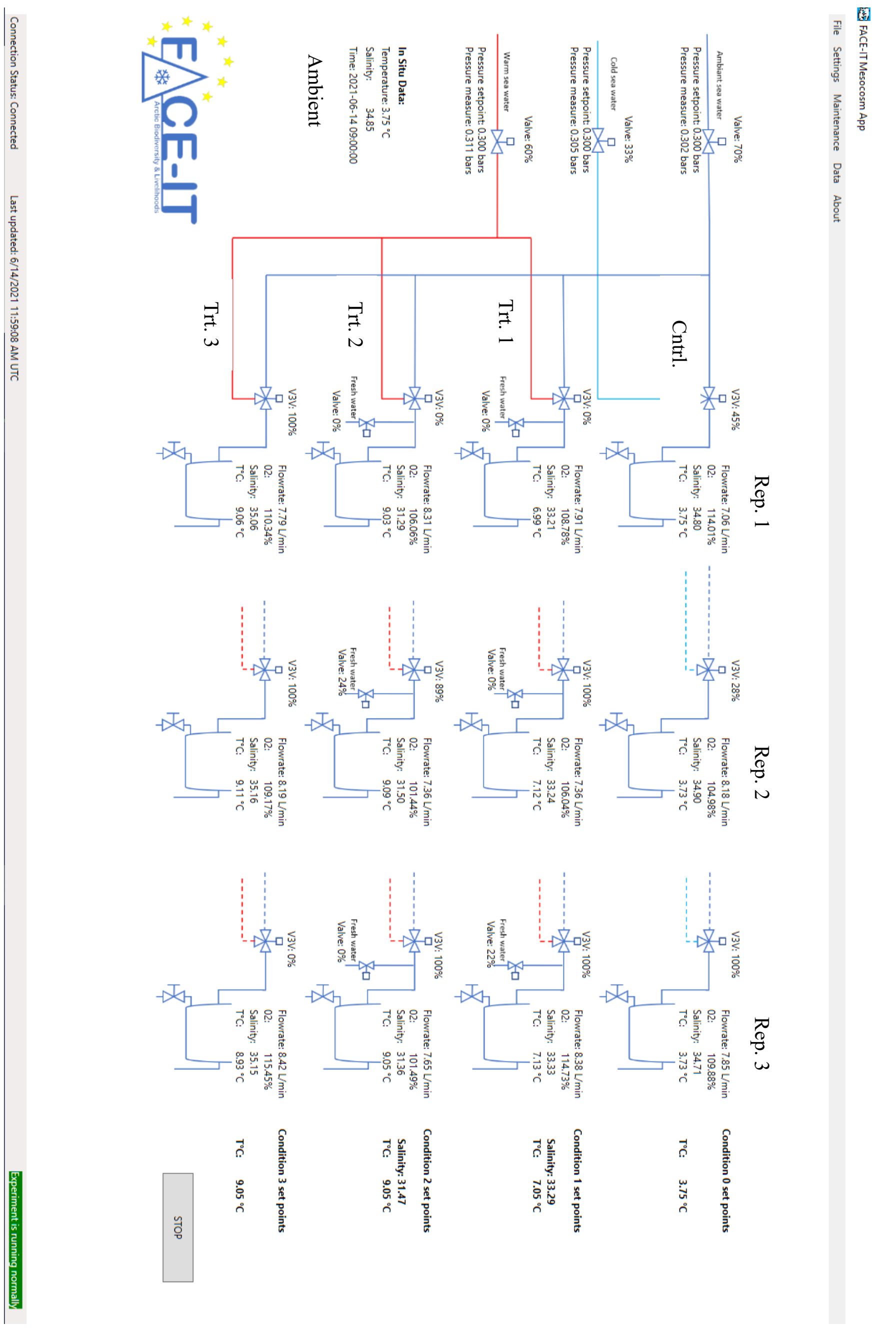

669 **Figure A3.** Application interface displaying real-time monitoring of ambient conditions as well
670 and control (Cntrl.), and treatment (Trt.) conditions for each replicate (Rep.) in each mesocosm.

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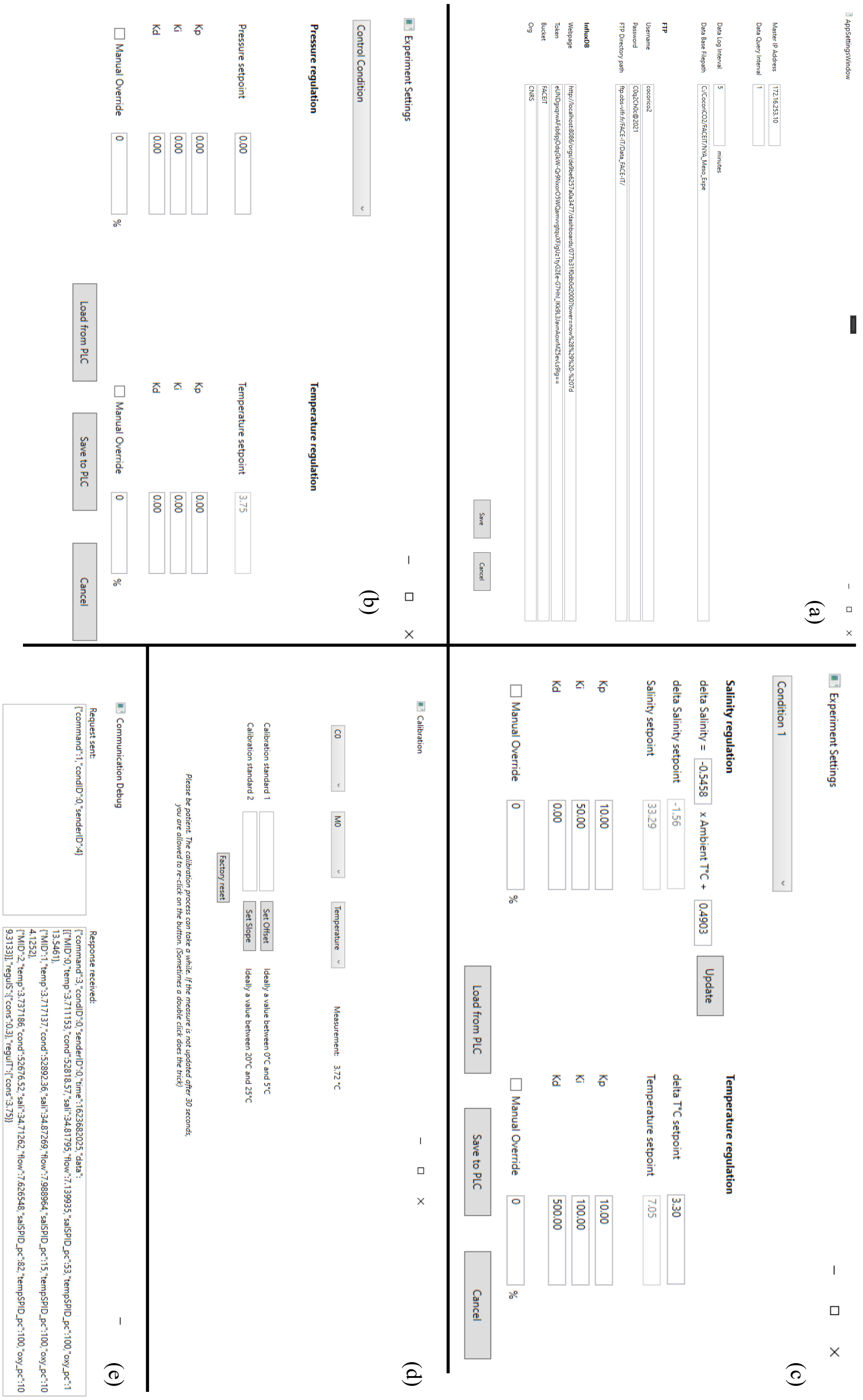

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Figure A4. Operation windows for the application and experimental settings (**a-c**). These windows are found under the 'settings' tab. Operation windows for sensor calibration and debugging (**d, e**). These are found under the 'maintenance' tab.

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Figure A5. Mean temperature (**a**) and salinity (**b**)over the entire operation of the experimental system.

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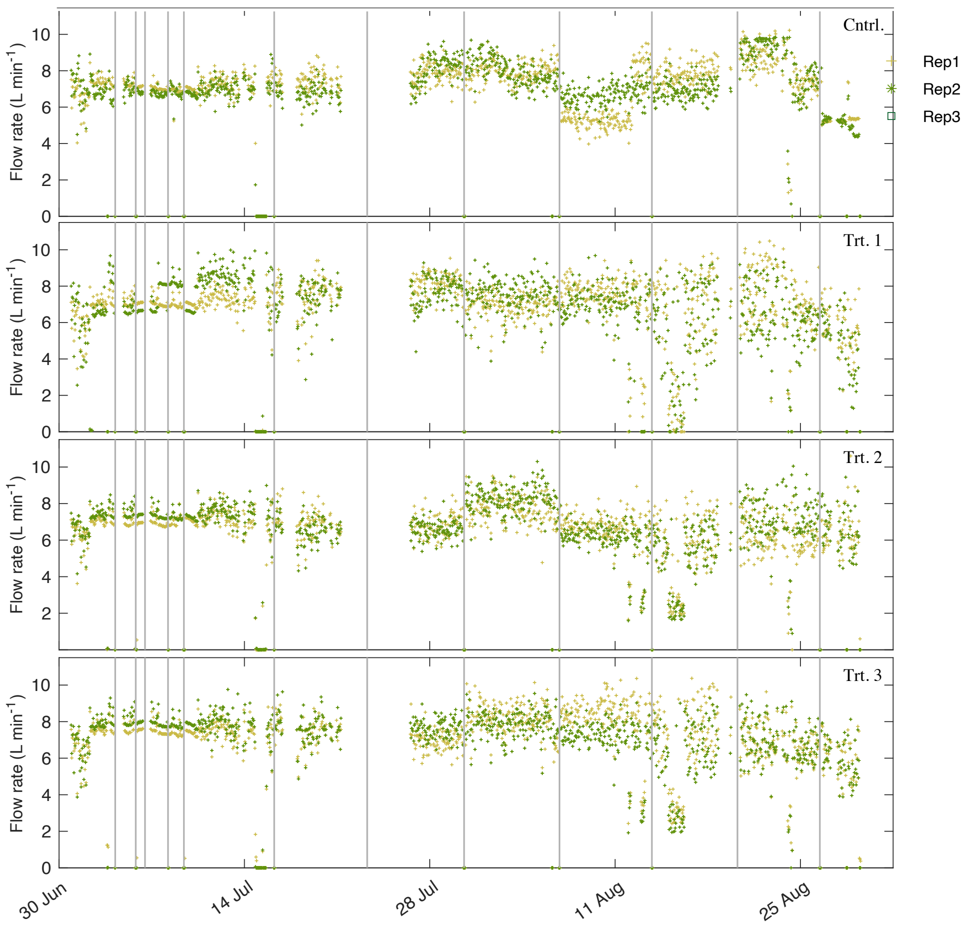

Figure A6. Flow rates for ambient-control, and treatments 1-3 for the entirety of the system operation. Black isoclines are when incubations were performed and the system was shut-off for a period of 3 h. Flow rates went to zero at these times.

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Figure A7. Electrical cabinet used for SalTExPreS

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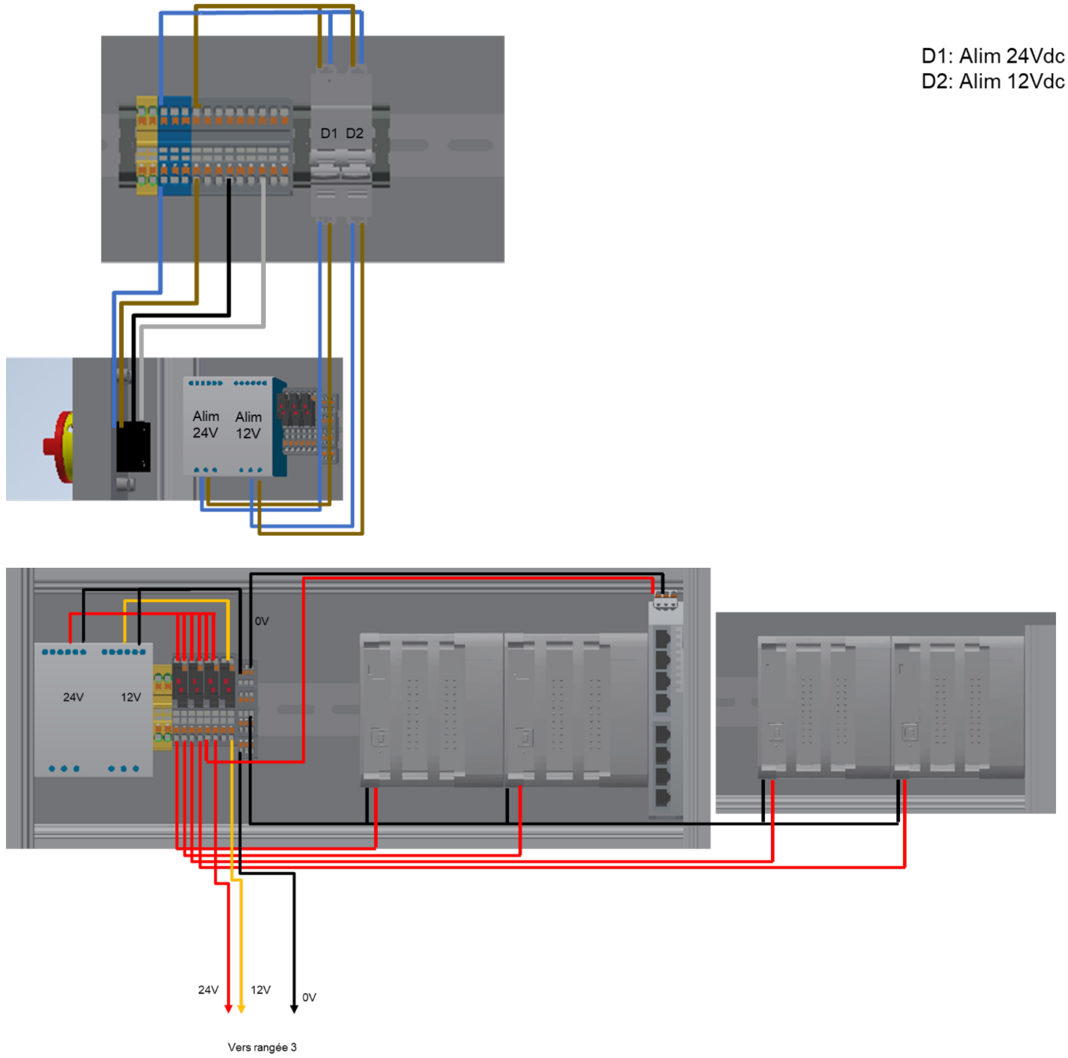
Figure A8. Electrical schematic for wiring within the electrical box.



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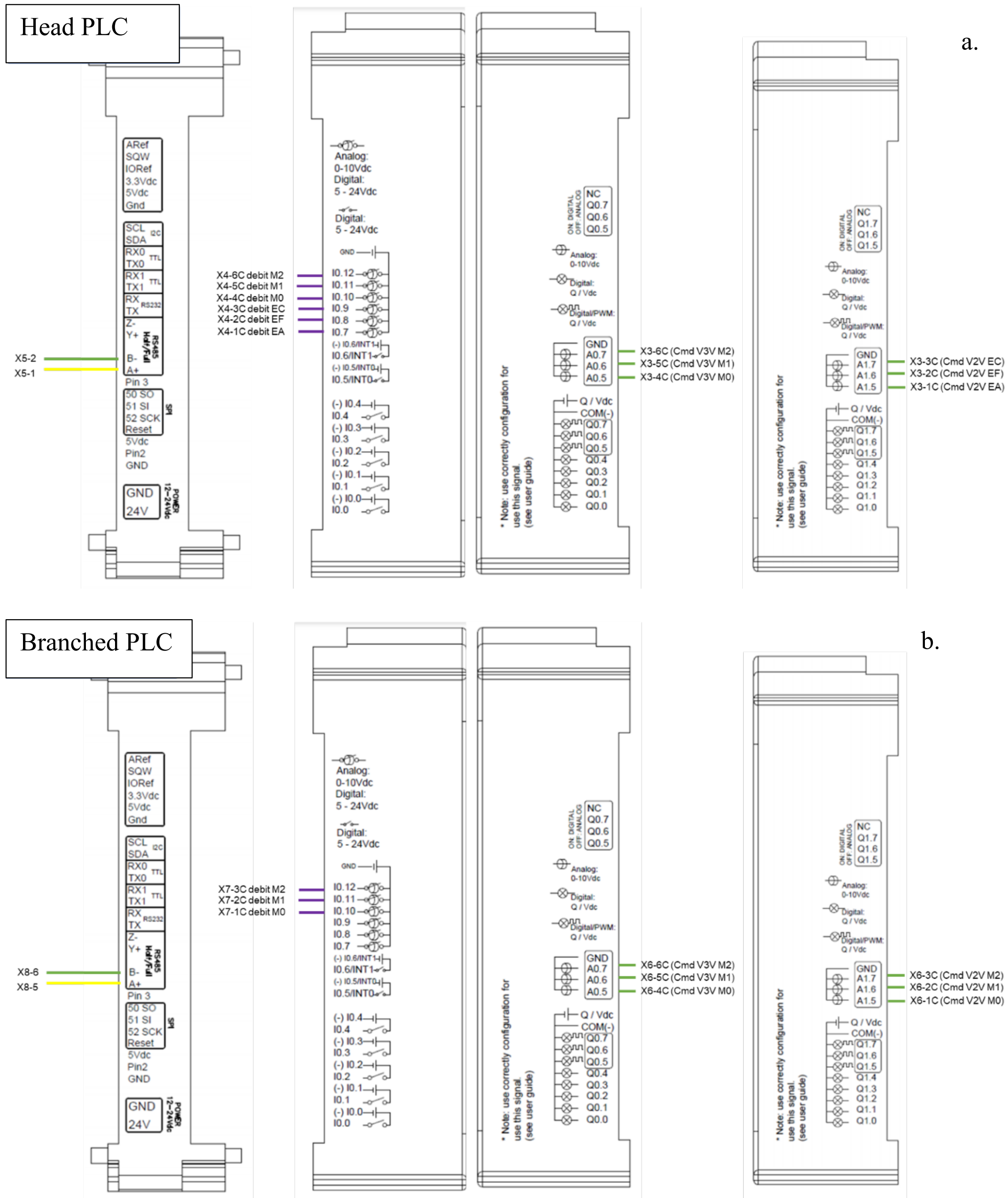

Figure A9. PLC controller diagram for Head (a) and Branched (b) operations.

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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	Albatros F13T	1
	PVC-U tubing and fittings		20mm, 32mm & 50mm diameter	–
	Insulated flexible hose		19mm diameter	100 m
Sensors				
	Conductivity / temperature	Aqualabo	PC4E	12
	Oxygen	Aqualabo	PODOC	12
	Pressure	Siemens	7MF1567-3BE00-1AA1	3
	Flow rate	IFM	SV3150	12
Actuators				
	Pressure regulation valves	BELIMO	R2025-10-S2 with LR24A-SR motor	3
	Temperature regulation valves	BELIMO	R3015-10-S2 with LR24A-SR motor	12
	Salinity regulation valves	BELIMO	R2015-10-S2 with LR24A-SR motor	6
Automation cabinet				
	Cabinet	Fibox	FIB8120017N	1
	Security switch	KRAUS-NAIMER	KNA002245	1
	12 vdc power supply	Lambda	LAMDRL30-12-1	1
	24vdc power supply	Lambda	LAMDRB240-24-1	1
	PLC	Industrial shields	Mduino-42+	4
	Ethernet switch	HIRSCHMANN-INET	HIR942132002	1