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Technical Note: Artificial coral reef mesocosms for ocean acidification investigations

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Abstract

The design and evaluation of replicated artificial mesocosms are presented in the context of a thirteen month experiment on the effects of ocean acidification on tropical coral reefs. They are defined here as (semi)-closed (i.e. with or without water change from the reef) mesocosms in the laboratory with a more realistic physico-chemical environment than microcosms. Important physico-chemical parameters (i.e. pH, pO_2, pCO_2, total alkalinity, temperature, salinity, total alkaline earth metals and nutrients availability) were successfully monitored and controlled. Daily variations of irradiance and pH were applied to approach field conditions. Results highlighted that it was possible to maintain realistic physico-chemical parameters, including daily changes, into artificial mesocosms. On the other hand, the two identical artificial mesocosms evolved differently in terms of global community oxygen budgets although the initial biological communities and physico-chemical parameters were comparable. Artificial reef mesocosms seem to leave enough degrees of freedom to the enclosed community of living organisms to organize and change along possibly diverging pathways.

1 Introduction

Over the last century, anthropogenic atmospheric carbon dioxide (CO_2) emissions have raised (IPCC, 2013). One of the consequences is ocean acidification (OA) as CO_2 dissolves in seawater. The carbonate chemistry equilibrium is thus modified and pH is decreased. In parallel, the interest of the scientific community for OA has raised in the last decade. Several strategies were used to understand OA effects and possible acclimation or adaptation of marine organisms (Widdicombe et al., 2010). Studies have been conducted in aquaria to understand the physiological effects of OA on organisms (Fig. 1). A single (most often) or a few species were maintained together under different controlled pH conditions. Results provided first insights to understand future OA effects and mechanisms. Laboratory experiments in aquaria are relatively easy to set up. They are replicable and can be finely controlled. However, results are hardly transposable to field conditions, due to the very artificial environment and to the absence of species interactions. The opposite strategy is to study the effects of pH in environments characterized by a naturally low pH 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) or the deep sea (Park, 1966; Roberts et al., 2006; Turley et al., 2007). However, several environmental parameters differ in these situations from the ongoing OA in most of the open ocean and benthic zones. For instance, the pH decrease in rocky tidal pools occurs over short periods of time and varies in intensity over seasons. Around volcanic vents and in upwelling zones, many marine organisms could escape from pH stress. Indeed, the biomass of less CO_2 tolerant sessile species decreases there (Hall-Spencer et al., 2008). Conversely, recruitment of juveniles from outside could mask other potential effects. There is generally no possibility of replication. Measurements can be performed on different organisms but as they originate all from the same location, they cannot be considered as independent replicates, leading to some degree of pseudo-replication and biased statistical analysis (Hurlbert, 1984). Finally, other physico-chemical factors (i.e. nutrients, trace elements, heavy metals and other pollutants, temperature, pressure) may create unwanted interactions with pH in the natural environment. For example, Vizzini et al. (2013) observed a trace element contamination around the CO_2 vents from Vulcano Island. The change in species distribution and the prospective physiological effects could therefore be falsely attributed to the pH decrease, due to the existence of other confounding factors. Microcosm and mesocosm studies represent compromises between aquaria experiments in the laboratory and field surveys. Historically, a “microcosm” was defined as an artificial, simplified ecosystem that was used to simulate and predict the behavior of natural ecosystem under controlled conditions (Odum, 1983). These are usually built in the laboratory for easy access. On the other hand, “mesocosm” was defined as partially enclosed outdoor

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experimental setup that closely simulates the natural environment (Odum, 1984). The advantages of these setups are numerous: possible replication, consideration of species interactions, tight (microcosms) or realistic (mesocosms) control of physico-chemical parameters and limitation of confounding factors.

The growing concern about the effects of OA on marine ecosystems, including on tropical coral reefs, led scientists to favor a mesocosm approach since 1995 (Stewart et al., 2013). In the following part, only studies about effects of ocean acidification on tropical coral reef ecosystem are considered. 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 at long-term (nine months) (Andersson et al., 2009; Jokiel et al., 2008; Kuffner et al., 2008). Mesocosms like this one, used for long-term experiments allow also to take into account acclimation in a naturally fluctuating environment: seasonal and daily variations of physico-chemical parameters (i.e. light, temperature, carbonate system parameters, oxygen). The mesocosm daily cycle followed the natural cycle thanks to a high flow rate of seawater input pumped from the adjacent reef. Natural recruitment was also possible through this seawater inflow. However, the high flow rate required that OA conditions were reached by HCl addition and total alkalinity (A_T) was therefore lower in the acidified mesocosms[1] compared to control ones. Yet, OA does not imply such a change in A_T (at least, not when due to an increased $p\mathrm{CO_2}$) which is known to affect biogenic calcification, particularly in corals (Jury et al., 2009). Recent studies using CO_2 manipulation to modify pH were also conducted in relatively small containers (150 L) which were called "open mesocosm" at short time scales (less than three months) (Comeau et al., 2013a, b; Leclercq et al., 2000, 2002). Moreover, field-like daily variations were not applied to the system. These studies were indeed not performed in mesocosms, according to Odum's definition (1983), but in microcosms. A recent study by Dove et al. (2013) proposed a longer experiment (nine months) in open artificial mesocosms where small communities were submitted to different $p\mathrm{CO_2}$ conditions. These different conditions were applied progressively during 2.5

[1]A solution was recently proposed to solve this technical problem (Jokiel et al., 2014b).

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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). Being artificial, it does not strictly match Odum's definition of a mesocosm. The proposal is thus to refer to this type of "mesocosm" as an "artificial mesocosm" (Fig. 1) as it also differs from microcosms (still according to Odum's definition) by daily variations of physico-chemical parameters which closely mimic the water environment in the field. This was achieved through community activity (respiration and photosynthesis mainly) and not through inflow of water from the corresponding natural ecosystem. However, Biosphere 2 was a large and costly project, leading to little possibilities of replication. Therefore, the method used was a "time series intervention analysis" with time intervals of stable conditions of no longer than 2 months and physico-chemical parameters, like A_T and $p\mathrm{CO_2}$, being changed randomly for each time interval and applied to the same artificial ecosystem. Unfortunately, this does not allow for long-term acclimation of the studied organisms and communities. Recent literature revealed new tools to investigate the impact of OA at the ecosystem level in situ. The "Free CO_2 Enrichment System" (FOCE) experimental devices (Kline et al., 2012; Gattuso et al., 2014) were recently developed. They are encapsulated open natural ecosystems with modern and sophisticated techniques to simulate a pH decrease similar to the one due to OA. Numerous advantages are highlighted: field recruitment, field physico-chemical daily variations, natural community, precise control of stress condition, replication, etc. Nevertheless some of these promising tools encompass other disadvantages such as elevated costs and more limited accessibility than laboratory-based systems. Being run at relatively large scales and most of the time in the field, it is hard 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). For this particular case, aquaria, microcosms and artificial mesocosms may be better suited. Among these alternatives, the artificial mesocosms best mimicks the natural environment variations and interspecific interactions in the ecosystem. In the present paper, the design and

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evaluation of small-scale artificial reef mesocosms are described. The objective was to construct an experimental design which combines most of the advantages of both microcosms and (field) mesocosms at a relatively low cost, which does not necessarily require water input from the natural environment, and which is easily replicable.

2 Design

2.1 Artificial reef mesocosm

The main concern in the design of an artificial reef mesocosm is to build a closed-system in the laboratory (microcosms characteristics: relatively cheap, replicable and easy to access for measurements and observations) with more realistic variations of main physico-chemical characteristics of the water environment (closer to properties usually attributable to field mesocosms), including daily variations. The challenge can thus be summarized by the following question: given data from the monitoring of 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? And if yes, how would the living community organize in such a system? Finally, how useful would it be for scientific investigations, like OA studies?

Figure 2 presents a simplified diagram of the system that was designed. Two identical artificial mesocosms were built in 2005 at UMONS (http://econum.umons.ac.be) and refined/tested until end of 2006. Each one consists of a closed system constituted of one main tank (500 L), 2 experimental aquaria (300 L) and common parts (sump, skimmer, etc.). The main tank is holding 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 (*Seriatopora hystrix*, *Acropora muricata*, *A. digitifera*, *A. tenuis*, *A. millepora*, *Pocillopora damicornis*, *Montipora patula*, *Stylophora pistillata*, etc.). It also contains algivorous animals in such density that it avoids the overgrowth of algae and maintains coral cover (echinoderms, mollusks, crustaceans, reef fishes, etc.). Detritivorous animals 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 installation of the reef community took two years before some ecological equilibrium was reached, characterized by a natural control of N and P macronutrients to concentrations close to those observed in the field (proxy used to assess that N and P cycles were established and stabilized, see results). The flow rate between the sump and the main tank is $14 \pm 0.1\,\mathrm{L\,min^{-1}}$. Experimental aquaria (300 L each) are connected to the common part, which makes the system a paired design. Each experimental aquarium is connected to the sump, but physico-chemical parameters such as pH, $p\mathrm{CO_2}$ 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 $0.8 \pm 0.5\,\mathrm{L\,min^{-1}}$. According to the present definition of an artificial mesocosm, the whole setup must be carefully controlled, either by biological ways, or by technical systems, to mimic changes in temperature, lighting, pH, $p\mathrm{O_2}$, $p\mathrm{CO_2}$, total alkalinity and macronutrients as observed in natura. The following part of the design section explains how this was possible. The reference location is a lagoon at 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 on the site (Cuet, personal communication TS1, see also Chauvin et al., 2011).

2.2 Real-time monitoring

The artificial mesocosm is fully monitored and controlled using IKS Aquastar devices. These devices are connected to a computer and record the main physico-chemical parameters every 20 s (i.e. temperature and pH of each aquarium). In order to be able to check the stability of the system at any time, temperature and pH plots are created automatically and continuously using the R software (R Core Team, 2013). These plots are continuously displayed in the mesocosm room as well as a web page through the internet using a free file hosting service (Dropbox folder update). They are thus available to every mesocosm user.

2.3 Light, temperature, water flow

Light is provided via T5 8 fluorescent lamps (39 W per lamp, 25 : 75 actinic blue 420 nm : trichromatic 10 000 K, Aqua Medic, Germany) for more flexibility. This allows to switch light on and off progressively by managing groups of T5 lamps with different light duration (Fig. 3) to mimic natural intensity and spectral variation of light. A closed-system allows an easy control of temperature in each experimental aquarium and in the main tank independently. Temperature probes (Aquastar, Germany) are connected to a computer that control both heaters (Eheim Jäger, Germany) and air fans (allowing for slight temperature decrease by water evaporation) or cooling units (for larger temperature decrease, not necessary in our 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 target value as a function of the time of the day. Moreover, water motion is really important in tropical reefs. Many physiological parameters rely on water flow around coral colonies (Badgley, 2006; Carpenter et al., 2007; Finelli et al., 2006; Sebens et al., 1998, 2003; Schutter, 2010). Each aquarium was equipped with two variable speed Tunze – Turbelle stream 6100 driven by a Tunze wave maker to simulate action of waves (from 0 to 40 $m^3\,h^{-1}$ of water flow). The reference site being located in a lagoon, hydrodynamism is relatively low in comparison to, say, the reef crest. It is thus more easily simulated in aquarium.

2.4 Seawater composition and salinity

As the experiment was run far away from tropical reefs, two alternatives were available to obtain seawater: natural seawater from a temperate coast, or artificial seawater.

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According to the constraint that the system should be easily replicable (including elsewhere), artificial seawater was preferred. It was 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 mesocosms, newly prepared artificial seawater was mixed and aerated overnight. Ten percent of the mesocosm water volume was changed every two weeks. The evaporation was compensated by addition of the same ASTM type II water using a Tunze 5017 osmolator. This device allows to keep water volume constant, and thus, also stabilizes salinity. Salinity was also checked every two days using a WTW 340i salinometer (WTW, Germany).

2.5 Oxygen

Oxygen concentration in the field follows a daily cycle around saturation (Kline et al., 2012; see also Fig. 7). It is mainly driven by biological activities (net photosynthesis of photoautotrophs 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 vs. night pO_2, each experimental aquarium is coupled with a 80 L-refugium. The refugia contain photoautotrophs (*Caulerpa* spp., *Halimeda* spp., etc.) and no grazers (hence the name, refugium) and are lighted with an inverted nycthemeral cycle compared to the mesocosm (T5 fluorescent lamps, $2 \cdot 39$ W, 10 000 K trichromatic, Aqua Medic, Germany). This setup limits the oxygen fluctuations between day and night in the experimental units. Its effects can be modulated through (1) the water flow between the aquarium and the refugium, (2) the biomass of photoautotroph and (3) the irradiance and duration over the refugium.

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2.6 Macronutrients

Experience from aquariology and public aquaria demonstrates that it is possible to reproduce to some extend the natural cycles of macronutrients 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, and enough anaerobic zones in the substrate for denitrification, can lead to stabilized concentrations in ammonium/ammoniac, nitrites, nitrates and orthophosphates. However, stabilization of these inorganic species close to their natural levels is an additional challenge. Here, the goal is to obtain and maintain near micro-molar concentrations of $NH_3 + NO_2^- + NO_3^-$, and submicromolar concentrations in orthophosphates. The approach used here is thus to set up progressively an equilibrated community of organisms in order to establish N and P cycles close to those observed in the ocean (of course, exchanges with other ecosystems like plankton arriving on a reef with the water currents, or exportation of sinking organic matter to the deep sea have to be simulated by artificial means – e.g., feeding and mechanical filters or settling tank, respectively). This adjustment takes time and is probably one of the hardest and longest stage in the establishment of an equilibrated artificial mesocosm. In our test system, it took two years to reach stability together with correct inorganic N and P concentrations.

Macronutrients cycles are established thanks to four items:

- Feeding the main tank with plankton (frozen Artemia and mysids) to simulate plankton importation from the open ocean. The amount of food provided is dictated by inorganic nitrogen and phosphorus concentrations resulting in the water (more food increases them, whereas less food has the opposite effect over a few weeks period).
- Simulating exportation of a fraction of the particulate matter produced out of the reef by a simple mechanical filter (perlon filter inside the sump weekly changed).

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- Simulating the dilution of the organic matter produced in the water column by the living organisms in a similar way as on the reef is impossible, because the ratio water volume to biomass is much lower in the artificial mesocosms. However, a skimmer is a filter able to eliminate a portion of this organic matter, especially amphiphilic molecules. To date, it is the best system available to lower the loading in organic matter in a seawater aquarium (Delbeek and Sprung, 2007), and it is considered as an effective system to keep scleractinian corals healthy in aquarium conditions. One Deltec AP850 skimmer was thus installed in each artificial reef mesocosm.

- Finally, the most important part is the community of photoautotrophs, heterotrophs and bacteria, as well as their respective biomasses. With a correct adjustment of the community, by trial and error, we were finally able to stabilize inorganic N and P concentrations to target values and to maintain them over several years.

2.7 Carbonate system

Recent studies highlighted the importance of daily pCO_2 fluctuations in the field (Comeau et al., 2014; Shaw et al., 2013). These daily variations are mainly driven by biological activities, as for oxygen. The aim here was once more to simulate these natural fluctuations, also mainly by biological activities. Furthermore, a pH difference had to be installed between the two experimental aquaria for the purpose of OA experimentations. Therefore, CO_2 bubbling in 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 had to be slightly increased. Calcium hydroxide saturated ASTM type II water was added, also computer-controlled by a pH probe in the control aquarium. Nevertheless, calcium hydroxide has a side-effect as it also increases A_T following:

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

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

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In order to keep the same A_T in each experimental aquarium, the problem was solved by adding the same amount of $Ca(OH)_2$ in the high pCO_2 aquarium as well. Overall, regular addition of alkalinity through $Ca(OH)_2$ is not a problem since, in these artificial mesocosms, alkalinity tends to decrease regularly due to bioaccretion by corals, urchins, mollusks, crustose coralline algae, and other calcifying organisms. Since bioaccretion still remains higher than $Ca(OH)_2$ additions, alkalinity was further stabilized in each artificial mesocosm by the use of a calcium reactor. The later is a container with solid calcium carbonate material maintained at low pH (around 6, or even less) by CO_2 bubbling controlled by a pH probe. In these conditions, the calcareous material progressively dissolves. A very slow water flow between the reactor and the mesocosm allows an increase of alkalinity in the water. The compensation of alkalinity is controlled by two parameters: the water flow between the reactor and the artificial mesocosm, and the pH maintained inside the reactor. These are adjusted by trial and error following alkalinity measurements in the mesocosms.

2.8 Test case in the experimental aquaria

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 at 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*, *Acropora digitifera*, *Pocillopora damicornis*) and the other half of substrate were collected at Réunion Island in the back-reef of La Saline fringing reef (21°70′ S, 55°32′ E). Permits were obtained before field collections from “Réserve Naturelle Marine de La Réunion” (RNN164) and “Direction de l’Environnement, de l’Aménagement et du Logement” (DEAL). Organisms collected at Réunion Island were transported to the mesocosm facilities in Belgium (transport duration: 24 h) in seawater using styrofoam boxes. They were acclimated in control conditions during seven months before the beginning of the experiment. Sixteen sea urchins, 0.8 kg of hermatypic scleractinians and 20 kg of reef calcareous substrate were installed in each experimental aquarium. The main unit of each artificial mesocosm contained the same organisms as the experimental aquaria but sea urchins were green *Echinometra* sp. B-like (Arakaki et al., 1998). The main tank was fed five times a week with frozen Artemia and mysis aquarium food (5 g; Ocean Nutrition) and dehydrated red algae (1 g; Nori). 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 stabilized pH. Major parameters such as temperature, pH, A_T, oxygen, nutrients, calcium and magnesium were monitored/controlled during the whole duration of the experiment.

2.9 Physico-chemical measurements

2.9.1 Seawater physico-chemical parameters

The electromotive force (e.m.f) was measured daily using a 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 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). The salinity and temperature were measured daily using a salinometer pH/Cond 340i WTW (USA). These measurements of pH/T°/salinity were used as 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 a potentiometric titration using 0.01 M HCl with 0.7 M NaCl following Dickson et al. (2007) but adapted for a smaller volume (25 mL). Each titration was automatically performed by computer using a Titronic Universal automatic titrator (SI Analytics, Germany), a C3010 multi parameter analyzer to record pH (Consort, Belgium), a TW

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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, Dickson, batch 94). The $p\mathrm{CO}_2$ 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_4^+, $\mathrm{NO}_3^- + \mathrm{NO}_2^-$ and PO_4^{3-} were analyzed through an automated colorimetric analysis using a QuAAtro nutrient analyzer coupled to a XY-2 auto sampler (Seal Analytical, Mecquon, Wisconsin, USA). Calibrations were done using standard solutions. Calcium and total alkaline earth metals (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 analyzer to record e.m.f (Consort, Belgium) and a TW Alpha Plus auto sampler (SI Analytics, Germany) using a custom software. Calcium concentration was measured by an 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). The total alkaline earth metals were determined by an 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).

2.9.2 Modelling of oxygen fluctuations

The oxygen daily cycle was checked over 5 days at the beginning and monitored in each experimental aquarium at the end of the experiment. Oxygen was recorded using Clark oxygen electrodes connected to the IKS control system. Each probe was calibrated before the monitoringusing 100 % O_2 air and 0 % O_2 (NaSO_2 solution in

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seawater kept away from air contact). Moreover, each probe signal was one-point corrected every day using a WTW oxymeter and an WTW Oxycal probe, calibrated using 100 % O_2 air and 0 % O_2 ($NaSO_2$ solution in seawater kept away from air contact). Oxygen net fluxes (i.e. net photosynthesis and dark respiration) at the ecosystem level were estimated by calculation using the R statistical software and the simecol package (Petzoldt and Kline, 2007). Net photosynthesis was defined from the following equation:

$$P = P_{max} \cdot (1 - e^{E/\mathrm{EK}}) + R_{dark}, \tag{1}$$

where P is the net photosynthesis in mmol O_2 min^{-1}, Pmax is the maximum net photosynthesis in mmol O_2 min^{-1}, E is the irradiance in PAR (µmol photons 2^{-1} s^{-1}TS2), EK is a constant that defines the efficiency of the photosynthesis as a function of irradiance and R_{dark} is the dark respiration in mmol O_2 min^{-1}. Considering one aquarium, the oxygen carried in or out of the aquarium by seawater change in the tank is define as:

$$\frac{dO_{2_{exp}}}{dt} = \frac{\mathrm{waterchange}}{\mathrm{Vol}} \cdot \left(O_{2_w} - O_{2_{in}}\right), \tag{2}$$

where water change is the volume of water exchanged between the aquarium and the main unit (in L min^{-1}), Vol is the volume of water in the aquarium (in L), O_{2_w} is the oxygen concentration in the aquarium and $O_{2_{in}}$ is the oxygen concentration of the water entering the aquarium. Since that water is pumped off the skimmer, O_2 is very close to saturation at any time (checked using the WTW oxymeter) and its O_2 concentration is computed from 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 is calculated as:

$$\frac{dO_{2_{air}}}{dt} = \frac{O_{2_{in}} - O_{2_w}}{\tau}, \tag{3}$$

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where the variation of oxygen inside the aquarium is then defined as:

$$\frac{O_{2_w}}{dt} = \frac{P}{Vol} - \frac{dO_{2_{exp}}}{dt} - \frac{dO_{2_{air}}}{dt}. \tag{4}$$

The mathematical model was fitted to measured oxygen concentration data and an optimizer was used to find best estimates for Pmax end Pdark (package R simecol, Petzoldt and Kline, 2007).

2.9.3 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 analyzed 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. Residuals analyses were graphically performed for each model to check normality, homoscedasticity and linearity of the residuals. Comparisons of pH during day and night were performed using paired t test with the Welch approximation to the degrees of freedom for unequal variances. Comparison of A_T, alkaline earth metals, nitrates, nitrites, ammonium and orthophosphates concentrations between control and treatment aquaria were performed using paired t tests with the Welch approximation to the degrees of freedom. When possible (enough replicates), normality and homoscedasticity of the residuals were verified using, respectively, a quantile-quantile plot and a Bartlett test.

3 Results

3.1 Carbonate sytem

3.1.1 Diurnal variations of pH

The major physico-chemical parameters are presented in Table 1 averaged by periods: during the acclimation period before the pH decrease (3 months), during the six months decrease and after the decrease, during the seven months stabilized period. It was possible to reasonably simulate natural diurnal variation of pH in each experimental aquarium during the OA experiment (Fig. 4), although it is hard to obtain realistic daily pH changes in acidified conditions. Both mesocosms presented a significantly different pH during the day and the night for each aquarium (paired t tests, all p values < 0.001), obtained mainly by 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 between night and day in control aquaria was equal to 0.2 pH unit while it was equal to 0.1 pH unit in high pCO_2 aquaria. The amplitude of the pH variations observed in the control aquaria was just slightly larger than that recorded in the reference lagoon as it was adjusted to get an average daily pH_T around 8.1 (Fig. 4). Community-driven pH changes appear lower in acidified aquaria than in 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. pH in each control aquarium showed a very small, but significant, increase throughout the experiment (Fig. 5, linear model, slope p values < 0.001). Nevertheless, this increase is lower than 0.02 units $year^{-1}$ TS3. The acidified aquaria showed a stable pH during the seven last months (stable conditions, linear model, slope p values ≥ 0.07). During the pH

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decrease in the acidified mesocosms, the pH slope was fixed to −0.03 unit every two weeks, which is much lower than the diurnal variation. Despite the increasing difference in pH between control and treatment aquaria, total alkalinity (Fig. 6) showed no significant difference (paired t test, all p values ≥ 0.11) and remained stable during the thirteen month experiment (linear models, all slope p values ≥ 0.15). Moreover, total alkalinity in the two mesocosms remained very close (within 5 % of variation).

3.1.3 Salinity and temperature

Temperature remained within 1° of variation over the whole experiment in all experimental aquaria (Table 1). 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 difference was observed between experimental aquaria (paired t tests, p values ≥ 0.06). A slight diurnal variation was observed, but only during the period when the highest temperatures were recorded. In this OA experiment test case, slight seasonal changes in temperature (and light) were not taken into account to avoid interference with the decreasing vs. stabilized pH phases. However, adjustments for seasonal changes would not be a technical problem. Salinity was also monitored throughout the experiment (Table 1). One mesocosm showed no significant variation through time (slope p values ≥ 0.21). The second mesocosm showed a slight and significant increase in salinity (slope p values ≤ 0.001), but under 1 PSU year^{-1}. No difference was observed between experimental aquaria (paired t tests, p values ≥ 0.43).

3.1.4 Nutrients

The inorganic nitrogen concentration was studied throughout the experiment. Nitrates, nitrites as well as ammonium concentration remained at concentration levels comparable to that observed in the field (Table 2) and did not vary significantly during the 13 months experiment (slope p values ≥ 0.07) except for ammonium, which was

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slightly higher at the beginning of the experiment. The inorganic phosphorus, i.e. orthophosphates, concentration also remained comparable to target concentration levels and did not vary during the experiment (slope p value ≥ 0.07). No difference was observed between aquaria (paired t tests, p values ≥ 0.30).

3.1.5 Calcium and total alkaline earth metals

The calcium concentration (Table 1) did not vary significantly according to time throughout the experiment in all aquaria, nor the total alkaline earth metals concentration (slope p values ≥ 0.07). Similarly, the ratio Ca/total alkaline earth metals was constant throughout the experiment in all aquaria (slope p values ≥ 0.19). The mean value recorded in mesocosms (5.71 ± 0.09) was lower than that in field (6.38). All these parameters did not vary significantly between contrasted pH conditions in both mesocosms (paired t tests, p values > 0.11).

3.1.6 Oxygen

The oxygen concentration in each aquarium was monitored over 5 days at the end of the experiment (Fig. 7). It followed a daily cycle mainly driven by biological activities in each aquarium. Oxygen saturation state oscillated between 85 and 130 %. The oscillations were larger in the control aquaria for both mesocosms. The overall balance of net oxygen fluxes were modeled for each aquarium (Table 3). Biological systems in each aquarium were global sources of O_2, except for the acidified aquarium of the mesocosm B. No difference was observed between control and treatment aquaria (Table 3) for dark respiration as well as for net photosynthesis (paired t tests, p values > 0.05).

4 Discussion

Here "artificial mesocosms" are defined as intermediary systems between laboratory-based microcosms and in situ mesocosms. Artificial mesocosms can be laboratory-based closed systems (or semi-closed systems on site), but unlike microcosms, they must mimic the physico-chemical environment as closely as possible, including daily changes. This requires first to define a target site in natura and to get it instrumented to obtain records of temperature, oxygen, pH, nutrients, etc. The test case here used a station in the fringing reef of Réunion Island at La Saline as a reference site (Cuet, personal communicationTS4). A second requirement is to obtain such variations in the most natural way possible in order to obtain an artificial ecosystem that behaves as much as possible similarly to a natural one. Conequently, the community of living organisms that establish and thrive in the artificial mesocosms must be directly responsible of much of the daily fluctuations of oxygen, $p\mathrm{CO_2}$ and thus, pH. This is only achieved by a correct balance between photosynthesis and respiration, meaning that photoautotrophs vs. heterotrophs biomasses must be carefully adjusted. Macronutrients (N and P in the test case, but also Si if diatoms play a major role in the ecosystem) must also be community-controlled as much as possible. N and P cycles must establish as completely as possible and the resulting concentrations in ammonium, nitrites, nitrates and orthophosphates must adjust at levels close to those found in the field. This is a challenge for very oligotrophic ecosystems, like tropical coral reefs, but present results highlighted its feasability. Obtaining realistic values of oxygen, carbon dioxide and macronutrients was not possible without a small technical input: (1) a refugium with inverted photoperiod was used to limit oxygen (and carbon dioxide) amplitude between day and night due to a lower water volume *per* biomass unit in the mesocosm than in the field, (2) artificial systems and manipulations were also required to mimic parts of N and P natural cycles: feeding with plankton to simulate imports, and filtering water mechanically (perlon) and chemically (skimmer) to simulate exports and dilution of the cocktail of organic matter produced in the water column. However, these are minimal interventions in comparison to heavy aerobic biological filters that lead to the accumulation of nitrates, or artificial denitrification filters or chemical phosphate filters that are proposed in the aquarium and aquaculture markets. A mainly community-driven regulation of the environment is thus possible and can mimic physico-chemical conditions in the field, even in small (1700 L in total) artificial mesocosms that are relatively cheap and easily replicable. Moreover, the twin mesocosms have been in a steady state now for over five years, indicating that such an equilibrium is sustainable on the long-term. The community had to be adjusted manually from time to time, by eliminating a part of the organisms that grew too much, such as a few coral colonies, or *Caulerpa* algae out of the refugia; or by adding missing components, as replacing dead fishes, mollusks or echinoderms. This simulates predation and recruitment that lack for obvious reasons in such artificial mesocosms. It should be noted that the equilibration of an artificial mesocosm takes time. Two years were required to achieve stable conditions in the described experimental device. However, with experience gathered here, it may be possible to obtain it faster in the future (but probably in no less than one year). Due to the sometimes excessive use of the term "mesocosm" in the literature to qualify poorly equilibrated communities in more or less artificial environments, the need to preserve Odum's original definitions of microcosms and mesocosms is here emphasized (Odum, 1983), as well as the lack of a term for items in between those two definitions. "Artificial mesocosm" is proposed. To qualify for the artificial mesocosm "label", a system must contain fully acclimated living organisms, be community-driven as much as possible (artificial filtration techniques limited to a strict minimum and justified to mimic environmental or ecological compartments impossible to maintain otherwise, or to simulate inputs and outputs to and from the ecosystem), and be equilibrated on the long-term (several months, if not years). Most importantly, it should also match physico-chemical changes of a reference site in the field (at least for major chemical parameters like oxygen, carbon dioxide, pH, alkalinity, and macronutriments). Clearly, this rules out many laboratory-based systems that must rather be called microcosms. The next key question, once one got a working artificial mesocosm, is what to do with it? Is it possible to run OA experiments with it? What happens if pH is lowered in the whole artificial mesocosm, or in a part of it like it was done here? Would this break completely the equilibrium, or would the artificial ecosystem be resilient enough to withstand such a change with organisms that remain observable in good conditions? To answer these questions, an original paired design was used with acidified and control conditions installed inside the same artificial mesocosms, using experimental aquaria connected to the main unit. It was shown that a paired design is technically possible, and a way to achieve it was proposed by addition of CO_2 or $Ca(OH)_2$. The latter also brings alkalinity to the system, which we would have had to do anyways to counterbalance net bioaccretion in the coral reef community. However, stripping CO_2 is probably a viable alternative that does not impact alkalinity in systems with no (positive) bioaccretion (Dickson et al., 2007). The main physico-chemical parameters did not vary between experimental aquaria of the same type, nor between mesocosms during the experiment. Moreover, at the beginning of the experiment, the same simplified biological community was introduced in 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 achievable in artificial mesocosms and the living community in the main tank remained stable despite these changes. It should be noted that pathogens are part of the community and episodes of white band disease were observable from time to time. Metagenomic analyses of the coral mucus revealed a large quantity of herpes-like viruses during one such episode (Laghdass and Gillan, personal communicationTS5). 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 make also part of the community to maintain in those artificial mesocosms. During this OA experiment, the ecophysiological response

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of the scleractinians and the sea urchins were also studied. Some of these results were published study (Moulin et al., 2014) and more will be published soon. This demonstrated that such community and long-term OA experiments can be run in artificial mesocosms. However, the word “artificial” must be kept in mind. Indeed, whatever the degree of realism, observations are only cautiously transposable to the field and must be verified thanks to in situ observations or experiments. Moreover, the replication of mesocosms have also to be considered: two mesocosms were identically prepared in term of technical devices, biomass each important species, substratum, etc. ... and can be considered as replicates at the beginning of the experiment. Nevertheless the simplified ecosystems inside each mesocosm had the opportunity to follow their own “evolution” during more than one year ... It is thus hard to consider both mesocosms as true replicates at the end of the experiment. For instance, the episode of white band disease did appear in both mesocosms, but was more intense in the mesocosm B, leading to change the total biomass of scleractinians in this mesocosm compared to the mesocosm A. So such complex systems can be considered as replicates for short term experiments, but the individual variability/change shouldn’t be underestimated when testing longer term experiments.

One particular point concerns the macronutrients. Tropical coral reefs require very low nutrient concentrations (oligotrophic waters; Cooper et al., 2009). Generally, very low nutrient concentrations can be difficult to maintain in closed systems and can rapidly rise to unrealistic concentrations, due to very limited volumes and water changes. 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; Ferrier-Pagés et al., 2000), or by increasing the severity of coral diseases (Bruno and Petes, 2003). The present artificial mesocosms did not suffer from these effects because low concentrations of macronutrients were obtained and maintained over several years. On the contrary, limitation of macronutrients is another possibility in such a closed system. The only N and P input here was through feeding (mainly with frozen plankton). Determination of correct feeding levels was done

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by indirect observations of macronutrient concentrations in order to come close to target levels. This required adjustments over a week, or even multi-weeks time scale. It should also be possible to fix a feeding level *per* time unit to a given N and P influx (for instance, to match values measured or estimated in the field), and then, to adjust the biomass of the various trophic components of the living community to reach target macronutrient concentrations in the water. This kind of approach was not tested in the present study. The concentrations of Ca^{++} and Mg^{++} TS6 ions in seawater partly affect the calcification rate of scleractinian corals and the nature of the precipitated mineral of sea urchins. Indeed, several studies showed that the calcium concentration influenced the calcification rate of corals (Langdon et al., 2000; Marshall and Clode, 2002). Furthermore, their concentration could even increase biological/metabolic effects (Mitsuguchi et al., 2003). Therefore, it is crucial to maintain constant concentration of calcium independently of pH conditions in order to avoid the effect of confounding factors on the calcification rate of corals. The mesocosm approach used here allowed to prevent this problem as concentrations of Ca^{++} TS7 ions remained constant throughout the experiment. However, the artificial seawater salt we used (Reef Crystal) contains a slightly larger amount of Ca^{++} TS8 than in naturel seawater (Atkinson and Bingman, 1997). This problem may certainly be overcome by selecting another brand of sea salt with a more balanced composition.

Daily changes in pH is undetermined for future OA conditions. A recent study showed that this fluctuation could be amplified by ocean acidification (Shaw et al., 2013). Jokiel et al. (2008) worked with an open system and observed 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 a scleractinians coral to OA (Comeau et al., 2014). In the present artificial mesocosms, the same refugia were used to buffer oxygen and carbon dioxide day/night fluctuations in both control and acidified conditions. The acidified conditions exhibited lower amplitude, but that may be linked with a slightly lower photosynthesis rate at the community level (thus considering both zooxanthellate

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corals and algae on and inside the substrate). In any way, these results should be considered carefully because the 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 in the experimental aquaria (Porzio et al., 2011). This constitutes an unwanted side-effect that may be eliminated by deciding amplitudes to use and tuning the refugia (algae biomasses, intensity and duration of light, and water flow to the aquaria) separately in the different subsystems. The same warning probably applies for different temperature conditions.

Species interactions can be very important when dealing with OA. Andersson et al. (2009) working on hermatypic coral calcification showed that the net balance of $CaCO_3$ accretion was declining in higher $p$$CO_2$ conditions. This shift demonstrated how the balance between calcifiers and eroders is important, highlighting the importance to conduct experiments at the ecosystem level to take into account interspecific interactions (Kroeker et al., 2012). Balance of calcifiers and bioeroders in the community must be considered, especially when $Ca(OH)_2$ is used to increase pH, because consumption of alkalinity by the community must be higher than the alkalinity introduced to the system by $Ca(OH)_2$ additions. The resulting unbalance is better compensated by an additional calcium reactor connected to the main unit. In the present study, algae, sea urchins, scleractinians corals and all the other organisms had the opportunity to acclimatize to new physico-chemical conditions before the OA experiment started. Sea urchins, for instance, were placed in each mesocosm 7 months before to start the experiment. Scleractinians corals were also introduced 6 months before the start of the experiment. Moreover only neo-formed coral branches (i.e. calcification occuring within the artificial mesocosm) were used for ecophysiological measurements. It ensures as much as possible that observed effects are due to the treatment. Artificial reef mesocosms can be designed, maintained and used for OA experiments. It is certainly possible to connect more experimental aquaria to test a suite of pH values (say 8.2, 8.0, 7.6 and 7.3, for instance). This would allow a much more powerful statistical approach of the analysis than with ANOVAs

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by means of linear or nonlinear modeling of biological responses in function of pH. On the other hand, four experimental aquaria would also allow to combine pH and temperature changes in a cross-factorial design with mesocosm as a repeated factor (being a paired design). Coral reef mesocosm used in ocean acidification studies until now (see references in the introduction section) require water input from the natural environment. Our system differ from these ones as it does not necessarily require it. Moreover, thanks to its flexibility, replicability, easy access and independence from extreme meteorological factors in the field, artificial mesocosms are a complementary tool to observations and experiments undertaken directly in the field (for instance future studies with FOCE systems; Gattuso et al., 2014).

5 Conclusions

The present study highlighted that artificial reef mesocosms are a complementary tool of field experiments, allowing an easy manipulation of seawater physico-chemistry and the study of ecophysiological effects on simplified reef ecosystems.

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References

Adey, W. and Loveland, K.: Dynamic Aquaria, Building and Restoring Living Ecosystems, 3rd edn., Academic Press, Elsevier, New York, 528 pp., 2011. 10

Andersson, A. J., Kuffner, I. B., Mackenzie, F. T., Jokiel, P. L., Rodgers, K. S., and Tan, A.: Net Loss of $CaCO_3$ from a subtropical calcifying community due to seawater acidification: mesocosm-scale experimental evidence, Biogeosciences, 6, 1811–1823, doi:10.5194/bg-6-1811-2009, 2009. 4, 25

Arakaki, Y., Uehara, T., and Fagoonee, I.: Comparative studies of the genus *Echinometra* from Okinawa and Mauritius, Zool. Sci., 15, 159–168, 1998. 12, 13

Atkinson, M. J. and Bingman, C.: Elemental composition of commercial seasalts, Journal of Aquariculture and Aquatic Sciences, 8, 39TS9, 1997. 24

Atkinson, M. J., Barnett, H., Aceves, H., Langdon, C., Carpenter, S. J., McConnaughey, T., Hochberg, E., Smith, M., and Marino, B. D. V.: The Biosphere 2 coral reef biome, Ecol. Eng., 13, 147–172, 1999. 5

Badgley, B., Lipschultz, F., and Sebens, K.: Nitrate uptake by the reef coral *Diploria strigosa*: effects of concentration, water flow, and irradiance, Mar. Biol., 149, 327–338, 2006. 8

Bruno, J. and Petes, L.: Nutrient enrichment can increase the severity of coral diseases, Ecol. Lett., 6, 1056–1061, 2003. 23

Calosi, P., Rastrick, S. P. S., Graziano, M., Thomas, S. C., Baggini, C., Carter, H., and Spicer, J. I.: Distribution of sea urchins living near shallow water CO_2 vents is dependent upon species acid-base and ion-regulatory abilities, Mar. Pollut. Bull., 73, 470–484, 2013. 3

Carpenter, L. W. and Patterson, M. R.: Water flow influences the distribution of photosynthetic efficiency within colonies of the scleractinian coral *Montastrea annularis* (Ellis and Solander, 1786), implications for coral bleaching, J. Exp. Mar. Biol. Ecol., 351, 10–26, 2007. 8

Chauvin, A., Denis, V., and Cuet, P.: Is the response of coral calcification to seawater acidification related to nutrient loading?, Coral Reefs, 30, 911–923, 2011. 7, 40

Chazottes, V., Le Campion-Alsumard, T., Peyrot-Clausade, M., and Cuet, P.: 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, 2002. 35

Cigliano, M., Gambi, M. C., Rodolfo-Metalpa, R., Patti, F. P., and Hall-Spencer, J. M.: Effects of ocean acidification on invertebrate settlement at volcanic CO_2 vents, Mar. Biol., 157, 2489–2502, 2010. 3

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Artificial coral reef mesocosms for ocean acidification investigations

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

Comeau, S., Carpenter, R. C., and Edmunds, P. J.: Effects of feeding and light intensity on the response of the coral *Porites rus* to ocean acidification, Mar. Biol., 160, 1127–1134, 2013a. 4

Comeau, S., Edmunds, P. J., Spindel, N. B., and Carpenter, R. C.: The responses of eight coral reef calcifiers to increasing partial pressure of CO_2 do not exhibit a tipping point, Limnol. Oceanogr., 58, 388–398, 2013b. 4

Comeau, S., Edmunds, P., Spindel, N., and Carpenter, R.: Diel $p$$CO_2$ oscillations modulate the response of the coral *Acropora hyacinthus* to ocean acidification, Mar. Ecol.-Prog. Ser., 501, 99–111, 2014. 11, 24

Cooper, T., Gilmour, J., and Fabricius, K.: Bioindicators of changes in water quality on coral reefs: review and recommendations for monitoring programmes, Coral reefs, 28, 589–606, 2009. 23

DelValls, T. A. and Dickson, A. G.: The pH of buffers based on 2-amino-2-hydroxymethyl-1,3-propanediol ("TRIS") in synthetic sea water, Deep-Sea Res. Pt. I, 45, 1541–1554, 1998. 13

Delbeek, J. C. and Sprung, J.: The Reef Aquarium, vol. 3, Ricordea Publishing, 680 pp., 2007TS10. 11

Dickson, A. G.: Standard potential of the reaction: $AgCl(s) + H_2(g) = Ag(s) + HCl(aq)$, and the standard acidity constant of the ion HSO_4 in synthetic sea water from 273.15 to 318.15 K, J. Chem. Thermodyn., 22, 113–127, 1990. 14

Dickson, A. G.,Sabine, C. L., and Christian, J. R.: Guide to Best Practices for Ocean CO_2 Measurements, PICES Special Publication 3, 191 pp., 2007. 13, 22

DOE: Handbook of methods for the analysis of the various parameters of the carbon dioxide system in seawater, Version 2, Department of Energy, ORNL/CDIAC-74, 1994TS11. 13

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

Dove, S. G., Klinea, D. I., Pantosa, O., Angly, F. E., Tyson, G. W., and Hoegh-Guldberg, O.: Future reef decalcification under a business-as-usual CO_2 emission scenario, P. Natl. Acad. Sci. USA, 110, 15342–15347, 2013. 4

Please note the remarks at the end of the manuscript.

Egilsdottir, H., Noisette, F., Noël, L. M.-L. J., Olafsson, J., and Martin, S.: Effects of pCO_2 on physiology and skeletal mineralogy in a tidal pool coralline alga *Corallina elongata*, Mar. Biol., 160, 2103–2112, 2012. 3

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.: Losers and winners in coral reefs acclimatized to elevated carbon dioxide concentrations, Nature Climate Change., 1, 165–169, 2011. 3

Feely, R. A., Sabine, C. L., Hernandez-Ayon, J. M., Ianson, D., and Hales, B.: Evidence for upwelling of corrosive "acidified" water onto the continental shelf, Science, 320, 1490–1492, 2008. 3

Ferrier-Pagés, C., Gattuso, J. P., Dallot, S., and Jaubert, J.: Effect of nutrient enrichment on growth and photosynthesis of the zooxanthellate coral *Stylophora pistillata*, Coral Reefs, 19, 103–113, 2000. 23

Finelli, C. M., Helmuth, B. S. T., Pentcheff, N. D., and Wethey, D. S.: Water flow influences oxygen transport and photosynthetic efficiency in corals, Coral Reefs, 25, 47–57, 2006. 8

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.: Free-ocean CO_2 enrichment (FOCE) systems: present status and future developments, Biogeosciences, 11, 4057–4075, doi:10.5194/bg-11-4057-2014, 2014. TS12 5, 26

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.: Volcanic carbon dioxide vents show ecosystem effects of ocean acidification, Nature, 454, 96–99, 2008. 3

Hurlbert, S. H.: Pseudoreplication and the design of ecological field experiments, Ecol. Monogr., 54, 187–211, 1984. 3

Intergovernmental Panel on Climate Change IPCC: Climate Change 2013: the Fifth Assessment Report of the IPCC, Cambridge University Press, Cambridge, 2013. 2

Jokiel, P., Rodgers, K., Kuffner, I., Andersson, A., Cox, E., and Mackenzie, F.: Ocean acidification and calcifying reef organisms: a mesocosm investigation, Coral Reefs, 27, 473–483, 2008. 4, 24

Jokiel, P. L., Bahr, K. F., and Rodgers, K. S.: Low-cost, high-flow mesocosm system for simulating ocean acidification with CO_2 gas, Limnol. Oceanogr.-Meth., 12, 313–322, 2014a.

Jokiel, P. L., Jury, C. P., and Rodgers, K. S.: Coral-algae metabolism and diurnal changes in the CO_2-carbonate system of bulk sea water, Peer J., 2, e378TS13, 2014b. 4

Please note the remarks at the end of the manuscript.

Jury, C. P., Whitehead, R. F., and Szmant, A. M.: Effects of variations in carbonate chemistry on the calcification rates of *Madracis auretenra* (= *Madracis mirabilis sensu* Wells, 1973): bicarbonate concentrations best predict calcification rates, Glob. Change Biol., 16, 1632–1644, 2009. 4

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

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.: A short-term *in situ* CO_2 enrichment experiment on Heron Island (GBR), Scientific Reports, 2, 413, doi:10.1038/srep00413, 2012. 5, 9

Kuffner, I. B., Andersson, A. J., Jokiel, P. L., Rodgers, K. S., and Mackenzie, F. T.: Decreased abundance of crustose coralline algae due to ocean acidification, Nat. Geosci., 1, 114–117, 2008. 4

Kroeker, K. J., Micheli, F., and Gambi, M. C.: Ocean acidification causes ecosystem shifts via altered competitive interactions, Nature Climate Change, 3, 156–159, 2012. 25

Langdon, C., Takahashi, T., Sweeney, C., Chipman, D., Goddard, J., Marubini, F., Aceves, H., Barnett, H., and Atkinson, M. J.: Effect of calcium carbonate saturation state on the calcification rate of an experimental coral reef, Global Biogeochem. Cy., 14, 639–654, 2000. 5, 24

Langdon, C., 5

Broecker, W. S., Hammond, D. E., Glenn, E., Fitzsimmons, K., Nelson, S. G., Peng, T.-H., Hajdas, I., and Bonani, G.: Effect of elevated CO_2 on the community metabolism of experimental coral reef, Global Biogeochem. Cy., 17, 1011, doi:10.1029/2002GB001941, 2003.

Lavigne, H. and Gattuso, J.-P.: Seacarb: Seawater Carbonate Chemistry with R (R package version 2.4.3), 2012TS14. 14

Leclercq, N., Gattuso, J.-P., and Jaubert, J.: CO_2 partial pressure controls the calcification rate of a coral community, Glob. Change Biol., 6, 329–334, 2000. 4

Please note the remarks at the end of the manuscript.

Leclercq, N., Gattuso, J., and Jaubert, J.: Primary production, respiration, and calcification of a coral reef mesocosm under increased CO_2 partial pressure, Limnol. Oceanogr., 47, 558–564, 2002. 4

Lueker, T. J., Dickson, A. G., and Keeling, C. D.: 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, Mar. Chem., 70, 105–119, 2000. 14

Marshall, A. T. and Clode, P. T.: Effect of increased calcium concentration in sea water on calcification and photosynthesis in the scleractinian coral *Galaxea fascicularis*, J. Exp. Biol., 205, 20107–2113, 2002. 24

Marubini, F. and Davies, P. S.: Nitrate increases zooxanthellae population density and reduces skeletogenesis in corals, Mar. Biol., 127, 319–328, 1996. 23

Marubini, F., Barnett, H., Langdon, C., and Atkinson, M.: Dependence of calcification on light and carbonate ion concentration for the hermatypic coral *Porites compressa*, Mar. Ecol.-Prog. Ser., 220, 153–162, 2001. 5

Mitsuguchi, T., Matsumoto, E., and Uchida, T.: Mg/Ca and Sr/Ca ratios of porites coral skeleton: evaluation of the effect of skeletal growth rate, Coral Reefs, 22, 381–388, 2003. 24

Mortensen, T. H.: A monograph of *Echinoida*, 3. *Camarodonta*, II *Echinoidae*, *Strongylocentrotidae*, *Parasaleniidae*, *Echinometridae*, vol. 3, CA Reitzel, Copenhagen, 277–439, 1943. 12

Moulin, L., Catarino, A. I., Claessens, T., and Dubois, P.: Effects of seawater acidification on early development of the intertidal sea urchin *Paracentrotus lividus* (Lamarck, 1816), Mar. Pollut. Bull., 62, 48–54, 2011. 3

Moulin, L., Grosjean, P., Leblud, J., Batigny, A., and Dubois, P.: 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, J. Exp. Mar. Biol. Ecol., 457, 97–104, 2014. 23

Odum, E.: Systems Ecology: an Introduction, Wiley, New York, 644 pp., 1983. 3, 4, 21

Odum, E.: The mesocosm, BioScience, 34, 558–562, 1984. 4

Park, K.: Deep-sea pH, Science, 154, 1540–1542, 1966. 3

Perez, F. F. and Fraga, F.: Association constant of fluoride and hydrogen ions in seawater, Mar. Chem., 21, 161–168, 1987. 14

Petzoldt, T. and Rinke, K.: simecol: an object-oriented framework for ecological modeling in R, J. Stat. Software, 22, 1–31, 2007. 15, 16

Porzio, L., Buia, M. C., and Hall-Spencer, J. M.: Effects of ocean acidification on macroalgal communities, J. Exp. Mar. Biol. Ecol., 400, 278–287, 2011. 25

R Core Team: R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria, available at: http://www.R-project.org/TS15, 2013. 8, 14

Roberts, J. M., Wheeler, A. J., and Freiwald, A.: Reefs of the deep: the biology and geology of cold-water coral ecosystems, Science, 312, 543–547, 2006. 3

Turley, C. M., Roberts, J. M., and Guinotte, J. M.: Corals in deep-water: will the unseen hand of ocean acidification destroy cold-water ecosystems? Coral Reefs, 26, 445–448, 2007. 3

Schutter, M.: The Influence of Light and Water Flow on the Growth and Physiology of the Scleractinian Coral *Galaxea fascicularis*, Ph.D. thesis, Wageningen University, Wageningen, Netherlands, 232 pp., 2010. 8

Sebens, K., Grace, S., and Helmuth, B.: Water flow and prey capture by three scleractinian corals, *Madracis mirabilis*, *Montastrea cavernosa* and *Porites porites*, in a field enclosure, Mar. Biol., 131, 347–360, 1998. 8

Sebens, K., Helmuth, B., Carrington, E., and Agius, B.: Effects of water flow on growth and energetics of the scleractinian coral *Agaricia tenuifolia* in Belize, Coral Reefs, 22, 35–47, 2003. 8

Shaw, E. C., McNeil, B. I., Tilbrook, B., Matear, R., and Bates, M. L.: Anthropogenic changes to seawater buffer capacity combined with natural reef metabolism induce extreme future coral reef CO_2 conditions, Glob. Change Biol., 19, 1632–41, 2013. 11, 24

Soetaert, K., Petzoldt, T., and Meysman, F.: marelac: Tools for Aquatic Sciences, R package version 2.1.2, available at: http://CRAN.R-project.org/package=marelacTS16, 2012. 15

Soffer, N., Brandt, M. E., Correa, A. M. S., Smith, T. B., and Thurber, R. V.: Potential role of viruses in white plague coral disease, International Society for Microbial Ecology Journal, 8, 271–283, 2014. 22

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.: Mesocosm Experiments as a Tool for Ecological Climate-Change, 1st edn., Elsevier Ltd., 2013TS17. 4

Please note the remarks at the end of the manuscript.

Szmant, A.: Nutrient enrichment on coral reefs: is it a major cause of coral reef decline?, Estuaries, 25, 743–766, 2002. 23
Vizzini, S., Di Leonardo, R., Costa, V., Tramati, C. D., Luzzu, F., and Mazzola, A.: Trace element bias in the use of CO_2 vents as analogues for low pH environments: implications for contamination levels in acidified oceans, Estuar. Coast. Shelf S., 134, 19–30, 2013. 3
Widdicombe, S., Dupont, S., and Thorndyke, M.: Laboratory experiments and benthic mesocosm studies, in: Guide to Best Practices for Ocean Acidification Research and Data Reporting, edited by: Riebesell, U., Fabry, V. J., Hansson, L., and Gattuso, J.-P., Publications Office of the European Union, Luxembourg, 2010TS18. 2
Wisshak, M., Schönberg, C., Form, A., and Freiwald, A.: Ocean acidification accelerates reef bioerosion, PLoS One, 7, 3–10, 2012. 24

Please note the remarks at the end of the manuscript.

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11, 1–43, 2014

Table 1. Mean physico-chemical parameters recorded in each aquarium before the pH decrease (three months monitoring), during the pH decrease (6 months monitoring) and after the pH decrease (7 months monitoring). Values represent means ±SDs. Mean temperature and pH were calculated from the measurements recorded every 20 s. 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.

Parameter	Mesocosm A – Control			Mesocosm A – Acidified		
	Before decrease	During decrease	After decrease	Before decrease	During decrease	After decrease
pHT	8.04 ± 0.02	8.06 ± 0.02	8.08 ± 0.03	8.07 ± 0.01	7.82 ± 0.11	7.63 ± 0.02
pCO_2 (ppm)	420 ± 21	388 ± 61	381 ± 40	378 ± 18	784 ± 224	1294 ± 125
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
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
CO_3 (mmol kg^{-1})	0.215 ± 0.014	0.229 ± 0.013	0.236 ± 0.021	0.229 ± 0.014	0.148 ± 0.034	0.101 ± 0.009
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
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
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
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
Ca (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
Ca + Mg + Sr (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
	Mesocosm B – Control			Mesocosm B – Acidified		
	Before decrease	During decrease	After decrease	Before decrease	During decrease	After decrease
pHT	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)	402 ± 23	388 ± 61	356 ± 47	484 ± 32	806 ± 249	1263 ± 92
Total alkalinity (mmol kg^{-1})	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})	1.824 ± 0.095	1.899 ± 0.259	1.766 ± 105	1.886 ± 0.089	2.115 ± 0.228	2.140 ± 0.121
CO_3 (mmol kg^{-1})	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	3.936 ± 0.266	4.629 ± 0.786	4.289 ± 0.298	3.487 ± 0.322	2.864 ± 0.374	1.760 ± 0.105
Ω calcite	5.979 ± 0.402	7.018 ± 1.190	6.511 ± 0.451	5.299 ± 0.488	4.344 ± 0.567	2.672 ± 0.159
Temperature (°C)	25.13 ± 0.12	25.36 ± 0.35	25.10 ± 0.22	25.03 ± 0.11	25.31 ± 0.33	25.04 ± 0.10
Salinity	34.50 ± 0.43	34.70 ± 0.46	34.50 ± 0.43	34.50 ± 0.42	34.70 ± 0.45	34.50 ± 0.42
Ca (mmol kg^{-1})	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 + Mg + Sr (mmol kg^{-1})	63.74 ± 0.44	67.16 ± 2.07	66.02 ± 1.22	63.78 ± 1.16	66.93 ± 2.05	66.39 ± 1.69

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Table 2. Mean nutrients 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 ±SDs. Nutrients were quantified every 2 weeks. Maximum field measurements are from Chazottes et al. (2002).

Nutrient	Meso. A-Ctrl.			Meso. A-Acid.		
	Before decrease	During decrease	After decrease	Before decrease	During decrease	After decrease
NO_3	0.52 ± 0.78	0.96 ± 0.78	0.87 ± 0.89	0.74 ± 0.49	0.43 ± 0.63	0.47 ± 0.69
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
NH_4	0.80 ± 0.88	0.44 ± 0.32	0.52 ± 0.69	0.90 ± 1.24	0.73 ± 1.04	0.44 ± 0.56
PO_4	0.42 ± 0.18	0.17 ± 0.24	0.30 ± 0.44	0.30 ± 0.35	0.18 ± 0.17	0.31 ± 0.44
	Meso. B-Ctrl.			Meso. B-Acid.		
	Before decrease	During decrease	After decrease	Before decrease	During decrease	After decrease
	1.30 ± 1.25	0.55 ± 0.67	0.84 ± 0.77	0.67 ± 1.11	1.31 ± 0.95	0.80 ± 0.74
NO_2	0.14 ± 0.15	0.13 ± 0.06	0.15 ± 0.09	0.15 ± 0.11	0.17 ± 0.11	0.18 ± 0.22
NH_4	1.20 ± 1.20	0.50 ± 0.49	0.32 ± 0.45	0.91 ± 0.81	0.73 ± 0.66	0.56 ± 0.64
PO_4	0.13 ± 0.32	0.49 ± 0.60	0.25 ± 0.34	0.76 ± 0.90	0.18 ± 0.18	0.34 ± 0.44
	Max field measurement					
NO_3	2.26 ($NO_3 + NO_2$)					
NO_2						
NH_4	1.08					
PO_4	0.33					

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Table 3. Result from the oxygen net fluxes modeling. Values are the best estimates for the parameters used in the model described at Eq. (1).

	Mesocosm A		Mesocosm B	
	Control	Acidified	Control	Acidified
Net photosynthesis (P, mmol h^{-1})	11.2	7.7	10.3	7.7
Dark respiration (Rdark; mmol h^{-1})	−7.2	−5.8	−4.7	−7.9
Daily balance (mmol h^{-1})	4	1.9	5.65	−0.2

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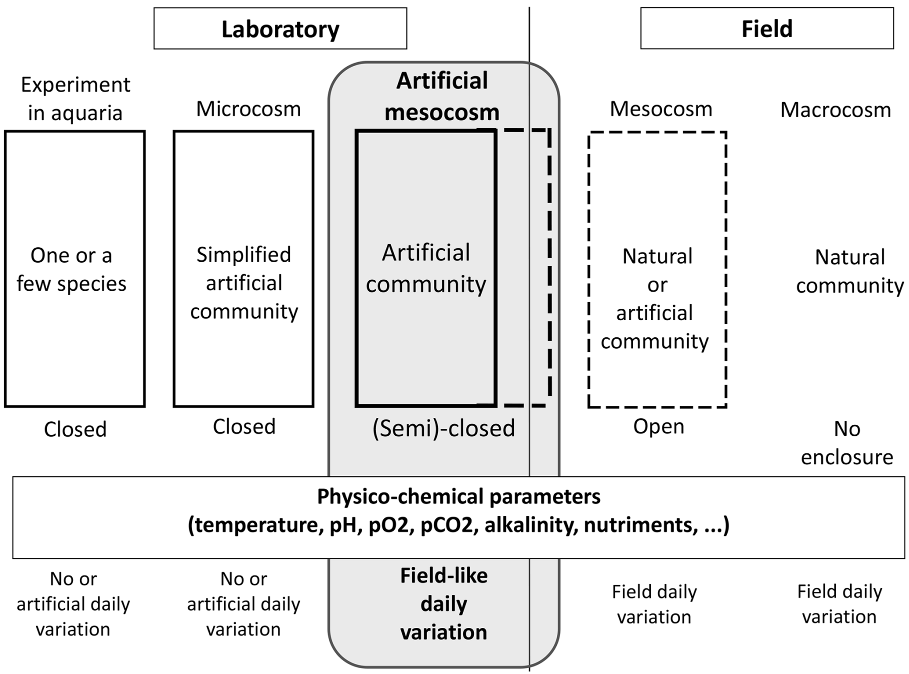

Figure 1. Different systems used in OA studies (as discussed in the text). The artificial mesocosm (in gray) appears as a compromise between more realism and complexity in one hand, and ease of maintenance and replication in the laboratory, on the other hand.

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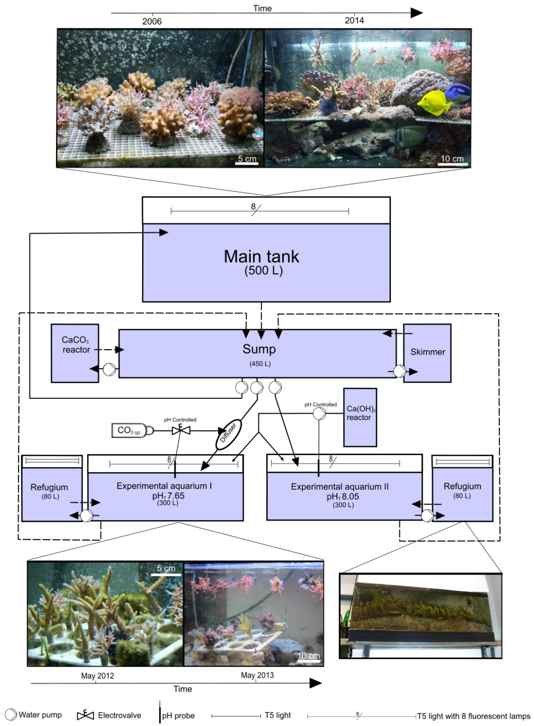

Figure 2. Artificial reef mesocosm. Main tank is connected to the sump (water change = $14\,\mathrm{L\,min^{-1}}$). A_T is stabilized using a $CaCO_3$ reactor. A skimmer eliminates the excess of dissolved, colloidal and particulate organic molecules in the water column. pH in experimental aquaria is controlled using CO_2 bubbling and $Ca(OH)_2$ additions. Each of the two experimental aquaria is connected to the sump (water change = $0.8\,\mathrm{L\,min^{-1}}$). Refugia connected to each experimental aquarium limit daily oxygen fluctuations. Experimental aquaria as well as main tank temperature are controlled with resistances and electric fans. Pictures illustrate the different parts and their evolution with time.

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Artificial coral reef mesocosms for ocean acidification investigations

J. Leblud et al.

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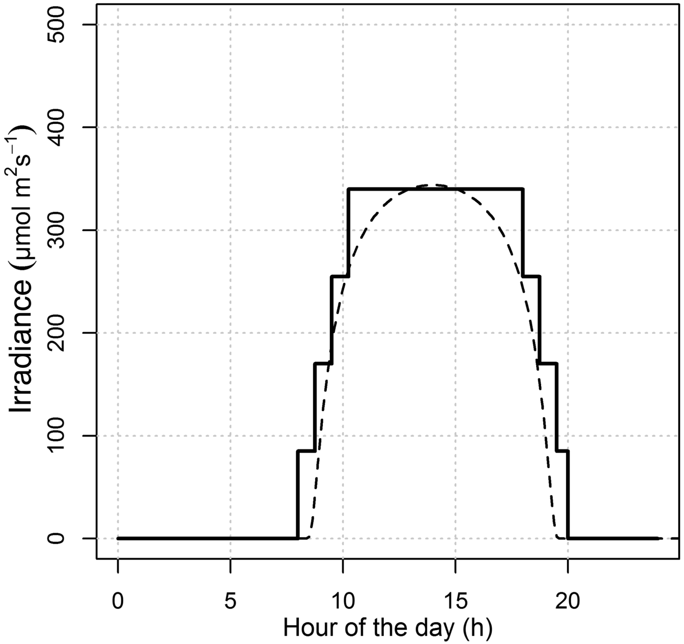

Figure 3. Irradiance daily cycle. Black line represents the irradiance measured with an Apogee Quantum Meter inside the main tank. Dotted line represents the Réunion theorical solar irradiance ajusted with field measurements at 1 m depth (as performed with the same Quantum Meter). Total day/night time is 12/12 h.

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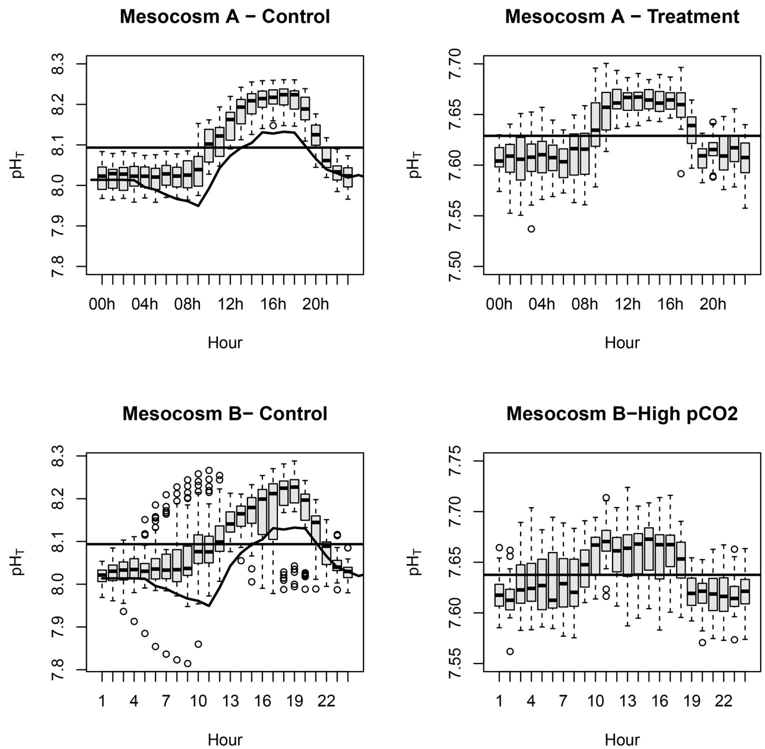

Figure 4. pH_T diurnal variations inside each experimental aquarium. Box-plots represent median (blackline), interquartile range (box), 1.5 times the interquartile range from the box edges (whiskers) and outliers (individual points). Each box-plot corresponds to data recorded every hour of each day over the 3 months after establishment of contrasted pCO_2 conditions. Medians were calculated from measurements recorded every 20 s. Black horizontal lines represent the global pH_T mean. Black curves in the control aquaria graphs represent the median field variation per hour (La Saline Lagoon, Réunion Island, Cuet, personal communicationTS19, see also Chauvin et al., 2011). Light is provided from 8 to 20 h.

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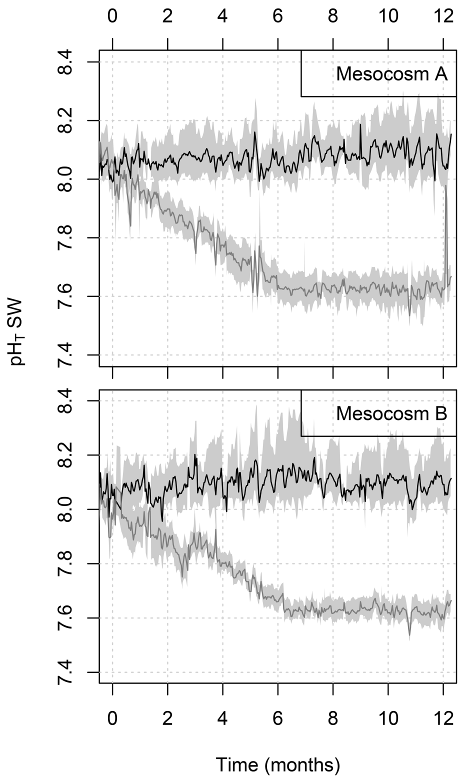

Figure 5. pH$_T$ time course in each experimental aquarium of both mesocosms during the experiment. Values represent pH$_T$ recorded every 20 s and then averaged by days (black lines for controls and grey lines for treatment aquaria). Envelopes correspond to minimum and maximum values per day.

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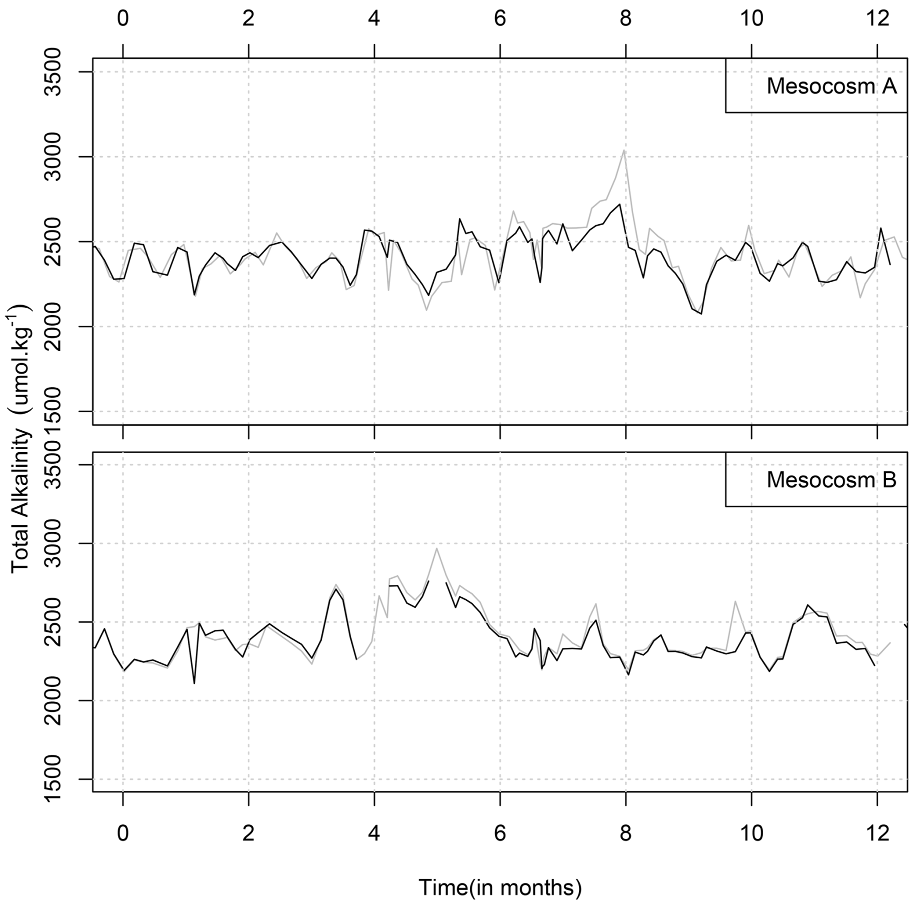

Figure 6. Total alkalinity (A_T) time course in each experimental aquarium of both mesocosms. Black lines represent the control aquaria, grey lines represent treatment aquaria. Total alkalinity was measured every 2 days.

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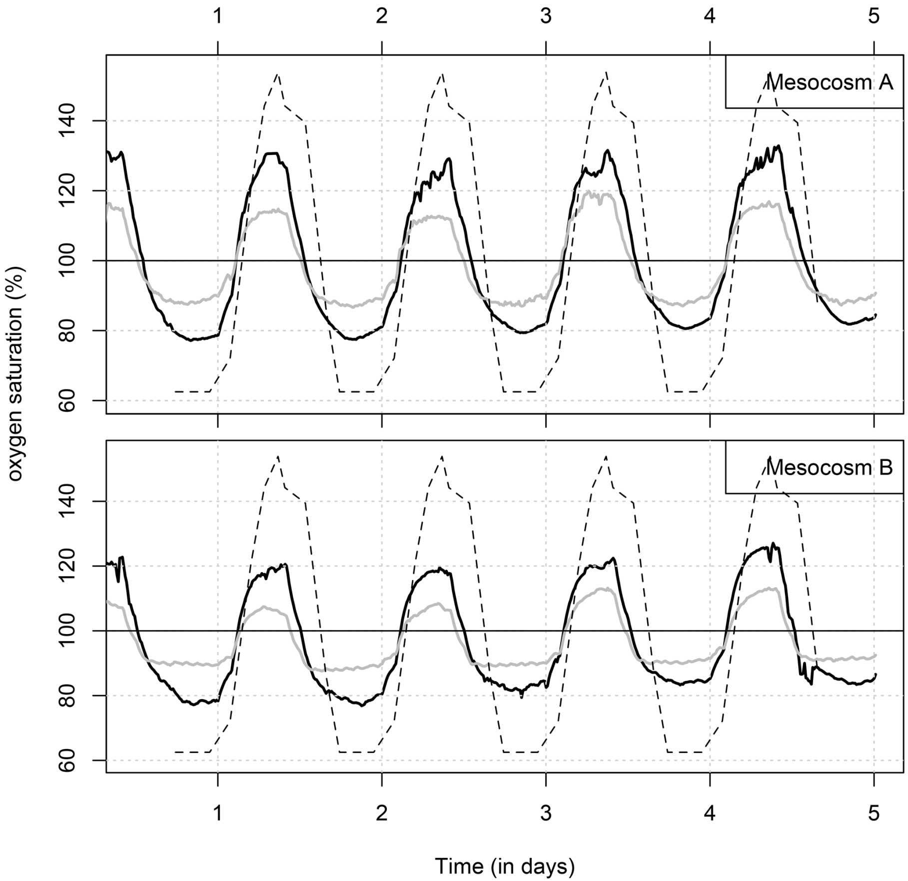

Figure 7. Oxygen saturation in each experimental aquarium of both mesocosms over a 5 day monitoring at the end of the experiment. Black lines represent the control aquaria, grey lines represent the acidified aquaria. Dotted lines represent field measurements from Clavier et al. (2013) at La Saline Lagoon, La Reunion. Oxygen concentration was recorded every 20 s.

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