Manuscript prepared for Biogeosciences
with version 2014/07/29 7.12 Copernicus papers of the LaTeX class copernicus.cls.
Date: 21 February 2015

Technical note: Laboratory-controlled coral reef minicosms for ocean acidification investigations

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Abstract. The design and evaluation of replicated laboratory-controlled artificial ecosystems (which are referred to here as minicosms to differentiate them from micro- and mesocosms) are presented in the context of a thirteen-month experiment on the effects of ocean acidification on tropical coral reefs. Minicosms are defined here as artificial communities maintained in (semi)-closed systems, i.e. with limited and controlled exchanges with the external environment, which are run in the laboratory, or on site, with realistic levels, and field-like daily fluctuations, of the main physico-chemical parameters. Physico-chemistry is controlled thanks to a combination of biological controls and self-regulations and, where this is not possible, technical means. The realism of the habitat is assessed by comparison to a reference site in the field which, in the present case, is a lagoon on Réunion Island. The results highlight that it is possible to study a simplified reef community in such minicosms over more than one year . On the other hand, two initially identical minicosms evolved differently in terms of the global community oxygen budgets. Minicosms, like many complex systems, seem to leave enough degrees of freedom to the enclosed community of living organisms to organise and change along possibly diverging pathways, as also happens in natural ecosystems.

1 Introduction

Over the last century, anthropogenic atmospheric carbon dioxide (CO_2) emissions have risen (IPCC (2013)). One of the consequences of this is ocean acidification (OA) as CO_2 dissolves in seawater and pH is decreased. In parallel, the interest of the scientific community in OA has risen in the last

decade. Several strategies have been used to understand OA effects and the possible acclimation or adaptation of marine organisms (Widdicombe et al. (2010)). The effects of pH on marine organisms have been studied in natural environments characterised by a naturally low pH such as intertidal zones (e.g. Moulin et al. (2011); Egilsdottir et al. (2012)), CO_2 seeps (or vents) (for example, Hall-Spencer et al. (2008); Cigliano et al. (2010); Fabricius et al. (2011); Calosi et al. (2013)), upwelling zones (Feely et al. (2008)) and the deep sea (Park (1966); Roberts et al. (2006); Turley et al. (2007)). Such studies provide many useful insights into the effect of low pH on marine organisms. The downside is that several environmental parameters differ in these situations from the ongoing OA. There is generally no possibility of replication. Measurements can be performed on different organisms, however, as they all originate from the same location they cannot be considered to be independent replicates, which therefore leads to some degree of pseudo-replication and biased statistical analysis (Hurlbert, 1984).

Studies have also been conducted in aquaria to understand the physiological effects of OA on organisms in tightly controlled experimental conditions. The complexity and realism of the environment recreated in these aquaria varies from bare tanks, containing just one individual organism, to sophisticated systems that mimic the natural environment and contain a community of animals, plants and microbes (Fig. 1). According to Odum, a microcosm is defined as an artificial, simplified ecosystem that is used in the laboratory to simulate and predict the behaviour of a natural ecosystem under controlled conditions (Odum (1983)). On the other hand, a mesocosm is defined as a partially enclosed outdoor experimental setup that closely simulates the natural environment (Odum (1984)). Thirty years on, more sophisticated devices are available to recreate more realistic conditions in the laboratory. Yet, the two terms coined by Odum are still used in a somewhat loose way (see examples later in this introduction). In this paper, the concept of minicosms is proposed as a supplement to Odum's definitions of micro- and mesocosms.

Considering the fact that natural habitats can be simulated in aquaria (Adey and Loveland (2011)), recent laboratory-based systems are now much closer to natural conditions than the definition of microcosms suggests. Mesocosms were sometimes used to represent these systems. However, the initial definition specifies that these are outdoor setups, thus excluding such aquaria in the laboratory. Should a redefinition, based on its actual use, be considered? This would be a pity because the distinction between laboratory-based and outdoor setups is easy to make. Daily fluctuations in the temperature, light, pH, pCO_2 and pO_2 that occur in the aquaria have major physiological impacts on the living organisms. Yet, they are often poorly documented in literature: average values and standard deviations over the whole period of the experiment are most often provided. Techniques like pH-stats, that stabilise the pH at a target value prevent or limit such daily fluctuations if the target value is constant. Daily changes occur in systems where water at target pH is introduced into the aquaria at a constant rate. However, day and night pH values can depart from realistic levels both towards higher or lower amplitudes. If the flow of the reference water at constant pH is very high,

fluctuations are limited. On the other hand, if the setup is densely populated with photoautotrophs, pH can raise very high during the day and drop rapidly at night. In both cases, daily changes of pH are not realistic and probably interfere with the experiment. Given that it is possible to get enough control of these daily changes in artificial ecosystems in the laboratory, the suggestion here is to consider this as the key criterium to characterise a minicosm and to differentiate it from a microcosm. This implies equiping a reference site in the field with pH, pO_2, temperature and light probes in order to document how these parameters fluctuate there. Then, the setup must be designed carefully enough to exhibit similar values. Finally, the same parameters must be recorded in the aquarium and the comparison must be reported. These additional steps ensure more realism in the habitat artificially recreated in a minicosm than in a microcosm.

To give a more complete definition, minicosms are artificial communities maintained in (semi)-closed systems, i.e. with limited and controlled exchanges with the external environment, which recreate a realistic habitat including daily fluctuations of the main physico-chemical parameters. Physico-chemistry is controlled thanks to a combination of biological controls and self-regulations and, where this is not possible, technical means. The realism of the habitat must be assessed by comparison to a reference site in the field, as it is operated in the laboratory.

With such minicosms, five different categories of experimental setups exist (Fig. 1). Single-species experiments in bare aquaria are the simplest ones, and also the most replicable ones. On the other hand, experiments directly in the field, or using the macrocosm approach, are the less artificial, but also less replicable and manipulable. Between these two extremes, microcosms, minicosms and mesocosms constitute complementary artificial systems that provide an increased level of realism, but also perhaps, more difficulty in terms of control and replication.

Since 1995, the growing concern about the effects of OA on marine ecosystems, including on tropical coral reefs, has led scientists to favour a mesocosm approach (Stewart et al. (2013)). In the following part, studies on the effects of OA on tropical coral reef ecosystems will be summarised. For instance, a continuous flow coral reef mesocosm (475 L) was used in studies investigating the impact of OA on a natural coral reef community over nine months. (Andersson et al. (2009), Jokiel et al. (2008); Kuffner et al. (2008)). Mesocosms like this one, used for long-term experiments over several months or years, mean that acclimation in a naturally fluctuating environment can also be taken into account, namely seasonal and daily variations of physico-chemical parameters. The mesocosm's daily cycle followed the natural cycle thanks to a high input flow rate of seawater pumped from the adjacent reef. Natural recruitment was also possible through this seawater inflow. In these studies, OA conditions were reached by HCl addition and total alkalinity (A_T) was therefore lower in the acidified mesocosms compared to the controls. Yet, OA does not imply such a change in A_T (at least, not when due to an increased pCO_2) which is known to affect biogenic calcification, particularly in corals (Jury et al., 2009). A solution was proposed to solve this technical problem ((Jokiel et al., 2014)). Recent studies using CO_2 manipulation to modify pH were also conducted in relatively

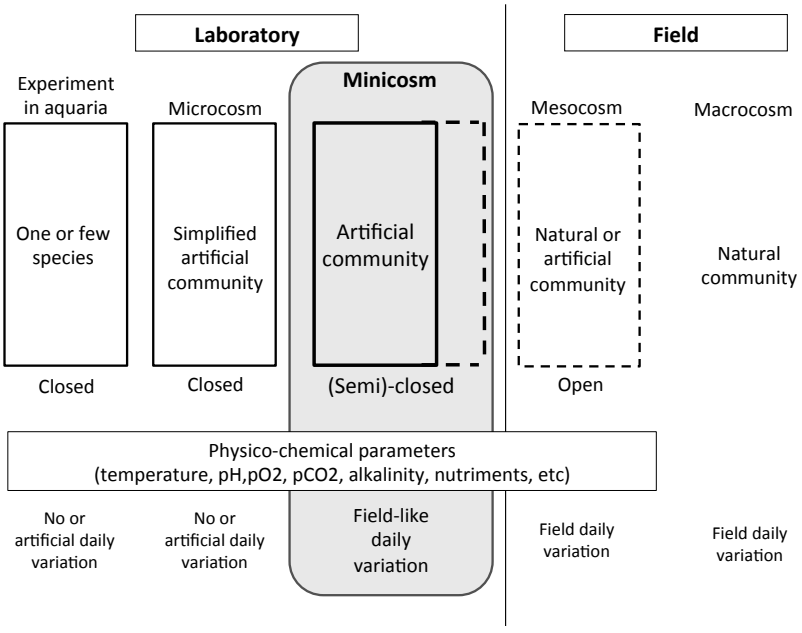

Figure 1. Different systems used in OA studies (as discussed in the text). The minicosm (in grey) serves as a compromise between more realism and complexity on the one hand, and ease of maintenance and replication in the laboratory, in the other hand.

small containers (150 L), which were called "open mesocosm" (Comeau et al. (2013a, b); Leclercq et al. (2000, 2002)), although these do not match Odum's definition of a mesocosm. Field-like daily variations were not applied to the system. It is therefore, better to refer to these setups as microcosms. A recent study by Dove et al. (2013) proposed a longer experiment (nine months) in a similar system where small communities were subject to different pCO_2 conditions. These different conditions were applied progressively over two and a half months.

In the nineties, a large closed reef "mesocosm" was developed (Biosphere 2, Atkinson et al. (1999); Langdon et al. (2000, 2003); Marubini et al. (2001)). Again, being on land, it does not strictly match Odum's definition of a mesocosm, but it may well be the first minicosm ever designed. Realistic daily changes of physico-chemical parameters were achieved through community activity, mainly respiration and photosynthesis and not through the manipulated inflow of water.

Kline et al. (2012) and Gattuso et al. (2014) recently described new tools to investigate the impact of OA at the ecosystem level *in situ*: "Free CO_2 Enrichment System" (FOCE) experimental devices. These are encapsulated open natural ecosystems with modern and sophisticated techniques to simulate a pH decrease similar to that caused by OA. These are true mesocosms, according to Odum's definition. Numerous advantages can be highlighted: field recruitment, field physico-chemical daily variations, natural community, precise control of stress conditions, replication, etc. Nevertheless some of these promising tools encompass a few disadvantages such as elevated costs and more lim-

ited accessibility than laboratory-based systems. Being run on relatively large scales, and most of the time in the field, it is difficult to combine pH decrease with another key factor in global change studies: temperature. Indeed, heating the large seawater masses that run through most FOCE systems would require too much energy (Gattuso et al., 2014). In this particular case, minicosms may provide an alternative.

In the present paper, the design and evaluation of tropical coral reef minicosms will be described. The objective of creating these minicosms was to construct an experimental design which provides a reef-like environment at a relatively low cost, which does not necessarily require natural seawater input, and which is replicable.

2 Design

The challenge in designing a reef minicosm is to answer the following question: given data for field physico-chemical parameters from the monitoring of say, oxygen, pH, total alkalinity and major nutrients (like N and P) in a given location in a natural tropical coral reef, is it possible to closely mimic these values in an artificial system in the laboratory? If so, how would the living community organise itself in such a system? Finally, how useful would it be for scientific investigations, such as OA studies?

Figure 2 presents a simplified diagram of one minicosm. Two identical setups were built in 2005 at UMONS (http://econum.umons.ac.be) and were refined and tested until the end of 2006. This system has been used since this initial test period and the design will be described below. Each minicosm consists of a closed system with one main tank (500L), two experimental aquaria (300L each) and common parts (sump, skimmer, etc). The main tank holds a diverse community of coral reef microbes, plants and animals. It contains reef substrate, handled with the same care as for fish or coral transportation to the laboratory, and its surface is almost completely covered with fast growing coral colonies (*Acropora digitifera*, *A. muricata*, *A. millepora*, *A. tenuis*, *Montipora patula*, *Pocillopora damicornis*, *Seriatopora hystrix*, *Stylophora pistillata*). It also contains algivorous animals in such density that the overgrowth of algae is avoided and coral cover is maintained (echinoderms, mollusks, crustaceans, reef fish, etc). Detritivores complete the community to recycle organic matter. The size of this tank is unfortunately not large enough to contain a few predators, hence the biomasses of the different ecological components are controlled manually (addition or elimination of items depending on the change of the community over several months). The main tank is mainly used to control the average water quality, by means of biological activity, as much as possible. The community is modifiable without impacting the organisms under manipulation in the experimental aquaria. It also contains mother colonies of corals and other organisms not currently included in the experiments. This configuration allows the nutrients to be controlled (by feeding fish to increase, or by adding more photoautotrophs to decrease, N and P concentrations in the water). The sump, filled

with reef substrate, is used as a convenient unit to collect seawater from the other tanks and to place various technical parts (Delbeek and Sprung (2007)).

The flow rate between the sump and the main tank is 14 ± 0.1 L min^{-1}. Experimental aquaria (300L each) are also connected to the sump, meaning that this is a paired design. Each experimental aquarium is connected to the sump, but physico-chemical parameters such as pH, pCO_2 or temperature can be controlled independently for each of them. Of course, more experimental aquaria can be connected to the system if required. The flow rate between the sump and each experimental aquarium is 0.8 ± 0.5 L min^{-1}. The following part of the design section explains how it was attempted to achieve realistic physico-chemical parameters. The reference location is a lagoon on Réunion Island: the back reef of La Saline fringing reef (21°70'S, 55°32'E). This lagoon offers a great diversity of reef organisms, and environmental data are available thanks to monitoring devices deployed at the site (Cuet, pers. comm., see also Chauvin et al. (2011)).

2.1 Minicosm monitoring

The minicosm is monitored and controlled using IKS Aquastar devices. These devices are connected to a computer and record the temperature and pH in each aquarium every 20 seconds. Plots are produced in real-time using the R software (R Core Team, 2013). These plots are displayed in the minicosm room and are also remotely visible via the internet. This information is thus available to every minicosm user at any time.

2.2 Light, temperature, water flow

Light is provided via eight T5 fluorescent lamps (39 W per bulb, 25:75 actinic blue 420 nm:trichromatic 10000 K, Aqua Medic, Germany) for each experimental aquarium and by sixteen of them for the main tank. This means that the light can be switched on and off progressively by managing groups of T5 bulbs with different light durations (Fig. 3) to mimic natural intensity and the spectral variation of light. Maximum photosynthetically active radiation (PAR) is 450±30 µmol.m^{-2}.s^{-1}. Each bulb is replaced every 6 months, to avoid the effect of ageing.

Temperature probes (Aquastar, Germany) are connected to a computer that controls heaters (Jäger, Germany) and air fans (allowing for slight temperature decrease by water evaporation) or cooling units (for larger temperature decrease, not necessary in the temperate lab) in each experimental aquarium and the main tank. Temperature hysteresis is equal to 0.3 °C. Differential day and night temperatures are obtained by changing the target value as a function of the time of the day.

Water motion is an important parameter in tropical reefs. Physiology of scleractinian corals is deeply influenced by water flow (Badgley (2006); Carpenter et al. (2007); Finelli et al. (2006); Sebens et al. (1998, 2003); Schutter (2010)). All aquaria are equipped with two variable speed Tunze - Turbelle Stream 6100S driven by a Tunze wave maker to simulate the action of the waves (pulses

from 0 to 40 m^3/h of water flow). The reference site being located in a lagoon, hydrodynamism is relatively low in comparison to, say, the reef crest and is more easily simulated in an aquarium.

2.3 Seawater composition and salinity

As the minicosm is run far away from tropical reefs, two alternatives were available to obtain seawater: natural seawater from a temperate coast or artificial seawater. Artificial seawater was chosen because it is more easily available and does not depend on natural variations in the field. It is prepared from ASTM type II water (Milli-G Direct, Millipore, Germany) and a mixture of mineral salts (Reef Crystals, Instant Ocean, USA). Before adding it into the minicosms newly prepared artificial seawater is mixed and aerated overnight. Ten percent of the minicosm water volume is changed every two weeks. Evaporation is compensated by addition of the same ASTM type II water using a Tunze 5017 osmolator. This device allows the water volume to be kept constant and stabilises salinity. Salinity is checked every other days using a WTW 340i salinometer (WTW, Germany).

2.4 Carbonate system

Recent studies have highlighted the importance of daily pCO_2 fluctuations on living organisms in the field (Comeau et al. (2014); Shaw et al. (2013)). These daily variations are mainly driven by biological activities, such as oxygen. The aim here is to simulate these natural fluctuations while maintaining a pH shift between the two experimental aquaria for the purpose of OA experiments. Therefore, CO_2 bubbling into the inflow water of the high pCO_2 aquarium was used. This bubbling is computer-controlled by a pH probe (Aquastar, Germany) by means of a solenoid valve. In the control aquaria, the pH has to be slightly increased. Calcium hydroxide saturated ASTM type II water is added, and this is also computer-controlled with a pH probe in the control aquarium. The pH probes are replaced every year. These systems do not act as pH-stats, but are only used to maintain a difference in the average pH (thus constraining the lowest value in the control and the highest level in the acidified aquaria respectively). Much of the daily fluctuation is due to biological activity in the experimental aquaria and in the refugia. It is certainly possible to use CO_2-free seawater to stabilise pH in the control experimental aquaria in replacement of $Ca(OH)_2$ additions, but this configuration has not been tested.

Calcium hydroxide also increased A_T following:

$$Ca(OH)_2 \leftrightarrow Ca^{2+} + 2OH^-$$
$$(Ca^{2+} + 2OH^-) + 2CO_2 \rightarrow Ca^{2+} + 2HCO_3^-$$

In order to keep the same A_T in each experimental aquarium, the same amount of $Ca(OH)_2$ is also added in the high pCO_2 aquarium as well. This method also helps to maintain A_T which drops rapidly otherwise, due to high calcification rate of the corals, sea urchins, mollusks, crustose coralline algae and other calcifying organisms. Bioaccreation is here the main factor that affects alkalinity. In

addition, nitrification also decreases alkalinity and bioerosion counterbalances these effects, to a minor extent, by producing alkalinity. In the initial trials, alkalinity still dropped rapidly, despite $Ca(OH)_2$ additions. Therefore alkalinity has had to be further stabilised by the use of a calcium reactor from the aquarium market (Aqua Medic, Germany). This is a container with solid calcium carbonate material maintained at low pH (around 6, or less) by CO_2 bubbling controlled by a pH probe. In these conditions, calcareous material progressively dissolves. A very slow water flow between the reactor and the mesocosm allows the alkalinity in the water to increase. The compensation of alkalinity is controlled by two parameters: the water flow between the reactor and the minicosm, and the pH maintained inside the reactor. These are adjusted according to alkalinity measurements in the minicosms.

2.5 Oxygen

Oxygen concentration in the field follows a daily cycle around saturation (Kline et al. (2012); see also Fig. 8). It is mainly driven by biological activities (net photosynthesis during the day, respiration of all organisms during the night). Daily fluctuations in pO_2 are also observed in closed systems (aquarium, microcosm). Nevertheless, the amplitude of oxygen variations in the laboratory can easily excess natural fluctuations, because of the water volume to biomass ratio that is much lower inside an aquarium than in the field. To avoid unnatural extremes in day *versus* night pO_2, each experimental aquarium is coupled with an 80L-refugium (Delbeek and Sprung (2007)) with a flow rate of 1.2 $L.min^{-1}$. In short, refugia are lit with an inverted day/night cycle (T5 fluorescent lamps, 2*39 W, 10000 K trichromatic, Aqua Medic, Germany). This setup limits the oxygen fluctuations between the day and night in the experimental units. Since higher flow rates are needed between the experimental aquaria and the refugia than between the experimental aquaria and the main tank, a single refugium for the whole minicosm is a suboptimal design in term of oxygen buffering in the experimental units.

2.6 Macronutrients

Studies from aquariology and public aquaria demonstrate that it is possible to reproduce, to a certain extend, the natural cycles of macronutrients, such as N and P, in closed systems (Adey and Loveland (2011)). A good balance between photoautotrophs, grazers and possibly some predators, together with efficient recycling of the organic matter is required. Moreover, anaerobic zones in the substrate promote denitrification. This lowers total inorganic nitrogen, possibly down to micromolar or submicromolar concentrations. Such concentrations are compatible with the values observed in the reference site (Chazottes et al. (2002)). The establishment of such conditions means that a balanced community of organisms has to be set up in order to establish N and P cycles. The exchanges with other ecosystems, like plankton importation from the open ocean, or organic matter exported in the form of sinking solid particles, have to be simulated by artificial means: feeding and mechanical fil-

Table 1. Balance between nitrogen and phosphorus inputs and outputs in the minicosm. Values represent means ± standard deviations. Inputs through feeding were estimated by using dry weights and the average elemental composition of zooplankton according to Anger et Dietrich (1984). Water change output is water eliminated from the system; input is freshly prepared water added to the system.

Nutrient	Source	Average output (µmol.day^{-1})	Average daily inputs (µmol.day^{-1})	Balance (µmol.day^{-1})
Nitrogen	Feeding	-	4700 ± 700	4700 ± 700
	Water change	-14.3 ± 0.8	26.6 ± 1.4	12.3 ± 2.0
Phosphorus	Feeding	-	290 ± 45	290 ± 45
	Water change	- 2.9 ± 0.2	3.6 ± 0.3	0.7 ± 0.4

tration, respectively. Adjustment like this take several months and this is probably one of the hardest, and longest, stages in establishing a minicosm.

The concentration of macronutrients in the water is controlled by two inputs and two outputs (Fig. 4). The main tank is fed with plankton (frozen Artemia and mysids; 0.6±0.1 g.day^{-1} dry weight), the amount is dictated by inorganic nitrogen and phosphorus concentrations in the water. Water changes (10% of the total volume every two weeks) also provide essential (micro)nutrients consumed by the coral reef community. These inputs are summarised in Table 1. Exportation of a fraction of the particulate matter produced is simulated by a mechanical filter (a perlon filter inside the sump, which is changed weekly). Dilution of the organic matter in the water column is simulated thanks to its partial elimination by a skimmer (Deltec AP850, Delbeek and Sprung (2007)). Unlike in many other aquaria, water change is not the main technique to keep N and P concentrations within acceptable levels in a well-balanced minicosm. The fresh artificial seawater contains 0.46 ± 0.01 µmol.L^{-1} PO_4^{3-}, 2.02 ± 0.01 µmol.L^{-1} NO_2^- + NO_3^- and 2.01 ± 0.01 µmol.L^{-1} NH_3. Indeed, N and P concentrations in the minicosms are very close to these levels, or slightly lower, resulting in a quasi neutral impact of the water change in term of inorganic N and P concentrations.

2.7 Test case OA experiment

For the test case OA experiment, a simplified reef community equal in biomass, was introduced progressively in each experimental aquarium. Sea urchins *Echinometra mathaei (E. mathaei violacea)* (Mortensen, 1943), violet *Echinometra* (see Arakaki et al. (1998)) were collected on Réunion Island in the Indian Ocean, in the back-reef of Saint-Pierre fringing reef (21°33'S, 55°47'E). Corals *Seriatopora hystrix*, *Acropora tenuis* and a half of the coral reef substrate (rocks) came from the aquarium market (Dejong Marinelife, Holland). Other coral species (*Acropora muricata*, *A. digitifera*, *Pocillopora damicornis*) and the other half of substrate were collected at Réunion Island in the back-reef of the La Saline fringing reef (21°70'S, 55°32'E). Permits were obtained before field collections from the "Réserve Naturelle Marine de La Réunion" (RNN164) and the "Direction de

l'Environnement, de l'Aménagement et du Logement" (DEAL). Organisms collected at Réunion Island were transported to the minicosm facilities in Belgium (transport duration: 24h) in seawater using styrofoam boxes. They were acclimated in control conditions for seven months before the beginning of the experiment. Sixteen sea urchins, 0.8 kg of hermatypic scleractinians and 20 kg of reef calcareous reef substrate were installed in each experimental aquarium. The main unit of each minicosm contained the same organisms as the experimental aquaria. Moreover the main tanks also contained reef fish: *Zebrasoma flavescens*, *Zebrasoma veliferum*, *Zebrasoma xanthurum*, *Paracanthurus hepatus* and *Amphirion ocellaris*. Sea urchins fed on macro algae and coralline algae attached to the reef substrate.

The OA experiment consisted of six months of progressive pH decrease in the acidified aquaria followed by seven months of stabilised pH. Major parameters such as temperature, pH, A_T, oxygen, nutrients, calcium and magnesium were monitored/controlled throughout the experiment.

2.8 Physico-chemical measurements

2.8.1 Seawater physico-chemical parameters

The electromotive force (e.m.f) was measured daily using an 827 pH Lab Metrohm meter (Switzerland) with a combined glass electrode (Metrohm 6.0228.010 with temperature sensor). The e.m.f was then converted to a total scale pH value (pH_T) using calibration curves of standard buffers of known pH, 2-aminopyridine/HCL (AMP) and tris/HCL (TRIS) (DOE (1994); DelValls et al. (1998); Dickson et al. (2007)). Salinity and temperature were measured daily using a salinometer pH/Cond 340i WTW (USA). These measurements of pH/T°/salinity were used as the one-point recalibration data for the continuous pH and temperature controllers. Seawater samples (50 mL) were collected daily and immediately filtered (0.22 µm GSWP, Millipore). Total alkalinity was measured by potentiometric titration using 0.01M HCl with 0.7M NaCl following Dickson et al. (2007), but adapted for a smaller volume (25 mL). Each titration was automatically performed by a computer using a Titronic Universal automatic titrator (SI Analytics, Germany), a C3010 multi parameter analyser to record pH (Consort, Belgium), a TW Alpha Plus autosampler (SI Analytics, Germany) and a laptop running a custom-made software piloting all three devices. Calibration was performed using certified reference seawater provided by A. G. Dickson (Scripps Institute of Oceanography, batches 94 and 120; difference between measurements and CRM before correction was lower than 24 µmol.kg^{-1}). Repeatability of alkalinity measurements was ± 6 µmol.kg^{-1}. The pCO_2 was calculated from A_T, pH_T, temperature and salinity data using the R software (R Core Team, 2013) and the package seacarb (Lavigne and Gattuso (2012); Lueker et al. (2000)'s constants for K1 and K2; Perez and Fraga (1987)'s constant for Kf; Dickson (1990)'s constant for Ks).

Every two weeks, seawater was sampled, filtered through a 0.22 µm filter (MilliPore), stored in polyethylene bottles and frozen at -20 °C until analysis. NH_3, NO_3^- + NO_2^- and PO_4^{3-} were

analysed using automated colorimetric analysis with a QuAAtro nutrient autoanalyser with an XY-2 autosampler (Seal Analytical, Mecquon, Wisconsin, USA). Calibrations were done using standard solutions.

Calcium and total alkaline earth metal (magnesium + calcium + strontium) concentrations were determined monthly by a potentiometric titration method adapted from Kanamori and Ikegami (1980). The titration was automatically performed by computer using a Titronic Universal automatic titrator (SI Analytics, Germany), a C3010 multi parameter analyser to record e.m.f (Consort, Belgium) and a TW Alpha Plus autosampler (SI Analytics, Germany) using custom software. Calcium concentration was measured by EGTA (molecular biological grade, VWR) titration using a calcium-selective electrode (Orion, Thermo Fisher Scientific, USA) and a calomel reference electrode (Schott B3510 Ch0, Germany). Total alkaline earth metals were determined by EDTA (Merck) titration using a divalent cation electrode (Consort, Belgium) and a reference electrode (Schott B3510 Ch0, Germany). Calibrations were performed using certified reference seawater (High-purity standards, USA).

2.8.2 Modelling of oxygen fluctuations

Oxygen was monitored every two months by using Clark electrodes connected to the IKS system. At the end of the experiment, a more detailed analysis of oxygen fluxes was performed performed by recording the data continuously over a five-day period using oxygen probes in each experimental aquarium, in the sumps and in the main tanks. Each probe was recalibrated daily (0 and 100 % O_2). Oxygen net fluxes (i.e. net photosynthesis and dark respiration) at the ecosystem level were calculated using the R statistical software and the simecol package. (Petzoldt and Kline (2007)). Net photosynthesis was defined from the following equation:

$$P = P_{max}.(1 - e^{E/EK}) + R_{dark} \text{ (1)}$$

Where P is the net photosynthesis in mmol O_2.min^{-1}, P_{max} is the maximum net photosynthesis in mmol O_2.min^{-1}, E is the irradiance in PAR (µmol photons 2^{-1}s^{-1}), EK is a constant that defines the efficiency of the photosynthesis as a function of irradiance and R_{dark} is the respiration in absence of light in mmol O_2.min^{-1}. Considering one aquarium, the oxygen carried in or out of the aquarium by water flow was defined as:

$$\frac{dO2_{exchange}}{dt} = \frac{V_{input}}{V_{aqua}} * (O2_{aqua} - O2_{input}) \text{ (2)}$$

Where $O2_{exchange}$ is the variation of oxygen due to water exchange between the aquarium and the other tanks in the minicosm, V_{input} is the volume of water input from the main tank (in L.min^{-1}), V_{aqua} is the volume of water in the aquarium (in L), $O2_{aqua}$ is the oxygen concentration in the aquarium and $O2_{input}$ is the oxygen concentration in the input water. Since this water is pumped out of the skimmer, $O2_{input}$ is very close to saturation at any time (checked by oxygen probes) and its

concentration is computed from the salinity and temperature of the tank using the R package marelac (Soetaert et al., 2012).

The oxygen exchanged with the air at the surface of the aquarium was calculated as:

$$\frac{dO2_{air}}{dt} = \frac{O2_{aqua} - O2_{sat}}{\tau} \quad (3)$$

Where $O2_{sat}$ is the oxygen saturation concentration depending on the seawater salinity and temperature, $O2_{aqua}$ is the aquarium oxygen concentration and τ is a coefficient depending on the water-air exchange efficiency, and determined experimentally.

The variation of oxygen inside the aquarium was then defined as:

$$\frac{O2_{aqua}}{dt} = \frac{P}{Vol} - \frac{dO2_{exchange}}{dt} - \frac{dO2_{air}}{dt} \quad (4)$$

The mathematical model was fitted to measured oxygen concentration data and an optimiser (Pseudo-random search algorithm of (Price , 1977)) was used to find best estimates for P_{max} end R_{dark} (package R simecol, Petzoldt and Kline (2007)).

2.8.3 Data analysis

Statistical analyses were performed using the statistical software R, and α was fixed to 0.05 for all tests. A_T, pH, salinity, alkaline earth metals and temperature were each analysed using linear models. Each parameter was tested as a dependent variable of the model and time was the independent variable. Slopes were then tested using t-tests to check if changes with time were significant. Residual analyses were graphically performed for each model to check the normality, homoscedasticity and linearity of the residuals. Comparisons of pH during the day and night were performed using paired t-tests with the Welch approximation for the degrees of freedom for unequal variances. Comparison of A_T, alkaline earth metal, nitrate, nitrite, ammonium and orthophosphate concentrations between the control and treatment aquaria were performed using paired t-tests with the Welch approximation for the degrees of freedom. Where there were enough replicates, the normality and homoscedasticity of the residuals were verified using quantile-quantile plots and Bartlett tests respectively.

3 Results

3.1 Carbonate sytem

3.1.1 Diurnal variations of pH

The major physico-chemical parameters are presented in Table 2 averaged by periods: the three-month acclimation period before the pH decrease, the six months during and after the decrease, and the seven-month stabilised period. It was possible to reasonably simulate the natural diurnal variation of pH in each experimental aquarium during the OA experiment (Fig. 5). Both minicosms presented

a significantly different pH during the day and night for each aquarium (paired t-tests, all p-values < 0.001), mainly as a result of biological activities (net photosynthesis during the day, dark respiration during the night, calcification), and just slightly facilitated by increased CO_2 and $Ca(OH)_2$ additions when needed (computer monitored and controlled). The amplitude of the pH variations between night and day in the control aquaria was equal to 0.2 pH unit while it was equal to 0.1 pH unit in the high pCO_2 aquaria.This was slightly higher than that recorded in the reference lagoon as it was adjusted to get an average daily pH_T around 8.1 (Fig. 5). Community-driven pH changes appeared lower in the acidified aquaria than in the control (most of the changes account for a day/night switch in the trigger level for CO_2 and $Ca(OH)_2$ additions).

3.1.2 Control of pH and alkalinity

The pH was recorded during the thirteen months of the experiment. The pH in each control aquarium showed a very small, but significant, increase throughout the experiment (Fig. 6, linear model, slope p-values < 0.001). Nevertheless, this increase was lower than 0.02 units.year^{-1}. The acidified aquaria showed a stable pH during the last seven months (stable conditions, linear model, slope p-values $\geq$ 0.07). During the pH decrease in the acidified aquaria, the pH slope was fixed at -0.03 unit every two weeks, which is much lower than the diurnal variation.

Despite the increasing difference in pH between the control and treatment aquaria, total alkalinity (Fig. 7) showed no significant difference (paired t-test, all p-values$\geq$ 0.1) and fluctuated within ±150 µmol.kg^{-1} limits during the thirteen-month experiment (linear models, all slope p-values $\geq$ 0.15). Moreover, total alkalinity in the two minicosms remained very close (within 5 % of variation). The total alkalinity fluctuations (Fig. 7) were due to intense, but slightly variable calcification, which is hard to track with the calcium reactor adjustment. They were nevertheless always the same between the control and treatment aquaria.

3.1.3 Temperature and salinity

The temperature remained within 1 degree of variation over the whole experiment in all experimental aquaria (Table 2). All experimental aquaria showed a slight temperature decrease during the experiment (slope p-values < 0.001). Nevertheless this decrease was lower than -0.1 °C.year^{-1}. No significant difference was observed between the experimental aquaria (paired t-tests, p-values $\geq$ 0.06).

Salinity was also monitored throughout the experiment (Table 2). One minicosm showed no significant variation through time (slope p-values $\geq$ 0.2). The second minicosm showed a slight and significant increase in salinity (slope p-values $\leq$0.001), but under 1 PSU year^{-1}. No significant difference was observed between the experimental aquaria (paired t-tests, p-values $\geq$ 0.4) .

3.1.4 Nutrients

The inorganic nitrogen concentration was studied throughout the experiment. The NO_3^-, NO_2^- and NH_3 concentrations remained at levels comparable to that observed in the field (Table 3) and did not vary significantly during the thirteen-months experiment (slope p-values $\geq$0.07) except for NH_3, which was slightly higher at the beginning of the experiment. Orthophosphates also remained at comparable concentrations to target levels and did not vary during the experiment (slope p-value $\geq$0.07). No significant difference was observed between aquaria (paired t-tests, p-values $\geq$ 0.3).

3.1.5 Calcium and total alkaline earth metals

The calcium concentration (Table 2) did not vary significantly with time throughout the experiment in all aquaria, nor did the total alkaline earth metal concentrations (slope p-values $\geq$0.07). Similarly, the ratio Ca/total alkaline earth metals was constant throughout the experiment in all aquaria (slope p-values $\geq$0.19). The mean value recorded in the minicosms (5.71$\pm$ 0.09) was lower that recorded in the field (6.38). All these parameters did not vary significantly between the contrasted pH conditions in both minicosms (paired t-tests, p-values > 0.1).

3.1.6 Oxygen

The oxygen concentration followed a daily cycle mainly driven by biological activities in the aquaria (Fig. 8). Oxygen saturation state oscillated between 85% and 130%. The oscillations were larger in the control aquaria for both minicosms. The overall balance of net oxygen fluxes was modelled for each aquarium (Table 4). Biological systems in each aquarium were the global sources of O_2, except for the acidified aquarium of minicosm B. No difference was observed between the control and treatment aquaria (Table 4) for respiration during the night as well as for net photosynthesis (paired t-tests, p-values > 0.05).

4 Discussion

Here minicosms are defined as intermediary systems between laboratory-based microcosms and *in situ* mesocosms. Particular attention must be paid to the simulation of realistic physico-chemical parameters of the environment to differentiate them from microcosms. Even more realism could be achieved mainly through the community of living organisms itself. Artificial filtration techniques cannot be avoided completely, but here they were limited as much as possible. Whether a system meets this goal or not could, and should, be assessed by comparison to a reference site in the field, including day and night fluctuations.

In such minicosms, a correct balance between photosynthesis and respiration is required both for *p*O2 and pH daily variations and for stable N and P concentrations. The respective biomasses of photoautotrophs and heterotrophs must be carefully adjusted. Macronutrients (N and P in this test

case, but also Si if diatoms play a major role in the ecosystem) can be essentially under the control of the community. Without input, biomasses cannot increase in the long term due to a depletion in N and P. This was observed during the first two years in the described system when no plankton was added (but this was, unfortunately, not quantified). Once the community was established, an auto-control of N and P concentrations was observed. This control was not due to water changes, because the concentration of inorganic N and P in the new and old waters were similar. Keeping macronutrients under control is particularly sensitive for tropical coral reef ecosystems (oligotrophic waters; Cooper et al. (2009)). Generally, very low nutrient concentrations can be difficult to maintain in closed systems, and can rise rapidly to unrealistic concentrations, due to very limited volumes where waste accumulates. An increase in the concentration of macronutrients in the water threatens coral reefs (Szmant (2002)), by decreasing calcification rates of hermatypic scleractinians (Marubini and Davies (1996); FerrierPages et al. (2000)), or by increasing the severity of coral diseases (Bruno and Petes (2003)). The presented minicosms did not suffer from these effects: low concentrations of macronutrients were maintained over several years.

In the case of this tropical coral reef, a mechanical filter and a skimmer were added to keep the water clear and to eliminate a part of the dissolved and colloidal organic matter. Coral produce mucus, and a cocktail of defensive substances are rapidly diluted in a natural reef. In the confined environment of a closed system, these substances accumulate. The skimmer is the only efficient means to eliminate a part of these substances and artificially simulate such dilution. In other, less oligothrophic, ecosystems additional filters might not be needed.

Another possible consequence of the confinement is the increase in day and night oxygen and carbon dioxide fluctuations in the water. A refugium illuminated in an inverse phase, to counterbalance this effect, was tested. Daily changes in pH are difficult to estimate for future OA conditions because these depend on many factors (Jury et al. (2013)). A recent study showed that these fluctuations could be amplified by OA (Shaw et al. (2013)). Jokiel et al. (2008) worked with an open system and observed increased daily pH fluctuations. Wisshak et al. (2012) also took into account these fluctuations mediated through biological activities. More importantly, recent studies have also shown that these fluctuations could modulate the response of scleractinian corals to OA (Comeau et al. (2014)). In the presented minicosms, the same refugia were used to simultaneously buffer oxygen and carbon dioxide day/night fluctuations in both the control and acidified conditions. In the control, a tradeoff was necessary because pH/pCO_2 fluctuations tended to be larger than in the field, while oxygen did change with a lower amplitude at the same time. The chosen configuration was thus the best possible compromise. Identical refugia were used for the acidified aquaria. Once the acidified condition was established, it appeared that both pH and pO_2 fluctuations were lower in amplitude than in the control. Oxygen flux modelling indicated a lower net photosynthesis in the acidified conditions. Oxygen fluctuations being the consequences of respiration and photosynthesis at the community level, a lower amplitude is coherent with acidification induced changes in the community. Interpre-

tation of pH fluctuations is more difficult because of the CO_2/$Ca(OH)_2$ additions. Even for oxygen, the presence of refugia may interfere too. These refugia contain algae that are also impacted by OA. Higher photosynthesis activity in the acidified refugia may better buffer global O_2 decrease and CO_2 increase during the night (Porzio et al. (2011)). This clearly constitutes an unwanted side-effect that may probably be mitigated by resizing the refugia according to net photosynthesis and respiration in the experimental aquaria, or, more easily, by changing the intensity of the light in these refugia. This is a sensitive aspect that deserves further work.

The concentrations of Ca^{++} and Mg^{++} ions in seawater partly affect the calcification rate of scleractinian corals (Langdon et al. (2000); Marshall and Clode (2002)) and the nature of the precipitated mineral in sea urchins. Furthermore, their concentration could even increase biological/metabolic effects (Mitsuguchi et al. (2003)). Therefore, it is crucial to maintain a constant concentration of calcium independently of pH conditions in order to avoid the effect of confounding factors on the calcification rate of corals. This was relatively easy to do in the minicosms. However, the artificial seawater salt used (Reef Crystals) contains a slightly higher concentration of Ca^{++} than in natural seawater (Atkinson and Bingman (1997)). This problem may certainly be overcome by selecting another brand of sea salt with a better balanced composition. The control of alkalinity was slightly difficult with such a heavy calcifying community. The observed variations of more than 100 $mmol.kg^{-1}$ are large. Clearly, a better control of this parameter would be appreciable in the context of OA experiments. However, due to the paired design, variations in the control and acidified aquaria of the same minicosm were quasi identical. This mitigates a probable impact of these variations in terms of relative calcification and erosion in the control *versus* acidified sub-communities. Control of the alkalinity was mediated by both $CaOH_2$ additions and by the calcium reactor. Whether a combination of CO_2-free water and a calcium reactor better controls alkalinity still remains to be tested. The sensitivity of the minicosm to alkalinity balance could perhaps also be used indirectly for accurate measurements of the bioerrosion *versus* bioaccretion balance. Andersson et al. (2009), working on hermatypic coral calcification, showed that the net balance of $CaCO_3$ accretion was declining in higher pCO_2 conditions. Experiments, at the ecosystem level, to determine the impact of OA on the balance between calcifiers and eroders, including intraspecific interactions, are important (Kroeker et al. (2012)). Minicosms could also be used here.

The twin minicosms have been in a steady state now for over five years, indicating that such a system is sustainable in the long-term. The community has had to be adjusted manually from time to time, by eliminating part of the organisms that grew too much out of the refugia, such as a few coral colonies, or *Caulerpa* algae; or by adding missing components, like replacing dead fish, mollusks or echinoderms. This simulates the predation and recruitment that are lacking, for obvious reasons, in such minicosms. At the beginning of the experiment, the same simplified biological community was introduced into these aquaria (same species assemblages and biomasses). The test case OA experiment was a relatively long-term one, over more than one year, and with a gradual decrease

of pH in the acidified aquaria. The purpose of this study was to avoid a brutal change (stress) and to take into account acclimation over several months, both for individual species and for the whole community. Such an experiment is clearly realisable in minicosms, and the living community in the main tank remained healthy despite the pH changes. It should be noted that pathogens are part of the community and episodes of white band disease were observable from time to time on many coral colonies. Metagenomic analyses of the coral mucus revealed a large quantity of herpes-like viruses during one such episode (Laghdass and Gillan, pers. comm.). Herpes-like viruses have already been shown to be related to some forms of the white band disease in the field (Soffer et al. (2014)). Pathogens also make up part of the community in such minicosms. During this OA experiment, the ecophysiological response of the scleractinians and sea urchins was studied. Some of these results have already been published (Moulin et al. (2014)) and more will be published soon.

5 Conclusions and perspectives

Minicosms can be used for OA experiments. Due to divergent the evolution of identical replicates in the long term, the paired design used here is useful. It provides more statistical power in the analyses. Seasonal temperature changes could also be reproduced. It is possible to connect more experimental aquaria to test different pH values (for instance 8.2, 8.0, 7.6 and 7.3). This would allow even more powerful statistical approaches, such as linear or nonlinear modelling of biological responses in function of pH. On the other hand, four experimental aquaria would also allow to combine pH and temperature changes to be combined in a cross-factorial design with minicosms a repeated factor (being a paired design). Moreover, thanks to its flexibility, replicability, easy access and independence from extreme meteorological factors in the field, laboratory controlled minicosms are worth considering as a complementary tool to observations and experiments undertaken directly *in natura*, for instance using FOCE systems (Gattuso et al. (2014)). Minicosms will always remain complementary tools, because, being artificial, results cannot be extrapolated carelessly to natural ecosystems. They are, however, flexible and convenient enough for OA experiments at the ecosystem level.

Acknowledgements. The authors would like to thank DEAL and GIP of the Marine Nature Reserve of Réunion Island for their help and for authorisation to collect sea urchins and corals in the field. The authors are also grateful to Prof. Cuet and the lab ECOMAR at the University of Réunion Island for allowing them to quote their field data. The collection of field data was made possible through the financial support of the European program "RUNSeaScience" (IRD Réunion) and the programme "OT-RUN Mer" (Observatoire des Sciences de l'Univers de La Réunion, OSU-R). The authors would also like to thank Natacha Brion (Analytical and Environmental Chemistry lab, VUB) for the analysis of nutrients in seawater and M. Collard, F. Gazeau, J.-P. Gattuso, and two anonymous referees, for their constructive remarks on the manuscript and N. Dickson for proofreading it. L. Moulin holds a FNRS-FRIA PhD grant. P. Dubois is a Research Director of the National

Fund for Scientific Research (FRS-FNRS; Belgium). This work is supported by FRFC contract n°2.4587.11
(Coral Reef Ecology in Acidified Mesocosms).

References

Adey, W. and Loveland, K. (2011) Dynamic aquaria. Building and restoring living ecosystems. 3rd Edition, Academic Press, Elsevier, New York, pp. 528.

Andersson, A.J., Kuffner, I., Mackenzie, P. L. Jokiel, Rodgers K. S. and Tan A. (2009) Net Loss of $CaCO_3$ from a subtropical calcifying community due to seawater acidification: mesocosm-scale experimental evidence. Biogeosciences, 6, 1811-1823.

Anger, K. and Dietrich, A. (1984). Feeding rates and gross growth efficiencies in Hyasaraneus L. larvae (Decapoda: Majidae) Reared in the laboratory. Journal of experimental marine biology and ecology, 77(1), 169-181.

Arakaki, Y., Uehara, T. and Fagoonee, I. (1998) Comparative studies of the genus *Echinometra* from Okinawa and Mauritius. Zoological science, 15, 159–168

Atkinson, M. J. and Bingman, C. (1997). Elemental composition of commercial seasalts. Journal of Aquariculture and Aquatic Sciences, 8, 39.

Atkinson, M.J., Barnett, H., Aceves, H., Langdon, C., Carpenter, S.J., McConnaughey, T., Hochberg, E., Smith, M. and Marino, B.D.V. (1999) The Biosphere 2 coral reef biome. Ecological Engineering, 13, 147–172.

Badgley, B., Lipschultz, F. and Sebens, K. (2006) Nitrate uptake by the reef coral *Diploria strigosa*: effects of concentration, water flow, and irradiance. Marine Biology,149, 327–338.

Bruno, J. and Petes, L. (2003) Nutrient enrichment can increase the severity of coral diseases. Ecology Letters, 6, 1056–1061.

Calosi, P., Rastrick, S.P.S., Graziano, M., Thomas, S.C., Baggini, C., Carter, H. and Spicer, J.I. (2013) Distribution of sea urchins living near shallow water CO_2 vents is dependent upon species acid-base and ion-regulatory abilities. Marine Pollution Bulletin, 73, 470–484.

Carpenter, L.W. and Patterson, M.R. (2007) Water flow influences the distribution of photosynthetic efficiency within colonies of the scleractinian coral *Montastrea annularis* (Ellis and Solander, 1786); implications for coral bleaching. Journal of Experimental Marine Biology and Ecology, 351, 10 – 26.

Chauvin, A., Denis, V. and Cuet, P. (2011) Is the response of coral calcification to seawater acidification related to nutrient loading? Coral Reefs, 30, 911–923.

Chazottes, V., Le Campion-Alsumard, T. , Peyrot-Clausade, M. and Cuet, P. (2002) The effects of eutrophication-related alterations to coral reef communities on agents and rates of bioerosion (Reunion Island, Indian Ocean). Coral reefs, 21, 375–390.

Cigliano, M., Gambi, M.C., Rodolfo-Metalpa, R., Patti, F.P. and Hall-Spencer, J.M. (2010) Effects of ocean acidification on invertebrate settlement at volcanic CO_2 vents. Marine Biology, 157, 2489–2502.

Clavier, J., Chauvaud, L., Cuet, P., Esbelin, C., Frouin, P., Taddei, D. and Thouzeau, G. (2008) Diel variation of benthic respiration in a coral reef sediment (Reunion Island, Indian Ocean). Estuarine, Coastal and Shelf Science, 76, 369-377.

Comeau, S., Carpenter, R.C. and Edmunds, P.J. (2013a) Effects of feeding and light intensity on the response of the coral *Porites rus* to ocean acidification. Marine Biology, 160, 1127–1134.

Comeau, S., Edmunds, P.J., Spindel, N.B. and Carpenter, R.C. (2013b) The responses of eight coral reef calcifiers to increasing partial pressure of CO_2 do not exhibit a tipping point. Limnology and Oceanography, 58, 388–398.

Comeau, S., Edmunds, P., Spindel, N. and Carpenter, R. (2014) Diel pCO_2 oscillations modulate the response of the coral *Acropora hyacinthus* to ocean acidification. Marine Ecology Progress Series, 501, 99–111.

Cooper, T., Gilmour, J. and Fabricius, K. (2009) Bioindicators of changes in water quality on coral reefs: review and recommendations for monitoring programmes. Coral reefs, 28, 589–606.

DelValls, T.A. and Dickson, A.G. (1998) The pH of buffers based on 2-amino-2-hydroxymethyl-1,3-propanediol ("TRIS") in synthetic sea water. Deep-Sea Research I, 45, 1541-1554.

Delbeek, J.C. and Sprung, J. (2007). The reef aquarium, Volume 3. Ricordea Publishing, pp. 680.

Dickson, A.G. (1990) Standard potential of the reaction: AgCI(s) + ½ H2(g) = Ag(s) + HCI(aq), and the standard acidity constant of the ion HSO4 in synthetic sea water from 273.15 to 318.15 K. J Chem Thermodyn, 22, 113-127.

Dickson, A.G., Sabine, C.L. and Christian, J.R. (2007) Guide to best practices for ocean CO_2 measurements. PICES Special Publication 3, pp 191.

DOE, 1994. Handbook of methods for the analysis of the various parameters of the carbon dioxide system in seawater. Department of Energy. ORNL/CDIAC-74. Version 2.

Doney, S.C., Fabry, V.J., Feely, R.A. and Kleypas, J.A. (2009) Ocean acidification: The other CO_2 problem. Annual Review of Marine Science, 1, 169–192.

Dove, S.G., Klinea, D.I., Pantosa, O, Angly F.E., Tyson, G.W. and Hoegh-Guldberg, O. (2013) Future reef decalcification under a business-as-usual CO_2 emission scenario. Proceedings of the National Academy of Sciences, 110, 15342–15347.

Egilsdottir, H., Noisette, F., Noël, L.M.-L.J., Olafsson, J. and Martin, S. (2012) Effects of pCO_2 on physiology and skeletal mineralogy in a tidal pool coralline alga *Corallina elongata*. Marine Biology, 160, 2103–2112.

Fabricius, K.E., Langdon, C., Uthicke, S., Humphrey, C., Noonan, S., De'ath, G., Okazaki, R., Muehllehner, N., Glas, M.S. and Lough, J.M. (2011) Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations. Nature Climate Change, 1, 165-169.

Feely, R. A., Sabine, C.L., Hernandez-Ayon, J.M., Ianson, D. and Hales, B. (2008) Evidence for upwelling of corrosive "acidified" water onto the continental shelf. Science, 320, 1490–1492.

Ferrier-Pagès, C., Gattuso, J.P.,Dallot, S. and Jaubert, J. (2000) Effect of nutrient enrichment on growth and photosynthesis of the zooxanthellate coral *Stylophora pistillata*. Coral Reefs, 19, 103–113.

Finelli, C.M., Helmuth, B.S.T., Pentcheff, N.D. and Wethey, D.S. (2006) Water flow influences oxygen transport and photosynthetic efficiency in corals. Coral Reefs, 25(1), 47-57.

Gattuso, J.-P., Kirkwood, W., Barry, J.P., Cox, E., Gazeau, F., Hansson, L., Hendriks, I., Kline, D.I., Mahacek, P., Martin, S., McElhany, P., Peltzer, E.T., Reeve, J., Roberts, D., Saderne, V., Tait, K., Widdicombe, S. and Brewer, P.G. (2014) Free Ocean CO_2 Enrichment (FOCE) systems: present status and future developments. Biogeosciences Discussions, 11, 4001–4046.

Hall-Spencer, J.M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, S.M., Rowley, S.J., Tedesco, D. and Buia, M.-C. (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. Nature, 454, 96–99.

Hurlbert, S. H. (1984) Pseudoreplication and the design of ecological field experiments. Ecological monographs 54 , 187-211.

Intergovernmental Panel on Climate Change IPCC (2013) Climate Change 2013: the fifth assessment report of the IPCC. Cambridge University Press, Cambridge.

Jokiel, P.L., Bahr, K.F. and Rodgers, K.S. (2014) Low-cost, high-flow mesocosm system for simulating ocean acidification with CO_2 gas. Limnology and Oceanography: methods, 12, 313-322.

Jokiel, P., Rodgers, K., Kuffner, I., Andersson, A., Cox, E. and Mackenzie, F. (2008) Ocean acidification and calcifying reef organisms: a mesocosm investigation. Coral Reefs, 27, 473–483.

Jokiel, P.L., Jury, C.P. and Rodgers, K.S. (2014) Coral-algae metabolism and diurnal changes in the CO_2 - carbonate system of bulk sea water. PeerJ, 2, e378.

Jury, C.P., Whitehead, R.F. and Szmant, A.M. (2009) Effects of variations in carbonate chemistry on the calcification rates of *Madracis auretenra* (= *Madracis mirabilis sensu* Wells, 1973): bicarbonate concentrations best predict calcification rates. Global Change Biol, 16, 1632–1644.

Jury, C.P., Thomas, F.I., Atkinson, M.J., Toonen, R.J. Buffer Capacity, Ecosystem Feedbacks, and Seawater Chemistry under Global Change. Water 2013, 5, 1303-1325.

Kanamori S. and Ikegami H. (1980) Computer-processed potentiometric titration for the determination of calcium and magnesium in seawater. Journal of the Oceanographical Society of Japan, 36, 177-184.

Kline, D.I., Teneva, L., Schneider, K., Miard, T., Chai, A., Marker, M., Headley, K., Opdyke, B., Nash, M., Valetich, M., Caves, J.K., Russell, B.D., Connell, S.D., Kirkwood, B.J., Brewer, P., Peltzer, E., Silverman, J., Caldeira, K., Dunbar, R.B., Koseff, J.R., Monismith, S.G., Mitchell, B.G., Dove, S. and Hoegh-Guldberg, O. (2012) A short-term in situ CO_2 enrichment experiment on Heron Island (GBR). Scientific reports, 2, 413.

Kuffner, I.B., Andersson, A.J., Jokiel, P.L., Rodgers, K.S. and Mackenzie, F.T. (2008) Decreased abundance of crustose coralline algae due to ocean acidification. Nature Geoscience, 1, 114–117.

Kroeker, K.J., Micheli, F. and Gambi, M.C. (2012) Ocean acidification causes ecosystem shifts via altered competitive interactions. Nature Climate Change, 3, 156–159.

Langdon, C., Takahashi, T., Sweeney, C., Chipman, D., Goddard J., Marubini, F., Aceves, H., Barnett, H. and Atkinson, M.J. (2000) Effect of calcium carbonate saturation state on the calcification rate of an experimental coral reef. Global Biogeochemical Cycles, 14, 639 – 654.

Langdon, C., and others. 2003. Effect of elevated CO2 on the community metabolism of experimental coral reef. Global Global Biogeochemical Cycles, 17,1011.

Lavigne, H. and Gattuso, J.-P. (2012). Seacarb: Seawater carbonate chemistry with R (R package version 2.4.3).

Leclercq, N., Gattuso, J.-P. and Jaubert, J. (2000) CO_2 partial pressure controls the calcification rate of a coral community. Global Change Biology, 6, 329-334.

Leclercq, N., Gattuso, J. and Jaubert, J. (2002) Primary production, respiration, and calcification of a coral reef mesocosm under increased CO_2 partial pressure. Limnology and Oceanography, 47, 558–564.

Lueker, T.J., Dickson, A.G. and Keeling, C.D. (2000). Ocean pCO_2 calculated from dissolved inorganic carbon, alkalinity, and equations for K-1 and K-2: validation based on laboratory measurements of CO_2 in gas and seawater at equilibrium. Marine Chemistry, 70, 105–119.

Marshall, A.T. and Clode, P.T. (2002) Effect of increased calcium concentration in sea water on calcification and photosynthesis in the scleractinian coral *Galaxea fascicularis*. Journal of Experimental Biology, 205, 20107-2113.

Marubini, F., Barnett, H., Langdon, C. and Atkinson, M. (2001) Dependence of calcification on light and carbonate ion concentration for the hermatypic coral *Porites compressa*. Marine Ecology Progress Series, 220, 153–162.

Marubini, F. and Davies, P.S. (1996) Nitrate increases zooxanthellae population density and reduces skeletogenesis in corals. Marine Biology, 127, 319–328.

Mitsuguchi, T., Matsumoto, E. and Uchida, T. (2003) Mg/Ca and Sr/Ca ratios of Porites coral skeleton: Evaluation of the effect of skeletal growth rate. Coral Reefs, 22, 381–388.

Mortensen T.H. (1943) A monograph of Echinoida. Vol.III, 3. Camarodonta. II Echinoidae, Strongylocentrotidae, Parasaleniidae, Echinometridae. CA Reitzel, Copenhagen, pp. 277-439.

Moulin, L., Catarino, A.I., Claessens, T. and Dubois, P. (2011) Effects of seawater acidification on early development of the intertidal sea urchin *Paracentrotus lividus* (Lamarck 1816). Marine Pollution Bulletin, 62, 48–54.

Moulin, L., Grosjean, P, Leblud, J, Batigny, A and Dubois, P. (2014) Impact of elevated pCO_2 on acid-base regulation of the sea urchin *Echinometra mathaei* and its relation to resistance to ocean acidification: a study in mesocosms. Journal of Experimental Marine Biology and Ecology, 457, 97-104.

Odum, E. (1983) Systems Ecology: An Introduction. Wiley, New York, pp. 644.

Odum, E. (1984) The mesocosm. BioScience, 34, 558–562.

Park, K. (1966) Deep-sea pH. Science, New Series, 154, 1540-1542.

Perez, F.F. and Fraga, F. (1987). Association constant of fluoride and hydrogen ions in seawater. Marine Chemistry, 21, 161-168.

Petzoldt, T. and Rinke, K. (2007). simecol: An Object-Oriented Framework for Ecological Modeling in R. Journal of Statistical Software, 22(9), 1–31. URL http://www.jstatsoft.org/v22/i09

Porzio L.,Buia M. C. and Hall-Spencer J.M. (2011). Effects of ocean acidification on macroalgal communities. Journal of Experimental Marine Biology and Ecology, 400, 278-287.

Price, W. L. (1977). A controlled random search procedure for global optimisation. The Computer Journal, 20, 367-370.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.URL http://www.R-project.org/.

Roberts, J.M., Wheeler A.J. and Freiwald A. (2006) Reefs of the deep: the biology and geology of cold-water coral ecosystems. Science, 312,547

Turley, C.M., Roberts, J.M. and Guinotte, J.M. (2007) Corals in deep-water: will the unseen hand of ocean acidification destroy cold-water ecosystems? Coral Reefs, 26, 445–448.

Schutter, M. (2010) The Influence of Light and Water Flow on the Growth and Physiology of the Scleractinian Coral *Galaxea fascicularis*. Thesis, Wageningen University, Wageningen, Netherlands, 232 pp.

Sebens, K., Grace, S. and Helmuth, B. (1998) Water flow and prey capture by three scleractinian corals, *Madracis mirabilis, Montastrea cavernosa and Porites porites*, in a field enclosure. Marine Biology, 131, 347–360.

Sebens, K., Helmuth, B., Carrington, E. and Agius, B. (2003) Effects of water flow on growth and energetics of the scleractinian coral *Agaricia tenuifolia* in Belize. Coral Reefs, 22, 35–47.

Shaw, E.C., McNeil, B.I., Tilbrook, B., Matear, R. and Bates, M.L. (2013) Anthropogenic changes to seawater buffer capacity combined with natural reef metabolism induce extreme future coral reef CO_2 conditions. Global Change Biology, 19, 1632–41.

Szmant, A. (2002). Nutrient enrichment on coral reefs: is it a major cause of coral reef decline? Estuaries, 25(4), 743–766.

Soetaert, K., Petzoldt, T. and Meysman, F. (2012). marelac: Tools for Aquatic Sciences. R package version 2.1.2. URL http://CRAN.R-project.org/package=marelac.

Soffer, N., Brandt, M.E., Correa, A.M.S., Smith, T.B. and Thurber, R.V. (2014) Potential role of viruses in white plague coral disease. International Society for Microbial Ecology Journal, 8, 271-283

Stewart, R.I.A., Dossena, M., Bohan, D.A., Jeppesen, E., Kordas, R.L., Ledger, M.E., Meerhoff, M., Moss, B., Mulder, C., Shurin, J.B., Suttle, B., Thompson, R., Trimmer, M. and Woodward, G. (2013) Mesocosm Experiments as a Tool for Ecological Climate-Change, 1st ed. Elsevier Ltd.

Vizzini, S., Di Leonardo, R., Costa, V., Tramati, C.D., Luzzu, F. and Mazzola, A. (2013) Trace element bias in the use of CO_2 vents as analogues for low pH environments: Implications for contamination levels in acidified oceans. Estuarine, Coastal and Shelf Science, 134, 19–30.

Widdicombe, S., Dupont, S. and Thorndyke, M. (2010) Laboratory experiments and benthic mesocosm studies. In: Riebesell, U., Fabry, V.J., Hansson, L. and Gattuso, J.-P. (eds) Guide to best practices for ocean acidification research and data reporting. Publications Office of the European Union, Luxembourg.

Wisshak, M., Schönberg, C., Form, A. and Freiwald, A. (2012) Ocean acidification accelerates reef bioerosion. PLoS One, 7, 3–10.

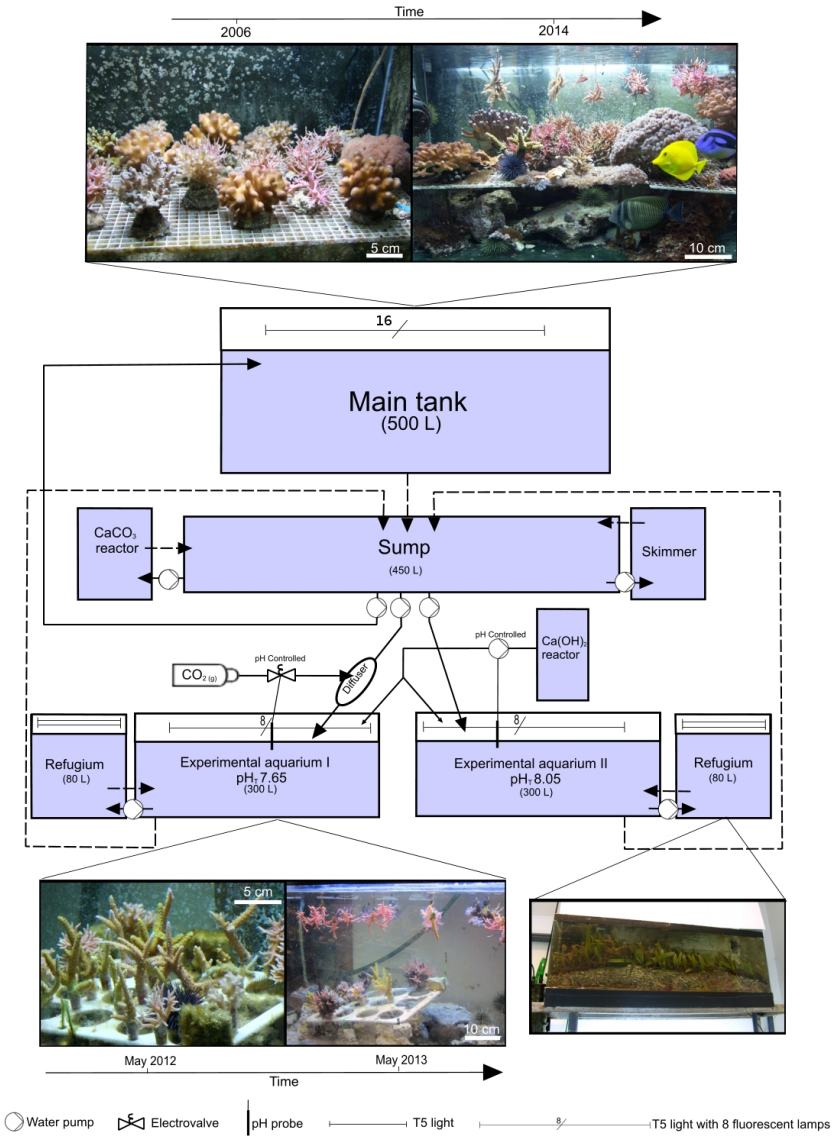

Figure 2. Coral reef minicosm. The main tank contains a community of reef organisms whose role is to biologically control the water quality. Experimental aquaria contain a community of organisms according to the experiment, and are thus less alterable. For instance, if, after starting an experiment, the whole setup needs adjustment of the biomass of photoautotrophs, it is in the main tank that changes are made. A sump collects water flowing from all items (water flow: 14 L.min^{-1} between the main tank and the sump and 0.8 L.min^{-1} between each experimental aquarium and the sump). The sump also contains technical devices and filters. A_T is stabilised using a $CaCO_3$ reactor. A skimmer eliminates the excess of dissolved, colloidal and particulate organic molecules in the water column. pH in experimental aquaria is controlled using CO_2 bubbling and $Ca(OH)_2$ additions. Refugia connected to experimental aquaria control daily oxygen fluctuations (water flow: 1.2 L.min^{-1}). Temperature is controlled with electric resistances and fans with a constant room temperature around 20°C. Pictures illustrate the different parts and their evolution with time.

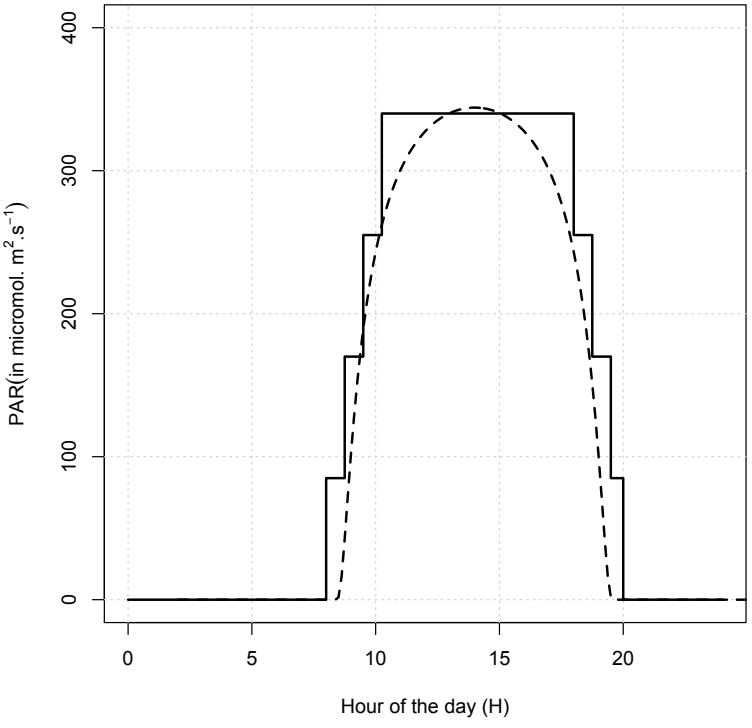

Figure 3. Daily cycle of light as photosynthetically active radiations (PAR). Black line represents PAR measured with an Apogee Quantum Meter inside the main tank. Dotted line is the theoretical PAR on Réunion Island, adjusted with field measurements at 1 m depth (using the same Quantum Meter). Total day/night time is 12h/12h in the minicosms.

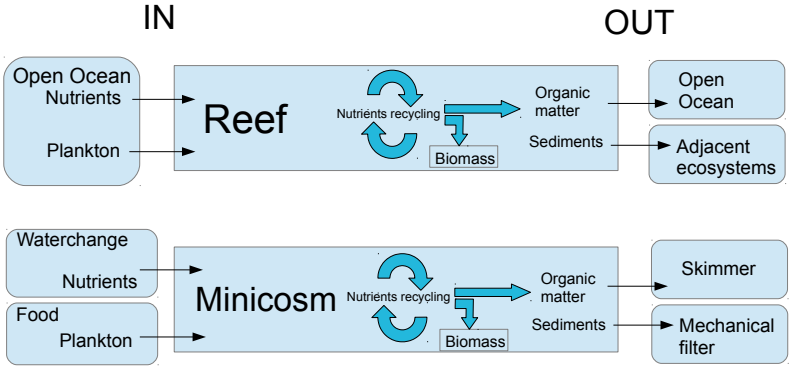

Figure 4. Comparison between a natural reef ecosystem and the minicosm. Natural inputs are simulated in the minicosm by feeding organisms with plankton and by performing water changes. Natural outputs are simulated by filters: skimmer and mechanical filter. When the minicosm is well-balanced, nutrients added to the system by inputs are either converted to biomass, or eliminated by outputs. The concentration of the macronutrients in the water remain stable with time and no massive water change is required to lower them periodically.

Table 2. Mean physico-chemical parameters recorded in each aquarium before the pH decrease (3 months monitoring), during the pH decrease (6 months monitoring) and after the pH decrease (7 months monitoring). Values represent means ± standard deviations. Mean temperature and pH were calculated from the measurements recorded every 20 seconds. Mean salinity, A_T and pCO_2 were calculated from the daily measurements. Calcium and total alkaline earth metals (Ca + Mg + Sr) were calculated from the monthly measurements.

	Minicosm A - Control			Minicosm A - Acidified			Minicosm B - Control			Minicosm B - Acidified		
Parameter	Before decrease	During decrease	After decrease	Before decrease	During decrease	After decrease	Before decrease	During decrease	After decrease	Before decrease	During decrease	After decrease
pH_T	8.04±0.02	8.06±0.02	8.08±0.03	8.07±0.01	7.82±0.11	7.63±0.02	7.99±0.02	8.09±0.03	8.09±0.04	8.05±0.03	7.83±0.09	7.62±0.02
pCO_2 (ppm)	420±21	388±61	381±40	378±18	784±224	1294±125	402±23	388±61	356±47	484±32	806±249	1263±92
Total alkalinity (mmol.kg^{-1})	2.363±0.110	2.400±0.118	2.406±0.147	2.353±0.107	2.372±0.141	2.447±0.200	2.373±0.123	2.511±0.331	2.350±0.109	2.373±124	2.495±0.204	2.379±0.131
HCO_3^- (mmol.kg^{-1})	1.833±0.084	1.836±0.102	1.826±0.128	1.789±0.081	2.009±0.141	2.225±0.177	1.824±0.095	1.899±0.259	1.766±105	1.886±0.089	2.115±0.228	2.140±0.121
CO_3^{2-} (mmol.kg	0.215±0.014	0.229±0.013	0.236±0.021	0.229±0.014	0.148±0.034	0.101±0.009	0.223±0.016	0.251±0.039	0.237±0.015	0.199±0.019	0.156±0.022	0.098±0.006
Ω aragonite	3.843±0.228	4.164±0.246	4.269±0.381	4.406±0.217	2.664±0.561	1.819±0.166	3.936±0.266	4.629±0.786	4.289±0.298	3.487±0.322	2.864±0.374	1.760±0.105
Ω calcite	5.841±0.344	6.323±0.373	6.480±0.578	6.174±0.327	4.047±0.856	2.764±0.254	5.979±0.402	7.018±1.190	6.511±0.451	5.299±0.488	4.344±0.567	2.672±0.159
Temperature (°C)	25.07±0.19	25.24±0.32	25.13±0.19	24.98±0.07	25.22±0.32	25.06±0.11	25.13±0.12	25.36±0.35	25.10±0.22	25.03±0.11	25.31±0.33	25.04±0.10
Salinity	34.47±0.44	34.14±0.79	34.47±0.44	34.37±0.95	34.13±0.78	34.37±0.95	34.50±0.43	34.70±0.46	34.50±0.43	34.50±0.42	34.70±0.45	34.50±0.42
Ca^{2+} (mmol.kg^{-1})	11.35±0.02	11.49±0.32	11.55±0.15	11.37±0.01	11.49±0.35	11.56±0.14	11.14±0.09	11.70±0.28	11.54±0.20	11.13±0.09	11.70±0.29	11.56±0.20
$Ca^{2+} + Mg^{2+} + Sr^{2+}$ (mmol.kg^{-1})	64.22±0.52	65.29±2.47	66.08±1.41	64.54±0.85	65.38±2.13	65.79±1.10	63.74±0.44	67.16±2.07	66.02±1.22	63.78±1.16	66.93±2.05	66.39±1.69

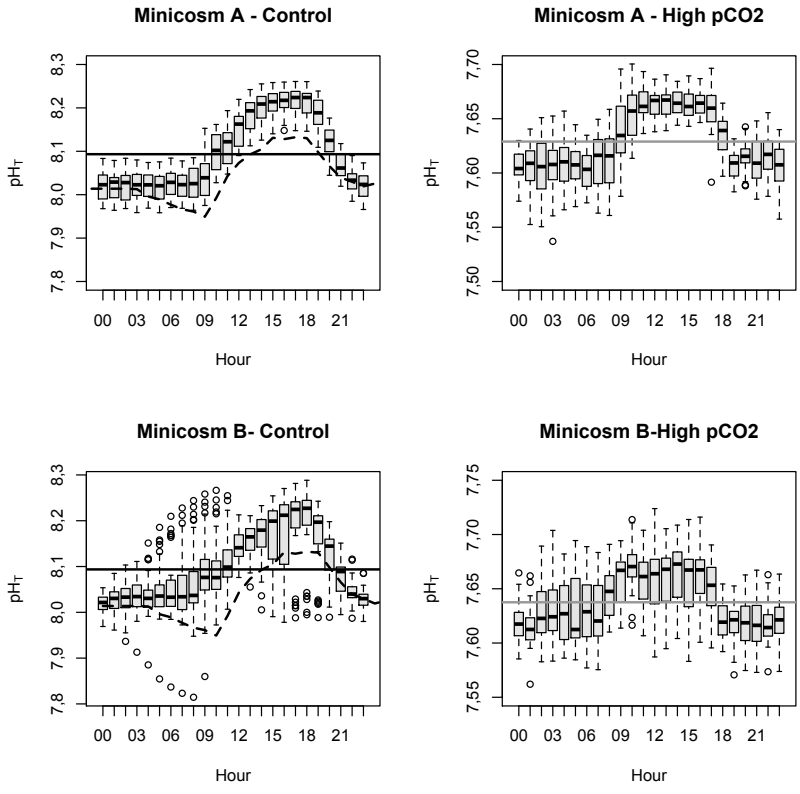

Figure 5. pH_T diurnal variations inside each experimental aquarium. Box-plots represent median (blackline), interquartile range (box), 1.5 times the interquartile range from the box edges (whiskers) and outliers (individual points). Each box-plot corresponds to data recorded every hour of each day after establishment of contrasted pCO_2 conditions. Medians were calculated from measurements recorded every 20 seconds. Horizontal lines represent the global pH_T mean. Dotted lines in the control aquaria graphs represent the median field variation per hour (La Saline Lagoon, Réunion Island, Cuet, pers. comm., see also Chauvin et al. (2011)). Light was provided from 8h to 20h.

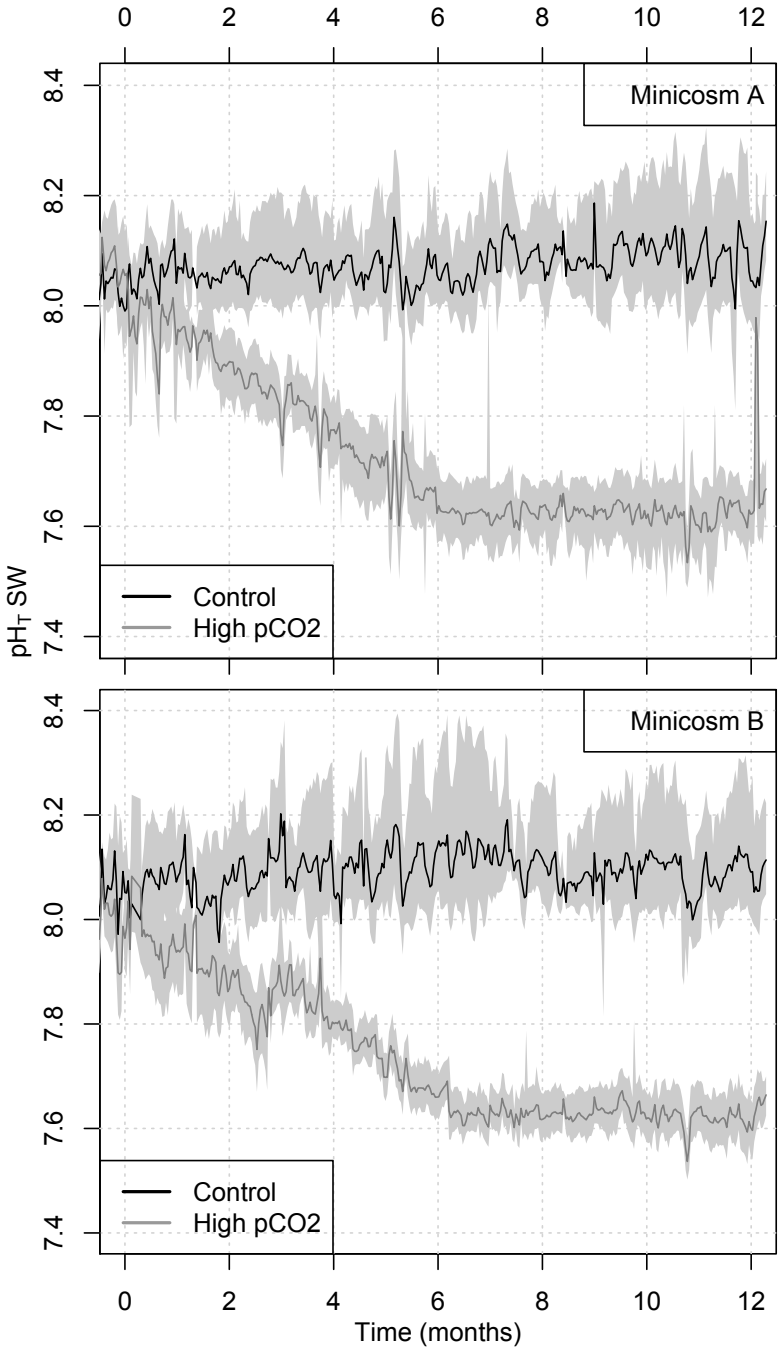

Figure 6. pH$_T$ time course in each experimental aquarium of both minicosms during the experiment. Values represent pH$_T$ recorded every 20 seconds and then averaged by days (black lines for controls and grey lines for treatment aquaria). Envelopes in light grey correspond to minimum and maximum values per day.

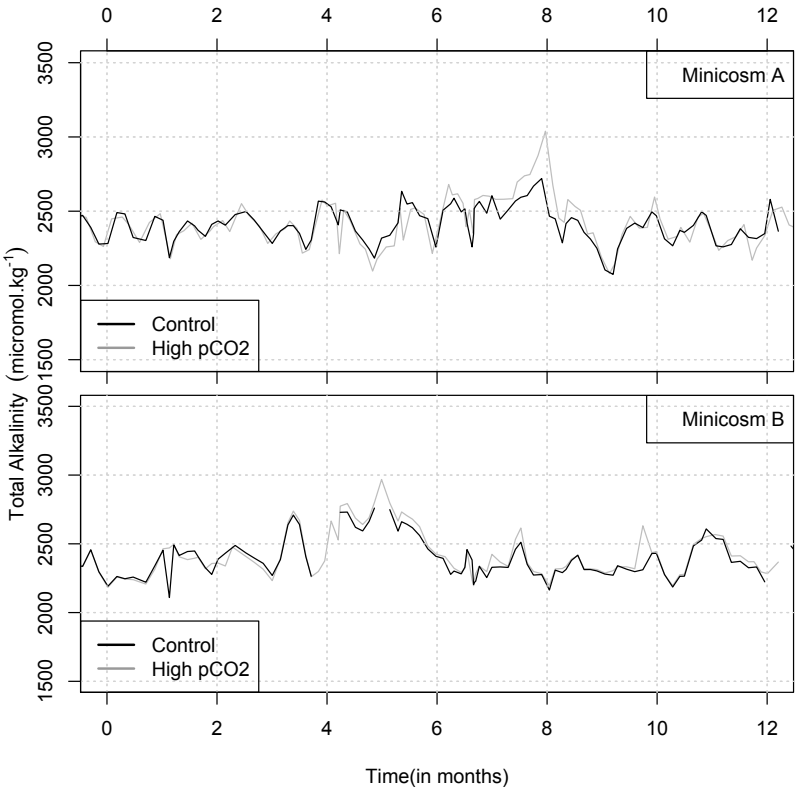

Figure 7. Total alkalinity (A_T) time course in each experimental aquarium of both minicosms. Black lines represent the control aquaria, grey lines represent acidified aquaria. Total alkalinity was measured every two days.

Table 3. Mean nutrient concentrations (in µmol kg^{-1}) in each aquarium before the pH decrease (3 months monitoring), during the pH decrease (6 months monitoring) and after the pH decrease (7 months monitoring). Values represent means ± standard deviations. Nutrients were quantified every two weeks. Maximum field measurements are from Chazottes et al. (2002).

	Mesocosm A - Control			Mesocosm A - Acidified			Mesocosm B - Control			Mesocosm B - Acidified			
	Before decrease	During decrease	After decrease	Before decrease	During decrease	After decrease	Before decrease	During decrease	After decrease	Before decrease	During decrease	After decrease	Max field measurement
	0.52±0.78	0.96±0.78	0.87±0.89	0.74±0.49	0.43±0.63	0.47±0.69	1.30±1.25	0.55±0.67	0.84±0.77	0.67±1.11	1.31±0.95	0.80±0.74	2.26 (NO_3 + NO_2)
	0.11±0.08	0.18±0.12	0.17±0.12	0.11±0.05	0.15±0.06	0.16±0.09	0.14±0.15	0.13±0.06	0.15±0.09	0.15±0.11	0.17±0.11	0.18±0.22	
	0.80±0.88	0.44±0.32	0.52±0.69	0.90±1.24	0.73±1.04	0.44±0.56	1.20±1.20	0.50±0.49	0.32±0.45	0.91±0.81	0.73±0.66	0.56±0.64	1.08
	0.42±0.18	0.17±0.24	0.30±0.44	0.30±0.35	0.18±0.17	0.31±0.44	0.13±0.32	0 .49 ± 0.60	0.25±0.34	0.76±0.90	0.18±0.18	0.34±0.44	0.33
P ratio	3.75/1	9.29/1	5.2/1	3.1/1	7.3/1	3.45/1	20.31/1	2.41/1	5.2/1	2.27/1	12.22/1	5/1	10.18/1

Table 4. Result from the oxygen net fluxes modelling. Values are the best estimates for the parameters used in the model described in eq. 1

	Minicosm A - Control	Minicosm A - Acidified	Minicosm B - Control	Minicosm B - Acidified
Net photosynthesis (P mmol.h^{-1})	11.2	7.7	10.3	7.7
Respiration (R_{dark}; mmol.h^{-1})	-7.2	-5.8	-4.7	-7.9
Daily balance (mmol.h^{-1})	4	1.9	5.65	-0.2

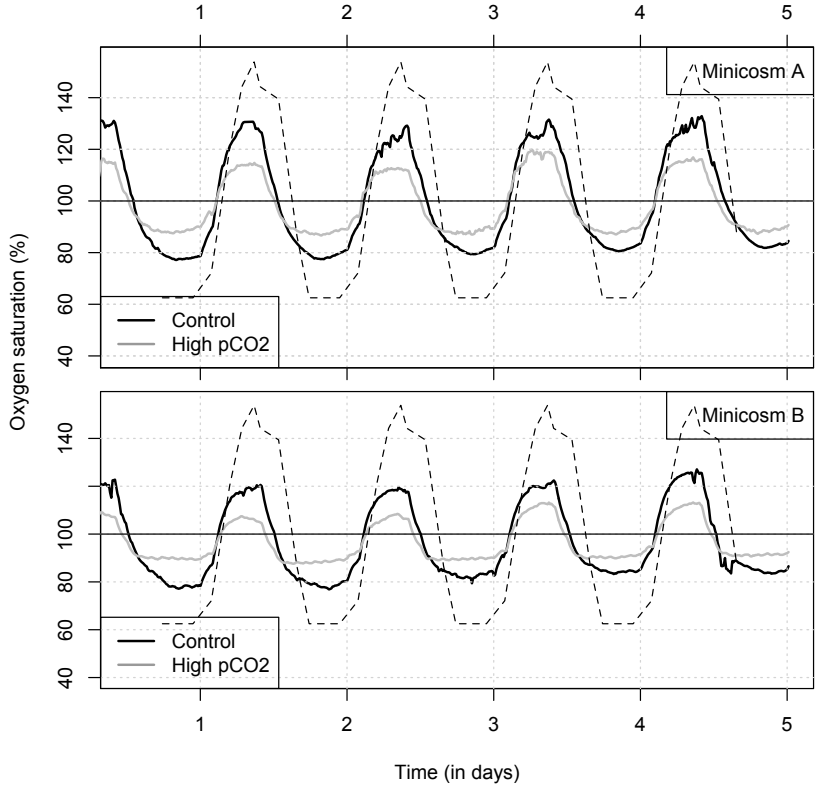

Figure 8. Oxygen saturation in each experimental aquarium of both minicosms over a five day monitoring period at the end of the experiment. Black lines represent the control aquaria, grey lines represent the acidified aquaria. Dotted lines represent field measurements from Clavier et al. (2013) at La Saline Lagoon, La Réunion. Oxygen concentration was recorded every 20 seconds.