

ERANet-LAC - JOINT CALL 2015-2016

Climate driven Changes in the Habitat Suitability of Marine Organisms (CLIMAR)

ELAC2015/T010495

D1.1. Report on methodological standardization and state of the art

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Deliverable D1.1. Summary

This deliverable provides a summary of the review of the state of the art, the standardization of methodology for CO₂ and temperature treatment, and thermal tolerance experiments as well as a standardized methodology for coupling ecophysiological and oceanographic data. A review on the effects of Ocean Acidification on fish has been written and published, and literature database for a further review on the effects of Ocean Acidification and temperature on fish and invertebrates is being gathered. Standardization of methodology for the CO₂ exposure and for thermal tolerance is presented for each partner. While the same principles were used, specific details differed between partners due to logistic constraints or differences between the characteristics of the species investigated. Standardization of methodology for oceanographic coupling was accomplished based on one area (Southern Argentina) that will be used as standard example for all the other areas of study.

1. Review and compilation of available data

Review and compilation of available data on the interactive effects of temperature and ocean acidification has been accomplished. A review paper on the effect of ocean acidification on fish was published in the journal *Ecological Monographs* (IF =8.86) (Cattano et al. 2018, see summary below), and data on the interactive effects of temperature and ocean acidification have been collected (see summary below). The write-up of this component is under way (**WPI**).

a) *The review paper on the component of ocean acidification effect on fish*

Title: Cattano C, Claudet J, Domenici P, Milazzo M (2018) Living in a high CO₂ world: a global meta-analysis shows multiple trait-mediated fish responses to ocean acidification *Ecological Monographs* 88(3): 320–335

Link: <https://esajournals.onlinelibrary.wiley.com/doi/pdf/10.1002/ecm.1297>

Abstract. Understanding how marine organisms will be affected by global change is of primary importance to ensure ecosystem functioning and nature contributions to people. This study meets the call for addressing how life-history traits mediate effects of ocean acidification on fish. We built a database of overall and trait-mediated responses of teleost fish to future CO₂ levels by searching the scientific literature. Using a meta-analytical approach, we investigated the effects of projected CO₂ levels by IPCC for 2050–2070 and 2100 on fish eco-physiology and behavior from 320 contrasts on 42 species, stemming from polar to tropical regions. Moreover, since organisms may experience a mosaic of carbonate chemistry in coastal environments (e.g., in estuaries, upwelling zones and intertidal habitats), which may have higher *p*CO₂ values than open ocean waters, we assessed responses from additional 103 contrasts on 21 fish species using *p*CO₂ levels well above IPCC projections. Under mid-century and end-of-century CO₂ emission scenarios, we found multiple CO₂-dose-dependent effects on calcification, resting metabolic rate, yolk, and behavioral performances, along with increased predation risk and decreased foraging, particularly for larvae. Importantly, many of the traits considered will not confer fish tolerance to elevated CO₂ and far-reaching ecological consequences on fish population replenishment and community structure will likely occur. Extreme CO₂ levels well above IPCC projections showed effects on fish mortality and calcification, while growth, metabolism, and yolk were unaffected. CO₂ exposures in short-term experiments increased fish mortality, which in turn decreased in longer-term exposures. Whatever the elevated CO₂ levels considered, some key biological processes (e.g., reproduction, development, habitat choice) were critically understudied. Fish are an important resource for livelihoods in coastal communities and a key component for stability of marine ecosystems. Given the multiple trait-mediated effects evidenced here, we stress the need to fill the knowledge gap on important eco-physiological processes and to expand the number and duration of ocean acidification studies to multi-generational, multiple stressor (e.g., warming, hypoxia, fishing), and species interactions experiments to better elucidate complex ecosystem-level changes and how these changes might alter provisioning of ecosystem services.

b) *Collection of literature data on the effect of ocean acidification and temperature on marine fish and invertebrates*

Published papers were categorized into larval fish, fish, invertebrates (benthic) and invertebrates (pelagic) topics. In total, >200 papers were found using a semi-structure literature reviews and data were extracted. Partners discussed and agreed on the variables to be used in the meta-analysis. These variables are: Taxon, family, species, climatic zone, habitat type, life stage, wild type (Yes/No), development type (direct vs larval), CO₂ treatment, Temperature treatment, animal response metric (e.g. lateralization, metabolic rate), acclimation time and significance vs control. The data analysis is underway and we foresee that it should be finished within the next six months.

2. Establishment of a standard methodology

The methodology used for the experiments was discussed during the first CLIMAR meeting. The protocols are all based on the same principles, both for CT_{max} and for the CO₂ exposure, although some specific adjustments were implemented, due to local specificities such as i) species-specific types of end points to be used when determining the critical thermal minimum and maximum (CT_{min}

and CTmax), ii) the local/regional in situ present-day patterns of in situ $p\text{CO}_2$ experienced by specific species. Here, a summary of the approach used in each laboratory is outlined.

2.1. CNR, Italy

Establishment of a standard methodology and protocol for testing the thermal tolerance of marine organisms exposed to various combinations of temperature and $p\text{CO}_2$ (acidification). The methodology for the acclimation to a constant $p\text{CO}_2$ and a constant temperature was discussed collectively during the first meeting and details of the set up are presented below (Fig 1).

a) Acidification set-up

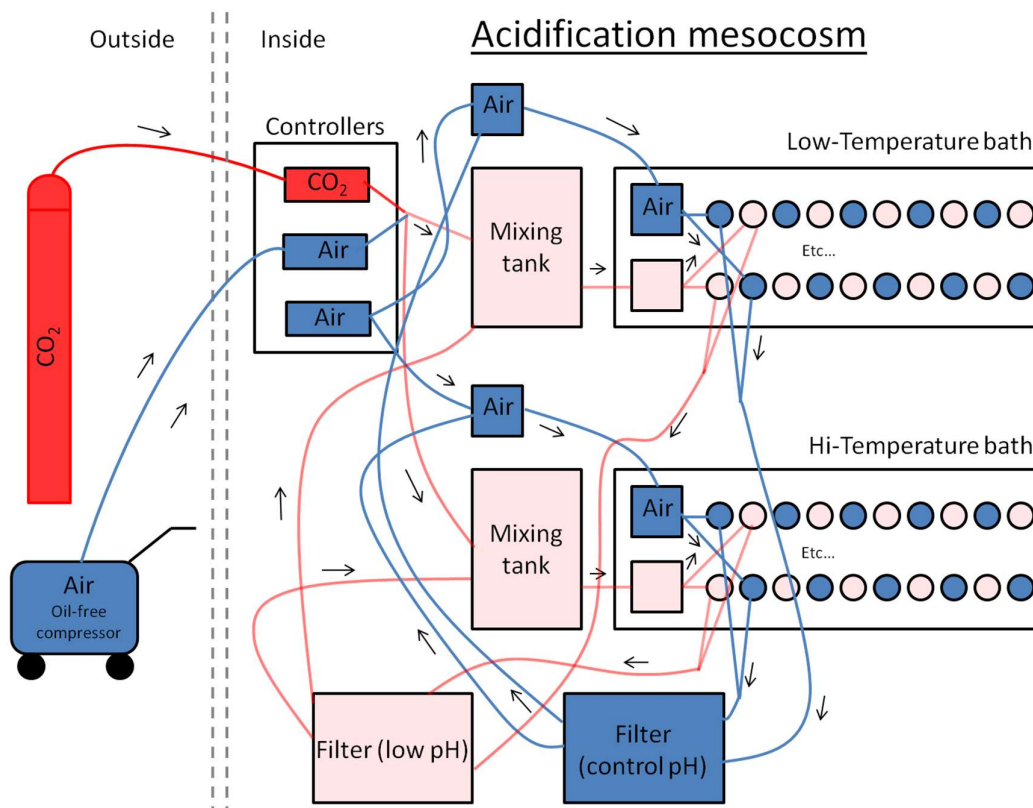


Fig. 1. Schematic of the mesocosm that was built at the CNR lab to simulate the ocean acidification conditions at two different water temperatures. The mixing tank corresponds to an “equilibration tank”. The “equilibration tanks” for (1) seawater and air and (2) seawater and air+ CO_2 blend will be of the same size.

Regarding the rearing and condition set-up for the experimental animals, this system has been developed successfully (see figure above). Four different filtration systems were built in order to provide good quality water supply in semiclosed circuits (30% of water renewal per week). All circuits are composed by a mechanic and biological filter, a chiller for the control of the water temperature and a mixing tank set-up, where the water is/was acidified according to the planned $p\text{CO}_2$ level. The systems can hold a total of 192 individuals in four different environmental conditions (two different temperatures and two different $p\text{CO}_2$ levels). An agreement with IMC Foundation (attached in the financial report) was established in order to provide the analysis of the all the chemical parameters needed to describe the $p\text{CO}_2$ treatments. The whole system is now ready for use.

b) The methodology for the CTmax/CTmin (thermal tolerance) experiments
 The methodology for the CTmax/CTmin experiments was tested in order to verify if the righting reflex could be used as a metric for thermal tolerance. For the experiments on sea urchin, preliminary work showed that the righting reflex in *Paracentrotus lividus* does not cause any habituation when individuals are tested at intervals of 10 minutes. The rate of increase in temperature was tested and the set-up available at CNR allows us to use 3 °C every hour.

2.2. CEAZA, Chile

a) The standard CO₂ mixing system
 A flow-through CO₂ mixing system similar to that described by Torres et al. (2013) was used to manipulate *p*CO₂ levels in the rearing tanks (Fig. 2). However, to meet the needs of CLIMAR this system was complemented (depending on the model species) with two or three independent temperature-controlled seawater units. Briefly, mass flow controllers (Aalborg ®, model GFC) were used to blend ambient air with pure CO₂ gas to obtain CO₂-enriched air to approximately 1200 µatm for the future *p*CO₂ condition and 500 µatm for the present day condition, which was then bubbled into four large (230-L), independent plastic reservoirs filled with 1.0 µm filtered seawater (FSW). From these reservoirs, treated FSW was delivered to 20-L equilibration tanks to adjust water temperature and then further transported to each experimental unit (replicate) at a flow rate of ~1 L h⁻¹. The carbonate system parameters, i.e. total alkalinity (TA), pH, temperature and salinity were quantified in triplicate once a week in each experimental treatment on three randomly chosen tanks per treatment. Total alkalinity was measured using an automated, open-cell titration system, described by Haraldsson et al. (1997) and the accuracy was verified using certified reference material (CRM) supplied by Andrew Dickson (Scripps Institution of Oceanography, San Diego, USA). The correction factor was approximately 1.002, corresponding to a difference of <5 µmol kg⁻¹. Seawater pH was measured inside a 25 mL closed cell at 25 °C using a pH meter (Metrohm 713) with a glass combined double junction Ag/AgCl electrode (Metrohm, 6.0219.100) calibrated using 8.089 Tris buffer (DOE [US Department of Energy], 1994) at 25 °C. Values of pH are reported on the total hydrogen ion scale. Temperature and salinity were measured using an Idronaut Ocean Seven CTD. The pH, TA, temperature and salinity data were used to calculate *p*CO₂ and CO₃²⁻ and seawater saturation stages for calcite and aragonite using CO2SYS program for Microsoft Excel set with Mehrbach solubility constants as refitted by Dickson and Millero (1987).

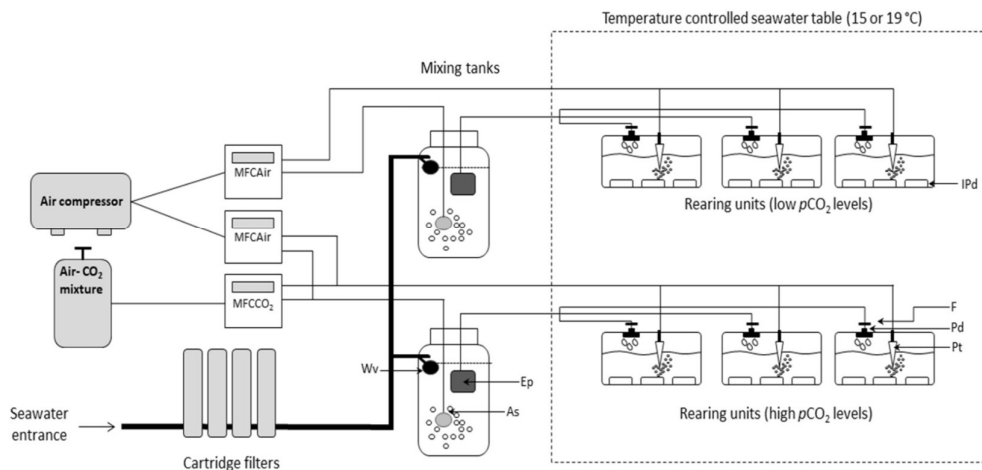


Fig. 2. Schematic representation (not to scale and for a single temperature) of the experimental mesocosm used to evaluate the combined effects of *p*CO₂ and temperature levels on the early developmental and reproductive traits of the model species in Chile. Figure taken from González et al. 2018, article published in the framework of CLIMAR.

b) The standard methodology for the CTmax/CTmin (thermal tolerance) experiments *Concholepas concholepas*. For the self-righting exercise, juveniles were placed upside down in the middle of the containers at the edge of a sloping Plexiglas surface covered with a Safety Walk® 3 M anti-slip tape (Fig. 3). Hence, the plane of the shell aperture was inclined by 30° with respect to the horizontal plane. This ensured that all individuals were in the same initial position and exposed to a similar degree of difficulty to self-right at the beginning of the CTmin/CTmax trials (Figure 3). Inability to self-right within 20 min was considered as the end-point since it involves a relatively simple procedure (Manríquez et al., 2016, 2017). Those individuals that were unable to self-right but displaying feet movement were given a second chance to self-right at the next measurement temperature. The CTmax and CTmin end-points were defined as the temperatures at which individuals first lost the ability to self-right within 20 min and could not self-right after being tested again at three successive (colder or warmer) temperatures. Time to self-right was measured with a digital stopwatch.

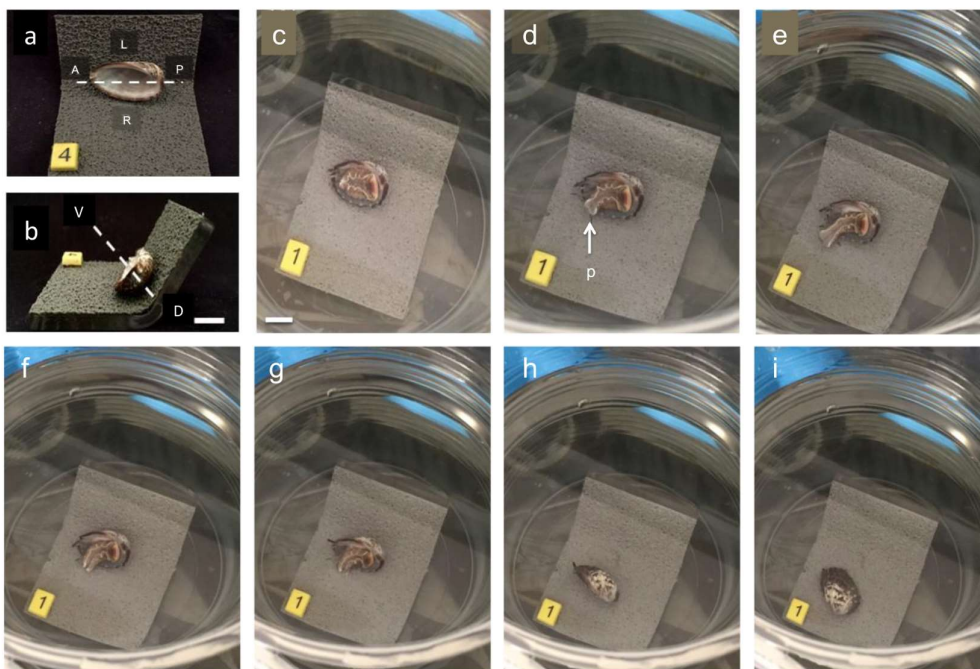


Fig. 3. Photographs of the experimental sloping Plexiglas surface used in the self-righting measurements and the sequence showing the self-righting behaviour of a small juvenile of *Concholepas concholepas*

Loxechinus albus. To determine CTmin and CTmax, sea urchins were placed into plastic beakers suspended within a 30 L thermo-stated bath connected to a temperature-controlled, circulating water bath. During the trials, sea urchins were exposed to a constant rate of change (increase or decrease) of $\sim 0.05 \text{ } ^\circ\text{C min}^{-1}$ ($3.00 \text{ } ^\circ\text{C h}^{-1}$; Table 1) and observed continuously until they reached the end-point (Fig. 4). From each exposure container, one sea urchin was selected at random and placed individually in another 1 L plastic container filled with FSW at the corresponding starting temperature for 5 min of acclimation under vigorous aeration. After a $1 \text{ } ^\circ\text{C}$ change in water temperature, self-righting success time and time to self-right was measured at the new temperature. After successful self-righting, the container was aerated to avoid oxygen depletion. All measurements were conducted under standard conditions of $p\text{CO}_2$ (current-day levels). Inability to self-right within 20 min was considered as the end-point since it involves a relatively simple procedure. Urchins were carefully placed upside down in the middle of the container. The CTmax and CTmin end-point were defined as the temperatures at which sea urchins first lost the ability to self-right and could not self-right after

being tested again at two successive (colder or warmer) temperatures. Self-righting behaviour was monitored continuously and the time needed for full righting was noted.

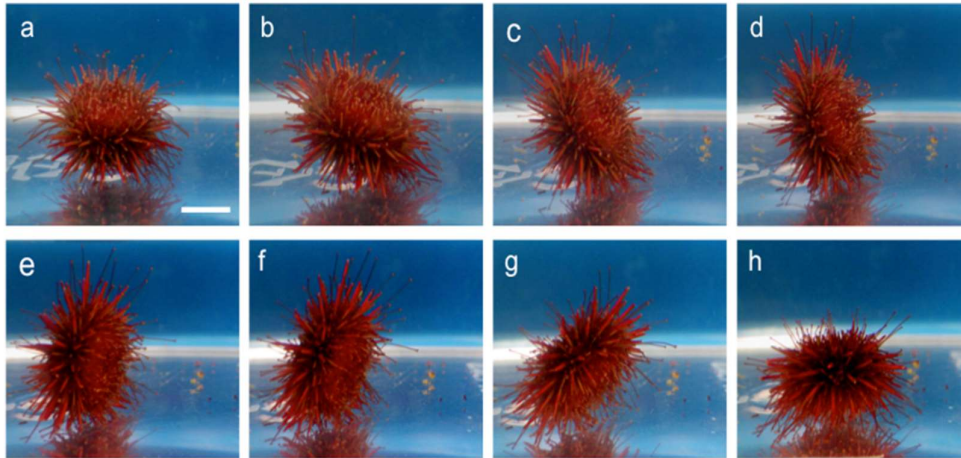


Fig. 4. Small juvenile of Loxechinus albus displaying the self-righting behaviour (a-h) to return to their normal posture (h) after being placed upside down (a).

2.3. University of Hamburg, Germany

a) Experimental set-up

In the experimental set-up built by the University of Hamburg (Fig 6), air was pumped through a bubble counter into a bottle containing soda lime in water to remove CO₂ from air (1). Then the air was pumped into another bottle containing water in which also pure CO₂ was pumped through a bubble counter (2). Then the mixture of CO₂-free air and pure CO₂ was pumped into an empty bottle to dry the air-CO₂ mixture (3). From here the air-CO₂ mixture was pumped through a CO₂ analyser which sends an electrical impulse to a motor (4) that was then regulating the bubble counter of the pure CO₂ (2) to adjust CO₂ to the desired high (green) or low (salmon) value. The air-CO₂ mixture was also pumped into rearing tanks (n=5 per treatment) which were housed in warm (red) and cold (blue) thermal bath (5). A separate second feed-back system (6) consisted of a computer with pH and temperature sensors. Pure CO₂ was bubbled into the tow high CO₂ tanks (at low and high temperature) until the desired pH was reached which corresponds to the pCO₂ of the rearing tank system. This pre-conditioned water from these reservoir tanks was used for daily manual water change in the rearing tanks (5).

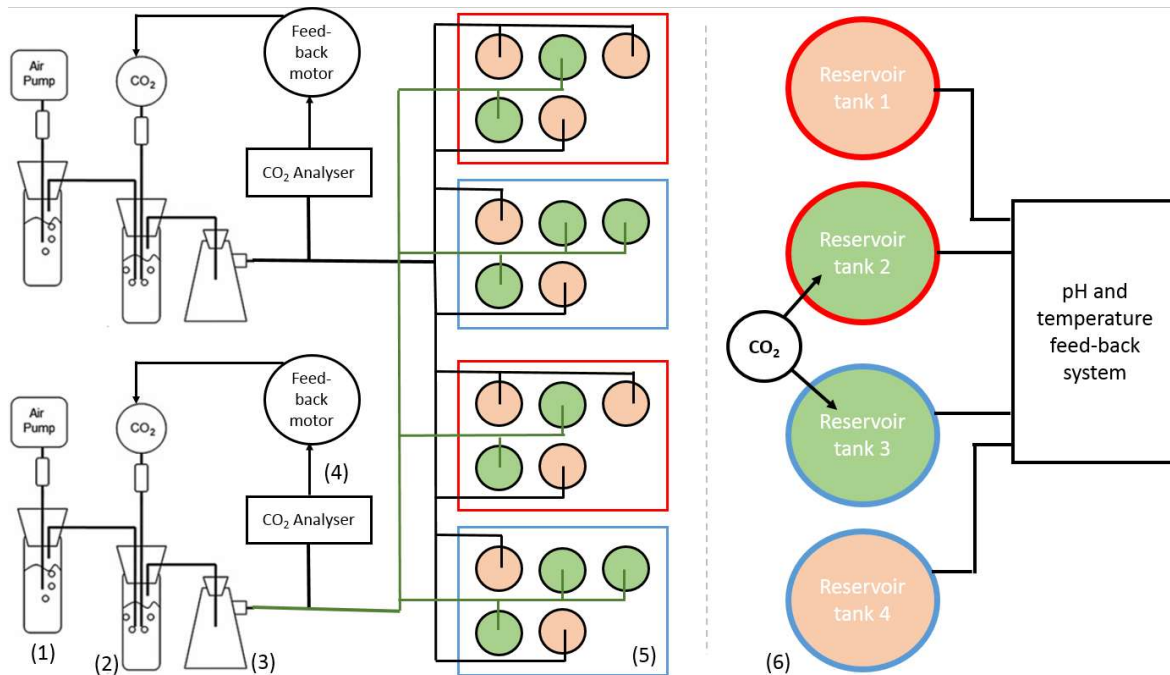


Fig. ... Schematic of the experimental set-up build by the University of Hamburg to simulate ocean acidification and ocean warming treatments. Modified according to Findlay et al. (2008).

b) The methodology for CT_{max} and CT_{min} (thermal tolerance) experiments:

Belone belone: CT_{min} and CT_{max} trials were conducted with one day old larvae. Larvae were carefully transferred from rearing tanks into individual test beakers (250 ml) containing water at the respective treatment condition. Beakers were randomly assigned to one of three water baths. Experimental runs commenced after an initial acclimation period of 15 min. One bath served as control unit and was kept at the treatment temperature while temperature in the other two baths was raised or dropped stepwise at a rate of $3^{\circ}C/h$ ($0.5^{\circ}C/10$ min). Each bath contained 10 test beakers with a mix of larvae from the high and low pCO_2 treatment. Larvae were constantly monitored by two observers who were blind to the pCO_2 condition. Yolk-sac larvae of *B. belone* swim constantly and near the surface. Notes were taken and experimental temperature was recorded when behaviour diverged from the usual surface swimming. CT_{min} and CT_{max} were considered to be the temperatures at which a larva showed clear signs of stress. At these endpoints, the beaker was removed from the bath and placed into a temperature-controlled room at the respective treatment temperature. After one hour larvae were checked for constant surface swimming which was considered as a recovery sign.

Clupea harengus: Heart rates of eye-staged embryos (80% eye pigmentation) were measured to determine CT_{min} and CT_{max} in *Clupea harengus*. Randomly chosen egg plates ($n=3$ per treatment) from rearing tanks were placed in individual observation chambers, housed in a Styrofoam block, and connected to a recirculating water bath. Within the bath, temperature and pCO_2 were kept stable using a chilling unit and an aquarium feed-back computer, respectively. The observation chambers were placed under a stereo microscope connected to a computer and camera system. After 15 min of incubation at the stable experimental temperature, 1-min videos were made of embryonic hearts in 10 randomly chosen embryos per plate. After a further 15 min at stable conditions, the temperature was increased (CT_{max}) or decreased (CT_{min}) by $3^{\circ}C$ within 20 min. This was repeated until the majority of embryos showed arrhythmia or cardiac arrest.

2.4. CONICET, Argentina

a) Experimental set-up

A flow-through CO₂ mixing system was built to manipulate *p*CO₂ levels in rearing containers. The system consists of three mass flow controllers (Aalborg®, model GdFC) used to blend pure CO₂ gas with the air provided by a Schulz MSV 12/100 air compressor. The obtained CO₂-enriched air is then bubbled into 230-L plastic reservoirs (mixing tanks) filled with seawater previously filtered (Hidroquil 1, 5, 10 µm). Finally, the water is delivered automatically to different rearing containers immersed in temperature-controlled water tables (Fig. 6). This set-up allow to expose up to 160 individuals to four treatments resulting from the combination of two different temperatures and two *p*CO₂ levels.

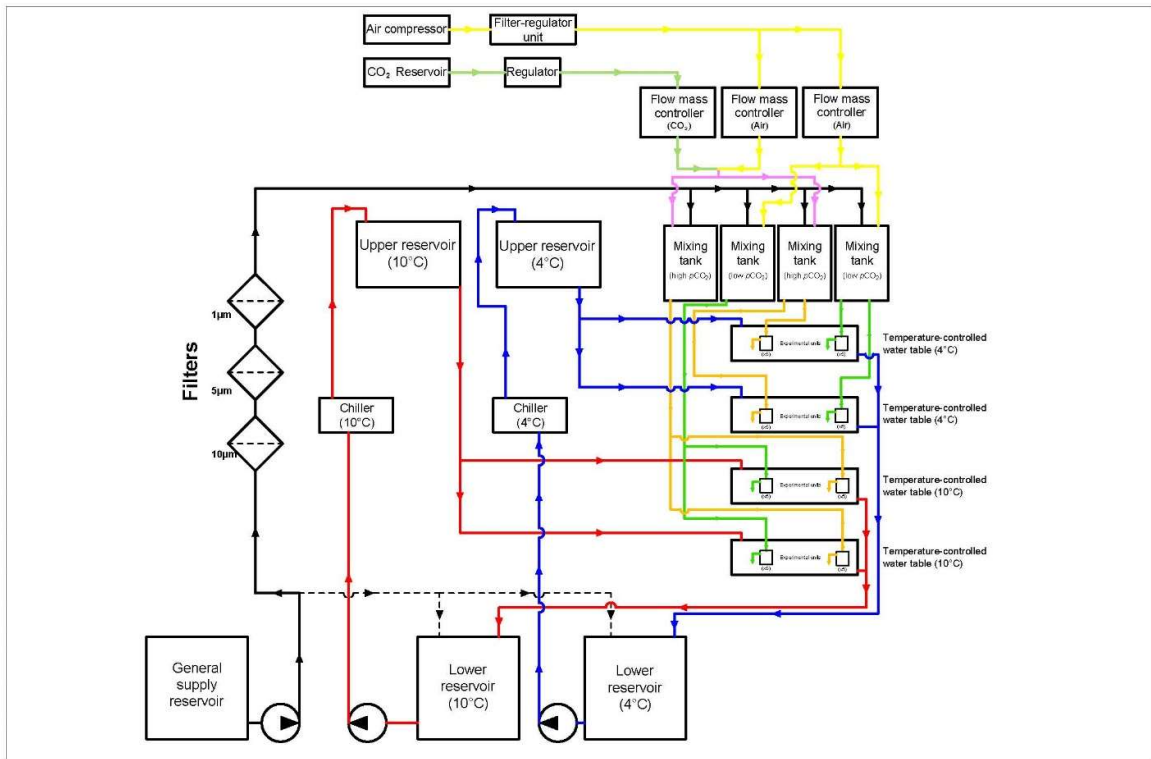


Fig. 6. Schematic of the experimental set-up built by CONICET to simulate the ocean acidification conditions at two different water temperature.

b) The methodology for the CT_{max}/CT_{min} (thermal tolerance) experiments

The methodology for the CT_{max}/CT_{min} experiments was previously developed and successfully employed with many fish species in our lab (Barrantes et al., 2017, Lattuca et al., 2018). However, in the present study a different heating/cooling rate was used, so many previous trials have been performed to test whether the available equipment in CADIC-CONICET could be employed. Finally, a heating/cooling rate of 3°C/hour was successfully achieved by means of a thermoregulator (Techno-TU20D) and a cooling chamber, respectively.

3. Standardization of methodology for oceanographic coupling.

Standardization of methodology for oceanographic coupling. This task has been accomplished for one of the CLIMAR study areas (Southern Argentina) for which oceanographic data were missing and this is presented below as the standard example that will be used for all the other areas of study. The oceanographic modeling has been set up in order to prepare the ground for the coupling with the biological data (WP1).

Reproducing the 3D water circulation and hydrodynamics in the Beagle Channel and surrounding coastal areas

Summary: The objective of this activity consists in reproducing the 3D water circulation and main hydrodynamics in the Beagle Channel and surrounding coastal areas. With this aim a tri-dimensional hydrodynamic numerical model based on the finite element method was adopted. The wind, tide and thermohaline forcing were considered in the numerical simulation. The tidal dynamic was correctly reproduced as highlighted by the comparison between simulation results were and available tidal data from gauges located along the Patagonia coast and the Beagle Channel. Meteorological forcing as well as large scale oceanographic data were collected. A set of calibration run was performed to test the model accuracy in simulating the full 3D general circulation in the area. In the following the adopted numerical method, dataset and simulation setup are described along with the preliminary results obtained by the runs.

3.1. The study site

The study area comprises the Beagle Channel and the surrounding coastal areas, part of the Atlantic Ocean and part of the Pacific Ocean (see figure 7). The wide extension of the selected domain is necessary to reproduce the main tidal dynamic in the Beagle Channel.

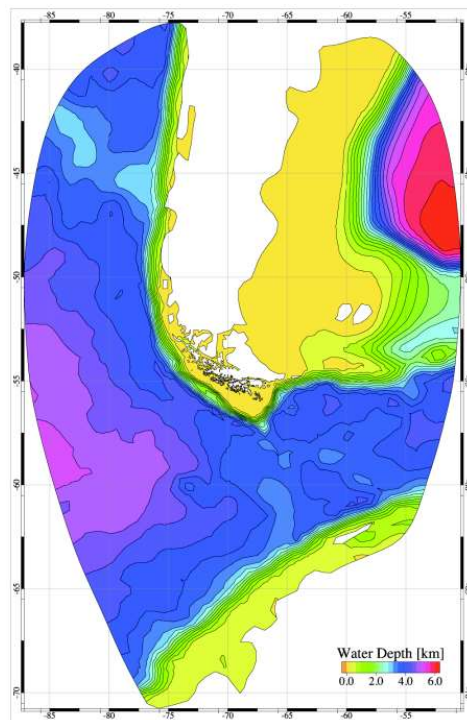


Fig. 7. Geometry and bathymetric features of the interested area.

The domain is characterized by complicated oceanographic features, including the Circumpolar Antarctic Current and the Malvinas current.

3.2. The numerical method

The tridimensional ocean model based on the finite element method, SHYFEM (Umgiesser et al., 2004) was applied to reproduce the water circulation in the BC. SHYFEM is a 3D hydrodynamic model based on the finite element method, which solves the shallow water equations integrated over each layer in their formulations with water levels and transports. It uses finite elements for horizontal spatial discretizations, z-layers for vertical discretizations and a semi-implicit algorithm for integration in time. It accounts for barotropic, baroclinic and atmospheric pressure gradients as well as wind drag and bottom friction, non linear advection and vertical turbulent processes. The model uses finite elements unstructured mesh for representing the domain. The investigated area was reproduced by means of a finite element grid composed by around 90000 nodes and 13000 triangular elements (see Fig. 8).

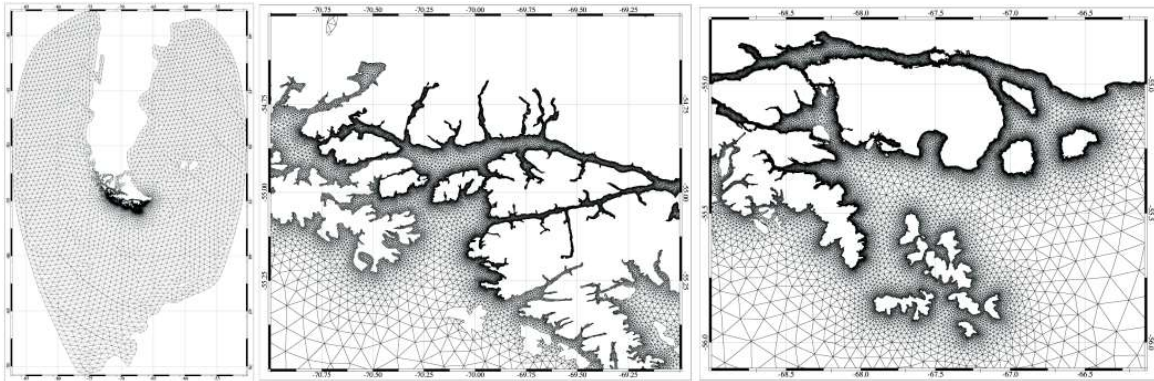


Fig. 8. Finite elements mesh adopted to reproduce the extended domain and the Beagle Channel.

The elements shape and size distribution was dependent on water depths, water depth gradients, coastline geometry and proximity to the area of interest. The lateral borders of the mesh were considered as open boundaries. At the open boundaries oceanographic data, including water elevation, currents, temperature and salinity fields from COPERNICUS (<http://www.copernicus.eu>) were used as model forcing. The model domain was vertically discretized in 25 zeta levels logarithmically spaced, with layer depths ranging between 5 and 6000 meters.

At the top boundary, the daily fields of wind speed, atmospheric pressure data, solar radiation, relative humidity and atmospheric temperature, from ECMWF (<https://www.ecmwf.int>) were used as surface forcing condition.

3.3. Results

A full year-simulation run was carried out to reproduce the tidal dynamic in the BC and surrounding area. The simulation is able to resolve the spatial distribution of the Phase and Amplitude of the main semidiurnal and diurnal astronomic tides M2 and K1(Fig 9).

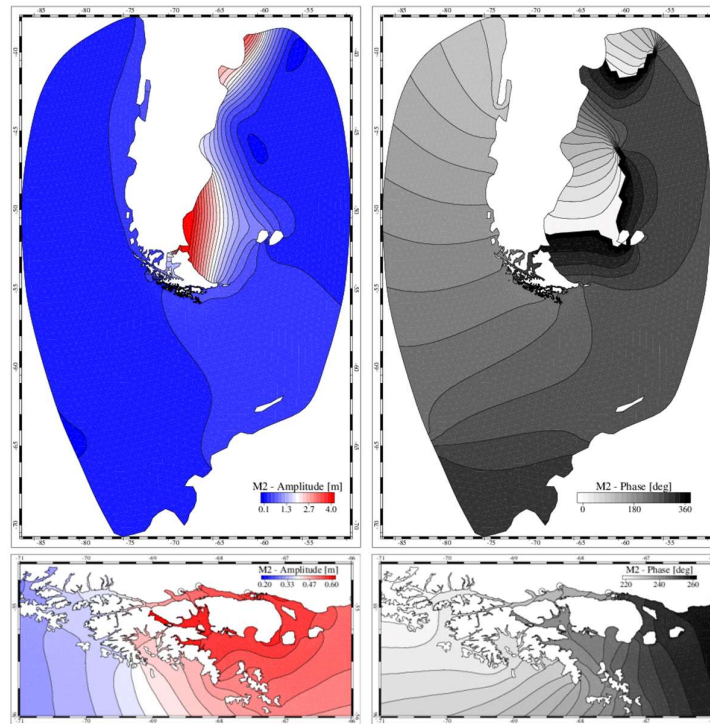


Fig. 9. Modeled co-range and co-tidal maps for M2 for the whole domain (upper panels) and for the Beagle Channel (lower panels).

The model results were compared with harmonics obtained from a set of 25 tidal gauges located along the Argentinean coasts on both Atlantic and Drake Channel sides. The accuracy of the model results were assessed in terms of Amplitude relative error (AE) and phase delay (D) between observed and modeled harmonic constants of the main constituents for the 26 tidal stations analysed by D’Onofrio et al. (2016).

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