Report on the monitoring of the Blusink deployment in Caniçal (Madeira/Portugal)

Revised and updated

AMACO

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1 Introduction

In order to assess the performance of the 'Blusink' technology in the ocean environment of the Madeira, an experimental deployment was conducted in Caniçal (Madeira, Portugal). The objectives of this project are: i) to evaluate the settlement and growth of local coralline algae species on 'Blusinkies' on the study site; ii) to evaluate the Carbon sequestration potential of colonized 'Blusinkies'.

2 Methods

2.1 Site and setup description

The experimental setup was deployed in a subtidal area in Caniçal (Madeira, Portugal — 32.73° N, 16.74° W) in March 2024 by an AMACO team. The site (Pedra d'Eira) is located close to the Port of Caniçal and is a shallow (16–22 m deep) basaltic-rocky area formed by an irregular lava flow. The rocky substrate is interspersed by sandy deposits (gravel and very coarse sand) where natural rhodolith beds form. Outside the rocky area (to E, S and W) the substrate is mainly composed by fine sand.

The experimental setup is composed of six plots of approximately 1.5 ×1.5 m, of which three plots (R1, R2 and R3) are located over rhodolith beds and the other three (A1, A2 and A3) are placed over coarse and fine sandy substrates at progressively longer distances from rhodolith beds — Figure 1.

The plots located over the rhodolith beds (R1, R2 and R3) were marked with one metal spike on each corner and the four spikes were connected with cave line (PES[1], 2 mm) to make them more visible. Inside each of these plots, 200 'blusinkies' were carefully placed over the rhodoliths (Figure 2).

In a central location relative to the three "R" plots (R1, R2 and R3), a sensor support was installed, where two data-loggers (one for temperature — HOBO Pendant MX2201 Water Temperature Data Logger, Onset Computer Corporation; and one for PAR — Odyssey Submersible Photosynthetic Active Radiation Logger, Dataflow Systems Limited) were deployed — Figure 3.

1: polyethersulfone

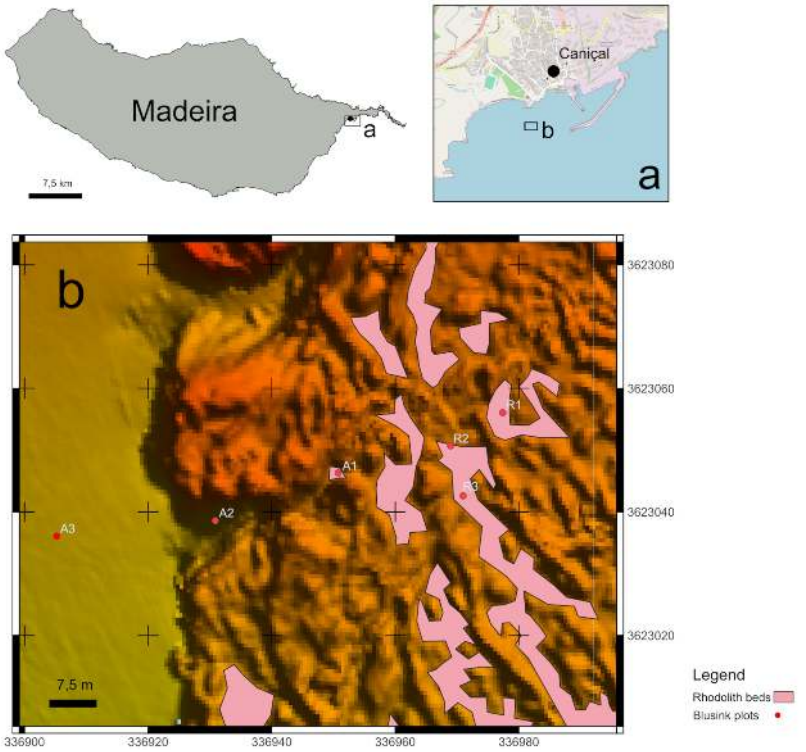


Figure 1: Location of the experimental setup in Caniçal (insert b) and its position in relation to Caniçal (insert a) and to Madeira island. CRS: EPSG5016.

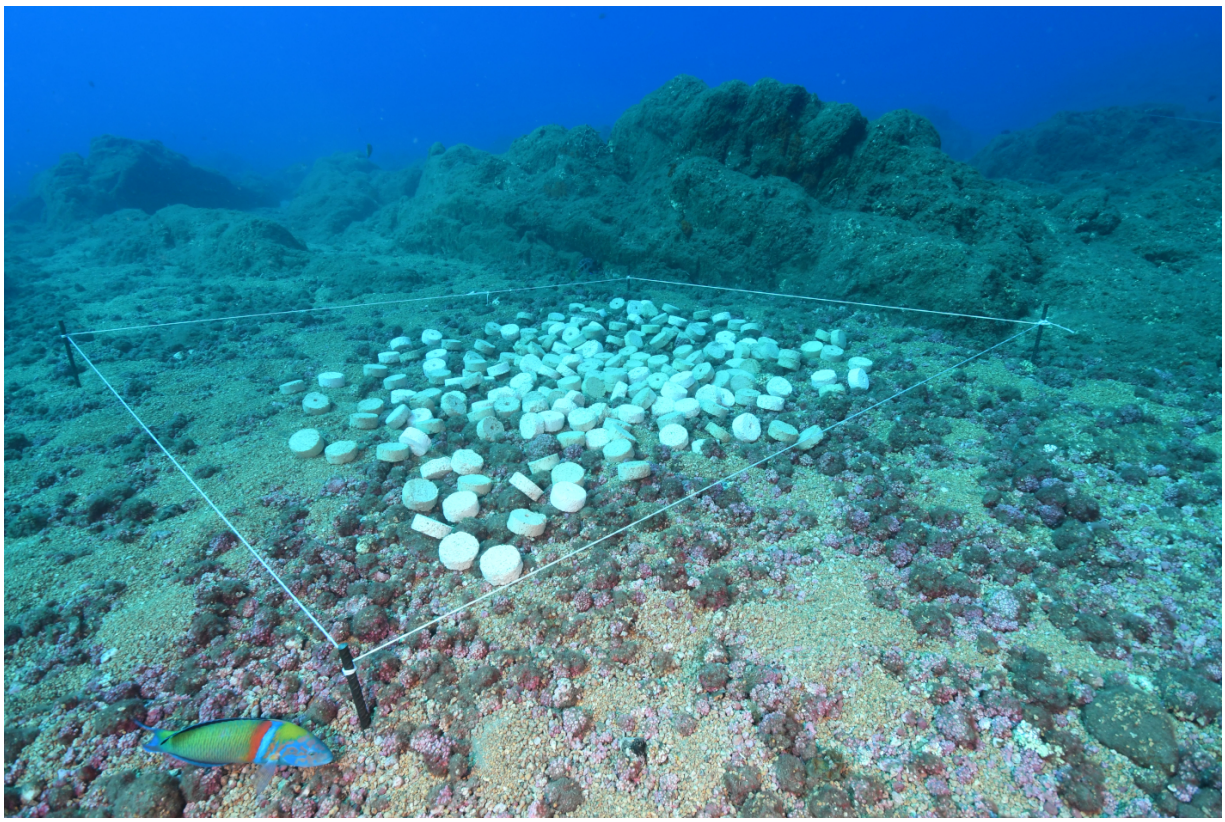


Figure 2: View of the R1 experimental plot, just after its deployment, with the 'blusinkies' placed over rhodoliths.

Another plot was created over a sandy substrate, adjacent to the rhodolith bed (plot A1). In this plot, the 'blusinkies' were placed inside a sandy patch delimited by rocks, which served as natural boundaries for this plot. Further from the rhodolith beds, over fine sandy substrates, two other plots were placed. One (A2) close to a rocky wall, and another (A3) further away from the rhodolith bed but in line of sight with A2. Both A2 and A3 were enclosed by a plastic mesh and held in place with metal spikes and tie-wraps.

After the deployment, several visits were made to the site on a monthly basis, on the following dates: April, 13; May, 11; June, 16 and July, 6, 2024.

Figure 3: Dataloggers in place, in the middle of the rhodolith bed.

2.2 Environmental monitoring

A HOBO Pendant MX2201 Water Temperature Data Logger (Onset Computer Corporation) was deployed on site set to measure water temperature every 30 min. Similarly a Photosynthetic Active Radiation Logger (PAR) — Odyssey Submersible (Dataflow Systems Limited) was deployed and set to take measurements every 15 min.

2.3 Colonization

The plots were visually assessed and photographed to evaluate i) the general state of the 'blusinkies' and ii) its colonization by organisms, namely macroalgae species. The visual assessment was complemented with pictures (Plots R2, R3, A1, A2, and A3) and video (plot R1).

2.4 Inorganic carbon (DIC) fluxes and calcification rates

Sampling and acclimation

A total of 15 blusinkies, five per treatment (control, sand and rock) were selected for laboratorial incubations. Units collected in the deployment site in Madeira were tagged for identification purposes and transported in a wet isothermic container to CCMAR lab in Faro, where they were placed in independent aquaria per treatment, in an open-circuit water system with dual mechanical filtration and UV sterilization, alongside continuous aeration. All aquaria were kept at a constant temperature of 20°C and exposed to uniform light intensity (120–150 µmol photons m^{-2} s^{-1}).

Incubations

Incubations were conducted in light and dark conditions, in a custom-made system, using 150 mL stirred plexiglas chambers, at a constant temperature of 20°C for a period of 2 hours. For incubations in the light, PAR (photosynthetic active radiation) was set at 125 µmol photons m^{-2} s^{-1}. At the beginning and at the end of each 2-hour incubation, in both light and dark conditions, water samples were collected from the incubation chambers and preserved for alkalinity analysis. At the end of the experiment, all blusinkies were weighted (wet weight) and then dried at 60°C for 48 hours to determine their final dry weight.

Calcification rates and DIC fluxes

Calcification rates were determined from the difference in alkalinity measurements of seawater samples taken at the beginning and end of the different incubations, according to the alkalinity anomaly principle (two equivalents of total alkalinity evolved per each mole of $CaCO_3$ precipitated) [3] and using the Gran titration method for alkalinity (TA) measurements [1, 2]. Water samples were titrated with HCl 0.1 M, using an automated

[3]: Smith et al. (1978), *Calcification and Organic Carbon Metabolism as Indicated by Carbon Dioxide*

[1]: Bradshaw et al. (1981), *Measurements of Total Carbon Dioxide and Alkalinity by Potentiometric Titration in the GEOSECS Program*

[2]: Hansson et al. (1973), *Evaluation of the Accuracy of Gran Plots by Means of Computer Calculations*

titration system (Titroline 7000, SI Analytics, Mainz, Germany) and the data was processed by the Titrisoft 3.2 software (SI Analytics, Mainz, Germany). Certified reference materials of known total alkalinity (CRMs, Batch No. 160; Scripps Institution of Oceanography, United States) were used to calibrate the results. Total Dissolved Inorganic Carbon (DIC) and its components (HCO^-_3, CO_3^{2-}, and CO_2) were calculated using Ernie Lewis' "CO2SYS.BAS" Visual Basic macro.

3 Results

3.1 Environmental data

On a routine visit to the site (May, 11th, 2024) it was found that the Hobo data-logger was not working and hence it was recovered. It was later found that the logger was flooded due to water ingress, and thus it was not possible to retrieve any data. Nevertheless, historical data for the same site is presented as a reference on Figure 4 and on Table 1.

The average monthly water temperatures recorded followed the usual pattern for the region, with increasing temperatures from April to August/September, followed by a decrease during autumn and winter months. Regarding the mean monthly values — Table 1, the maximum mean values varied between 24°C (September, 2020) and 18.5°C (April, 2022). The minimum mean water temperature varied between 17.8°C (March, 2022) and 22.7°C (September, 2020).

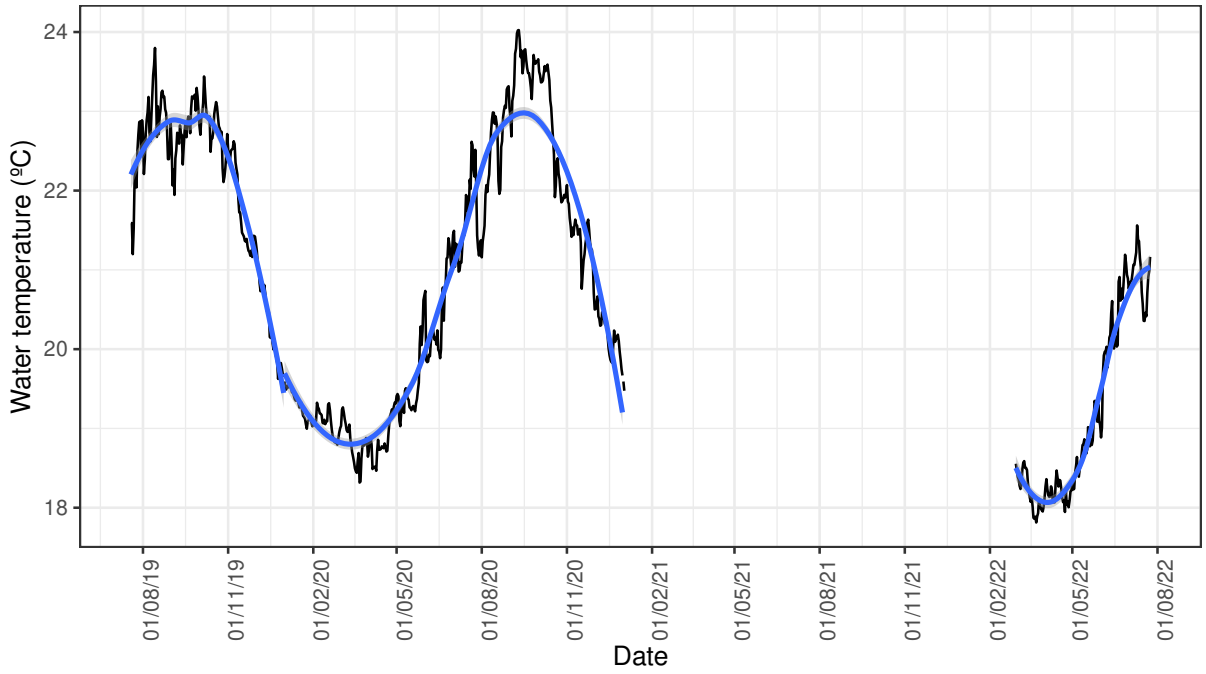


Figure 4: Daily water temperature variation on the site between 19/07/2019 and 02/01/2021 and between 01/03 and 24/07/2022.

The PAR data obtained on site is presented on Figure 5. There was a variation in PAR values during the sampled period and the data show that April was the month with the lowest irradiance values. The highest daily mean PAR value — 275 µmol·m^{-2}·s^{-1}, was recorded on 2024-03-06, while the minimum was recorded on April, 5th, 2024. Considering monthly means (Table 2), April and June were the months with lower recorded PAR mean values, while March and May were the months where mean PAR values were higher.

Date	Min	Max	Mean
07/2019	21,2	22,9	22,2
08/2019	22,2	23,8	22,9
09/2019	21,9	23,3	22,8
10/2019	22,1	23,4	22,8
11/2019	21,2	22,6	21,8
12/2019	19,6	21,3	20,3
01/2020	19,0	19,6	19,3
02/2020	18,8	19,3	19,1
03/2020	18,3	19,3	18,8
04/2020	18,5	19,3	18,9
05/2020	19,0	20,7	19,5
06/2020	19,8	21,4	20,4
07/2020	21,0	22,6	21,6
08/2020	21,2	23,3	22,5
09/2020	22,7	24,0	23,5
10/2020	21,9	23,7	22,8
11/2020	20,7	22,1	21,4
12/2020	19,7	20,7	20,2
01/2021	19,5	19,6	19,5
03/2022	17,8	18,6	18,2
04/2022	17,9	18,5	18,2
05/2022	18,2	19,4	18,8
06/2022	18,9	21,2	20,3
07/2022	20,4	21,6	20,9

Table 1: Monthly minimum (Min), maximum (max) and average water temperature means recorded in Caniçal. Min: minimum mean; Max: maximum mean; Mean: average monthly water temperature.

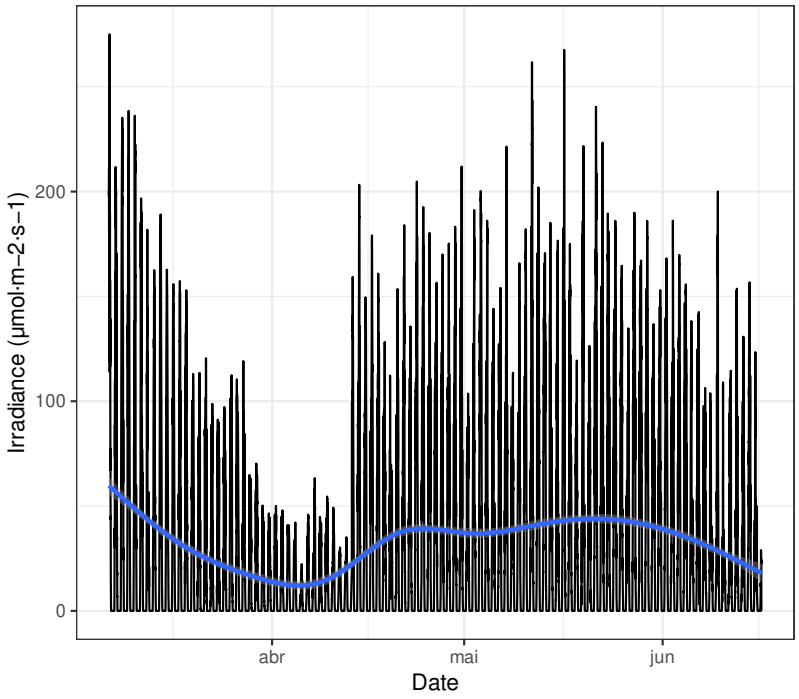


Figure 5: Daily PAR variation (µmol·m^{-2}·s^{-1}) on the site between 5 March and 16 June, 2024.

Month	Min	Max	Mean
March	0	275	32
April	0	212	26
May	0	268	41
June	0	200	29

Table 2: Monthly means for irradiance values (µmol·m^{-2}·s^{-1}).

3.2 Colonization

After four months of the initial deployment, there were visible changes, not only in the community associated with the rhodolith beds, but also on the species associated with the 'blusinkies'. In fact, between April and May, a considerable growth of *Asparagopsis taxiformis* was observed on the rhodolith bed and the associated plots (Figure 6). This species started to disappear in May and was not observed in July. By observing the 'blusinkies' it was possible to verify that they are being used as shelter by several species but also that they are providing substrate for the fixation of other — Table 3.

Table 3: List of taxa associated with the 'blusink' plots in Sand and in Rhodolith beds. "Substrate" refers to species directly attached to the 'blusinkies', and "Protection/shelter" refers to species seen using the 'blusinkies' as shelter.

'Blusink' plots — Sand		'Blusink' plots — Rhodolith beds	
Substrate	**Protection/shelter**	**Substrate**	**Protection/shelter**
Serpulidae	*Hermodice carunculata*	Serpulidae	*Bursa* sp.
Schizoporella dunkeri	*Bittium* sp.	Coralline rhodophyta	*Hermodice carunculata*
Padina pavonica	*Galathea* sp.	*Schizoporella dunkeri*	*Scorpaena maderensis*
Coralline rhodophyta	*Gobius gasteveni*	*Asparagopsis taxiformis*	
Dictyota sp. 1	*Scorpaena maderensis*	*Dictyota* spp.	
Dictyota sp. 2	*Chromis limbata*	*Rhynchozoon papuliferum*	
	Serranus atricauda		
	Sparisoma cretense		
	Calcinus tubularis		

The 'blusinkies' are providing substrate to bryozoans, algae and polychaetes, both on the plots located on the rhodolith beds and on the plots located over sand. Regarding the species using the 'blusinkies' as protection or shelter, these were mainly small fish and invertebrates (polychaeta,

crustaceans and mollusks). This effect was more pronounced on the plots located over sandy substrates, particularly on the ones located further away from the rhodolith beds (A2 and A3). Details on this colonization are pictured on Plates 1 and 2, respectively for the plots over sand and for plots over the rhodolith beds.

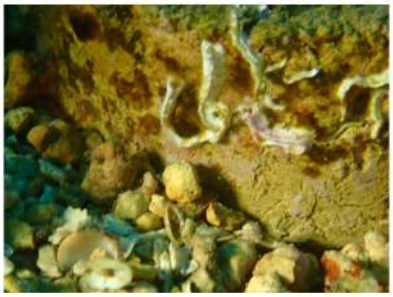
Due to the nature of the sediment, and to the fact that is further away from the basalt wall (and hence, less protected), some of the 'blusinkies' in the plot A3 were covered by sand.

Figure 6: *Asparagopsis taxiformis* growing over the rhodolih bed in April, 2024.

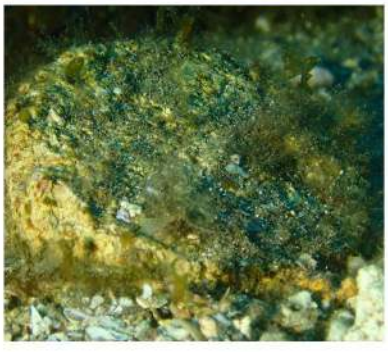






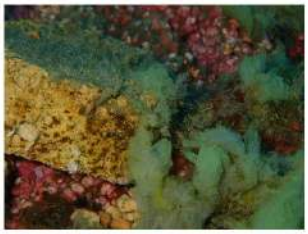




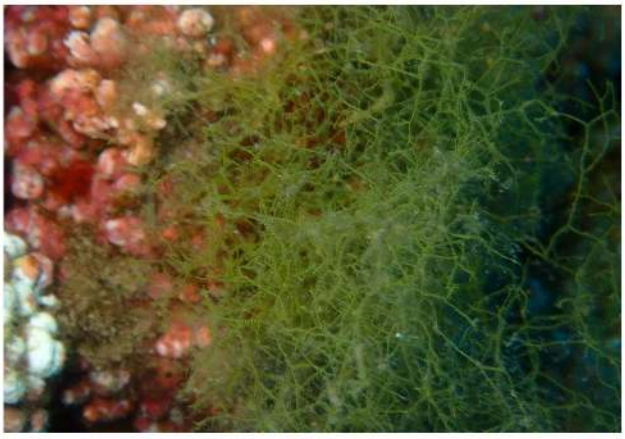




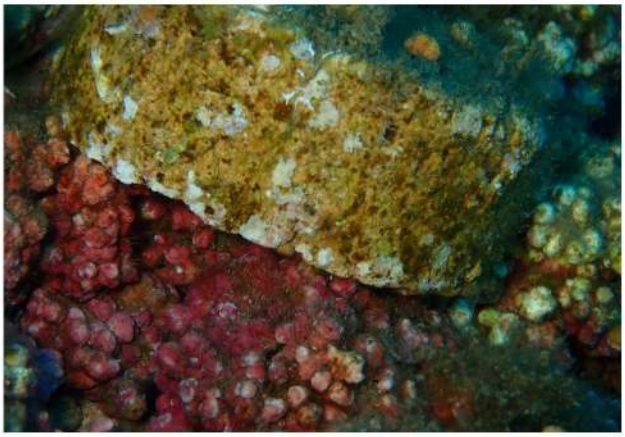










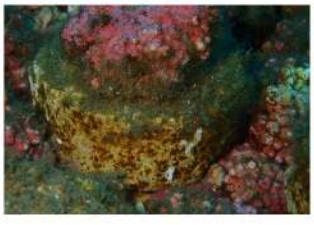



Table 4: Average ± standard deviation of Dissolved Inorganic Carbon (DIC) fluxes and Calcification (G) in mg per blusinkie unit per hour, for each group (control, sand and rock)

	Light Incubation		Dark Incubation	
	ΔDIC (mg/h)	**G** (mg $CaCO_3$/h)	**ΔDIC** (mg/h)	**G** (mg $CaCO_3$/h)
Control	-0.466±0.053	1.308±0.249	-0.577±0.037	1.505±0.296
Rock	-0.411±0.15	1.243±0.52	-0.096±0.187	0.089±0.816
Sand	-0.271±0.125	0.833±0.317	-0.108±0.127	0.113±0.315

3.3 Inorganic carbon (DIC) fluxes and calcification rates

Blusinkies from all groups (control, sand and rock) showed negative DIC fluxes in the light, meaning that units from all treatments were effectively removing DIC from seawater (Table 4). DIC uptake rates were higher in control units and in those deployed over rhodolith beds adjacent to rocky formations. As well, positive calcification rates were observed across all treatments in the light, with higher rates shown by control blusinkies and those deployed near rock. Incubations conducted in the dark revealed a less clear pattern in both sand- and rock-deployed blusinkies, while control units showed clear carbon sequestration and positive calcification also under dark conditions.

Figures 7 and 8 depict the chemical changes observed in seawater during the 2-hour incubations. In all treatments, and both in the light and in the dark incubations, it's clear that DIC decrease was mainly driven by the decrease in bicarbonate, while carbon dioxide also decreased, but with a neglectable contribution to DIC fluxes. Also noticeable was a significant increase of carbonate across treatments. This combined pattern of the three DIC species naturally resulted in the pH increase observed throughout.

DIC uptake, pH increase and calcification rates were all more prominent in control (new) blusinkies, but also very significative in units deployed in the field. The similar results obtained for control blusinkies under light and dark conditions show that the materials used in their fabrication react with water to promote calcification and DIC uptake, as expected, through an exclusively chemical process. What the present results show is that, after six months of being deployed, and with some biological colonization, blusinkies continued to perform positively in their anticipated function of sequestering carbon and reducing seawater acidity. Deployments near rhodolith beds and rocky formations appear to have performed more promisingly than those deployed in a sandy bottom. This pattern can probably be attributed to an apparently lower algae colonization of blusinkies deployed in the sandy bottom. However, the considerable variability in the results, especially in dark-incubated samples, prevents a more definitive conclusion at this stage. It is therefore suggested that these incubations be repeated over time in deployed blusinkies, to assess their longer-term performance in natural conditions and with additional biological colonization. Experimentally, it also became clear that the number of replicates needs to be increased to better incorporate the large individual variability observed in deployed units.

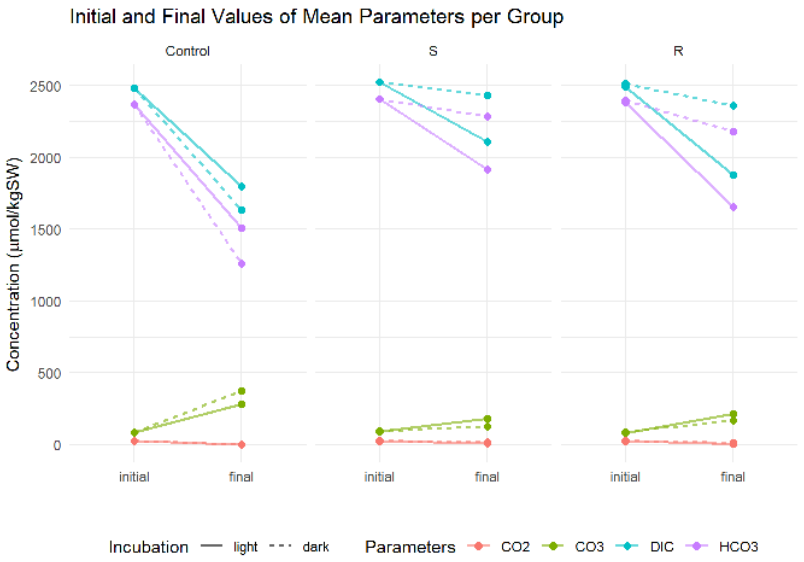


Figure 7: Blusinkie laboratorial incubations in light (solid lines) and dark conditions (dashed line) — Average initial and final concentrations of DIC and its major components (HCO^-_3, CO_3^{2-}, and CO_2) per group (control, sand (S), and rock (R)).

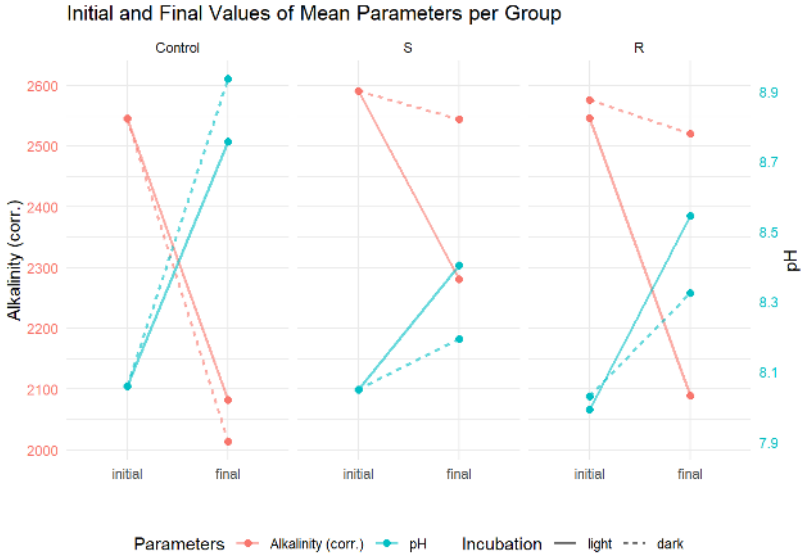


Figure 8: Blusinkie laboratorial incubations in light (solid lines) and dark conditions (dashed line) — Average initial and final alkalinity and pH per group (control, sand (S), and rock (R)).

4 Other aspects

After five months of deployment in Caniçal, the following problems were identified:

1. Flooding of temperature loggers. These were replaced by more robust models.
2. Some spikes disappeared from the plots, having been replaced by complementary ones. The fixation of the nets on the sandy plots was reinforced with additional spikes and cable ties.
3. Video doesn't seem to be the best approach to monitoring (resolution; time to process the data; difficulties in maintaining distance/angle to plots).

Bibliography

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