https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

Biogeosciences Discussions
Open Access
EGU

Intercomparison of four methods to estimate coral calcification under various environmental conditions

Miguel Gómez Batista[1], Marc Metian[2], François Oberhänsli[2], Simon Pouil[2], Peter W. Swarzenski[2], Eric Tambutté[3], Jean-Pierre Gattuso[4,5], Carlos M. Alonso Hernández[1], Frédéric Gazeau[4]

[1]Centro de Estudios Ambientales de Cienfuegos, Cuba
[2]International Atomic Energy Agency, Environment Laboratories, 4a Quai Antoine 1er, MC-98000 Monaco, Principality of Monaco
[3]Centre Scientifique de Monaco, Department of Marine Biology, MC-98000 Monaco, Principality of Monaco
[4]Sorbonne Université, CNRS, Laboratoire d'Océanographie de Villefranche, LOV, F-06230 Villefranche-sur-Mer, France
[5]Institute for Sustainable Development and International Relations, Sciences Po, 27 rue Saint Guillaume, F-75007 Paris, France

Correspondence to: Miguel Gómez Batista (mgomezbatista@gmail.com)

Keywords: Calcification; Coral; Alkalinity anomaly; Calcium anomaly; ^{45}Ca incorporation; ^{13}C incorporation

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

Biogeosciences Discussions Open Access EGU

Abstract

Coral reefs are constructed by calcifiers that precipitate calcium carbonate to build their shells or skeletons through the process of calcification. Accurately assessing coral calcification rates is crucial to determine the health of these ecosystems and their response to major environmental changes such as ocean warming and acidification. Several approaches have been used to assess rates of coral calcification but there is a real need to compare these approaches in order to ascertain that high quality and intercomparable results can be produced. Here, we assessed four methods (total alkalinity anomaly, calcium anomaly, ^{45}Ca incorporation and ^{13}C incorporation) to determine coral calcification of the reef-building coral *Stylophora pistillata*. Given the importance of environmental conditions on this process, the study was performed under two pH (ambient and low level) and two light (light and dark) conditions. Under all conditions, calcification rates estimated using the alkalinity and calcium anomaly techniques as well as ^{45}Ca incorporation were highly correlated. Such a strong correlation between the alkalinity anomaly and ^{45}Ca incorporation techniques has not been observed in previous studies and most probably results from improvements described in the present paper. The only method which provided calcification rates significantly different from the other three techniques was ^{13}C incorporation. Calcification rates based on this method were consistently higher than those measured using the other techniques. Although reasons for these discrepancies remain unclear, the use of this technique for assessing calcification rates in corals is not recommended without further investigations.

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019


Biogeosciences Discussions Open Access EGU

1. Introduction

Calcification is the fundamental biological process by which organisms precipitate calcium carbonate. Calcifying organisms take up calcium and carbonate or bicarbonate ions to build their biomineral structures (aragonite, calcite and/or vaterite) which have physiological, ecological and biogeochemical functions. Moreover, calcium carbonate plays a major role in the services provided by ecosystems to human societies.

The ocean has absorbed large amounts of anthropogenic CO_2 since the start of the industrial revolution and is currently sequestering about 22% of CO_2 emissions (average 2008-2017; Le Quéré et al., 2018). This massive input of CO_2 in the ocean impacts seawater chemistry with a decrease in seawater pH, carbonate ion concentrations [CO_3^{2-}] and an increase in CO_2 and bicarbonate concentrations [HCO_3^-]. These fundamental changes to the carbonate system are referred to as "ocean acidification" (OA; Gattuso and Hansson, 2011). Models project that the average surface water pH will drop by 0.06 to 0.32 pH units by the end of the century (IPCC, 2014).

The effect of OA on the ocean is currently the subject of intense research with particular attention to organisms producing $CaCO_3$. For instance, coral communities have already proven to be particularly vulnerable to rapidly changing global environmental conditions (e.g. Albright et al., 2018). In order to help project the future of coral reefs, accurate estimates of calcification rates during realistic perturbation experiments are necessary in order to produce high quality and intercomparable results (Langdon et al., 2010).

Several methods are available to quantify rates of coral calcification. Calcification can be measured as the increase of $CaCO_3$ mass (e.g. the buoyant weight technique; Jokiel et al., 1978) or following the incorporation of radio-labelled carbon or calcium in the skeleton

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019


Biogeosciences Discussions Open Access EGU

(Goreau, 1959), but also through the quantification of changes in a seawater constituent that is stoichiometrically related to the amount of $CaCO_3$ precipitated. For instance, the alkalinity anomaly technique (Smith and Key, 1975) has been widely used to estimate net calcification of organisms and communities, especially of corals and coral reef environments (e.g. Smith and Kinsey, 1978; Gazeau et al., 2015; Albright et al., 2016; Cyronak et al., 2018). Total alkalinity (A_T) is directly influenced by bicarbonate and carbonate ion concentrations together with a multitude of other minor compounds (Wolf-Gladrow et al., 2007). Calcification consumes carbonate or bicarbonate, following the reversible reaction:

$$Ca^{2+} + 2HCO_3^- \leftrightarrow CaCO_3 + CO_2 + H_2O \quad (1)$$

Calcification consumes two moles of HCO_3^-, hence decreasing A_T by two moles per mole of $CaCO_3$ produced (eq. 1). It is possible to derive the rate of net calcification (gross calcification - dissolution) by measuring A_T before and after incubating an organism or a community. This method must assume, however, that only calcification influences A_T (Smith and Key, 1975).

In contrast to A_T, the concentration of calcium (Ca^{2+}) in seawater is only biologically influenced by net calcification and a 1:1 relationship can be used to derive net calcification rates (eq. 1). The depletion of A_T and Ca^{2+} needs to be corrected for gains of A_T and Ca^{2+} resulting from evaporation. These corrections can be applied through the incubation of seawater in the absence of coral (Schoepf et al., 2017). Both the alkalinity anomaly and calcium anomaly methods are non-destructive and typically show a solid agreement (Chisholm and Gattuso, 1991; Murillo et al., 2014; Gazeau et al., 2015).

The ^{45}Ca incorporation technique has been used since the 1950's (Goreau and Bowen, 1955; Goreau, 1959). While earlier techniques showed low reproducibility, methodological improvements led to a significant reduction of the deviations between replicates (see

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

89 Tambutté et al., 1995, for more details). The strength of this method is that it is extremely
90 sensitive for measuring short-term variations in gross calcification rates. However, in contrast
91 to the A_T and Ca^{2+} anomaly techniques, it is a sample-destructive method.

92 Previous studies designed to compare calcification rate estimates using the ^{45}Ca
93 incorporation and A_T anomaly methods revealed subtle discrepancies. For example, Smith and
94 Roth in Smith and Kinsey (1978) reported an overestimation of rates based on the ^{45}Ca
95 method. In contrast, Tambutté et al. (1995) and Cohen et al. (2017) reported a decrease in A_T
96 without concomitant incorporation of ^{45}Ca, therefore suggesting an overestimation of
97 calcification derived from A_T measurements. However, during these studies, in order to avoid
98 radioactive contamination of laboratory equipment, estimates of calcification were not
99 performed during the same incubations, but rather during incubations performed over two
100 consecutive days.

101 In contrast to the ^{45}Ca incorporation method, to the best of our knowledge, no studies
102 have used carbon-based incorporation techniques to estimate coral calcification rates in the
103 framework of ocean acidification. Past studies that compared carbon and calcium
104 incorporation rates in coral skeletons based on a double labelling technique with $H^{14}CO_3$ and
105 ^{45}Ca showed that only a minor proportion of the labelled seawater carbon is incorporated in
106 the skeleton (e.g. Marshall and Wright, 1998) and that the major source of dissolved inorganic
107 carbon for calcification is metabolic CO_2 (70–75% of the total $CaCO_3$ deposition; Furla et al.,
108 2000). Consequently, under both light and dark conditions, the rate of ^{45}Ca deposition appears
109 greater than the rate of ^{14}C incorporation (Furla et al., 2000). To the best of our knowledge,
110 only one study estimated calcification rates of a benthic calcifier (coralline algae) using a
111 stable carbon isotopic technique through addition of ^{13}C-labelled bicarbonate (McCoy et al.,
112 2016).

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

113 The present study aimed at comparing calcification rates measured using the alkalinity
114 and calcium anomaly methods, as well as the ^{45}Ca and ^{13}C incorporation techniques.

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019


Biogeosciences Discussions Open Access EGU

2. Material and Methods

Colonies of the reef-building coral *Stylophora pistillata* were incubated in the laboratory, both in the light and dark, under ambient and lowered pH conditions. At ambient pH (experiment conducted in July-August 2017), two sets of incubations were performed using either ^{45}Ca or ^{13}C additions and calcification rates based on these techniques were compared to those derived, during the same incubations, by the alkalinity and calcium anomaly techniques. At lowered pH (experiment conducted in August 2018), no incubations with ^{13}C addition were conducted and only the three other techniques were compared.

2.1. Biological material and experimental set-up

Specimens used in this experiment originated from colonies of the coral *Stylophora pistillata* (Esper 1797) initially sampled in the Gulf of Aqaba (Red Sea, Jordan) and transferred to the Scientific Centre of Monaco where they were cultivated under controlled conditions for several years. In June 2017, terminal branches of *S. pistillata*, free of boring organisms, were cut and suspended with a nylon line to allow tissues to fully cover the exposed skeleton for at least five weeks (Tambutté et al., 1995; Houlbrèque et al., 2015). The nubbins were fed with rotifers (once a day) and artemia nauplii (twice a week) and kept under an irradiance of 200 µmol photons m^{-2} s^{-1} (12:12 light:dark photoperiod, light banks: HQI 250W Nepturion - BLV (Germany) / 200 µmol photons m^{-2} s^{-1}), a seawater temperature of 25 ± 0.5 °C and a salinity of 38 ± 0.5. Before the start of the experiment, specimens were transferred to the International Atomic Energy Agency (IAEA). For the second set of experiments in 2018, nubbins were prepared in June 2018 and cultured, under the conditions described above, at IAEA except that colonies were fed twice a week with newly hatched

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

brine shrimp nauplii (1 nauplius ml^{-1}). Biometrics parameters (size, weight) on the biological material are shown in Table 1.

Different types of incubations were conducted. In July-August 2017, one set of incubations was performed under ambient pH conditions with the addition of radioactive calcium dichloride ($^{45}CaCl_2$). During the same period, another set of incubations was performed, under ambient pH conditions, with addition of labelled ^{13}C-sodium bicarbonate (^{13}C-$NaHCO_3$ 99%). Finally, in August 2018, one set of incubations was performed under lowered pH conditions (see thereafter for more details) with the addition of $^{45}CaCl_2$. For all sets of incubations, organisms were incubated for 5 to 11 hours (Table 1), both in the light and dark, in 500 mL polyethylene beakers equipped with a magnetic stirrer (Fig. 1). Six and five replicates were used, respectively, at ambient a low pH. Furthermore, for all sets of incubations, one beaker was incubated, under the same conditions as the other beakers, without coral and served as a control.

For each set of incubations, 2.4 L of seawater, pumped continuous from offshore of the IAEA Monaco premises at 50 m depth, were filtered onto 0.2 µm (GF/F, 47 mm). For incubations performed at lowered pH condition, pure CO_2 was bubbled in the 2.4 L initial seawater batch using an automated pH-stat system (IKS Aquastar©) until the target pH was reached. The pH electrode from the pH-stat system was inter-calibrated using a glass combination electrode (Metrohm, Ecotrode Plus) calibrated on the total scale using a TRIS buffer solution with a salinity of 35 (provided by A. Dickson, Scripps Institution of Oceanography, San Diego). Initial pH_T (total scale) levels were set to ~7.2. It must be stressed that pH levels were not regulated during the incubations. For ^{45}Ca-incubations, this initial batch was spiked with ca. 10 µL of $^{45}CaCl_2$ to reach a nominal activity of 25 Bq mL^{-1}. Before distributing seawater to the experimental beakers, a one-milliliter aliquot of seawater was

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

161 removed for the precise determination of the initial activity. Samples were stored, in the dark,
162 in high-performance glass vials for 24 h before counting. For ^{13}C-incubations, to determine
163 seawater background isotopic level ($\delta^{13}C$) of the dissolved inorganic carbon pool ($\delta^{13}C$-C_T),
164 three 27 mL samples were collected and gently transferred to glass vials avoiding bubbles.
165 Then, ~8.95 mg of ^{13}C-$NaHCO_3$ were added to the batch of filtered ambient seawater to
166 increase $\delta^{13}C$-C_T to ca. 1,500‰. For the determination of $\delta^{13}C$-C_T after enrichment, two 27
167 mL samples were handled as described above. The vials were then sealed after being
168 poisoned with 10 µL of saturated mercuric chloride ($HgCl_2$) and stored upside-down at room
169 temperature in the dark for subsequent analysis.
170 For all sets of incubations, samples for the measurements of pH_T, A_T (200 mL), and
171 Ca^{2+} concentrations (50 mL) were taken before distributing seawater to the experimental
172 beakers. While pH_T was measured immediately after sampling, samples for A_T measurements
173 were poisoned with 40 µL of 50% saturated $HgCl_2$ and stored in the dark at 4 °C pending
174 analysis less than two weeks later. Samples for $[Ca^{2+}]$ measurements were not poisoned and
175 stored in the dark at 4 °C pending analysis less than two weeks after sampling.
176 Gravimetrically determined amounts of filtered seawater (ca. 300 g) were transferred
177 to the incubation containers which were placed in a temperature-controlled (IKS Aquastar©)
178 water bath maintained at 25 ± 0.5 °C. Coral nubbins were suspended with a nylon line in the
179 experimental beakers 4 cm below the water level covered with transparent film to limit
180 evaporation (Fig. 1). During the low pH incubations conducted in 2018, to avoid a
181 physiological stress, coral nubbins were acclimated by gradually lowering pH to the target
182 levels during 24 h. This acclimation was performed in an open-flow 20 L aquarium (one full
183 water renewal per hour) using a pH-stat system as previously described and with a pH
184 decrease of ca. 0.03 units h^{-1}.

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

Incubations in the light were performed at an irradiance of 200 µmol photons m^{-2} s^{-1} during daytime whereas dark incubations were conducted at night. Before the beginning of the incubations, all beakers (containing corals) were precisely weighed at ± 0.01 g (Sartorius BP 310S).

At the conclusion of the incubations, all beakers were precisely weighed to evaluate evaporation and seawater samples were analyzed for pH_T, A_T and $[Ca^{2+}]$ as well as for ^{45}Ca activity or $\delta^{13}C$-C_T depending on the type of incubations. pH_T was measured immediately and samples for A_T and $[Ca^{2+}]$ determinations were filtered onto 0.2 µm (GF/F, Ø 47 mm), poisoned with saturated $HgCl_2$ (only for A_T) and stored in the dark at 4 °C pending analysis (within two weeks). The corals were then removed from the beakers for the analysis of incorporated ^{45}Ca or ^{13}C. Three additional corals which were not incubated were processed for carbon isotopic composition of the previously accreted calcium carbonate (see section "2.3. Computations and statistics").

2.2. Analytical techniques

Immediately after sampling, pH_T was measured on a Metrohm 826 mobile pH-logger and a glass electrode (Metrohm, Ecotrode Plus) calibrated on the total scale using a TRIS buffer of salinity 35 (provided by A. Dickson, Scripps University, USA). A_T was determined in triplicate 50 mL subsamples by potentiometric titration on a titrator Titrando 888 (Metrohm) coupled to a glass electrode (Metrohm, Ecotrode Plus) and a thermometer (pt1000). The pH electrode was calibrated before every set of measurements on the total scale using a TRIS buffer of salinity 35 (provided by A. Dickson, Scripps University, USA). Measurements were carried out at a constant temperature of 25 °C and A_T was calculated as described in Dickson et al. (2007). Certified reference material (CRM; batches 143 and 156)

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

208 provided by A. Dickson (Scripps University, USA) were used to check precision (standard
209 deviation within measurements of the same batch) and accuracy (deviation from the certified
210 nominal value). Over the six series of A_T measurements performed during the experiment,
211 mean accuracy and precision (± SD) were respectively 7.2 ± 1.2 and 1.2 ± 0.2 µmol kg^{-1}.
212 $[Ca^{2+}]$ was determined in triplicate using the ethylene glycol tetra acetic acid (EGTA)
213 potentiometric titration (Lebel and Poisson, 1976). About 10 g of sampled seawater and 10 g
214 of $HgCl_2$ solution (ca. 1 mmol L^{-1}) were accurately weighed out. Then, about 10 g of a
215 concentrated EGTA solution (ca. 10 mmol L^{-1}, also by weighing) was added to completely
216 complex Hg^{2+} and to complex nearly 95% of Ca^{2+}. After adding 10 mL of borate buffer
217 (pH_{NBS} ~ 10) to increase the pH of the solution, the remaining Ca^{2+} was titrated by a diluted
218 solution of EGTA (ca. 2 mmol L^{-1}) using a tritrator (Titrando 888, Metrohm) coupled to an
219 amalgamated silver combined electrode (Metrohm Ag Titrode). Following Cao and Dai
220 (2011), the volume of EGTA necessary to titrate the remaining ca. 5% of Ca^{2+} were obtained
221 by manually fitting a polynomial function to the first derivative of the titration curve using the
222 function "loess" of the R software[1]. The EGTA solution was calibrated prior to each
223 measurement series using International Association for the Physical Sciences of the Oceans
224 (IAPSO) standard seawater (salinity = 38.005). Mean $[Ca^{2+}]$ precision obtained using this
225 technique was 2.9 µmol kg^{-1} (n = 40), corresponding to a coefficient of variation (CV) of
226 0.026%.
227 To determine the specific activity in radio-labelled seawater, the 1 mL aliquots were
228 transferred to 20 mL glass scintillation vials and mixed in proportion 1:10 (v:v) with
229 scintillation liquid Ultima Gold ™ XR. According to a method adapted from Tambutté et al.
230 (1995), at the end of incubation sampled nubbins were immersed for 30 min in beakers

[1]The R Development Core Team, R.: A language and environment for statistical computing, 2018.

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

containing 300 mL of unlabelled seawater to achieve isotopic dilution of the ^{45}Ca contained in the gastrovascular cavity. Constant water motion was provided in the efflux medium by magnetic stirring bars. Tissues were then dissolved completely in 1 mol L^{-1} NaOH at 90 °C for 20 min. The skeleton was rinsed twice in 1 mL NaOH and twice in 5 mL in MilliQ water. It was then dried for 72 h at 60 °C, weighed (referred thereafter to as skeleton dry weight), and dissolved in 12 N HCl. Three 200 µL aliquots from each skeleton dissolution were transferred to 20 mL glass scintillation vials and mixed with 10 mL scintillation liquid Ultima Gold ™ XR. Radioactive samples were thoroughly mixed to homogenize the solution and kept in the dark for 24 h before counting. The radioactivity of ^{45}Ca was counted using a Tri-Carb 2900 Liquid Scintillation Counter. Counting time was adapted to obtain a propagated counting error of less than 5% (maximal counting duration was 90 min). Radioactivity was determined by comparison with standards of known activities and measurements were corrected for counting efficiency and physical radioactive decay.

The analyses of seawater $\delta^{13}C$-C_T as well as of the ^{13}C signature of coral calcified tissues were performed at Leuven University. For $\delta^{13}C$-C_T analyses, a helium headspace (5 mL) was created in the vials and samples were acidified with 2 mL of phosphoric acid (H_3PO_4, 99%). Samples were left to equilibrate overnight to transfer all C_T to gaseous CO_2. Samples were injected in the carrier gas stream of an EA-IRMS (Thermo EA1110 and Delta V Advantage), and data were calibrated with NBS-19 and LSVEC standards (Gillikin and Bouillon, 2007). Corals were treated following the same protocol as for ^{45}Ca incorporation measurements and powdered. Triplicate subsamples of carbonate powder (~100 µg) were placed into gas-tight vials, flushed with helium, and converted into CO_2 with H_3PO_4. After 24 h, subsamples of the released CO_2 were injected into the EA-IRMS system as described above. Data were calibrated with NBS-19 and LSVEC. Carbon isotope data are expressed in

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

the delta notation (δ) relative to Vienna Pee Dee Belemnite (VPDB) standard and were calculated as:

$$R_{sample} = \frac{\delta^{13}C_{sample}}{1000 + 1} \cdot R_{VPDB} \quad (2)$$

2.3. Computations and statistics

The carbonate chemistry was assessed using pH_T and A_T and the R package seacarb[2]. Propagation of errors on computed parameters was performed using the new function "error" of the package seacarb (Orr et al., 2018) on the R software, considering errors associated to the estimation of A_T as well as errors on dissociation constants.

Estimates of coral calcification rates based on changes in A_T and $[Ca^{2+}]$ during incubations were computed following equations (3) and (4), respectively. As shown in these equations, initial levels of A_T and $[Ca^{2+}]$ are not necessary to compute calcification rates and only final values in the incubations with corals and without corals (controls) were used:

$$G_{AT} = -\frac{(A_{T2} - A_{T1}) - (A_{T2c} - A_{T1})}{2t} \cdot \frac{W_w}{W_c} = -\frac{(A_{T2} - A_{T2c})}{2t} \cdot \frac{W_w}{W_c} \quad (3)$$

$$G_{Ca} = -\frac{(Ca_2 - Ca_1) - (Ca_{2c} - Ca_1)}{t} \cdot \frac{W_w}{W_c} = -\frac{(Ca_2 - Ca_{2c})}{t} \cdot \frac{W_w}{W_c} \quad (4)$$

where A_{T1} and Ca_1 are A_T and Ca^{2+} concentrations at the start of the incubations (in µmol kg^{-1}), A_{T2}/A_{T2c} and Ca_2/Ca_{2c} are A_T and Ca^{2+} concentrations at the end of the incubations, respectively with and without corals, t is the incubation duration in h, W_w and W_c are respectively the mass of seawater (average between initial and final weights) and the coral skeleton dry weight (g; DW). G_{AT} and G_{Ca} are therefore expressed in µmol $CaCO_3$ g DW^{-1} h^{-1}. Error propagation was used to estimate errors:

[2]seacarb: seawater carbonate chemistry with R. Gattuso, J.-P., J. M. Epitalon, H. Lavigne, J. C. Orr, B. Gentili, M. Hagens, A. Hofmann, A. Proye, K. Soetaert and J. Rae, 2018. https://cran.r-project.org/package=seacarb

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

$$SE_{G_{AT}} = \frac{\sqrt{SE_{AT_2}^2 + SE_{AT_{2c}}^2}}{2t} \cdot \frac{W_w}{W_c} \quad (5)$$

$$SE_{G_{Ca}} = \frac{\sqrt{SE_{Ca_2}^2 + SE_{Ca_{2c}}^2}}{t} \cdot \frac{W_w}{W_c} \quad (6)$$

Coral calcification rates based on ^{45}Ca incorporation were estimated using measured seawater activity and activity recorded in the skeleton digest. Rates were then normalized per g skeleton dry weight using the formula:

$$G_{^{45}Ca} = \frac{Activity_{sample} \cdot \frac{Ca}{Activity_{seawater}}}{W_c \cdot t} \quad (7)$$

where $Activity_{sample}$ is the average of counts per minute (CPM) of three 200 µL aliquots from the dissolved skeleton sample, $Activity_{seawater}$ is the total CPM in the 1 mL seawater samples, Ca is the $[Ca^{2+}]$ measured in the corresponding samples (average between initial and final values, µmol kg^{-1}) and further converted to µmol L^{-1} considering a temperature of 25 °C and a salinity of 38, W_c is the skeleton dry weight (in g) and t the incubation duration (h). $G_{^{45}Ca}$ is therefore expressed in µmol $CaCO_3$ g DW^{-1} h^{-1}. The standard errors for these calcification rate estimates were propagated based on standard errors associated with the measurements of triplicate samples for both $Activity_{sample}$ and $[Ca^{2+}]$.

The precipitation of calcium carbonate minerals (G) during the incubation interval was also estimated using measured $\delta^{13}C$ values and isotope mass balance calculations [eq. (8) and (9) below]. The CO_2 released during phosphoric acid digestion is derived from two sources: new coral $CaCO_3$ and previously accreted skeletal carbonate mineral. The new carbon acquired in each measured nubbins ($\delta^{13}C_N$) was assumed to have the same carbon isotope composition as the labelled seawater C_T (average between initial and final level, $\delta^{13}C$-C_T ~

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

295 1,400-1,700‰). The previously accreted skeletal material was assumed to have a $\delta^{13}C$ value
296 equal to the measured value for the background sample ($\delta^{13}C_P$). The $\delta^{13}C$ value ($\delta^{13}C_M$),
297 representing the mixture of new calcified material and previously accreted carbonate mineral,
298 is then calculated the following mixing equation:

299 $$\delta^{13}C_M = f_G \cdot \delta^{13}C_N + (1 - f_G) \cdot \delta^{13}C_P \quad (8)$$

300 where f_G is the fraction of the calcium carbonate mineral precipitated during the experiment,
301 and $\delta^{13}C_N$ and $\delta^{13}C_P$ are the carbon isotope compositions of the newly precipitated and
302 previously accreted calcium carbonate, respectively. Equation (8) was solved for f_G to
303 determine the calcium carbonate precipitated during the incubation using:

304 $$G_{13_C} = \frac{f_G}{t \cdot M_{CaCO_3}} \cdot 1e^6 \quad (9)$$

305 where M_{CaCO3} is the molar mass of calcium carbonate (g mol^{-1}) and t is the incubation
306 duration in h. G_{13C} are therefore expressed in µmol $CaCO_3$ g DW^{-1} h^{-1}. The standard errors for
307 these calcification rate estimates were calculated based on standard errors associated with the
308 triplicate measurements of $\delta^{13}C_P$ and $\delta^{13}C_N$.
309 Model-II linear regressions (Sokal and Rohlf, 1995) were used to compare net
310 calcification rates obtained with the different methods. All regressions were performed using
311 function "lmodel2" of the package lmodel2[3] on the R software.

[3]lmodel2: Model II Regression, Legendre P. and J. Oksanen, 2018. https://cran.r-project.org/package=lmodel2

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

3. Results

Environmental conditions at the start of the different incubations are shown in Table 2. All incubations performed under ambient pH_T (~8.05) were conducted under carbonate chemistry favorable to calcification with saturation states with respect to aragonite (Ω_a) well above 1 (average of 4.0 ± 0.1 over the four incubations). In contrast, during experiments at low pH_T (initial pH_T ~ 7.2), seawater was corrosive with respect to aragonite (Ω_a ~ 0.75). However, as pH was not regulated during the incubations (see previous section), it increased, at lowered pH, to an average of 7.75 ± 0.03 (n = 5) in dark conditions and to an average of 7.84 ± 0.03 in light conditions (n = 5). Evolution of pH in control beakers (final pH_T of 7.78 and 7.48; n = 1 for both in the light and in the dark, respectively) showed that the observed increase in beakers with corals was due to the additive effects of biological control (photosynthesis minus respiration and calcification) and exchanges at the interface in the light, and mostly due to CO_2 exchange with air during the much longer incubations performed in the dark. Assuming linear variations with time, the average conditions of the carbonate chemistry in the lowered pH experiments were slightly favorable to aragonite production (Ω_a = 1.4 ± 0.2 in the dark, n = 5 and 1.6 ± 0.05 in the light, n = 5). Under ambient pH conditions (both for ^{45}Ca and ^{13}C incubations), pH did not change during incubations in the light (average final pH_T of 8.05 ± 0.03, n = 12, data not shown) while it decreased in the dark, due to respiration and calcification, to reach an average pH_T level of 7.62 ± 0.07, n = 12, data not shown). In control beakers under ambient pH, pH_T slightly increased in the light (8.09, n = 2) and did not change in the dark (8.05, n = 2).

^{45}Ca activities in seawater did not change during the incubations, reaching a final activity of 16.1 ± 1.2 (n = 12) and 28.5 ± 0.6 (n = 10) Bq mL^{-1} under ambient and lowered pH

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

conditions, respectively (including both dark and light incubations, data not shown). Furthermore, for all incubations, these values are similar to those measured in beakers without corals (control, data not shown). Under ambient pH levels (no incubation at lowered pH), seawater was enriched in ^{13}C ($\delta^{13}C$-C_T) from a background level of 0.26 ± 0.05‰ (n = 3) to 1,740 ± 4.7‰ (n = 2) and 1,634 ± 11‰ (n = 2) in the light and dark, respectively. During light condition incubations, $\delta^{13}C$-C_T levels decreased to an average of 1,636 ± 10‰ (n = 6, data not shown) while they decreased to an average of 1,466 ± 24‰ in dark conditions (n = 6, data not shown). Incubations in control beakers (without corals) showed that the majority of $\delta^{13}C$-C_T loss for both types of incubations (light and dark) was due to ^{13}C incorporation by corals with a minor effect of gas exchanges at the interface (data not shown).

Changes in A_T and $[Ca^{2+}]$ in beakers containing corals as compared to control beakers, during all sets of incubations, are shown in Table 3. Both variables declined in all incubations as a consequence of coral calcification. Changes in A_T during incubations in control beakers (data not shown) were comprised between 0.1 and 1.1% of the initial level. Similar results were observed for $[Ca^{2+}]$ with a relative change comprised between 0.05 and 1.15% of the initial value. These minimal changes were corroborated with no measurable changes in seawater weight between the start and the end of all incubations (data not shown), showing that evaporation, if any, was minimal using our experimental set-up over the considered incubation times. At ambient pH levels, decreases in A_T and $[Ca^{2+}]$ (average of -380 ± 97 and -194 ± 51 µmol kg^{-1} for both parameters, respectively, n = 24 including both ^{45}Ca and ^{13}C incubations) were roughly similar under light and dark conditions although coral specimen used for dark incubations were ca. 166% heavier (skeleton dry weight, see Table 1). Incubations performed under lowered pH levels showed much lower A_T and $[Ca^{2+}]$ net consumption rates than under ambient pH levels. Under these pH conditions, an extremely

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019


Biogeosciences Discussions Open Access EGU

359 high A_T consumption rate was observed in one beaker (dark incubation, see Table 3) while no
360 changes in [Ca^{2+}] was observed in a total of three beakers (see Table 3). These rates have
361 been considered as outliers and were not included in the following analyses.
362 ^{45}Ca activities in coral skeleton reached maximum levels under ambient pH and light
363 conditions (average of 87.5 ± 9.1 Bq, n = 6). Although seawater was more enriched in ^{45}Ca at
364 the lower pH levels (see above), ^{45}Ca activity in corals incubated under these conditions were
365 much lower with lowest values measured in the dark (average of 19.6 ± 9.1 Bq, n = 5). $\delta^{13}C$
366 levels measured in coral skeletons (-3.69 to 8.92‰) showed significant enrichment as
367 compared to background levels (-3.97 ± 0.35‰, n = 9).
368 Estimated rates of calcification using the different techniques are presented in Table
369 A1 and are compared in Figs. 2, 3 and 4 as well as in Table 4. Rates were higher in the light
370 than in the dark and much lower rates were estimated at lowered pH. The rates measured by
371 alkalinity anomaly (G_{AT}) and calcium anomaly (G_{Ca}) techniques were highly correlated (Fig.
372 2; $R^2 = 0.98$, $p < 0.01$, n = 34). No significant difference was observed between rates
373 measured by the two methods (see Table 4 for the 95% confidence intervals of the slope and
374 intercept). The ^{45}Ca method provided also very similar rates than the two previous approaches
375 (Fig. 3; G_{Ca} vs. G_{45Ca} not shown) although the slope and the intercept of the geometric
376 regression between G_{AT} and G_{45Ca} were significantly different from 1 and 0, respectively.
377 Finally, the only approach that did not provide similar rates to the others was the ^{13}C
378 incorporation technique. Calcification rates based on this method were systematically higher
379 than those measured using the other three techniques (see Table 4), and rates were not always
380 significantly related (e.g. $R^2 = 0.33$, $p > 0.05$, n = 12 for G_{AT} vs G_{13C}, see Fig. 4; other
381 relationships not shown).

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

4. Discussion

Under all experimental conditions (ambient pH vs low pH, light vs dark), significant consumption rates of A_T and Ca^{2+} as well as significant incorporation rates of ^{45}Ca and ^{13}C were observed in the zooxanthellate coral *Stylophora pistillata*. For all methods, calcification rates were lower in dark than in light conditions. Such trends are expected as it has long been established that calcification rates increase in zooxanthellate corals during periods in which photosynthesis is occurring (Yonge, 1931), a process known as light-enhanced calcification (e.g. Gattuso et al., 1999). Even under lowered pH conditions, at pH levels far below those predicted to occur in the next decades (starting pH_T of ca. 7.2, average pH_T during incubations of ca. 7.5), all corals appeared to produce calcifying structures under both light and dark conditions. The organisms selected for this experiment were fully coated with tissues with no exposed calcareous structures which can explain the absence of observable net dissolution such as reported by Cohen et al. (2017) in a similar study. Since our experimental protocol was not designed to address the potential impact of decreasing pH levels on calcification rates of this species (no control of carbonate chemistry during incubations, no acclimation of the organisms etc.), we will not discuss further the observed decrease of calcification rates identified by the three techniques used at these pH levels.

Under all experimental conditions, rates of calcification calculated using the alkalinity and the calcium anomaly techniques were highly correlated with a slope of 1 and no significant intercept. These results are consistent with previously published data on colonies of *Pocillopora damicornis* (Chisholm and Gattuso, 1991), *Cladocora caespitosa* (Gazeau et al., 2015) and several other coral species (Murillo et al. 2014). Although the precision obtained on Ca^{2+} measurements is among the highest reported to date (Gazeau et al., 2015),

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

405 the alkalinity anomaly technique appears as the most appropriate to estimate calcification
406 rates of isolated corals (better precision, stronger signals). As observed by Murillo et al.
407 (2014), this is not true when an entire community including sediment is investigated. The
408 occurrence of several processes in the sediment that can impact A_T prevents the use of this
409 technique. It is therefore recommended to use the calcium anomaly technique when working
410 in natural settings, assuming that Ca^{2+} concentrations are measured with an analytical
411 technique as precise as the one used in our study (CV < 0.05%). Similarly, although
412 corrections are possible when applying the alkalinity anomaly technique on organisms that
413 significantly release nutrients (echinoderms, bivalves etc.), the use of the calcium anomaly
414 technique is highly recommended instead (Gazeau et al., 2015).

415 Calcification rate estimates based on changes of A_T or Ca^{2+} were highly correlated
416 with estimates based on ^{45}Ca incorporation in corals. These results are not consistent to those
417 reported by Smith and Roth (in Smith and Kinsey, 1978), Tambutté et al. (1995) and Cohen et
418 al. (2017). These studies revealed discrepancies between the alkalinity anomaly and the ^{45}Ca
419 incorporation techniques. Smith and Roth found that rates measured with the ^{45}Ca method
420 were higher than those measured using the alkalinity anomaly technique (significant ^{45}Ca
421 incorporation at $\Delta A_T = 0$). Results from both Tambutté et al. (1995) and Cohen et al. (2017)
422 suggested the opposite with a decrease in A_T consumption without any concomitant ^{45}Ca
423 incorporation. A number of reasons may explain these discrepancies. First, the present study
424 is the first one comparing these techniques in the same incubations, in contrast to the other
425 ones in which incubations for A_T anomaly and ^{45}Ca incorporation were performed over two
426 consecutive days (due to radioactive contamination issues). Second, calcification expressed as
427 absolute changes in A_T during incubations, measured during our experiment, were at least one
428 order of magnitude higher than measured during these studies (44,200 to 745,600 nmol vs

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

429 less than 4,000 nmol in previous experiments). Cohen et al. (2017) have shown that such
430 discrepancies were much higher at very low rates and that the ratio between rates estimated
431 based on ^{45}Ca incorporation and A_T consumption were getting closer to 1 with increasing
432 calcification rates. Nevertheless, even at the highest levels of calcification computed during
433 these studies, ^{45}Ca-based rates were still significantly different from ΔA_T-based rates, which is
434 in contrast with our results.

435 As already mentioned, although calcification rates of the present study were lower at
436 lowered pH levels, there was still a close to perfect agreement between the different
437 techniques. While the ^{45}Ca labelling technique is thought to provide rates of gross
438 calcification, there is no doubt that both the A_T and Ca^{2+} anomaly techniques allow the
439 estimation of net calcification rates (gross calcification – dissolution). A full agreement of
440 rates computed from these methods further suggest that no dissolution of previously
441 precipitated $CaCO_3$ structures occurred during our study, even under lowered pH conditions.
442 The corals used in our experiment were fully covered with tissues which is likely the reason
443 that no dissolution was measured.

444 Furthermore, we must note that the protocol for ^{45}Ca incorporation considered in our
445 study differed from the one used in the above-mentioned past studies. A much smaller activity
446 was used (0.025 kBq mL^{-1}) compared to Tambutté et al. (1995; 40 kBq mL^{-1}) and Cohen et al.
447 (2017; 9 kBq mL^{-1}). Moreover, in contrast to Cohen et al. (2017), rates were not corrected for
448 ^{45}Ca incorporation on the skeleton of dead corals. This choice was motivated by the absence
449 of detectable radioactivity on bare skeletons exposed for 7 h and treated with the same
450 protocol than one used in our study (Lanctôt, pers. comm.).

451 To the best of our knowledge, this is the first study comparing calcification rates
452 measured using the ^{13}C labelling technique to the more widely used alkalinity and calcium

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

anomaly techniques. It shows that ^{13}C-derived rates were systematically higher and much more variable (with large uncertainties) than the ones estimated using the two other techniques. As already mentioned, several studies have shown that most of the carbon precipitated in the skeleton comes from coral and its symbiotic zooxanthellae (e.g. Erez, 1978; Furla et al., 2000), leading to an underestimation of calcification rates based on labelled, radioactive carbon incorporation. As there is no reason for ^{13}C to behave differently, our results appear inconsistent with a metabolic source of carbon. As the nubbins were treated following the same protocol as for ^{45}Ca incorporation measurements, it is unclear why much stronger ^{13}C incorporation were obtained and why variability is so high. Before better insights on such discrepancies can be developed, we recommend to avoid this technique to estimate coral calcification rates.

Although our study was designed to compare different techniques to estimate calcification rates and not to define the best experimental approach to study the effects of ocean acidification on coral species using these different approaches, our results provide some insights that we further discuss in the following section. Measuring and comparing calcification rates of organisms under varying pH conditions requires the careful choice of a volume and a time interval such that the precision of the calcification rate measurement is large enough to observe significant signals and that the change in carbonate chemistry parameters between the beginning and end of the incubation is small compared to the range of these parameters in the different treatments (Langdon et al. 2010).

Table 5 illustrates the incubation time necessary to obtain measurable changes considering the ratio between incubation volume and coral size chosen for our study. As the ^{13}C incorporation method did not provide reliable rates, this technique was not considered in this analysis. The threshold for significant signals was set at 10-fold the analytical precision

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

of the instruments (Langdon et al. 2010) for A_T and Ca^{2+} measurements (1.2 and 2.9 µmol kg^{-1}, respectively) and above the detection limit of 15 cpm for ^{45}Ca activity estimated. Maximum incubation times have been determined considering a maximum decrease of C_T by 10% (Langdon et al. 2010).

Under light and ambient pH conditions, even if the ratio between incubation volume and nubbin size is much higher than for previous similar studies (e.g. Cohen et al. 2017), all methods would allow a precise estimation of calcification rates over very short incubation times (~15 min to 1 h, depending on the method) while leading to moderate changes in C_T (< 10%). In the dark, and at ambient pH conditions, in the absence of C_T increase by photosynthesis, the increase of C_T due to respiration, of which only a minor portion is compensated by calcification, narrows the possible incubation period to 12 h. However, this is still much larger than the incubation time allowing to obtain a significant signal with the three methods (~20 min to ~2 h). At lower pH, both under light and dark conditions, and using open systems without a continuous pH regulation as in our study, it is obvious that the calcium anomaly technique is not well adapted to this experimental protocol. Indeed, as a consequence of lower calcification rates at lower pH and important CO_2 degassing, incubation times necessary to obtain significant signals using this technique are too large to maintain the carbonate parameters within an acceptable range (ΔC_T < 10%). This is not insurmountable as a continuous regulation of pH using for instance pure CO_2 bubbling or incubations performed in a closed container (i.e. without contact to the atmosphere) would alleviate these problems. Nevertheless, if such experimental protocols cannot be followed, our results show that the alkalinity anomaly and ^{45}Ca incorporation techniques are still sensitive enough, at lowered pH, to estimate reliable calcification rates in zooxanthellate corals maintained in open-

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

500 systems without continuous pH regulation, while maintaining acceptable changes in the
501 carbonate chemistry.

502 In conclusion, the present study is the first one allowing a direct (i.e. during the same
503 incubations) comparison of three methods used to estimate coral calcification rates, the
504 calcium and alkalinity anomaly techniques and the ^{45}Ca incorporation technique. These
505 methods provided very consistent calcification rates of the coral *Stylophora pistillata*
506 independently of the conditions set for the incubations (light vs dark, ambient vs low pH).
507 Among these three methods, the alkalinity anomaly and the ^{45}Ca incorporation techniques
508 appear to be the most sensitive allowing the quantification of coral calcification rates without
509 significant changes in targeted environmental conditions. In contrast, the ^{13}C incorporation
510 technique did not provide reliable calcification rates and its use is not recommended until
511 further investigations clarify the discrepancies. Finally, this study was restricted to a single
512 coral species and used nubbins fully covered with tissues. Conducting similar comparison
513 studies with other coral species as well as other major calcifying groups widely studied in the
514 context of ocean acidification (e.g. coralline algae, molluscs etc…) would be necessary.

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

Acknowledgements

This work was supported by the IAEA's Ocean Acidification International Coordination Center (OA-ICC) and the IAEA-ICTP Sandwich Training Educational Programme (STEP) and Project "Strengthening the National System for Analysis of the Risks and Vulnerability of Cuban Coastal Zone Through the Application of Nuclear and Isotopic Techniques" National Program PNUOLU /4-1/ 2 No. /2017 of the National Nuclear Agency (AENTA). We thank the Monaco government, the Centre Scientifique de Monaco for propagating and maintaining the coral nubbins and Samir Alliouane for technical assistance for total alkalinity and calcium measurements.

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

REFERENCES

Albright, R., Caldeira, L., Hosfelt, J., Kwiatkowski, L., Maclaren, J. K., Mason, B. M., Nebuchina, Y., Ninokawa, A., Pongratz, J., Ricke, K. L., Rivlin, T., Schneider, K., Sesboüé, M., Shamberger, K., Silverman, J., Wolfe, K., Zhu, K., and Caldeira, K.: Reversal of ocean acidification enhances net coral reef calcification, Nature, 531, 362, 10.1038/nature17155, 2016.

Albright, R., Takeshita, Y., Koweek, D. A., Ninokawa, A., Wolfe, K., Rivlin, T., Nebuchina, Y., Young, J., and Caldeira, K.: Carbon dioxide addition to coral reef waters suppresses net community calcification, Nature, 555, 516, 10.1038/nature25968, 2018.

Cao, Z., and Dai, M.: Shallow-depth $CaCO_3$ dissolution: Evidence from excess calcium in the South China Sea and its export to the Pacific Ocean, Global Biogeochemical Cycles, 25, doi:10.1029/2009GB003690, 2011.

Chisholm, J. R. M., and Gattuso, J.-P.: Validation of the alkalinity anomaly technique for investigating calcification and photosynthesis in coral reef communities, Limnology and Oceanography, 36, 1232-1239, 1991.

Cohen, S., Krueger, T., and Fine, M.: Measuring coral calcification under ocean acidification: methodological considerations for the ^{45}Ca-uptake and total alkalinity anomaly technique, PeerJ, 5, e3749, 10.7717/peerj.3749, 2017.

Cyronak, T., Andersson, A. J., Langdon, C., Albright, R., Bates, N. R., Caldeira, K., Carlton, R., Corredor, J. E., Dunbar, R. B., Enochs, I., Erez, J., Eyre, B. D., Gattuso, J.-P., Gledhill, D., Kayanne, H., Kline, D. I., Koweek, D. A., Lantz, C., Lazar, B., Manzello, D., McMahon, A., Meléndez, M., Page, H. N., Santos, I. R., Schulz, K. G., Shaw, E., Silverman, J., Suzuki, A., Teneva, L., Watanabe, A., and Yamamoto, S.: Taking the

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

metabolic pulse of the world's coral reefs, PLOS ONE, 13, e0190872, 10.1371/journal.pone.0190872, 2018.

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

Erez, J.: Vital effect on stable-isotope composition seen in foraminifera and coral skeletons, Nature, 273, 199-202, 10.1038/273199a0, 1978.

Furla, P., Galgani, I., Durand, I., and Allemand, D.: Sources and mechanisms of inorganic carbon transport for coral calcification and photosynthesis, Journal of Experimental Biology, 203, 3445-3457, 2000.

Gattuso, J.-P., and Hansson, L.: Ocean acidification: background and history, in: Ocean acidification, edited by: Gattuso, J.-P., and Hansson, L., Oxford University Press, Oxford, 1-20, 2011.

Gazeau, F., Urbini, L., Cox, T. E., Alliouane, S., and Gattuso, J. P.: Comparison of the alkalinity and calcium anomaly techniques to estimate rates of net calcification, Marine Ecology Progress Series, 527, 1-12, 2015.

Gillikin, D. P., and Bouillon, S.: Determination of $\delta^{18}O$ of water and $\delta^{13}C$ of dissolved inorganic carbon using a simple modification of an elemental analyser-isotope ratio mass spectrometer: an evaluation, Rapid Communications in Mass Spectrometry, 21, 1475-1478, doi:10.1002/rcm.2968, 2007.

Goreau, T. F.: The physiology of skeleton formation in corals. I. A method for measuring the rate of calcium deposition by corals under different conditions, Biological Bulletin, 116, 59-75, 1959.

Goreau, T. F., and Bowen, V. T.: Calcium uptake by a coral, Science, 122, 1188-1189, 10.1126/science.122.3181.1188, 1955.

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

571 Houlbrèque, F., Reynaud, S., Godinot, C., Oberhänsli, F., Rodolfo-Metalpa, R., and Ferrier-
572 Pagès, C.: Ocean acidification reduces feeding rates in the scleractinian coral
573 *Stylophora pistillata*, Limnology and Oceanography, 60, 89-99, doi:10.1002/lno.10003,
574 2015.
575 IPCC: Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III
576 to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change,
577 edited by: Team, C. W., Pachauri, R. K., and Meyer, L. A., IPCC, Geneva, Switzerland,
578 151 pp., 2014.
579 Jokiel, P. L., Maragos, J. E., and Franzisket, L.: Coral growth: buoyant weight technique, in:
580 Coral reefs: research methods, edited by: Stoddart, D. R., and Johannes, R. E., Paris:
581 Unesco, 379-396, 1978.
582 Langdon, C., Gattuso, J.-P., and Andersson, A. J.: Measurements of calcification and
583 dissolution of benthic organisms and communities, in: Guide to Best Practices for
584 Ocean Acidification Research and Data Reporting, 213-234, 2010.
585 Le Quéré, C., Andrew, R. M., Friedlingstein, P., Sitch, S., Hauck, J., Pongratz, J., Pickers, P.
586 A., Korsbakken, J. I., Peters, G. P., Canadell, J. G., Arneth, A., Arora, V. K., Barbero,
587 L., Bastos, A., Bopp, L., Chevallier, F., Chini, L. P., Ciais, P., Doney, S. C., Gkritzalis,
588 T., Goll, D. S., Harris, I., Haverd, V., Hoffman, F. M., Hoppema, M., Houghton, R. A.,
589 Hurtt, G., Ilyina, T., Jain, A. K., Johannessen, T., Jones, C. D., Kato, E., Keeling, R. F.,
590 Goldewijk, K. K., Landschützer, P., Lefèvre, N., Lienert, S., Liu, Z., Lombardozzi, D.,
591 Metzl, N., Munro, D. R., Nabel, J. E. M. S., Nakaoka, S. I., Neill, C., Olsen, A., Ono,
592 T., Patra, P., Peregon, A., Peters, W., Peylin, P., Pfeil, B., Pierrot, D., Poulter, B.,
593 Rehder, G., Resplandy, L., Robertson, E., Rocher, M., Rödenbeck, C., Schuster, U.,
594 Schwinger, J., Séférian, R., Skjelvan, I., Steinhoff, T., Sutton, A., Tans, P. P., Tian, H.,

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

595 Tilbrook, B., Tubiello, F. N., van der Laan-Luijkx, I. T., van der Werf, G. R., Viovy, N.,
596 Walker, A. P., Wiltshire, A. J., Wright, R., Zaehle, S., and Zheng, B.: Global Carbon
597 Budget 2018, Earth Syst. Sci. Data, 10, 2141-2194, 10.5194/essd-10-2141-2018, 2018.
598 Lebel, J., and Poisson, A.: Potentiometric determination of calcium and magnesium in
599 seawater, Marine Chemistry, 4, 321–332, doi:10.1016/0304- 4203(76)90018-9, 1976.
600 Marshall, A. T., and Wright, A.: Coral calcification: autoradiography of a scleractinian coral
601 *Galaxea fascicularis* after incubation in ^{45}Ca and ^{14}C, Coral Reefs, 17, 37-47,
602 10.1007/s003380050092, 1998.
603 McCoy, S. J., Pfister, C. A., Olack, G., and Colman, A. S.: Diurnal and tidal patterns of
604 carbon uptake and calcification in geniculate inter-tidal coralline algae, Marine
605 Ecology, 37, 553-564, doi:10.1111/maec.12295, 2016.
606 Murillo, L. J. A., Jokiel, P. L., and Atkinson, M. J.: Alkalinity to calcium flux ratios for corals
607 and coral reef communities: variances between isolated and community conditions,
608 PeerJ, 2, e249, 10.7717/peerj.249, 2014.
609 Orr, J. C., Epitalon, J.-M., Dickson, A. G., and Gattuso, J.-P.: Routine uncertainty
610 propagation for the marine carbon dioxide system, Marine Chemistry, 207, 84-107,
611 https://doi.org/10.1016/j.marchem.2018.10.006, 2018.
612 Schoepf, V., Hu, X., Holcomb, M., Cai, W.-J., Li, Q., Wang, Y., Xu, H., Warner, M. E.,
613 Melman, T. F., Hoadley, K. D., Pettay, D. T., Matsui, Y., Baumann, J. H., and Grottoli,
614 A. G.: Coral calcification under environmental change: a direct comparison of the
615 alkalinity anomaly and buoyant weight techniques, Coral Reefs, 36, 13-25,
616 10.1007/s00338-016-1507-z, 2017.
617 Smith, S. V., and Key, G. S.: Carbon dioxide and metabolism in marine environments,
618 Limnology and Oceanography, 20, 493-495, 1975.

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

619 Smith, S. V., and Kinsey, D. W.: Calcification and organic carbon metabolism as indicated by
620 carbon dioxide, in: Coral reefs: Research methods, edited by: Stoddart, D. R., and
621 Johannes, R. E., Monogr. Oceanogr. Methodol. 5. UNESCO, 1978.
622 Sokal, R. R., and Rohlf, F. J.: Biometry, the principles and practice of statistics in biological
623 research, 3rd ed. W. H. Freeman, New York, 1995.
624 Tambutté, E., Allemand, D., Bourge, I., Gattuso, J.-P., and Jaubert, J.: An improved Ca^{45}
625 protocol for investigating physiological mechanisms in coral calcification, Marine
626 Biology, 122, 453-459, 10.1007/bf00350879, 1995.
627 Wolf-Gladrow, D. A., Zeebe, R. E., Klaas, C., Körtzinger, A., and Dickson, A. G.: Total
628 alkalinity: the explicit conservative expression and its application to biogeochemical
629 processes, Marine Chemistry, 106, 287-300, doi:10.1016/j.marchem.2007.01.006,
630 2007.

631 Table 1. Experimental details for the series of incubations of the coral *Stylophora pistillata* performed under ambient and low pH, and in
632 the light and dark following ^{45}Ca or ^{13}C labelling. The ratio W_w:W_c corresponds to the ratio between seawater weight (g) and skeletal dry
633 weight (g). Values represent mean ± standard deviation (SD); n is the number of true replicates considered for each experiment.

pH conditions	Ambient (n = 6)				Lowered (n = 5)	
Added label	^{45}Ca		^{13}C		^{45}Ca	
Light conditions	Light	Dark	Light	Dark	Light	Dark
Coral size (mm)	33.2 ± 1.5	44.7 ± 1.5	36.3 ± 2.2	50.2 ± 1.7	26.0 ± 1.6	28.9 ± 1.9
Coral Skeleton dry weight (g)	2.5 ± 0.5	3.8 ± 0.7	2.6 ± 0.5	4.7 ± 0.5	2.1 ± 0.2	2.8 ± 0.4
Ratio W_w:W_c	126.4 ± 25.6	81.9 ± 14.7	106.9 ± 24.5	67.8 ± 7.5	146.5 ± 14.3	110.0 ± 12.4
Incubation time (h)	8	8	9.12	9.12	5	11

Table 2. Environmental conditions at the start of incubations of the coral *Stylophora pistillata*. pH on the total scale (pH_T), partial pressure of CO_2 (pCO_2 in µatm), total alkalinity (A_T in µmol kg^{-1}), dissolved inorganic carbon (C_T in µmol kg^{-1}), saturation states with respect to aragonite (Ω_a) and calcite (Ω_c) as well as calcium concentrations ($[Ca^{2+}]$ in µmol kg^{-1}) are presented. Labelled seawater ^{45}Ca activity ($Activity_{seawater}$ in Bq mL^{-1}) and the isotopic level, after enrichment, of the seawater dissolved inorganic carbon pool ($\delta^{13}C$-C_T in ‰) are also shown. Means ± standard deviation of analytical triplicates (duplicates for $\delta^{13}C$-C_T) are shown when available.

pH conditions	Ambient				Lowered	
Added label	^{45}Ca		^{13}C		^{45}Ca	
Light conditions	Light	Dark	Light	Dark	Light	Dark
pH_T	8.05	8.05	8.06	8.05	7.21	7.24
pCO_2	427.6 ± 8.2	438.8 ± 8.5	425.6 ± 8.2	424.1 ± 8.2	3,727.2 ± 66.8	3,460.1 ± 62.1
A_T	2,556.0 ± 0.5	2,620.0 ± 0.7	2,615.2 ± 0.6	2,535.9 ± 1.8	2,558.4 ± 0.3	2,552.9 ± 2.4
C_T	2,206.4 ± 7.4	2,264.1 ± 7.6	2,252.9 ± 7.7	2,188.2 ± 7.6	2,597.1 ± 2.5	2,579.8 ± 3.5
Ω_a	3.9 ± 0.2	4.0 ± 0.2	4.1 ± 0.2	3.9 ± 0.2	0.7 ± 0.0	0.8 ± 0.0
Ω_c	5.9 ± 0.3	6.1 ± 0.3	6.2 ± 0.3	5.9 ± 0.3	1.1 ± 0.1	1.2 ± 0.1
$[Ca^{2+}]$	11,179.6 ± 0.0	11,164.0 ± 2.0	11,096.5 ± 13.4	11,098.5 ± 2.8	11,281.2 ± 5.5	11,277.6 ± 0.3
$Activity_{seawater}$	16.6	15.1	-	-	28.5	30.4
$\delta^{13}C$-C_T	-	-	1,740 ± 4.7	1,634 ± 11	-	-

Table 3. Changes in total alkalinity (A_T) and calcium concentrations ([Ca^{2+}]) during the different types of incubations as compared to control beakers: $\Delta A_T = A_{T2} - A_{T2c}$, $\Delta[Ca^{2+}] = Ca_2 - Ca_{2c}$, both expressed in µmol kg^{-1}. Standard errors (SE) have been calculated as $\sqrt{SE_{AT_2}^2 + SE_{AT_{2c}}^2}$ and $\sqrt{SE_{Ca_2}^2 + SE_{Ca_{2c}}^2}$ for A_T and [Ca^{2+}], respectively, where SE correspond to standard errors associated with the measurement of three analytical replicates per sample. ^{45}Ca activity ($Activity_{sample}$ in Bq) and ^{13}C incorporation ($\delta^{13}C_M$ in ‰) of sampled corals are also shown. Values of ^{45}Ca activity and $\delta^{13}C$ are mean ± standard error of the mean (SE) associated with the measurement of three aliquots for each coral.

Experiment	Beaker#	ΔA_T	SE ΔA_T	$\Delta[Ca^{2+}]$	SE $\Delta[Ca^{2+}]$	$Activity_{sample}$	$SEActivity_{sample}$	$\delta^{13}C_M$	SE $\delta^{13}C_M$
Ambient pH - ^{45}Ca - Light	1	-343.6	1.3	-166.0	6.0	78.5	1.9	-	-
	2	-368.9	0.9	-174.1	5.1	86.5	2.9	-	-
	3	-336.9	0.9	-181.3	2.7	78.2	2.3	-	-
	4	-364.3	0.9	-190.6	6.3	85.2	0.8	-	-
	5	-406.7	0.7	-225.6	1.4	95.7	2.6	-	-
	6	-407.5	1.2	-175.9	1.1	100.6	3.5	-	-
Ambient pH - ^{13}C - Light	1	-386.3	1.5	-195.0	3.8	-	-	-1.4	2.0
	2	-422.6	1.3	-206.8	4.2	-	-	1.8	3.2

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

	3	-405.4	1.9	-200.9	2.1	-	-	3.4	5.1
	4	-481.6	1.3	-253.2	2.0	-	-	1.1	2.0
	5	-498.4	1.3	-260.5	5.7	-	-	0.8	0.7
	6	-618.1	1.8	-317.7	4.4	-	-	0.1	1.8
Ambient pH - ^{13}C - Dark	1	-300.5	1.4	-168.9	0.6	-	-	-0.3	1.3
	2	-440.8	1.4	-220.7	2.5	-	-	-3.0	0.5
	3	-223.5	1.9	-135.1	0.8	-	-	-3.1	0.6
	4	-347.3	1.1	-185.3	0.2	-	-	0.5	5.4
	5	-571.7	1.3	-301.7	1.2	-	-	0.6	2.1
	6	-434.5	1.3	-224.6	3.7	-	-	0.7	6.1
Ambient pH - ^{45}Ca - Dark	1	-290.2	1.6	-157.9	2.2	56.44	1.24	-	-
	2	-274.3	1.2	-130.4	4.4	50.1	0.74	-	-
	3	-300.8	1.3	-168.3	0.9	57.17	1.75	-	-
	4	-327.0	2.7	-139.3	5.3	66.24	0.69	-	-
	5	-342.8	1.2	-172.6	3.0	68.37	3.11	-	-
	6	-228.3	1.8	-113.4	2.5	52.36	2.49	-	-

Biogeosciences Discussions
Open Access
EGU

Lowered pH - ^{45}Ca - Light	1	-59.3	2.2	-1.6*	6.9	20.2	0.7	-	-
	2	-44.2	2.2	-11.0	2.2	15.3	0.4	-	-
	3	-71.3	2.8	-28.0	5.9	22.5	0.3	-	-
	4	-70.2	2.4	-35.7	7.6	23.4	0.4	-	-
	5	-56.4	2.5	-19.6	7.1	20	0.9	-	-
Lowered pH - ^{45}Ca - Dark	1	-745.6*	13.2	0.8*	0.3	14.5	0.2	-	-
	2	-52.4	2.1	-1.0*	1.0	22.1	0.3	-	-
	3	-50.5	2.1	-22.5	2.8	22.1	0.1	-	-
	4	-54.3	2.1	-30.3	8.5	23.3	0.4	-	-
	5	-99.4	2.1	-32.8	4.1	16.1	0.1	-	-

646

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

Table 4. Model-II regression results of the comparison between calcification rates estimated using the different methods considered in this study: the alkalinity and calcium anomaly techniques (G_{AT} and G_{Ca}, respectively) as well as the ^{45}Ca and ^{13}C incorporation techniques (G_{45Ca} and G_{13C}, respectively). The number of samples (n), the regression coefficient (R^2), as well as the slope and intercept (including their 95% confidence intervals, 95% CI) are shown for each comparison. Few identified outliers (n = 4) have been removed from the analyses, see Table 3 and Table A1.

Methods compared	n	R^2	Slope			Intercept		
			Value	95% CI		Value	95% CI	
				Low	High		Low	High
G_{AT} *vs.* G_{Ca}	32	0.98	0.95	0.90	1.00	0.09	0.00	0.18
G_{AT} *vs.* G_{45Ca}	21	0.99	0.94	0.90	0.98	0.09	0.03	0.15
G_{Ca} *vs.* G_{45Ca}	20	0.97	1.00	0.91	1.09	-0.06	-0.20	0.07
G_{AT} *vs.* G_{13C}	12	0.33	0.49	0.05	1.2	0.77	-1.2	2.1
G_{Ca} *vs.* G_{13C}	12	0.32	0.46	0.03	1.1	0.94	-0.9	2.2

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

Table 5. Incubation times (t_{min}; h) necessary to obtain significant signals using the three methods: the alkalinity anomaly technique (A_T), the calcium anomaly technique (Ca^{2+}) and the ^{45}Ca incorporation techniques (^{45}Ca), see text for calculation procedures. t_{max} (h) is the maximum incubation time to maintain carbonate chemistry within an acceptable range (ΔC_T < 10%). The ratios between incubation volume (in mL) and the size of the nubbins (in cm), considered in our study for the different sets of incubations (Ambient pH vs Low pH; Light vs Dark), are also shown. t_{min} values are noted in bold when higher than t_{max}.

	Ratio V:S	t_{min} (h)			t_{max} (h)
		A_T	Ca^{2+}	^{45}Ca	
Ambient pH – Light	77-95	0.26	1.00	0.6	49.7
Ambient pH – Dark	59-69	0.33	2.10	1.5	12.1
Lowered pH – Light	109-121	1.25	**6.15**	1.1	2.8
Lowered pH – Dark	95-109	1.60	**11.20**	3.4	4.4

663 Table A1. Calcification rates estimated by the different methods considered in this study: the alkalinity and calcium anomaly techniques
664 (G_{AT} and G_{Ca}, respectively) as well as the ^{45}Ca and ^{13}C incorporation techniques (G_{45Ca} and G_{13C}, respectively). All rates are mean ±
665 standard errors of the mean (SE) and are expressed in µmol $CaCO_3$ g DW^{-1} h^{-1}.

Experiment	Beaker#	G_{AT}	SE G_{AT}	G_{Ca}	SE G_{Ca}	G_{45Ca}	SE G_{45Ca}	G_{13C}	SE G_{13C}
Ambient pH - ^{45}Ca - Light	1	3.28	0.01	3.17	0.11	3.41	0.08	NA	NA
	2	3.21	0.01	3.03	0.09	3.29	0.11	NA	NA
	3	2.69	0.01	2.89	0.04	2.77	0.08	NA	NA
	4	3.38	0.01	3.54	0.12	3.48	0.03	NA	NA
	5	2.41	0.00	2.68	0.02	2.53	0.07	NA	NA
	6	2.43	0.01	2.10	0.01	2.65	0.09	NA	NA
Ambient pH - ^{13}C - Light	1	3.26	0.01	3.29	0.06	NA	NA	1.92	1.35

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

2	3.30	0.01	3.23	0.07	NA	NA	4.27	2.27		
3	3.09	0.01	3.06	0.03	NA	NA	5.47	3.66		
4	2.98	0.01	3.14	0.02	NA	NA	3.74	1.36		
5	2.80	0.01	2.92	0.06	NA	NA	3.49	0.41		
6	2.73	0.01	2.81	0.04	NA	NA	3.00	1.22		
								0.79		
Ambient pH - ^{13}C - Dark	1	1.33	0.01	1.50	0.01	NA	NA	2.58		
	2	1.63	0.01	1.63	0.02	NA	NA	0.68	0.23	
	3	0.85	0.01	1.03	0.01	NA	NA	0.61	0.30	
	4	1.24	0.00	1.32	0.00	NA	NA	3.14	3.67	
	5	1.96	0.00	2.07	0.01	NA	NA	3.21	1.35	
	6	1.42	0.00	1.46	0.02	NA	NA	3.28	4.16	

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

Ambient pH - ^{45}Ca - Dark	1	1.59	0.01	1.72	0.02	1.54	0.03	NA	NA		NA
	2	1.39	0.01	1.32	0.04	1.26	0.02	NA	NA		NA
	3	1.46	0.01	1.64	0.01	1.43	0.04	NA	NA		NA
	4	1.29	0.01	1.10	0.04	1.33	0.01	NA	NA		NA
	5	1.44	0.01	1.45	0.03	1.44	0.07	NA	NA		NA
	6	0.75	0.01	0.75	0.02	0.89	0.04	NA	NA		NA
Lowered pH - ^{45}Ca - Light	1	1.00	0.04	0.05*	0.23	0.85	0.03	NA	NA		NA
	2	0.66	0.03	0.33	0.07	0.58	0.02	NA	NA		NA
	3	0.96	0.04	0.75	0.16	0.80	0.01	NA	NA		NA
	4	1.04	0.04	1.06	0.23	0.94	0.02	NA	NA		NA
	5	0.75	0.03	0.52	0.19	0.73	0.03	NA	NA		NA

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

Lowered pH - ^{45}Ca - Dark	1	4.05*	0.07	-0.01*	0.00	0.20	0.00	NA	NA
	2	0.22	0.01	0.01*	0.01	0.24	0.00	NA	NA
	3	0.25	0.01	0.22	0.03	0.30	0.00	NA	NA
	4	0.30	0.01	0.34	0.10	0.35	0.01	NA	NA
	5	0.48	0.01	0.32	0.04	0.21	0.00	NA	NA

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019

Figure captions

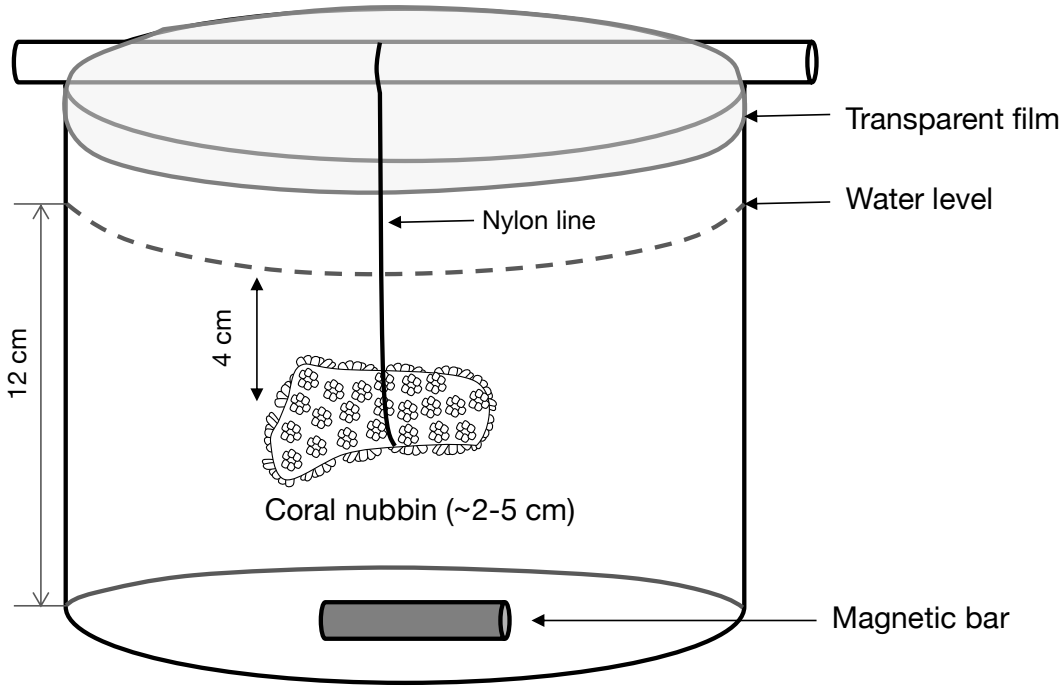
Fig. 1. Scheme of the polyethylene container in which a coral nubbin is suspended with a nylon line and covered with a transparent film.

Fig. 2. Calcification rates estimated based on the alkalinity anomaly technique (G_{AT}) as a function of calcification rates estimated based on the calcium anomaly technique (G_{Ca}). The dashed line represents the 1:1 relationship while the full line represents the model-II regression relationship. Horizontal error bars represent standard errors (SE) associated with the estimation of G_{Ca}. Vertical error bars representing SE associated with the estimation of G_{AT} are too small to be visible. The corresponding dataset can be found in Table A1.

Fig. 3. Calcification rates estimated based on the alkalinity anomaly technique (G_{AT}) as a function of calcification rates estimated based on the ^{45}Ca incorporation technique (G_{45Ca}). The dashed line represents the 1:1 relationship while the full line represents the model-II regression relationship. Horizontal error bars represent standard errors (SE) associated with the estimation of G_{45Ca}. Vertical error bars representing SE associated with the estimation of G_{AT} are too small to be visible. The corresponding dataset can be found in Table A1.

Fig. 4. Calcification rates estimated based on the alkalinity anomaly technique (G_{AT}) as a function of calcification rates estimated based on ^{13}C incorporation technique (G_{13C}). The dashed line represents the 1:1 relationship while the full line represents the model-II regression relationship. Horizontal error bars represent standard errors (SE) associated with the estimation of G_{13C}. Vertical error bars representing SE associated with the estimation of G_{AT} are too small to be visible. The corresponding dataset can be found in Table A1.

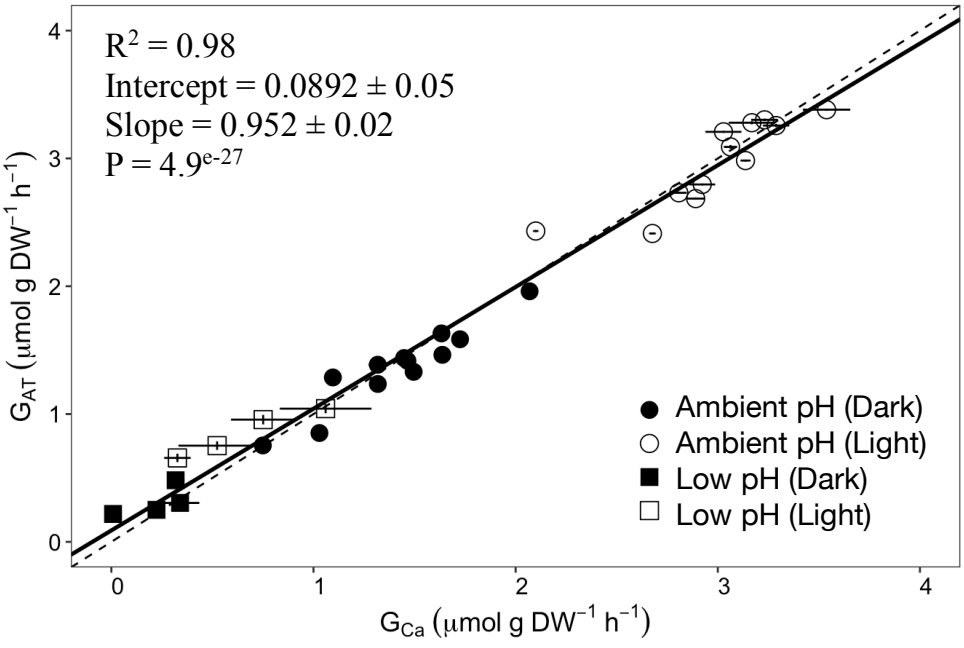
https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019



688

689 Fig. 1.

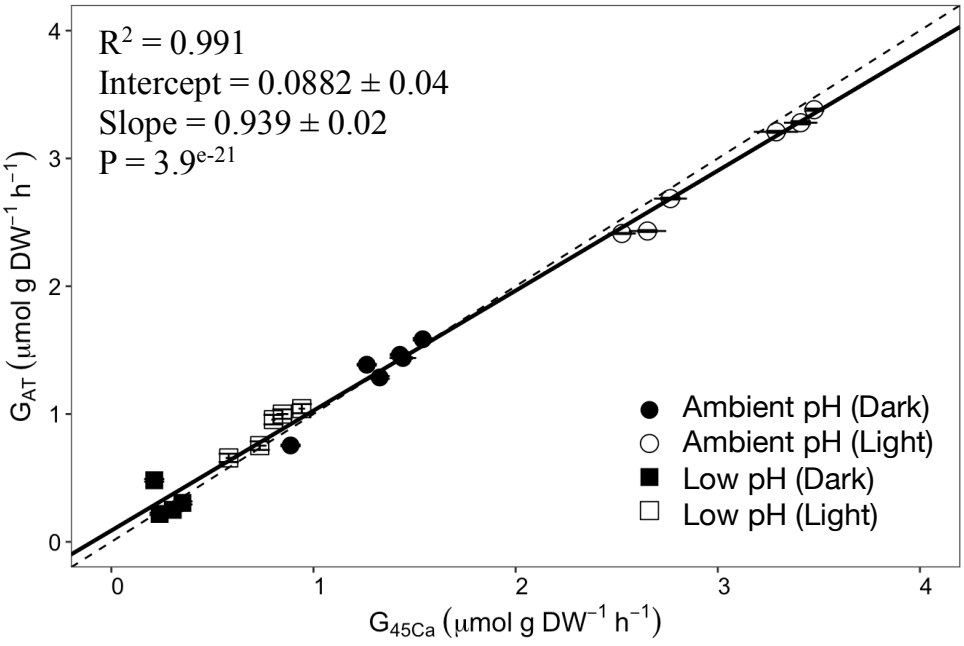
https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019



690

691 Fig. 2.

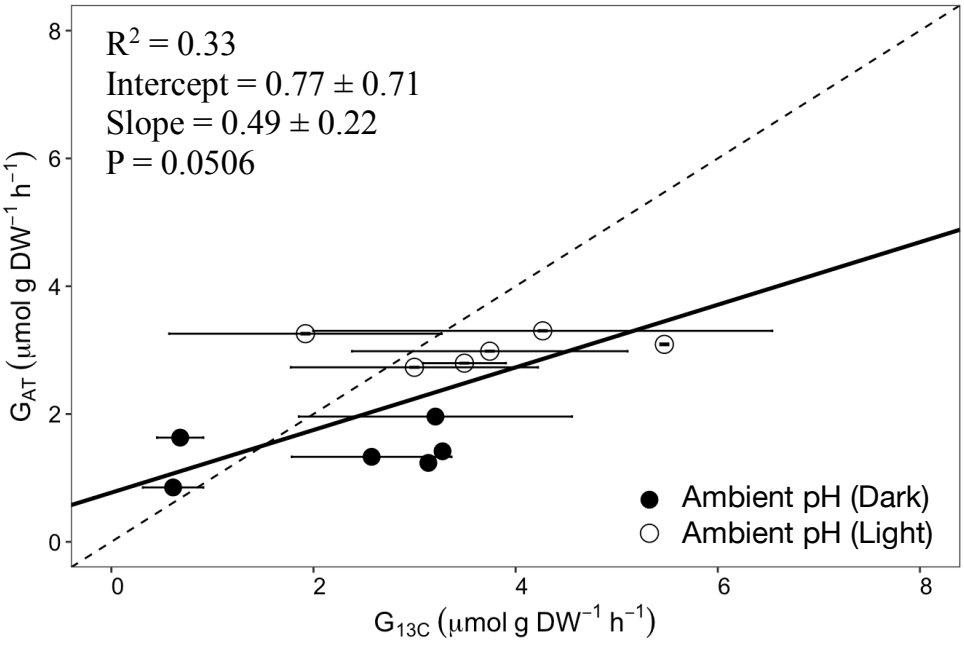
https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019



692

693 Fig. 3.

https://doi.org/10.5194/bg-2019-369
Preprint. Discussion started: 13 September 2019



694

695 Fig. 4.