ASSOCIATION OF EUROPEAN MARINE BIOLOGICAL LABORATORIES EXPANDED

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Title: Association of European Marine Biological Laboratories Expanded

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Technical design specifications for experimental systems

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Websites, patent filling, etc.	☐
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Confidential, only for members of the consortium (including the Commission Services)	☐

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Document properties

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Abstract

This deliverable provides a summary of technical design specifications for experimental systems developed during WP10 (Development and standardization of on-site instrumentation for experimental marine biology and ecology), presented in different categories of experimental system (tidal simulation systems, pH control systems, multi-parameter monitoring and regulation, pressure sensors, lighting systems, raceways).

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Table of contents

1. Introduction

Understanding the responses of biological entities (individuals, species, species assemblages) to single and/or combined environmental forcing(s) is a focal point of biological research. In addition to field sampling and *in situ* experiments, controlled *in vitro* experiments are crucial in this regard. European marine biological stations have a long history of providing facilities for field collection and *ex situ* observation of marine organisms, but modern marine biological research increasingly requires access to state-of-the-art on-site experimental facilities. In light of the diversity of marine organisms in terms of size, trophic modes and reproduction processes, there is significant demand for facilities for conducting on-site experiments at different scales, from a few litres up to several cubic metres. Experiments generally require precise regulation of multiple environmental parameters, including (but not exclusive to): temperature, light quality and quantity, salinity, dissolved and/or particulate nutrient availability, oxygen concentration, pCO_2 and other carbonate chemistry parameters, the alternation of emersion/immersion cycles (reproduction of tidal cycles), and physical water mass dynamics (flow speed and turbulence). Over the years, systems designed to regulate some (but rarely all) of these parameters have been developed in most of the marine stations involved in the consortium. However, these systems have most often been developed for specific research projects by in-house (and sometimes external) research teams, often by non-permanent staff. The result is that the technical knowledge and expertise required to maintain and operate (and therefore provide access to) these systems has often not been perpetuated beyond the duration of specific projects. Another consequence is that systems have been designed without concertation between marine stations, meaning that inter-site reproducibility of experiments is most often highly compromised.

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2. Objective

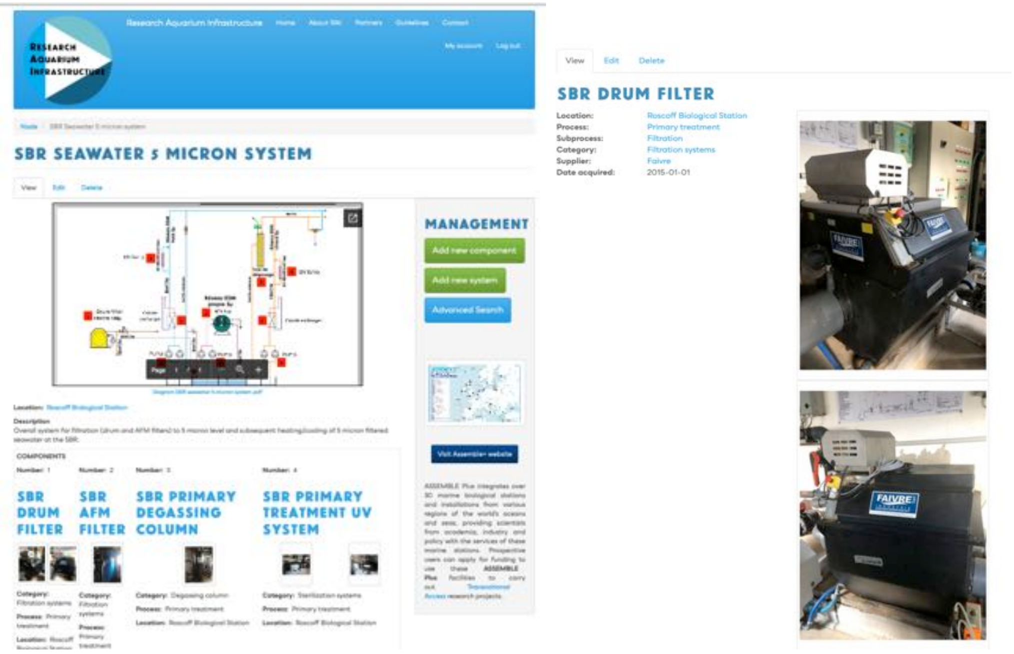
To produce a set of detailed technical specifications and guidelines to facilitate future cross-consortium implementation of standardized experimental systems for the culture of marine organisms for biological and ecological research.

3. WP partners

- Sorbonne University: Station Biologique de Roscoff (SBR) ; Observatoire Océanologique de Banyuls (OOB) ; Institut de la Mer de Villefranche (IMEV)
- University of Vigo ECIMAT marine station (UPV-EHU)
- Kristineberg marine station, University of Gotenborg (UGOT)
- University of Algarve (CCMAR)
- Stazione Zoologica Naples (SZN)
- Inter-University Institute Eilat (IUI)
- University of Helsinki (UH)
- Flanders Marine Institute, University of Gent (VLIZ)
- Marine Biological Association Plymouth (MBA)
- University of Galway (NUIG)

4. Design specifications

Collaborative R&D in this WP focussed on different categories of experimental equipment according to the interests of different partners. An on-line database (Fig. 1) was created in the context of this WP with the objective of cross-consortium recording of detailed technical information for experimental systems and associated infrastructure.



SBR SEAWATER 5 MICRON SYSTEM

COMPONENTS

SBR DRUM FILTER

SBR AFM FILTER

SBR PRIMARY DEGASSING COLUMN

SBR PRIMARY TREATMENT UV SYSTEM

SBR DRUM FILTER

Location: Roscoff Biological Station
Process: Primary treatment
Subprocess: Filtration
Category: Filtration systems
Supplier: Faivre
Date acquired: 2015-01-01

Figure 1: Web portal of the Research Aquarium Infrastructure on-line database

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It is planned to expand contributors to this database to other EMBRC partners and integrate the web interface into the EMBRC site. This report provides a summary of developments made on experimental systems in the following categories:

4.1. Tidal simulation systems

UPV-EHU tidal simulation system

The tidal simulation system controls water level in benthic tanks (0.7m^3 fiberglass tanks) with a continuous water input and proportional control of the drain. The system has an ultrasonic level sensor that constantly monitors water level in the tanks, with an electrovalve system adjusting it automatically and constantly to the selected curve. Tidal curves can be programmed in advance, selecting water level as speed of increase/decrease of water level.

Figure 2: Ultrasonic sensor for water level monitoring.

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Figure 3: Electrovalve system to control the drain and water level in mesocosm tanks.

In addition to control of tidal simulation, temperature and salinity can be monitored and controlled in thus system, thus allowing simulation of conditions found in estuaries.

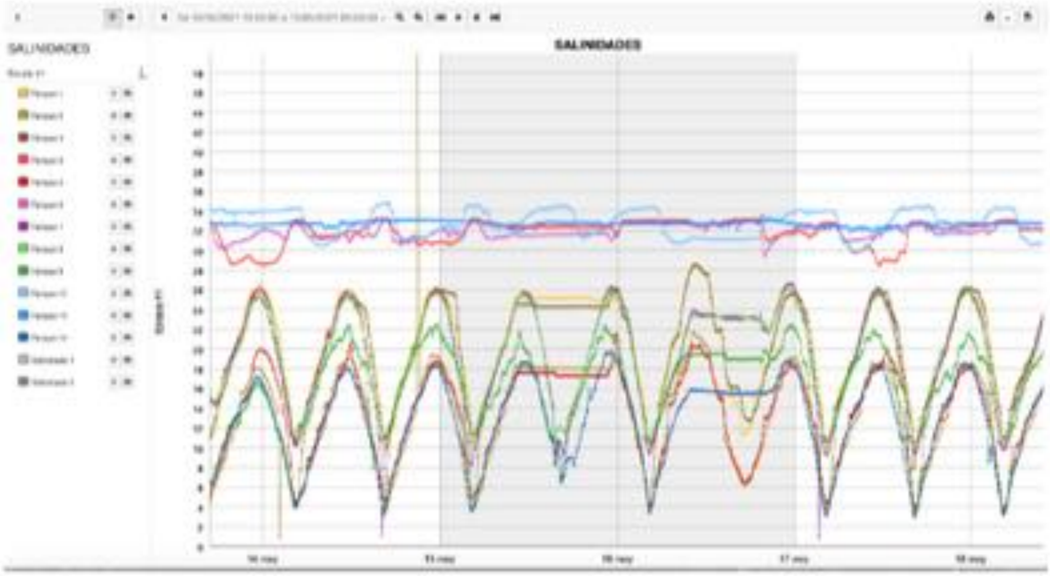

Figure 4: Differential salinity variation during the tidal cycle in different experimental tanks.

The cost of the tidal simulation system was ca 13k€.

SBR tidal simulation system

The SBR developed a gravity-based tide simulation (Fig 5).

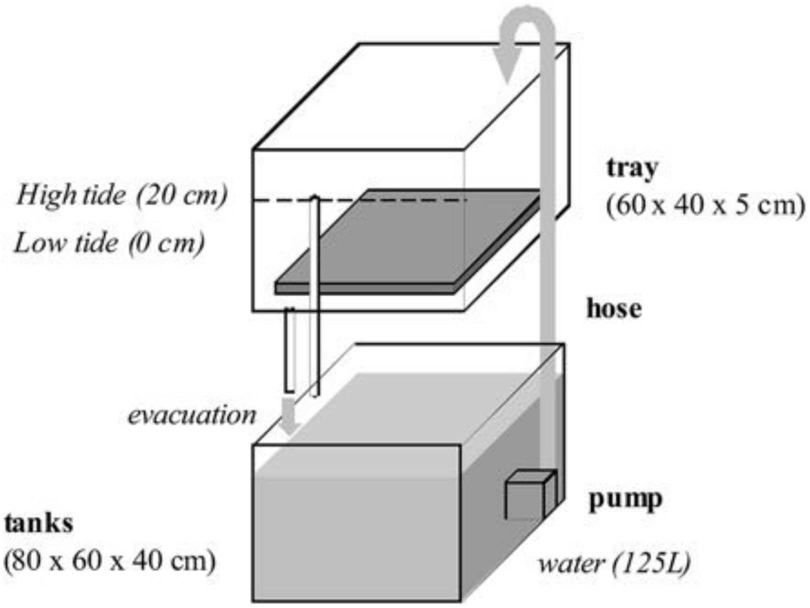

Figure 5: Principle of gravity-based tidal simulator

To simulate low tide, tank A flows into tank B by natural gravity draining through evacuation 1. Pump 3 is stopped and the arrival of fresh seawater is closed. To simulate high tide, Pump 3 starts, its flow is higher than the maximum flow of the evacuation 1. The level mounts in the tank A until the evacuation 2. The flow rate of the pump 3 is calculated to be absorbed by the evacuations 1 and 2 to avoid overflow.

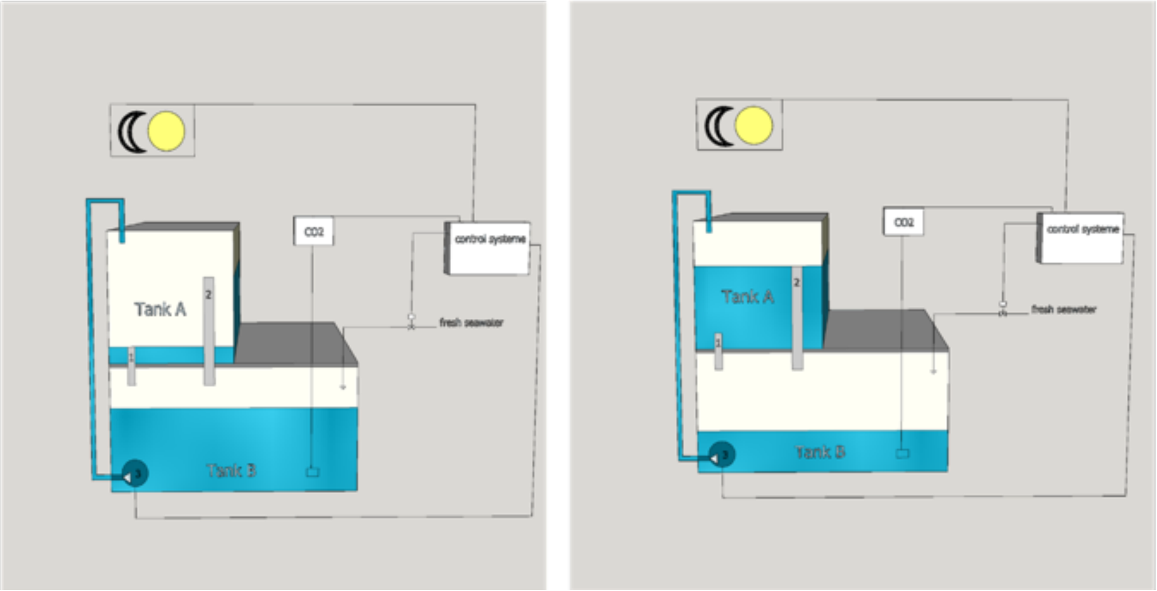

Figure 6: Schematic representation of gravity-based tidal simulator

Temperature (via Techo units), salinity and pH (via CO_2 injection) can be controlled in the header tank and regulated via an Aquatronica control module (Fig 7).

S 00-A PH TAMPON 7.63pH
S 01-A Densité 1165.8g/l
S 02-A TEMP ESTRAN 19.4°C
S 04-A PH ESTRAN 7.59pH
S 02-B Niveau BAS
S 03-A Redox 338mV
PU 00-D EDM NEUVE OFF
PU 00-F APPORT CO2 OFF
PU 00-A POMPE MAREE OFF
PU 00-B POMPE COURANT OFF
PU 00-C BRASSAGE TAMP OFF
PU 00-E ECLAIRAGE JOU ON

Figure 7: Aquatronica control module

Different versions of the system have been developed with tank volumes between 300 L and 25 L, with replicate tanks (up to 12) in versions with smaller volume tanks.

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Figure 8: 300L tank version of the gravity-based tidal simulator with pH control.

Figure 9: 50L tank version (with 6 replicate tanks) of the gravity-based tidal simulator.

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4.2. pH control systems

UGOT Experimental acidification systems

At KMRC, options were explored to create realistic natural variability in experimental systems that could be transferred to any site of the ASSEMBLE+ network as well as in any marine station, including in developing countries. This would significantly contribute to the achievement of the SDG 14.3 goal.

Two approaches were tested: (i) using existing technology (pH stats) available in several stations within the ASSEMBLE+ network; (ii) a simplified protocol using no technology and based on manual manipulation of seawater chemistry. Both approaches followed the established best practices in the field of ocean acidification for the manipulation and measurements of the carbonate chemistry (Riebesell et al. 2011).

(i) In natural coastal ecosystem, variability in carbonate chemistry can be driven by various processes including upwelling, pollution, currents. However, biology is often one of the main drivers through the balances between photosynthesis, respiration and calcification. For example, a diurnal cycle with high pH during the day (photosynthesis) and low pH during the night (respiration) is common in European coastal waters. We explored the possibility to take advantage of this natural process to create variability into experimental system.

- pH was manipulated using a pH stat system (AquaMedic) in a 50L header tank continuously fed with deep seawater pumped directly from the vicinity of the marine station and heavily aerated to equilibrate at atmospheric CO_2 (400ppm). Four different pH levels were tested ranging between 8.1 and 7.5, covering present and future pCO_2 under different IPCC scenarios.

- Water from this header tank was feeding experimental aquariums containing seagrass at the same density as observed in the field (Figure 10a). Aquariums were exposed to a 12:12 photoperiod.

- The carbonate chemistry was then monitored following best practices in both the header tank and the experimental aquariums.

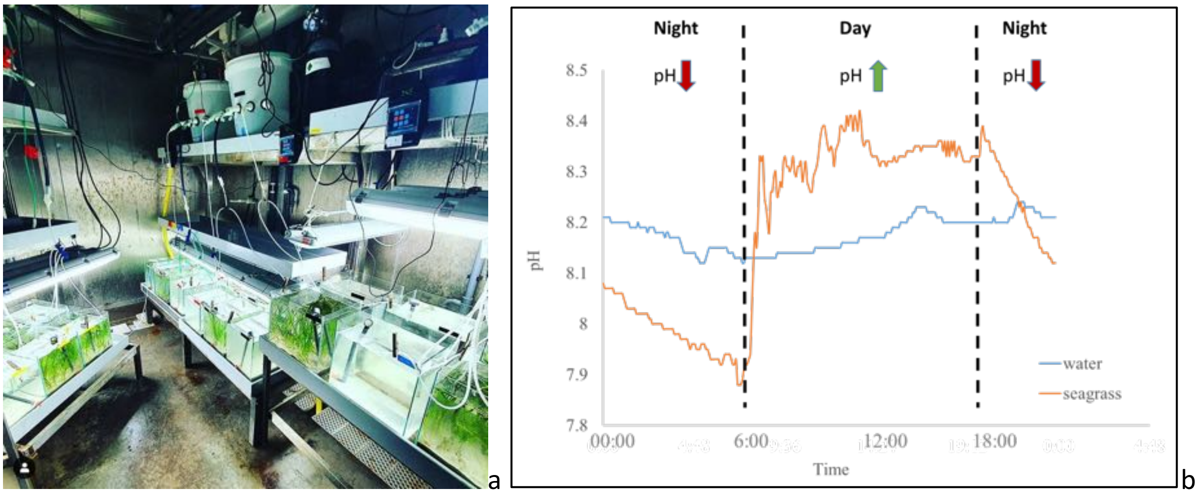

Figure 10 – a. Experimental set-up using pH stats and seagrass. B. Example of the carbonate chemistry measured in the header tank (water) and in the experimental aquarium (seagrass).

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The presence of seagrass led to a 0.5 pH variability like what was observed in the field (Figure 1b). This system was successfully tested to evaluate the impact of pH variability on sea urchin larvae. The details of the experimental set-up and the results of the experiments will be published in Cossa et al. (*in prep*).

ii. Not all laboratories are equipped with pH stats. As an alternative, we explored the possibility of manual water changes to create natural variability in an experimental unit (1L). This was tested for an experiment aiming at understanding the role of pH variability on sea urchin larvae. The water used for the experiment were equilibrated manually at different target pH in 30L tanks using CO_2. Water in the experimental unit was changed manually every 12 hours. Larvae were filtered on a 100mm mess ensuring that they were always under water and transferred to fresh seawater at the target pH before being returned to their aquarium.

Measurements of the carbonate chemistry confirmed that this approach allowed to maintain the pH at the desired levels and create a day-night pH variability (Table 1).

	pH_T	$p$$CO_2$	Ωcalcite	Ωaragonite
Target pH				
• 8.1	8.13±0.01	348±12	4.21±0.09	2.68±0.06
• 7.8	7.82±0.02	816±54	2.33±0.09	1.48±0.06
• 7.5	7.53±0.02	1655±97	1.26±0.05	0.80±0.03

Table 1 – pH in the experimental units after manual manipulation. No significant differences were observed between replicates (ANOVA, data not shown).

The details of the experimental set-up and the results of the experiments will be published in Duvane et al. (*in prep*).

Cossa D, Infantes E & Dupont S (*in prep*) Short-term pH Variability by Seagrass meadows: A hidden cost in marine calcifiers in future OA conditions.

Duvane J & Dupont S (*in prep*) Phenotypic plasticity on the sea urchin Echinus esculentus larvae under constant and fluctuating seawater pH conditions.

Riebesell U, Fabry VJ, Hansson L & Gattuso J-P (eds) (2011) Guide to best practices for ocean acidification research and data reporting. [reprinted edition including erratum]. Luxembourg, Publications Office of the European Union, 258pp. (EUR 24872 EN). DOI 10.2777/66906

Vargas CA, Cuevas LA, Broitman BR, San Martin VA, Lagos NA, Gaitán-Espitia JD & Dupont S (2022) Upper environmental *p*CO2 drives sensitivity to ocean acidification in marine invertebrates. Nature Climate Change. 12: 200-207.

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CCMAR Acidification Unit

An enhanced unit for ocean acidification experiments was developed at CCMAR, with CO_2 levels being controlled through pH. The overall characteristics of the Acidification Unit are:

- 3 independent systems: 2 CO_2 levels + Control
- Controlled CO_2 level between 410ppm and 2000ppm
  Control system with degassing column to assure 410 ppm of CO_2
- Each system's header tank can provide up to $4m^3/h$ of seawater to the experimental tanks
- Flow-through system
- Temperature control in 2 systems with heat pumps
- Continuous logging of pH and temperature of each header tank
- Continuous logging of pH, temperature, salinity, dissolved O_2, ORP and turbidity of the water entering each header tank
- Different types experimental tanks: 20L(48) ;110L (24); 650L(6)…

Before the water enters the acidification systems, salinity, dissolved oxygen (% sat, ppm), pH, temperature, salinity, ORP and turbidity are measured every minute. The values are obtained by an EXO1 multiparameter probe (YSI) and sent to a computer by serial communication (RS232). The data is received by a gateway, processed and sent to a web-based software (WebData Monitor) that collects the information and sends it to a postgres database. The pH and temperature values provided by the pH controllers are collected by ADVANTECH PLCs and sent by modbus ip to the aforementioned gateway and then gathered in the WebData monitor to be integrated in the same database. The PLCs also receive data regarding the operation of the system's water pumps and the water levels in each tank. The data can be accessed in real time through a web interface like GRAFANA that interrogates directly the database or through an opensource SCADA software (ScadaBR) that besides the real time access allows to obtain historical and hourly averages or the desired intervals. ScadaBR also allows the development of specific dashboards for each acidification system to define set points, failure alarms and alarms based on the combination of various information with the subsequent sending of warning emails.

Temperature control with heat pumps (KRIPSOL) was also installed in two of the three acidification systems, with a power of 9000BTUs, allowing, within certain limits, the maintenance of constant temperatures during the tests. To aid temperature control, the salt water supplied to each acidification system passes through a titanium plate heat exchanger using a water source with a constant temperature of 20ºC. In this way it is possible to significantly reduce the daily temperature fluctuations of the salt water.

A "Multiplexing in-line sensors array" is also planned to make possible measure in each experimental tank: temperature, salinity, pH and dissolved O2 values, every 2h. The SCADA software already in use will allow the integration and control of the "multiplexing of the sensors on line" with the integration of all the parameters measured in this and the other sensors. This aspect still needs further hardware development.

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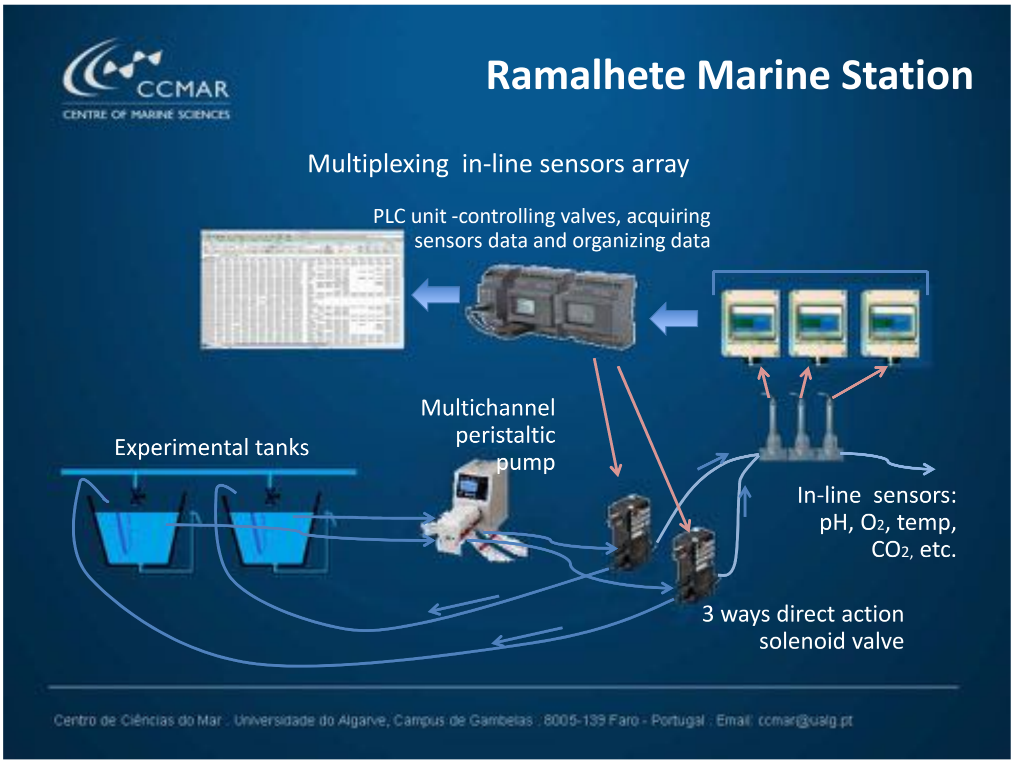

Ramalhete Marine Station

Multiplexing in-line sensors array

PLC unit -controlling valves, acquiring sensors data and organizing data

Experimental tanks

Multichannel peristaltic pump

In-line sensors: pH, O_2, temp, CO_2, etc.

3 ways direct action solenoid valve

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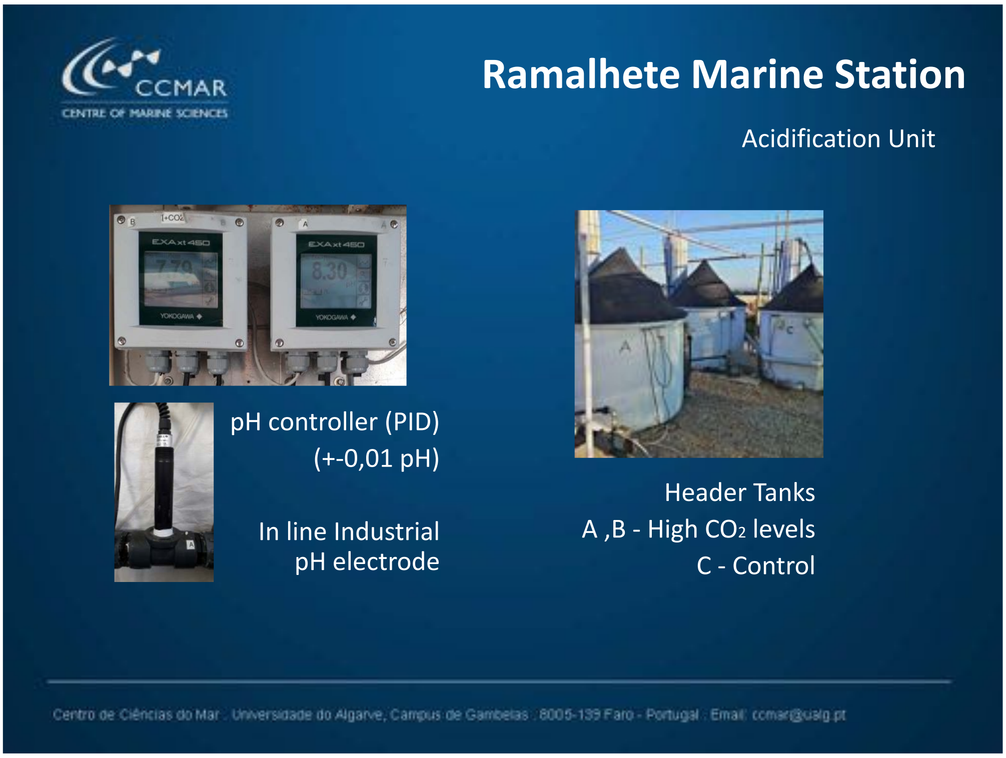
Figure 11: Overview of CCMAR Acidification Unit



Ramalhete Marine Station

Acidification Unit

pH controller (PID)
(+-0,01 pH)

In line Industrial
pH electrode

Header Tanks
A ,B - High CO_2 levels
C - Control

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Figure 12: CCMAR Acidification Unit: pH control and header tanks

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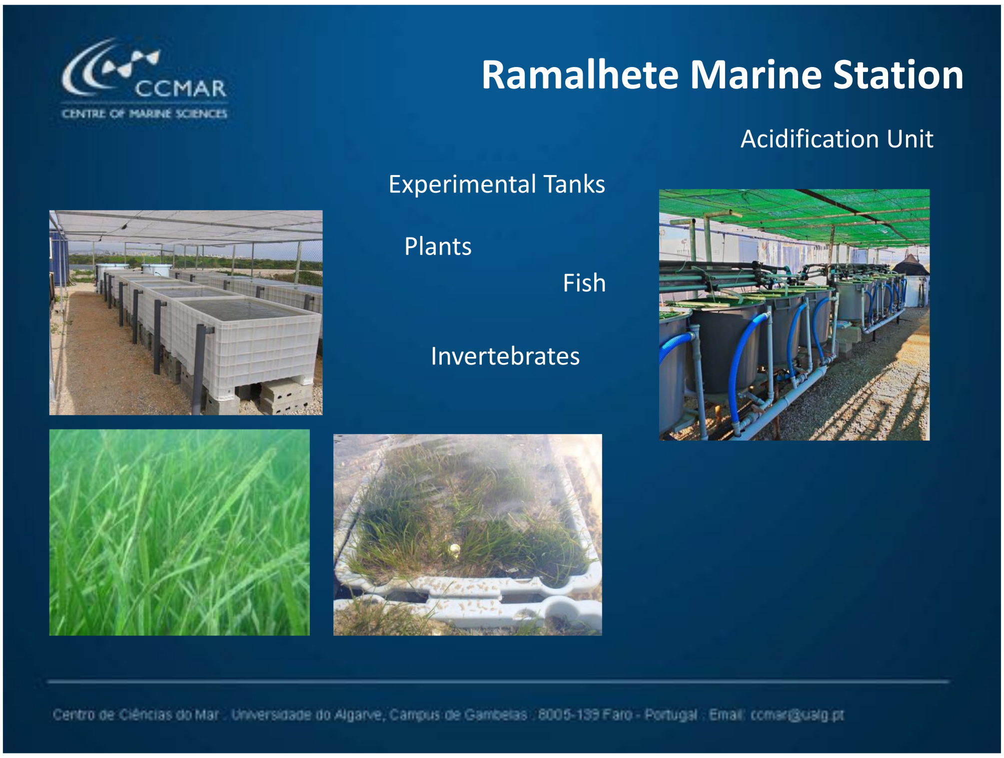

CCMAR
CENTRE OF MARINE SCIENCES

Ramalhete Marine Station

Acidification Unit

Experimental Tanks

Plants

Fish

Invertebrates

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Figure 13: Images of CCMAR Acidification Unit

MBA Mesocosm facility

MBA mesocosm facilities have been updated to install new Aqua Medic pH computers, and these are being tested in two long-term experiments on the effects of temperature, pH and salinity on the invasive oyster *M. gigas* and the *Mytilus edulis/galloprovincialis* hybrid.

4.3. Multi-parameter monitoring and regulation

IUI Red Sea Simulator

The Red Sea Simulator (RSS) facility at the Interuniversity Institute for Marine Science in Eilat, was designed to simulate climate change associated future conditions in the Red Sea. This *state-of-the-art*, large-scale, flow-through system comprises 80 independent experimental aquariums. pH and temperature in each aquarium can be set to a user-defined off-set from ambient reef values. The system is regulated and monitored by a two-armed robot, equipped with sensors on each arm. This facility formed a hub for international collaboration around corals response to environmental change with dozens of international scientists participating in research and has been viewed by hundreds of governmental scientific advisors, diplomats, and the public.

Work focused on improving continuous monitoring of the Red Sea Simulator (RSS) flow through aquaria system. A new monitoring robot was assimilated with better sensor- carrying capabilities and improved mechanics. The new robot which monitors 64 tanks (40L each) 24/7 was equipped with sensors for dissolved oxygen, pH, temperature, salinity, a camera on each arm and a Monitoring Pulse

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Amplitude Modulated (PAM) fluorometer (Fig 15). The new robot was tested and successfully assimilated to run during experiments on corals in the RSS.

Figure 14: Red Sea Simulator RSS

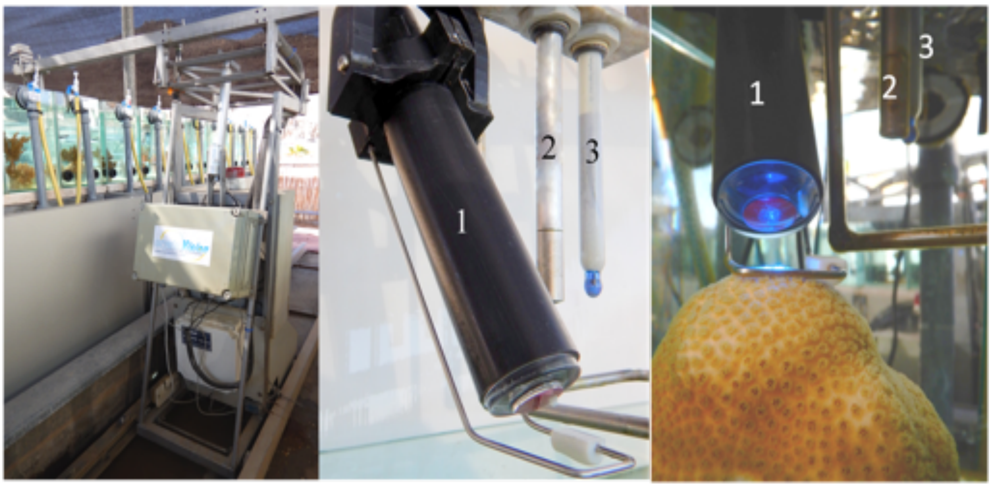

Figure 15: In situ PAM analysis integrated into RSS

A major challenge was overcoming a drift in the accuracy of sensors in the 80 experimental tanks, mainly temperature sensors. It was found out that over time, some sensors were drifting and not all at the same pace. This required improvement of hardware but also a software improvement. An algorithm based on replicate tanks was set to alert on any unexpected deviation from the treatment temperature value. Consequently, in case of a sensor drift, the experimentalist is notified of peculiarities in a given tank. The data and the sensor can be checked and in case of odd readings, the sensor gets calibrated and/or replaced. Better sensors were purchased to replace the existing ones. The new setup was already tested in a couple of large-scale experiments in the RSS and was found to be consistent and reliable in a range of environmental conditions.

The first experiment was used to study how corals respond to marine heatwaves (MHWs) at different scenarios of climate change (ocean warming and acidification). Simulations of RCP 8.5 demonstrated that corals were more sensitive to MHWs compared with corals under RCPs 2.6 and 4.5 and exceeded their bleaching threshold at a lower onset rate, lower intensity, and shorter duration of MHW. Similarly, recovery from each of the scenario’s RCP/MHV were examined. This section of the project

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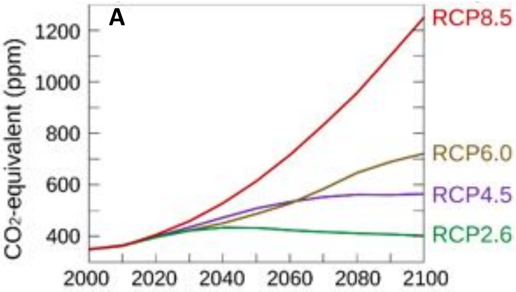
generated an enormous dataset on the species-specific responses of Red Sea corals to the combined effect of long-term climate change (warming and acidification) and shorter MHW scenarios and assist projections at reef scale.

In a second experiment the RSS was programmed to automatically run selected simulations of marine heatwaves. Since the RSS follows the diurnal and seasonal temp and pH at sea, the rise in temperature in spring to summer, is reflected in the experimental tanks. On top of the gradual rise in sea surface temperature, abrupt, MHW-like conditions were successfully simulated. Five MHWs were simulated with three RCP scenarios in the background (Figure 16).



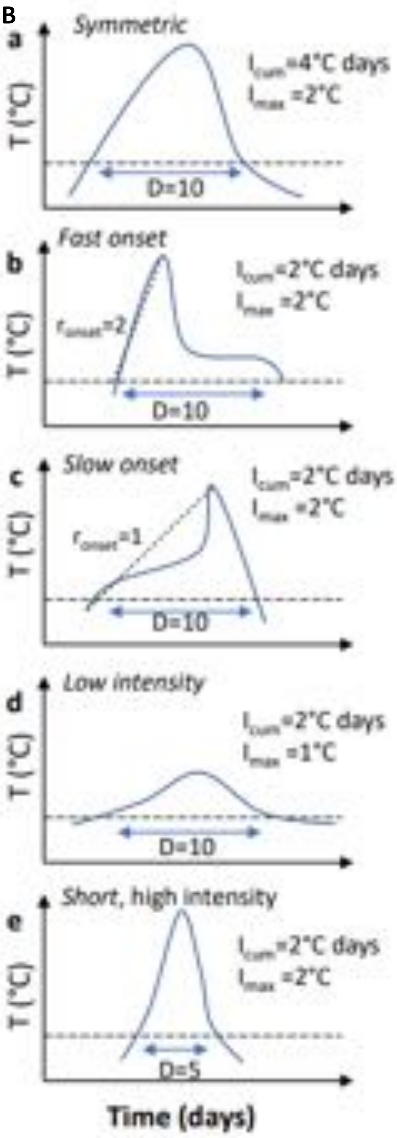


Figure 16, **A***: atmospheric CO2-equivalent concentrations (in parts-per-million-by-volume (ppmv)) according to the four RCPs used by the IPCC.* **B***: taxonomy of heatwaves distinguished by duration (D), maximum intensity (i_{max}), cumulative intensity (i_{cum}), and rate of onset (r_{onset}).*

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UH mesocosm systems

At the Finnish ASSEMBLE Plus partners (University of Helsinki, Tvärminne Zoological Station (UH-TZS), together with the Third Parties University of Turku, Archipelago Research Institute (UTU-ARI) and Åbo Akademi University, Husö Biological Station (AAU-HBS)), development of experimental facilities and related instrumentation focussed on a range of systems for testing the ecosystem effects of climate change, for example heatwaves.

At all three stations (TZS, ARI, HBS), existing indoor as well as outdoor experimental facilities have been upgraded, new mesocosms have been installed and functional systems for manipulation and monitoring of temperature and salinity have been developed.

The development at TZS included indoor mesocosms (12 x 600 litre), with lights and sensors for oxygen, conductivity and temperature (Fig 17). The effects of heatwaves are of particular interest. These systems are suitable for pelagic as well as benthic studies, including long-term incubation and manipulation of intact sediment cores, taken with a big box corer.

- For the mesocosms, Aqua Medic LEDspot 200 W flex lights are used (suitable also for e.g. seagrass work) and in the smaller climate rooms, Aqua Medic aquarius plant 120 lights are used.
- For monitoring dissolved oxygen levels, salinity and temperature in the water in both indoor and outdoor mesocosms, standard loggers were chosen: Onset HOBO U26-001 Dissolved Oxygen Logger, Onset HOBO U24-002-C Conductivity Logger.
- In addition to controlling room temperature in the experimental facilities (4-20°C), water temperature can be controlled with tank-specific heaters and chillers (TECO TK2000H water heater & chiller, TECO TK2000 water chiller, EHEIM Type 1250 219 water pump, Fluval FX4 water filter).

Figure 17: TZS Indoor mesocosm system

In addition, TZS developed and successfully tested a set of heated benthic chambers (Fig 18) for subtidal experimentation done using SCUBA diving, which allow us to study *in situ* the effects of e.g. marine heatwaves on the functioning of benthic ecosystems (including macrofaunal bioturbation, benthic nutrient cycling and the physiology of the fauna). The chambers were developed in

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collaboration with an underfloor heating company. The next step is to develop the chambers to work on hard substrates and in remote locations (e.g. solar panels for power).

Figure 18: Prototype benthic chamber for heatwave experiments

OOV Monitoring and control of environmental variables

OOV established a technical description for relatively small experimental systems (litre to several dozens of litre per tank) in order to control mono- or multi-parameters (temperature, pH and light…).

The configuration of experimental system (Fig 19) can be used for different types of macro-organisms, benthic and planktonic For benthic species (i.e. sessile) regular tanks can be used; however, regarding macroplanktonic species (jellyfish, salps…), specific tanks (Kreisel tank) are needed to maintain organisms alive during the experiment.

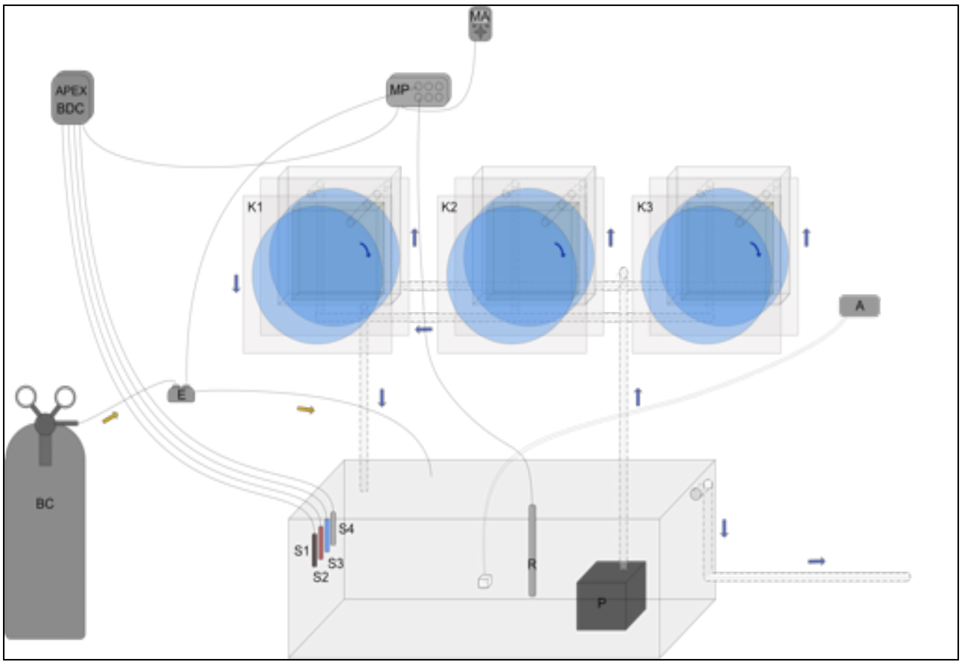

Figure 19: OOV multi-parametric experimental system design (with Kreisel tanks)

Legend:

BDC : Control board

MP : Multi-socket plug

MA : display monitor/module

BC : CO2 bottle

E : Solenoid valve

S1 : Temperature probe

P : Water pump

S2 : ORP probe

S3 : pH probe

S4 : Salinity probe

H : heater

A : Air pump

K1, K2, K3 : Kreisel 1, 2 et 3

Automatic controlled system is now accessible and democratized for the general public aquariologist. Monitoring of parameters and management of aquarium maintenance are maintained. So, to supervise one or several experimental systems, we used a controller named APEX from Neptune store (https://www.neptunestore.eu) (Fig 20).

This system allows to:

- control different parameters for a targeted value, configurable on site or remotely,
- define an acceptable threshold and send an alarm (notification) in case of exceeded threshold,
- monitor and acquire automatic data for different parameters (temperature, pH, conductivity…) with high frequency of measurement (definition of the time step).

The control system centralizes all data collected by the probes and transfers them to the server in real time via wireless connection. An Apex Fusion software or mobile phone application (iOS or Playstore) allows to remotely consult graphs after connection to a secured account (login and password). The Apex application can show graphs in real time and parameters can be modified. Recorded data of parameters can be downloadable.

A guideline of the system installation and its uses with tips was carried out.

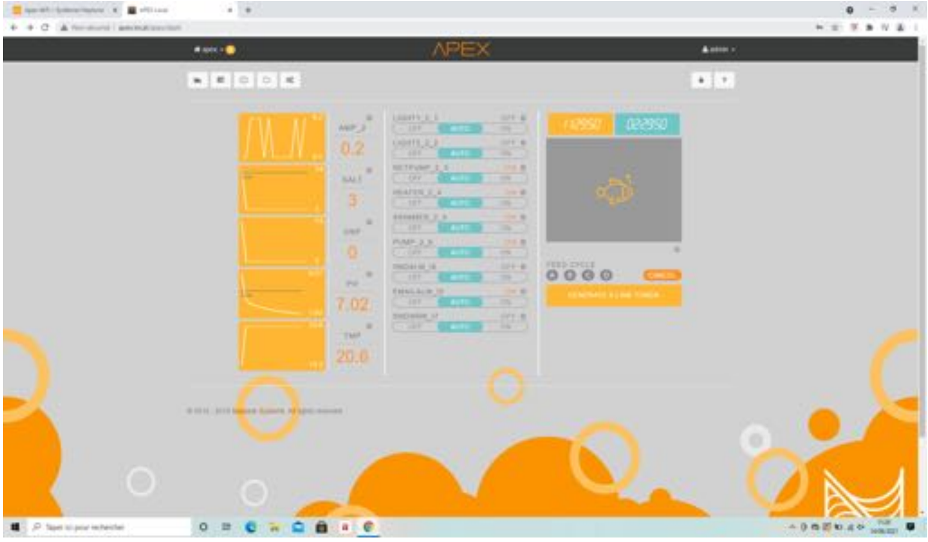

Fig 20: Screen of Apex Fusion software (computer version)

ECIMAT Monitoring and control of environmental variables

This system has been designed for real-time monitoring, visualization and data-base creation of the environmental variables in the experimental tanks at the ECIMAT mesocosm facility. The system has a central unit to compile data and 12 portable units (IP68) with wireless connection, to which sensors are connected allowing data to be sent in real-time to the central unit. Each of the portable units can connect to up to 4 sensors with 4-20mA signal communication. Aqualabo sensors for temperature, salinity, pH and dissolved oxygen are installed, but other variables (turbidity, PAR, etc) could also be monitored.

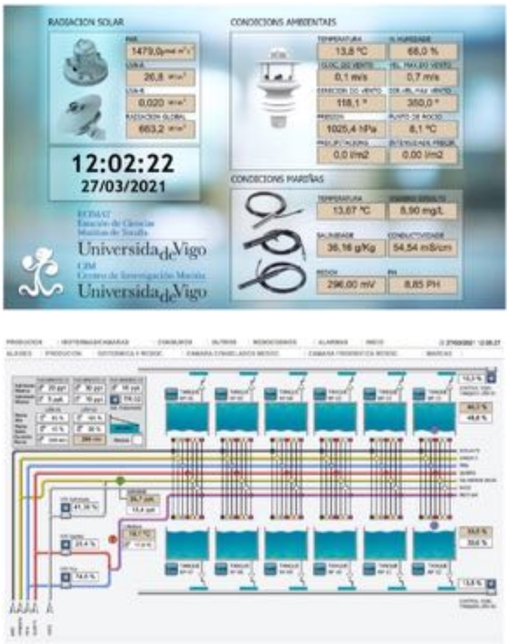

Figure 21: Screens for real-time monitoring of environmental variables and control of the tidal cycle and incoming water conditions in experimental tanks.

Figure 22: Temperature, salinity, pH and dissolved oxygen sensors in one of the experimental tanks.

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The 12 portable units will allow monitoring the different mesocosm tanks of the facility, from benthic mesocosm tanks (0.7m^3 fiberglass tanks) to the pelagic mesocosms (1-2m^3 polyethylene bags installed into a 70m^3 concrete pool). Monitoring of environmental conditions is completed with a meteorological station (MaxiMet GMX600, with temperature, humidity, pressure, wind and precipitation measurements) and Delta Ohm sensors for global radiation (PYRA03), PAR (LPPAR03), UV-A (LPUVA03) and UV-B (LPUVB03).

Figure 23. Sensors for global radiation, PAR, UV-A and UV-B in the ECIMAT mesocosm facility.

The cost of the whole system (including probes) was approximately 85k€.

OOB Experimental ecotoxicology facility

OOB worked on the development of new microcosms able to perform ecotoxicological tests according to health and safety standards regarding the use of pollutants. A room for “toxicological and ecotoxicological experiments” (20m^2) has been installed during the recent work in building A of the OOB. Several recommendations from different sources were followed, including “Biosafety in Microbiological and Biomedical Laboratories” (CDC/NIH publication) and CFR section on toxin safety from the US government (Title 32 CFR 627.28 and 627.29). Decisions on risk vs. cost vs. available floor space were made during the risk assessment process when planning new facilities. The room has a dual high-efficiency particular air (HEPA) filtration in the ventilation system, which allows working with powdered toxins or for operations which could generate aerosols. The secondary HEPA (in series) ensures that toxins are not released inadvertently into the environment (a typical HEPA filter is 99.97% efficient). The exhaust ventilation system has an emergency back-up power generator, which offers from 10 to 12 room volume air changes per hour, thus ensuring a reasonable amount of negative pressure (and proper airflow) for the toxin work area. Exhaust ventilation systems employ a visible and audible alarm for loss (or severe reduction) of exhaust air. The room maintained a negative pressure compared to adjoining rooms and corridors (airflow velocity 100 lfpm). An emergency shower, eye wash station, and hand-washing sink is readily available close to the room. The room (20m^2) can accommodate a maximum of 6 persons. It is thermostatically controlled (15-25°C), it has electricity

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and ceiling lighting. It is equipped with a tempered glass wet bench. A toxic chemical fume hoods was installed to safely ventilate volatile chemical fumes and vapors. The room is supplied with fresh water and sea water with mechanical filtration systems from 200 to 0.2 microns. It can accommodate a 300L tank for the decontamination of effluents by treatment with activated carbon, if needed. A good work practice booklet and specific formation for the safety conducting toxic tests has been developed for any person working in this room, including checklist and procedures in chronological order, use of protective clothing, decontaminating materials, etc.

4.4. Pressure sensors

VLIZ: Porewater pressure sensors to study behavior of benthic invertebrates

Behavioural activities of infauna are at the base of ecosystem functioning through their impact on seabed biodiversity, sediment dynamics and biogeochemical cycling. Studying such activities is however challenged by the hidden (i.e. subsurface) life habit of infauna. However, behaviours such as feeding, burrowing, defaecation, burrowing, pseudofaeces production, burrow ventilation, etc. can be inferred from the hydraulic changes these activities produce in the sediment pore water. To investigate infauna behaviour we built pressure sensors (Honeywell type 26PC) into a plastic tube (Ø = 1.2 cm, height = 11.5 cm) with a sealed bottom and open top (Figure 24). Each sensor contains a pressure port close to the wall of the tube and a reference port in seawater plenum inside the tube. Sensors are positioned at a specific depth distanced typically < 1 cm away from the study animal depending on the autoecological knowledge of the fauna and the sediment type. The hydraulic activities are recorded at a rate of 200 Hz (National Instruments with SignalExpress 2014 software). Sensors are calibrated before deployment by stepwise filling of a tank to different heights (i.e. pressures) of sea water used in the experiments to determine the relationship between sensor output (mV) and hydraulic head. In total hydraulic activities of 32 animals can be studied independently and simultaneously for several weeks depending on the sample frequency and data storage capacity. A r-script is produced for data processing, including smoothing using a running median per hour to reduce noise. Porewater hydraulic signatures (i.e. waveforms, Figure 25) are linked to different behavioural activities based on simultaneously recorded time-lapse footages and/or solute dynamics using microsensors.

The developed facility has been used to investigate the behavioral consequence of variable pressures in the marine environment, such as the ocean warming, acidification and noise pollution.

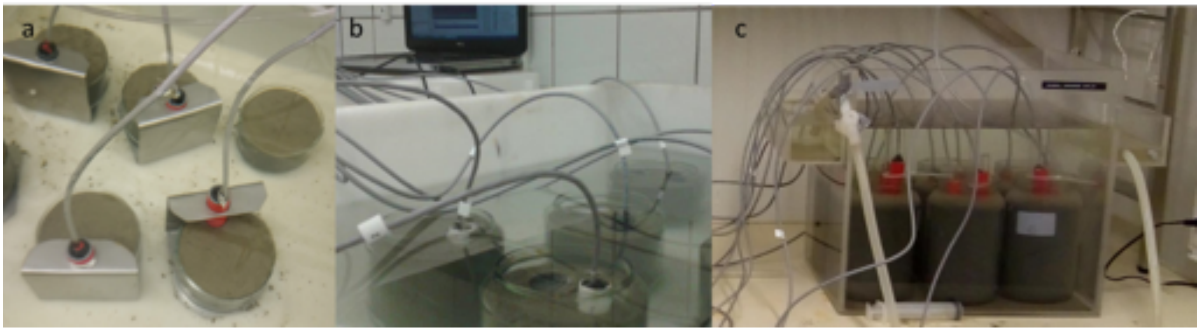

Figure 24. Pressure sensors deployed to study the hidden behaviour of the bivalves *Abra alba* (a) and *Scrobicularia plana* (b), and the polychaete *Lanice conchilega* (c).

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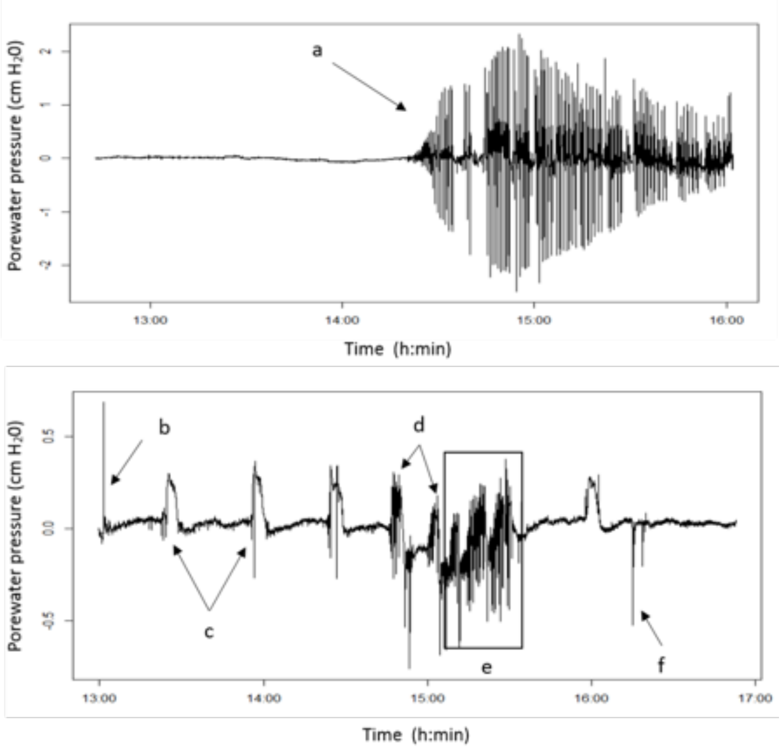

Figure 25. Porewater records showing different pressures characteristics related to behaviour of the infaunal clam *Scrobicularia plana* representing specific behaviours: (a) burrowing, (b) pressurisation during respiration, (c) suspension feeding, (d) deposit feeding, (e) defecation and (f) valve claps.

4.5. Lighting systems

OOB LED Microplate light simulator

OOB developed a system for measuring the growth of microorganisms and automated recording of luminescence under controlled conditions of light wavelength (red, blue green) provided by individual LED lights for each well of multi-well plates. Developments focussed on allowing a wide range of light intensities (1 to 1000 microEinsteins/m^2.s^1), improving homogeneity of light from well to well, integrating 4 LEDs for each well covering the whole light spectrum (white, blue, red, green), developing programmes for simulation of realistic sunlight conditions, integrating temperature control (6 plates) and a cheap and open source robot

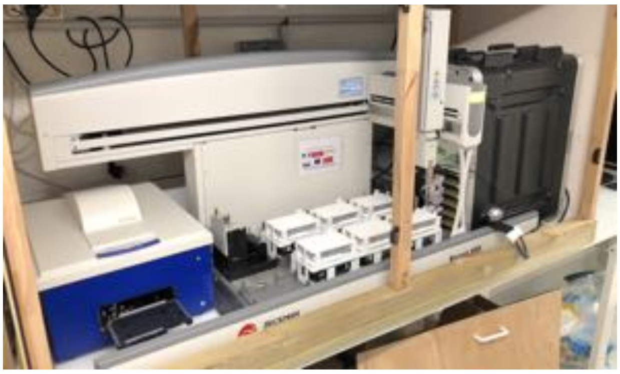

Figure 26: Microplate light simulator

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SZN Seagrass mesocosms

The system is composed of 4 modules, each with three 500 L experimental tanks and one 800 L conditioning tank. All modules are equipped with systems for controlling temperature and light quality and quantity from the surface to 30 m depth, with sinusoidal simulation of daily light cycle.

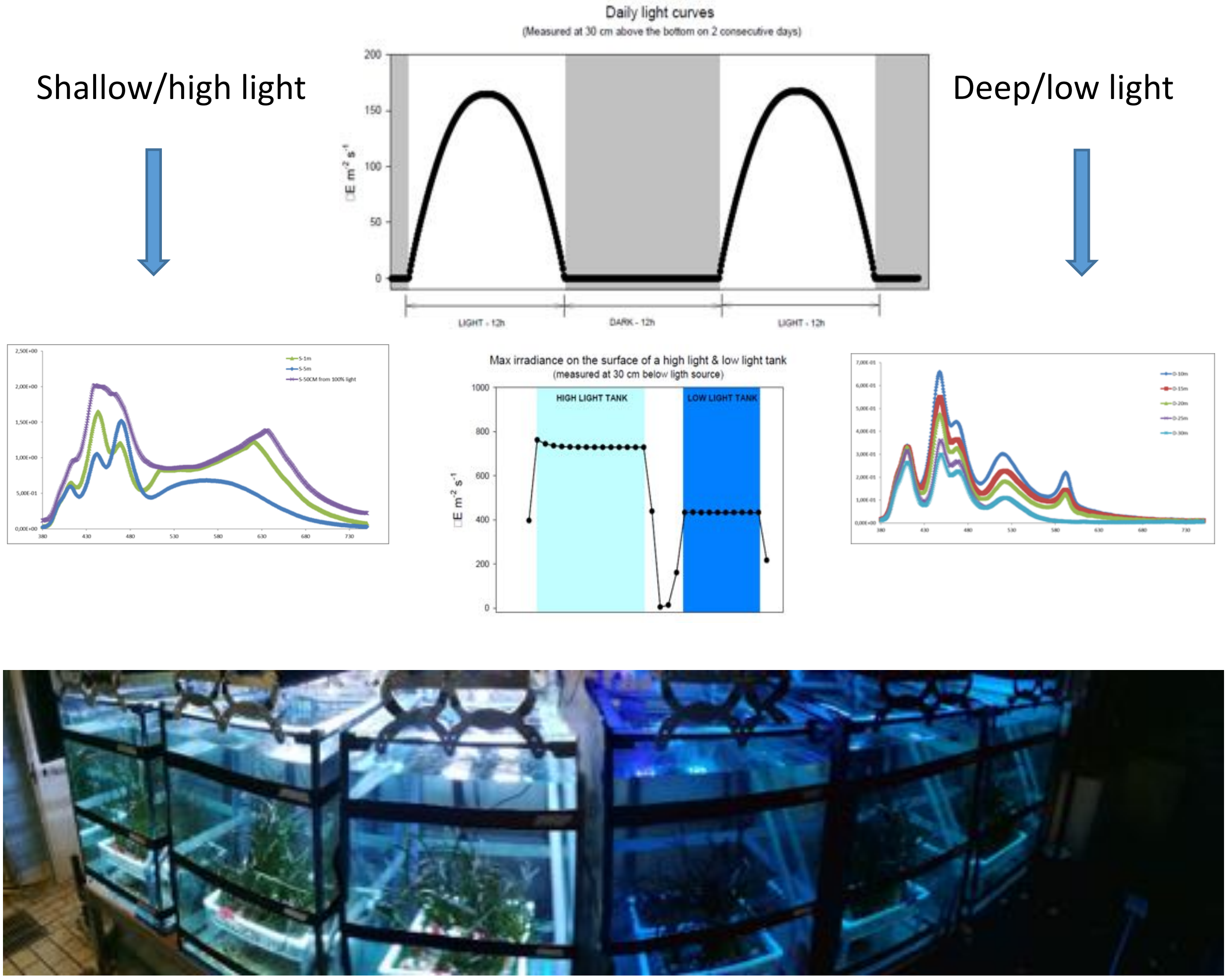

Figure 27: SZN seagrass mesocosm

SZN has also developed the Pholia light system composed by 3 three panels equipped with RGB diodes (LEDs), each diode providing the colors blue, red and green which can be independently modulated.

The system can mimic the light present in all marine environments (surface, bottom, etc.) in any season and the light that planktonic microorganisms can encounter - in terms of intensity and colors - during their movements in the water column.

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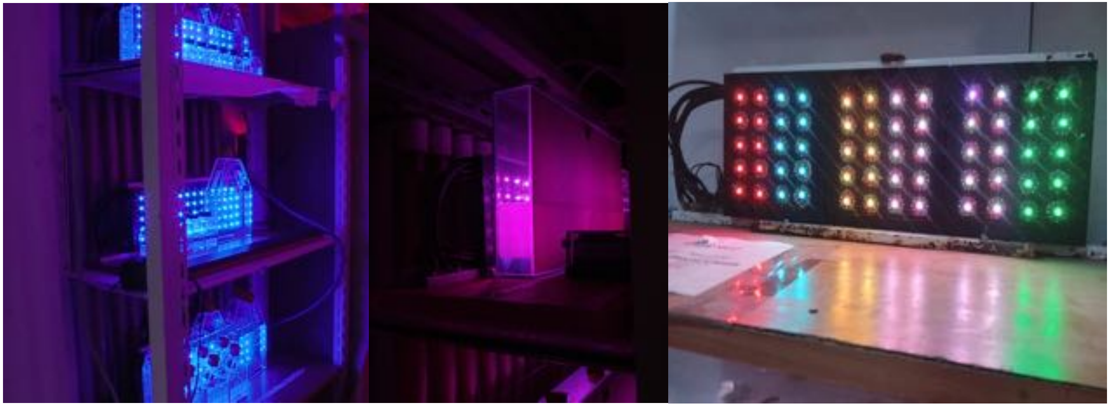

Figure 28: Pholia LED system

4.6. *Raceway system*

CCMAR Raceway system

CCMAR developed a system with rectangular tanks in a raceway configuration in semi-closed system, equipped with pumping capacity that allow performing of forced swimming experiments with marine fish. These tanks have been used to evaluate the performance of growth with Senegalese sole. Raceway tanks with controlled water current velocities are known to promote enhanced rates of growth and better welfare, as described for several species of fish as a consequence of sustained swimming, often accompanied by better feed conversion efficiency, improved muscular- skeletal development, improved osmoregulation and improved disease resistance. This is particularly useful in active species such as salmonids or breams.

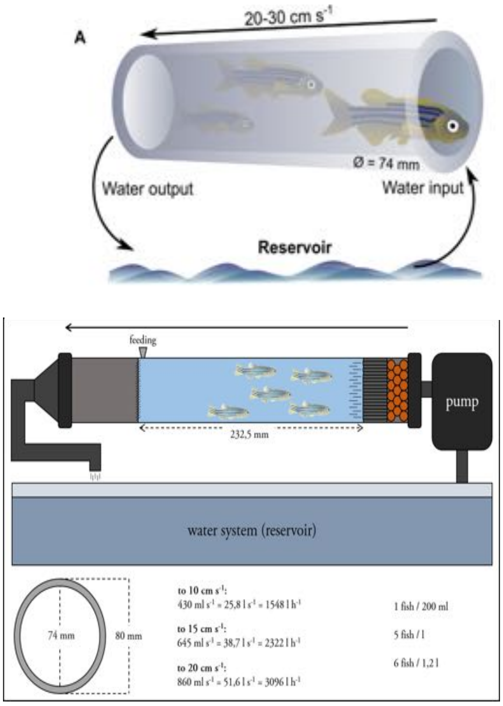

Figure 29. Technical overview of raceway system for forced swimming experiments

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Figure 30. Raceway system for forced swimming experiments

This system has been used for performing studies that allowed to better understand the swimming-induced contractile activity and the response of the skeletal tissues and cells to increased loads induced by forced swimming. This system can be used for studying other key aspects related to swimming in fish, ranging from a better understanding of swimming during migratory behaviors, investigating the molecular response of organs and tissues to swimming-induced forces.

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