RESEARCH ARTICLE

Evaluation of a semi-automatic system for long-term seawater carbonate chemistry manipulation

Evaluación de un sistema semiautomático para la manipulación del sistema carbonato en agua de mar durante periodos extensos

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ABSTRACT

The assessment of the effects of Ocean Acidification (OA) on marine life has received increasing attention in recent marine research. On a mesocosmic scale, the CO_2 levels in seawater can be manipulated to evaluate experimentally the consequences of OA on marine organisms (vertebrates and invertebrates). An ideal manipulation of carbonate chemistry should mimic exactly the changes to carbonate chemistry, which will occur in years to come. Although some methods have been described in the literature, here we describe in detail a simple, robust and inexpensive system to produce CO_2-enriched seawater by bubbling the seawater with air-CO_2 mixtures. The system uses mass flow controllers (MFC) to blend atmospheric air with pure CO_2 to produce two pCO_2 levels. The air-CO_2 mixtures are delivered continuously to seawater equilibration reservoirs, and simultaneously to an infrared CO_2 analyser to verify CO_2 levels in the air-CO_2 mixture delivered to the equilibration tanks. We monitored both pH and total alkalinity in the equilibration reservoirs over a period of one year in order to document the long-term performance of this system for simulating the future carbonate chemistry of seawater in a coastal laboratory. System performance was sufficient to maintain three contrasting (e.g., 397, 709 and 1032 matm) and relatively constant (the coefficient of variability was 11 %, 9 % and 9 % respectively) seawater pCO_2 during a year-long monitoring.

Key words: carbonate chemistry manipulation, ocean acidification.

RESUMEN

La evaluación de los efectos de la acidificación oceánica en la vida marina ha recibido creciente atención en la actual investigación marina. A escala de "mesocosmos" los niveles de CO_2 del agua de mar pueden ser manipulados para permitir la experimentación con organismos marinos (vertebrados e invertebrados). Una manipulación óptima de la química del carbono debiese emular exactamente los cambios esperado en la química del carbono. A pesar que algunos métodos han sido descritos en la literatura, el presente estudio describe en detalle un sistema simple, robusto y de bajo costo para acondicionar agua de mar enriquecida en CO_2 a través del burbujeo del agua de mar con mezclas de aire y CO_2. El sistema mezcla aire con CO_2 puro para producir dos niveles de pCO_2 utilizando para ello controladores de flujo másico. Las mezclas aire-CO_2 son enviadas a tambores con agua de mar y simultáneamente a un analizador de CO_2 para verificar los niveles de CO_2 en las mezclas aire-CO_2 burbujeadas en los recipientes. Monitoreamos el pH y la alcalinidad por más de un año con el objeto de documentar el desempeño de largo plazo de este sistema emulando la futura química del carbono en agua de mar en un laboratorio costero. Concluimos que el desempeño del sistema es suficiente para mantener niveles pCO_2 contrastantes (e.g., 397, 709, 1032 µatm) y relativamente constantes (el coeficiente de variación fue de 11 %, 9 % y 9 %, respectivamente) durante aproximadamente un año de monitoreo.

Key words: acidificación del océano, manipulación de la química de los carbonatos.

INTRODUCTION

Carbon dioxide (CO_2) released due to human activities has caused a rise in the concentration of atmospheric CO_2, and an increase in dissolved inorganic carbon content of oceanic surface waters. CO_2 reacts with seawater reducing its pH (a phenomenon named Ocean Acidification (OA)) and carbonate ion concentration (Caldeira & Wickett 2003; Orr et al. 2005; Raven et al. 2005). The carbonate ion reduction results in a decrease in the Aragonite (Ar) and Calcite (Ca) saturation state of seawater (Orr et al. 2005). Therefore, it is predicted that the primary effect of OA will be the undersaturation of seawater in Ar in a vast part of the world's oceans, in particular high-latitude regions which have naturally low carbonate ion concentrations (Fabry et al. 2009; Orr et al. 2005).

The evidence that OA is occurring is now irrefutable (Orr et al. 2005). OA allows to fundamentally alter marine ecosystems in time frames that are essentially irreversible from the perspective of human societies (Raven et al. 2005). Understanding the impacts of OA in ocean ecosystems will likely be a major subject in marine science in the coming decades. In this context, developing CO_2 systems that allows emulating the future conditions of an ocean with high CO_2 levels is essential. An ideal manipulation of carbonate chemistry (i.e. CO_2 system) should mimic exactly the changes to carbonate chemistry that will occur in years to come. During the last decade, experimental simulations of future marine carbonate chemistry have been conducted mainly in North America and Europe, to study the effect of OA on marine organisms (Fangue et al. 2010; Gattuso et al. 2010). A growing body of experimental evidence on OA effects is mainly derived from short-term experiments of typically less than 100 days duration (Kroeker et al. 2013), which normally corresponds to a small proportion of the life span of the respective model organisms. There is therefore a need to develop and test experimental facilities that can allow longer periods of experimentation (i.e. from several months to years) covering one or several life cycles of marine invertebrates, in order to know more about the physiological acclimation and the adaptation capacity of species to OA.

Seawater chemistry can be manipulated in various ways in order to alter the carbonate system (Gattuso et al. 2010). Probably the most common carbonate system manipulation techniques are "aeration techniques" bubbling CO_2-air mixtures or pure CO_2 to reach a target pH or pCO_2 level. Two major subgroups within these aeration techniques are (1) the "pH-stat" and (2) the continuous bubbling with pre-mixed gases (Gattuso et al. 2010). The first is based on a feedback system through continuous monitoring of seawater pH, and the intermittent injection of pure CO_2 or CO_2-free air to keep pH into a target range. The second is based on the continuous bubbling with an air-CO_2 mixture of a defined pCO_2.

These two aeration techniques share a common weakness: they do not compensate for changes in A_T resulting from precipitation and dissolution of $CaCO_3$ or those changes resulting from evaporation. Furthermore the "pH-stat" is limited by inherent variability associated to the feedback system, while bubbling with pre-mixed gases produces a less variable control on seawater pCO_2. An accurate comparison between systems are beyond the scope of this paper, however there is clearly a wide variability in the setting and the performance of these systems, necessitating a detailed description of both methods and carbonate chemistry (Gattuso et al. 2010).

The carbonate chemistry experimental setup described here corresponds with the "aeration with pre-mixed gases" group. This system was implemented at Calfuco Marine Laboratory (http://www.ciencias.uach.cl/unidades/calfuco/index.php), located in southern Chile (40° S) near the city of Valdivia and under the influence of the Valdivia River. The coastal water supplied to the aquaria may therefore experience variations in salinity and other parameters associated with freshwater inputs, in addition to marine variations such as coastal upwelling and tidal cycles.

In this paper we will describe the first laboratory facility to study the consequences of the OA on marine invertebrates in Chile and South America. Moreover we discuss the long-term performance of this system (operative since late 2010) and others considerations in maintaining incubations at different CO_2 levels for extended time periods (i.e. robustness and operational cost of the system).

METHODS

The pCO₂ generation system

This system manipulates carbon chemistry by changing Total Carbon (C_T) at constant Total Alkalinity (A_T), using the "Aeration to target pCO_2" technique (Gattuso et al.

2010). The pCO_2 regulation system described here (Fig. 1) has been adapted to generate air-CO_2 mixtures of 750 ppm and 1200 ppm in dry air. Those nominal levels correspond approximately with projected atmospheric levels for years 2079 and 2127 under RPC 8.5 scenario (Meinshausen et al. 2011; Riahi et al. 2007; Vuuren et al. 2011). Current (control) levels of CO_2 (approx. 387-391
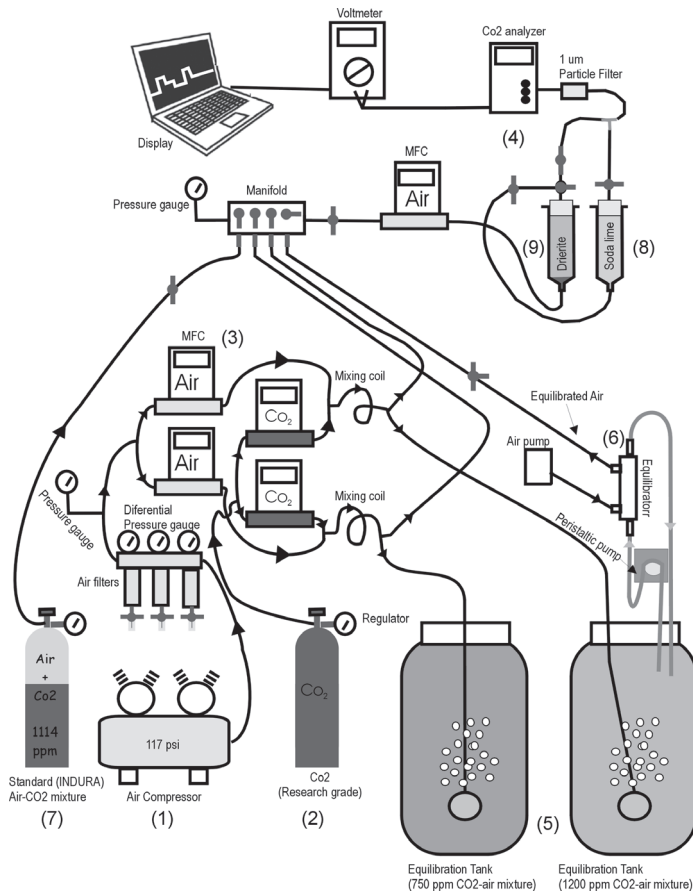

Fig. 1: Main characteristics of the Calfuco Marine Laboratory seawater-CO_2 equilibration system. Compressed air (1) is blended with pure CO_2 (2) using MFCs (3). The CO_2-air mixture is split into two branches, one going to the CO_2 analyser (4) to allow fine regulation of the MFCs, and the other to the seawater in the equilibration tanks (5). The pCO_2 of the seawater in the mixing reservoirs can be monitored using a seawater-air equilibrator (6). The equilibrated air is pumped to a CO_2 analyser, with analogue output. The voltage signal is displayed graphically to show the evolution of seawater equilibration with CO_2. The CO_2 analyser is calibrated with a standard CO_2-air mixture (7) and with CO_2-free air (CO_2 removed with soda lime, 8). The gas entering the CO_2 analyser is dried using a desiccant (9) and filtered (removing particles larger than 1 µm). The air-CO_2 mixture is injected into the 250 l equilibration tanks or directly into the experimental treatments. Samples for discrete analysis of pH and A_T are collected periodically from the equilibration tanks to verify the functioning of the system.

Características principales del sistema de equilibración de agua marina con CO_2 del laboratorio Marino de Calfuco. El Aire comprimido (1) se mezcla con CO_2 puro (2) usando Controladores de Flujo Másico CFM (3). La mezcla CO_2-aire se divide en dos ramas, una dirigida al analizador de CO_2 (4) para permitir la regulación fina de los CFM, y la otra rama es dirigida al agua de mar contenida en los tambores de mezcla (5). La pCO_2 del agua de mar en los tambores de mezcla puede ser monitoreada usando un equilibrador agua de mar-aire (6). El aire equilibrado es bombeado al analizador de CO_2, el que cuenta con una salida analógica. La señal de voltaje se muestra gráficamente para mostrar la evolución de la equilibración con CO_2. El analizador de CO_2 es calibrado con una mezcla aire-CO_2 estándar (7) y con aire libre de CO_2 (donde el CO_2 del aire fue removido con soda carbonatada, 8). El gas que ingresa al analizador de CO_2 es secado usando un desecante (9) y filtrado (removiendo la partículas mayores de una micra). La mezcla aire- CO_2 es inyectada en tambores de equilibración o directamente a los tratamientos de los experimentos. Periódicamente se recolectan muestras desde el tanque de equilibración para el análisis de pH y alcalinidad total de manera de verificar el funcionamiento del sistema.

ppm for the 2011 and early 2012 period at the Southern Hemisphere; http://www.csiro.au) were obtained by the equilibration of seawater with atmospheric air. For the 750 ppm and 1200 ppm treatments, we blended dry air with pure CO_2 to each target concentration using an air MFC (Aalborg, model GFC; http://www.aalborg.com) and a CO_2 MFC (Aalborg, model GFC). In total we used 4 MFC valves mounted on a board for horizontal gas flow. Dry and filtered air is generated by compressing atmospheric air (117 psi) using an oil-free, 4-piston air compressor (Schulz, model MSV12); the dry air passes through a particle filter rack (MTA; http://www.mta-it.com) to retain particles larger than 1µm before entering the air MFCs. Pressure in the air line is maintained at approximately 10 psi using a standard regulator before entering the MFC valves. Similarly, pure CO_2 (Research grade, INDURA; http://www.indura.cl) was regulated at 10 psi using a single stage CO_2 regulator. The air flow in the air MFCs was set at 5 litre min^{-1} for both treatments, and the CO_2 flows were set initially at 4.06 ml min^{-1} and 1.56 ml min^{-1}, to theoretically (assuming that the pCO_2 of the air used in the blend was already 388ppm of CO_2) produce a pCO_2 of 1200 ppm and 750 ppm, respectively. Finally we manually adjusted the CO_2 MFC flow until the required target pCO_2 of the air-CO_2 mixture was reached. The pCO_2 of the air-CO_2 mixture was continuously monitored using the system described below.

The gas blend pCO_2 monitoring system

The pCO_2 monitoring system was based on a CO_2 analyser (Qubit system, model S151), primarily for measuring the CO_2 content in the air-CO_2 mixture. The CO_2 analyser voltage output was displayed in a PC using a multimeter interface (RadioShack, model 22-812). Based on this monitoring methodology, we manually adjusted the CO_2 MFCs to achieve the target pCO_2, and we verified that the pCO_2 remained constant. All air samples passing through the CO_2 analyser were previously dried using a Drierite desiccant column (W. H. Hammond Drierite Co.; http://www.secure.drierite.com) and filtered (Millipore 1 µm filter) (Fig. 1). The CO_2 analyser was calibrated with air-CO_2 mixture standards of 1,114 ppm manufactured by INDURA (http://www.indura.cl), while zero was obtained by passing the air through a soda lime CO_2-removal column (Fig. 1). The flow rate of the CO_2-air mixture (samples and standard) entering the CO_2 analyser was kept at approximately 150 ml min^{-1} using an Air-MFC (Aalborg).

The seawater mixing reservoirs

Once the experimental treatment gases are produced, each gas is mixed with 1 µm filtered seawater (FSW hereafter) in a gas-mixing reservoir bucket. Each reservoir consists of a 250 litre food grade bucket (polyethylene HADAD plastics) into which the air-CO_2 mixture is injected though a large aquarium grade air diffuser. The air-CO_2 mixtures are injected at the bottom of the buckets at ca. 6-7 psi, using plastic tubing and an air-stone bubbler.

Since the air -CO_2 mix is water undersaturated due to condensation in the air compression tank, evaporation of seawater causes an increment in salinity and total alkalinity. The magnitude of this increment is variable, and depends on the intensity of the bubbling relative to the volume of the seawater to be equilibrated, as well as other factors (e.g., temperature). We expect that maximum A_T increments occur when a large dry air-CO_2 mixture equilibrates with a small volume of water. In order to assess the magnitude of this effect, we measured the A_T in one liter of filtered seawater before and after 24 hours of constant air-CO_2 bubbling. In these experimental trials the total alkalinity concentration rose 8-12 mmol l^{-1} d^{-1} (an increment of ca. 0.4 % of the initial A_T). Although this increment in alkalinity does not affect the final pCO_2 of the equilibrated water, it does affect other aspects of carbon chemistry (e.g., changes in Omega, usually < 1 %). It is calculated that Omega will increase by 6% for each unit of salinity increment, and therefore we used two strategies to prevent or reduce the effect of increment in salinity and alkalinity: 1) frequent changing of the equilibrated water (e.g., total or partial daily replacement of the equilibrated water at 250 L container); 2) bubbling the mixture into the water to allow the air-CO_2 mixture reach water saturation level, before injecting the air-CO_2 mixture into the experimental reservoirs.

Monitoring of pCO_2, pH and A_T in equilibration reservoirs.

The reservoir tanks were cleaned at regular intervals (approximately every 7 days) and the seawater was replaced with fresh FSW. Twelve hours after initiating the bubbling of the new seawater, we collected samples from the equilibration tanks and from the intertidal zone where the inlet of the seawater supply system is located, for analysis of the pH and A_T. The equilibrated seawater pCO_2 may also be monitored by pumping the seawater to a gas exchange column or equilibrator (Mini-Module Membrane Contactor; http://www.liqui-cel.com) and measuring the pCO_2 of the equilibrated air. This last feature is particularly useful for determining the equilibration period after renewal of the seawater, i.e. when the pCO_2-time relationship reaches a plateau (normally in the first 12 h). However this latter procedure does not play any role in the functioning or calibration of the air-CO_2 mixture generation system.

pH samples were collected in 50 mL syringes and immediately transferred to a close 25 mL cell, thermostatically controlled at 25.0 °C. The pH was measured at 25.0 °C with a Metrohm 713 pH meter (input resistance > 10^{13} Ohm, 0.1 mV sensitivity and nominal resolution 0.001 pH units) and a glass combined double junction Ag/AgCl electrode (Metrohm model 6.0219.100) calibrated with 8.089 Tris buffer (DOE 1994) at 25.0 °C; pH values are reported on the total hydrogen ion scale (DOE 1994).

Seawater samples for A_T were poisoned with 50µL of saturated $HgCl_2$ solution and stored in 250 ml polypropylene bottles in darkness at room temperature until analysis. A_T was determined by potentiometric titration in an open cell with 0.05M HCl (Merck Titrisol®) (Haraldsson et al. 1997). The accuracy was controlled against a certified reference material (CRM, supplied by Andrew Dickson, Scripps Institution of Oceanography, San Diego, USA).The correction factor was approximately 1.002, corresponding to a difference of about 5µmol kg^{-1}. Every sample was analysed with 2 or 3 replicates.

Omega Ar (Ω_{Ar}) and Omega Ca (Ω_{Ca}) were estimated from the pH-A_T pairs, in addition to temperature, salinity and pressure were obtained with a small CTD (Hydronaut). Carbonate system parameters calculations were performed using CO2SYS software (Lewis &Wallace 1998) available on http://cdiac.ornl.gov/ftp/co2sys/. Seawater pCO_2, Omega Aragonite and Omega Calcite were calculated using Mehrbach solubility constants (Mehrbach et al. 1973) refitted by Dickson and Millero (Dickson & Millero 1987). The calculations were performed on a total hydrogen ion scale (pH$_T$). For KSO_4 we used the constant determined by Dickson (1990).

Uses of CO₂ equilibrated seawater

Depending upon the experimental setting, the equilibrated seawater in the equilibration reservoirs was used in two distinct ways. In one type of experiments a special tubing system was used to allow seawater recirculation between the equilibration reservoirs and the rearing containers, the total volume of equilibrated water circulated through the rearing containers was approximately 10000 times the volume of the organisms. In other type of experiments the equilibrated seawater was used to fill the rearing containers and during the experimental rearing each container was connected with tubing to directly inject the required air-CO_2 mixture. In this last case the volume of rearing containers was typically 500-1000 times the volume of the organism.

RESULTS

Evaluation of the system to manipulate carbonate chemistry in the equilibration reservoirs.

Performance

Aeration to achieve a target CO_2 will change the amount and speciation of dissolved inorganic carbon in equilibration tanks, in turn modifying pH (Fig. 2A, B), pCO_2 (Fig. 2E) and Ω_{Ar} (Fig. 2F). The pCO_2 of the CO_2-enriched water
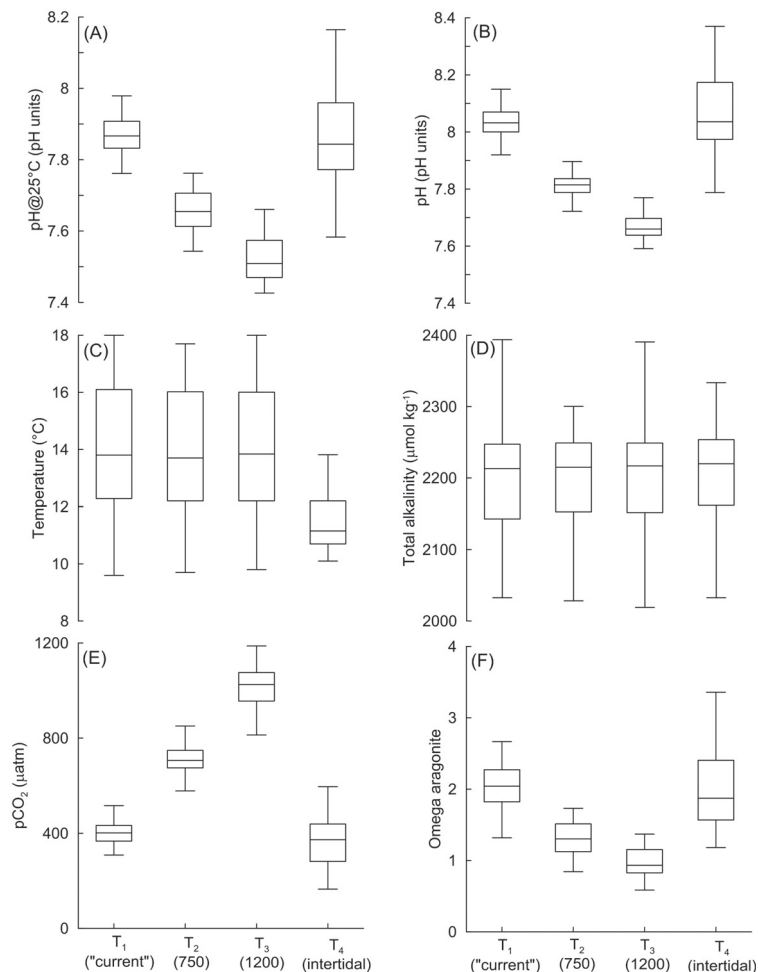

Fig. 2: Statistical parameters: pH measured at 25 °C, pH calculated at in situ temperature, temperature, total alkalinity, partial pressure of CO_2 and Omega Aragonite, in the equilibration reservoirs and intertidal (T_1 for atmospheric treatment, T_2 for 750 ppm treatment, T_3 for 1200 ppm treatment, T_4 for the intertidal site where the aquarium inlet is located) during 2011 and early 2012. The box plot indicates the smallest observation (sample minimum), lower quartile (Q1), median (Q2), upper quartile (Q3), and the largest observation (sample maximum).

Parámetros estadísticos: pH medido a 25°C, pH calculado a la temperatura in situ, temperatura, alcalinidad total, presión parcial de CO_2 y Omega aragonita, en los tanques de equilibración y en el intermareal (T1 es el tratamiento atmosférico, T2 es el tratamiento 750 ppm, T3 es el tratamiento de 1200 ppm, T4 es el intermareal donde se ubica la toma de agua del acuario) durante 2011 y comienzos del 2012. Los gráficos de caja indican la observación menor (el mínimo valor), primer cuartil (Q1), segundo cuartil ó mediana (Q2), tercer cuartil (Q3), y la mayor observación (el máximo valor).

(inferred from pH and At measurements) was relatively low compared to the pCO_2 of the air-CO_2 mixtures, particularly for the highest CO_2/air blend (1200 µatm) which results in a mean seawater pCO_2 of ca. 1032 µatm, i.e. 14 % lower (see Fig. 2E).

The temperature and A_T remained constant between treatments (Fig. 2C, D) but there was a conspicuous variation over the course of the year (Fig. 3B,C), caused by environmental factors such as variable influence of rivers, upwelling, annual temperature cycle, etc. The seawater A_T at both the intertidal (inlet) and the equilibration reservoirs was positively correlated with salinity (Fig. 4). However events of low salinity water at the intertidal of Calfuco (e.g., < 30) were rare (Fig. 4).
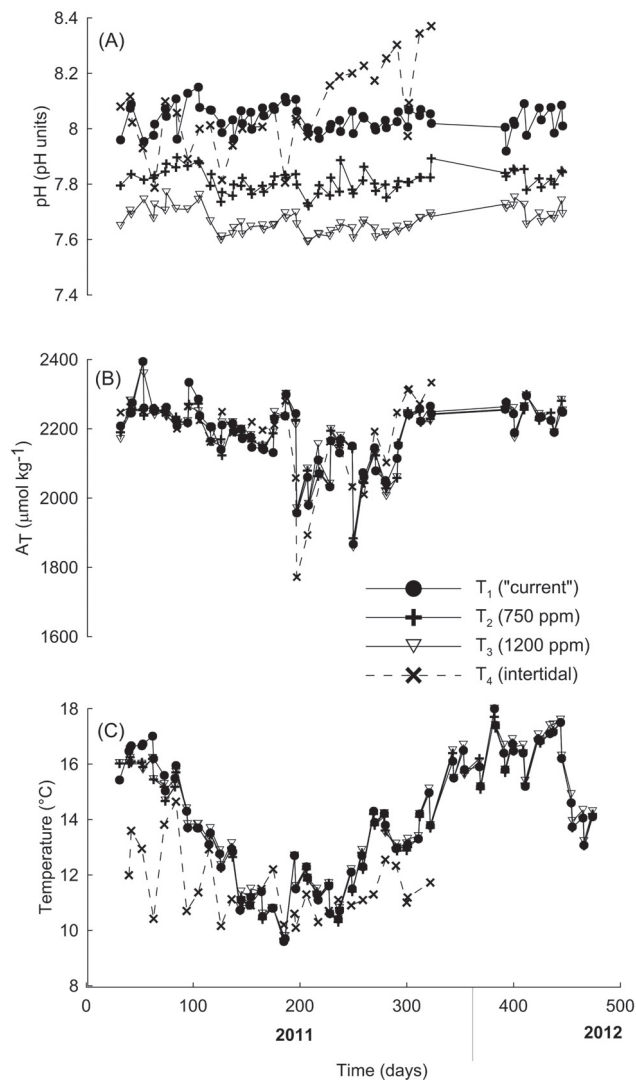

Fig. 3: Time series pH calculated at in situ temperature (i.e. the temperature in the equilibration tanks at the moment of sampling); A_T and temperature in the equilibration reservoirs and intertidal (T_1 for Atmospheric treatment, T_2 for 750 ppm treatment, T_3 for 1200 ppm treatment, T_4 for the intertidal site where the aquarium inlet is located) during 2011 and early 2012.

Serie de tiempo de pH calculado a la temperatura in situ (i.e. la temperatura en los tanques de equilibración al momento del muestreo); A_T y temperatura en los tanques de mezcla y en el intermareal (T1 es el tratamiento atmosférico, T2 es el tratamiento 750 ppm, T3 es el tratamiento de 1200 ppm, T4 es el intermareal donde se ubica la toma de agua del acuario) durante el 2011 y el comienzo del 2012.
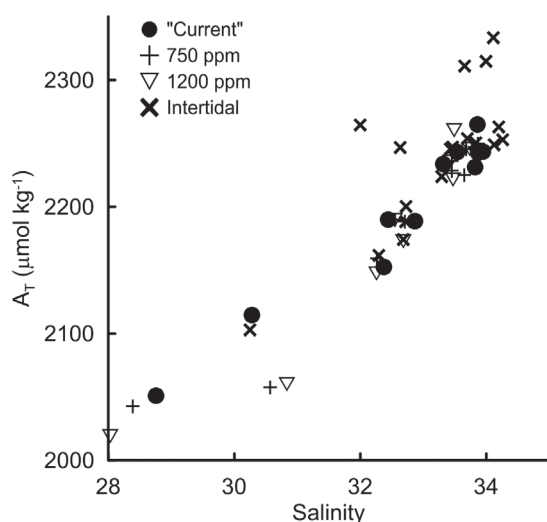
Fig. 4: A_T-Salinity relationship in the equilibration reservoirs and the intertidal (T_1 for Atmospheric treatment, T_2 for 750ppm treatment, T_3 for 1200ppm treatment, T_4 for the intertidal site where the aquarium inlet is located) during 2011 and early 2012.

Relación entre A_T y salinidad en los tambores de equilibración y en el intermareal (T1 es el tratamiento atmosférico, T2 es el tratamiento 750 ppm, T3 es el tratamiento de 1200 ppm, T4 es el intermareal donde se ubica la toma de agua del acuario) durante el 2011 y el comienzo del 2012.

Environmental variations in temperature, A_T and salinity explain most of the variability in pH and Aragonite (Ω_{Ar}) in the CO_2 enriched treatments. For example, based on the ranges of variation in temperature, salinity and A_T measured in the atmospheric treatment seawater (i.e. 8.4 °C, 9 and 526 µmol kg^{-1} respectively), we calculated that the maximum fluctuation in pH and Ω_{Ar} at constant pCO_2 (i.e. at ~380 µatm), should be ~0.13 pH units and ~1.7 respectively.

The lower variability of pH in the equilibration reservoirs when compared to the intertidal site (Fig. 2A) demonstrates the capacity of the system to manipulate carbonate parameters, even when critical variables (such as temperature) were not kept constant.

Robustness

The system requires little daily attention with the exception of: (1) draining the compressor reservoir (see Fig. 1) to remove condensed water, and (2) measuring pH and A_T in the equilibration reservoirs. The CO_2 mole fraction remained virtually constant for several weeks or even months, and so little adjustment of the MFCs was required. After more than a year of continuous functioning of this system we have not detected any malfunctions. Even during power failures the bubbling in the equilibration tanks restarted automatically when power was restored.

Operational cost

The operational costs of the experimental system are low (less than US$ 2000 per year), consisting mainly in: (1) ultra pure CO_2 (one CO_2 cylinder per year), (2) air-CO_2 standards (one every 1 or 2 years, depending on the frequency of IR calibration), and (3) maintenance or replacement of compressor pistons (probably after 1-2 years). The most significant cost is related to accurate monitoring of pH and A_T, including reagents, buffers, electrodes and reference seawater material (ca. US$ 5000 per year).

DISSCUSION

The "aeration to target pCO_2" system described here was sufficient to maintain different pH, pCO_2 and $CaCO_3$ saturation states in the treatments year round. However the air-CO_2 mixtures (pCO_2 of 750 µatm and 1200 µatm) injected into the equilibration tanks produce CO_2-enriched seawater with a lower pCO_2 (6 % and 14 % lower, respectively; see Fig. 2E). We estimate that the dilution effect of water moisture on seawater pCO_2 can explain 1-2% of the pCO_2 reduction from the originally dry air-CO_2 mixture. Seawater fully equilibrated with a dry air-CO_2 mixture with a pCO_2 = 1200 µatm should produce a maximum seawater pCO_2 of 1176 µatm at 18 °C. The rest of this discrepancy can be attributed to the incapacity of this system to fully equilibrate seawater at high pCO_2 levels in a period of 12 h. Seawater pCO_2 increment slows down as seawater pCO_2 approaches to the target pCO_2 levels, in asymptotic fashion, requiring longer equilibrations periods that the one used here (12 hours). However, in spite of the discrepancies discussed above, this system had the capacity to produce significantly different CO_2 levels in seawater (Fig. 2E) with

a relatively low variability (CV= 9 %). This overall variability includes the effect of the natural variability in salinity, alkalinity and temperature, as well as the bias in the pH and A_T measurements, and uncertainties in the pCO_2 calculation. The error of A_T analysis was constrained using reference material. The pH error was calculated to be lower than 0.006-0.009 pH units (Torres et al. 1999) however in the few occasions when salinity dropped below 30, additional bias, associated with larger differences in the liquid junction potential between the buffer and the sample, are expected to become significant (Wedborg et al. 2007). Tests on the same sample using TRIS buffer, at salinity 35 and 25, show minor discrepancies (< 0.005 pH units); we expect that even in this particular example (salinity range between 28-30) the pH error does not exceed 0.01 pH units. If we consider that maximum uncertainties of pH and alkalinity are in the order 0.01 pH units and ca. 9 in alkalinity, we expect a total error of ca.10 µatm for our pCO_2 estimations (Torres et al. 1999), which in turn corresponds with a small percentage of the pCO_2 of the CO_2-enriched treatments (ca. 1 %). Thus most of the overall variability reported here arises because of changes in temperature, salinity and alkalinity over time. Despite less than full control over these parameters, the system was sufficient for long term experimentation at contrasting pCO_2 (Fig. 2E), pH (Fig. 2A) and Omega Ar (Fig. 2F) levels.

The natural variability of pH and pCO_2 along the coast of Chile (Torres et al. 2011; Torres et al. 1999) is extreme when compared to other geographic areas (e.g., Tropical waters (Astor et al. 2005)), hence the fluctuation in carbonate system parameters in the different treatments shown here is not unusual for marine life in this region. Moreover a fully constant chemostat (for pH or Ω_{Ar}) might be considered unrealistic for simulating high-CO_2 scenarios to which coastal organisms in Eastern Boundary Current systems may be exposed. Therefore we conclude that the performance of the system described here is adequate to simulate high-CO_2 scenarios for Chilean coastal waters under laboratory conditions.

The robustness of this system makes it suitable for short and long-term experiments (months to years), necessary to adequately investigate the consequences of OA on marine invertebrates. Using the system described here we have been able to rear egg-capsules of *Concholepas concholepas* (Bruguière, 1789) during almost their entire developing period until hatching (i.e., 30-60 days of rearing), and small juveniles of the same species originating from competent larvae collected in the field (i.e. 1 to 2 years of rearing, (Manríquez et al. 2013)). Other species such as the mitilid *Mytilus chilensis* (Hupé, 1854) and the intertidal snail *Acanthina monodon* (Pallas, 1774) have also been reared in this system (Navarro et al. 2013). These experiments carried out so far, have shown significant effects of OA on the studied species, both positive and negative (e.g., Navarro et al. 2013). This highlights that the system is well suited for long-term experiments investigating the consequences of OA on the performance of early ontogenetic stages of marine invertebrate species. Finally, it is important to highlight that the relatively low-cost maintenance (ca. US$ 20 per day) makes this system economically feasible for a wide range of marine laboratory facilities.

LITERATURE CITED

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