

VARYING CONDITIONS IN INTERTIDAL POOLS: HIGH RESOLUTION pH DYNAMICS AND PRIMARY PRODUCTION

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ABSTRACT

Most studies designed to assess the effects of ocean acidification take place in coastal and intertidal environments, which are characterized by a great variability of its physical and chemical parameters. However, a great number of these studies use fixed pH levels predicted for the future, disregarding natural pH oscillations. In this work we studied the pH oscillations and primary productivity of intertidal rockpools in two rocky shore areas. To provide high resolution continuous pH data we used an autonomous pH measuring system which consisted of a pH sensor, a data logger and a battery encased in a waterproof container. Oxygen concentration and primary production from phytoplankton and macro-phytobentos were also measured. We found a range of pH variation in the pools of 0.07 pH units/day when water dynamics was high and of 0.26 pH units/day when conditions were more stable. Carbonate systems parameters, temperature and oxygen concentration were related and they responded to the day / night cycle and hydrodynamic conditions. We suggest that these natural oscillations in pH and temperature must be taken into account in ocean acidifications studies in order to obtain more accurate results.

KEYWORDS: pH variability, intertidal pools, macroalgae, phytoplankton.

CONDICIONES VARIABLES EN CHARCOS INTERMAREALES: DINÁMICAS DE
ALTA RESOLUCIÓN DEL pH Y PRODUCTIVIDAD PRIMARIA

RESUMEN

La mayoría de los estudios diseñados para evaluar los efectos de la acidificación oceánica son llevados a cabo en ambientes costeros e intermareales, que se caracterizan por una gran variabilidad de sus parámetros físicos y químicos. Sin embargo, una gran cantidad de estos estudios utiliza niveles fijos de pH previstos para el futuro, sin tener en cuenta las variaciones naturales de pH. En este trabajo estudiamos la variación del pH y la producción primaria en una serie de charcos intermareales de dos zonas costeras rocosas. Utilizamos un sistema autónomo de medida para obtener un registro continuo de pH. También se midieron la concentración de O₂ y la producción primaria del fitoplancton y del macrofitobentos. Encontramos una amplia variación diaria del pH en los charcos intermareales, de 0.26 unidades de pH / día en condiciones de baja hidrodinámica marina y de 0.07 unidades / día en condiciones de alta hidrodinámica. Los parámetros del sistema del carbonato, temperatura y concentración de O₂ se mostraron dependientes del ciclo día/noche y de las condiciones hidrodinámicas. Proponemos tener en cuenta estas oscilaciones de pH y temperatura en el diseño de los estudios sobre la acidificación del medio marino para obtener resultados más precisos y realistas.

PALABRAS CLAVE: variabilidad del pH, charcos intermareales, macroalgas, fitoplancton.

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1. INTRODUCTION

Since the beginning of the Industrial Revolution, with the increase in use of steam power with carbon as a main source of energy for human activities, followed by petroleum and natural gas, anthropogenic CO₂ emissions related to the use of fossil fuels have caused an increase of atmospheric CO₂ concentration from 280 ppmv (parts per million volume) (Le Querè *et al.* 2009) to 404 ppmv (NOAA-ESRL, 2016). This CO₂ concentration is likely to exceed 1000 ppmv by the year 2100 (Meehl *et al.* 2007; Fabry *et al.* 2008) if anthropogenic CO₂ emissions are not significantly reduced. Based on this increase of CO₂ and other greenhouse gasses, a rise in air temperature of 2.6 to 4.8°C (IPCC 2014) and of 1°C to 7°C in surface sea water temperature (Houghton *et al.* 2001) has been predicted for the year 2100. The increased concentration of atmospheric CO₂ has caused a shift in the oceans: from a net source of CO₂ to the atmosphere to a CO₂ sink. Since 1800, oceans have taken up about 24% of the anthropogenic CO₂ emissions (Canadell *et al.* 2007). This has produced a series of alterations in the oceanic carbonate system, known as Ocean Acidification (OA): when CO₂ dissolves in seawater, it reacts with water forming carbonic acid (H₂CO₃) which immediately dissociates and loses hydrogen ions (H⁺) resulting in the formation of bicarbonate (HCO₃⁻) and carbonate (CO₃²⁻) ions. This causes an increase of H⁺, which lowers the seawater pH. Over the last 200 years, ocean pH is thought to have decreased by approximately 0.1 units, from 8.21 to 8.07 (Royal Society 2005; Kleypas 2006). Current mean ocean pH is 8.07 (Hall-Spencer *et al.* 2008) and it is predicted that pH will decrease by a further 0.3-0.5 units by the end of the 21st century (Gattuso and Hansson 2011; Gruber *et al.* 2012). These changes in the chemical equilibrium of seawater have caused a decrease in the saturation states of calcite and aragonite, which can cause negative effects on calcifying organisms (Orr *et al.* 2005; Kroeker *et al.* 2011; Doney *et al.* 2012), many of which live in coastal habitats.

Coastal ecosystems are characterized by pronounced temporal and spatial variability in carbonate chemistry and pH (Hofmann *et al.* 2011; Mercado and Gordillo 2011; Duarte *et al.* 2013). In these environments in particular, variability may be amplified due to the ambient heterogeneity and biological activity (Middelboe and Hansen 2007). There is a relationship between the biological activity of primary producers and the variability of seawater pH. For example, Mediterranean *Posidonia oceanica* meadows are able to modify pH in the water column by 0.2 to 0.7 pH units in a day through photosynthetic activity and community respiration (Frankignoulle and Distèche 1984; Frankignoulle and Bouqueneau 1990; Invers *et al.* 1997). In the open ocean, diurnal variation is not as pronounced: an average

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pH range of 0.024 units is more typical (Hoffmann *et al.* 2011). The metabolic activity of primary producers in productive habitats can raise pH during the day to higher levels, providing mitigation from the negative effects of OA to the organisms inhabiting these systems. For example, high pH associated to photosynthesis has been related to enhanced calcification rates of calcareous algae in a tropical seagrass bed (Semesi *et al.* 2009).

The effects of ocean acidification on marine ecosystems have been an important research area during the last years. Numerous laboratory studies have been published showing evidence that ocean acidification influences development, growth, physiology and survival of marine organisms, especially calcifying species (Orr *et al.* 2005; Fine and Tchernov 2007; Ries *et al.* 2009; Dupont *et al.* 2013). These studies have demonstrated a wide range of responses to seawater acidification by different taxonomic groups (Doney *et al.* 2009; Kroeker *et al.* 2013). However, most studies designed to identify the effects of ocean acidification use already available average values of carbon chemistry parameters (pH, total alkalinity, $p\text{CO}_2$ and dissolved inorganic carbon), instead of measuring them *in situ* (McElhany and Busch 2012). Separate studies, specifically measuring pH and carbonate levels in the same habitats, often result in values that are inconsistent with the used $p\text{CO}_2$ averages. Experiments designed to assess the impact of ocean acidification should therefore also measure carbon chemistry within the studied habitats of the model species used.

Intertidal pools are one of the marine environments with greater temporal and spatial variability, as variables such as biota, pool size, and distance to the shore contribute to high diurnal changes of temperature, salinity, oxygen concentration and pH. Daily fluctuations in oxygen saturation, alkalinity and pH have previously been recorded due to biological processes in tide pools (Pyefinch 1943; McGregor 1965; Ganning 1971; Green 1971; Daniel and Boyden 1975; Morris and Taylor 1983). Huggett and Grigffiths (1986) recorded higher oxygen values in the daytime (when photosynthesis is occurring) and lower values at night.

Here, we quantify pH and carbonate system variability in several intertidal pools representative of intertidal rocky platforms common in the North Atlantic subtropical region, and characterize them in terms of temperature, oxygen concentration, primary productivity and water dynamics. Our main objective was to assess natural *in situ* pH values and daily cycles in these habitats, in order to provide information of this geographic area that will inform future ocean acidification studies.

2. MATERIAL AND METHODS

To assess temporal and spatial variability of temperature, pH, oxygen concentration and macroalgal and phytoplankton productivity, two intertidal locations were studied at the north of Tenerife, Canary Islands: «Punta del Hidalgo» and «Finca El Apio». Both locations were selected for having large intertidal platforms with large rockpools. Two large intertidal pools with similar characteristics were studied at each site plus 2 small pools with different distances to the subtidal zone, where high resolution temperature data was collected.



2.1. SALINITY, TEMPERATURE AND OXYGEN CONCENTRATION

Oxygen concentrations were measured directly at each pool using a portable O₂ sensor (VWR OX 4000 H portable). The O₂ sensor was calibrated daily using an open air calibration procedure, as dictated in the manual. Salinity and temperature were measured using a handheld conductimeter (WTW cond 315i). To acquire high resolution temperature data, submersible autonomous data loggers (HOBO Water Temperature Pro v2 Data Logger) were deployed at each pool.

2.2. PH VARIATION

To measure daily pH variability, a moored pH measuring system was deployed at four of the studied pools (table 1). Each system consisted of a Seabird SBE 18 pH sensor attached to a data logger and a lead battery protected inside a waterproof container. Each system was placed inside a plastic box with several openings to allow water circulation and this box was firmly attached to the bottom of the pool. The pH sensors were previously calibrated against NIST buffer solutions (4, 7 and 10 pH ± 0.02) using the software SEASOFT and its module pHfit. The loggers were programmed to take measurements once every 30 minutes and the systems were deployed for at least 24 hours.

TABLE 1. CHARACTERISTICS OF THE STUDIED INTERTIDAL POOLS. PH= «PUNTA DEL HIDALGO»; FA= «FINCA DEL APIO». POSITION IS THE RELATIVE DISTANCE TO THE SUBTIDAL ZONE, FROM CLOSER (1) TO FARTHER (4). IN BOLD LETTERS, POOLS WHERE THE PH SENSORS WERE DEPLOYED

POOL	POSITION	WIDTH (m)	LENGTH (m)	DEPTH (m)	VOLUME (m ³)
PH1	1	7.5	20.7	2.0	310.5
PH2	4	7.1	15.75	0.4	44.73
PH3	2	4.75	30.4	1.4	202.16
PH4	3	6.8	9.4	0.35	22.37
FA1	1	4.8	14.4	1.2	82.94
FA2	2	6.5	15.4	0.8	80.08
FA3	3	6.2	7.0	0.6	26.04
FA4	4	4.5	8.2	0.7	25.83

2.3. CARBON SYSTEM PARAMETERS

Seawater total alkalinity (TA) was measured in each of the studied pools using an open cell potentiometric titration with a Metrohm Dosimat 665 titrator using 0.01 N HCl with a salinity of about 35 (Dickson *et. al* 2007).



The rest of the carbonate chemistry parameters were calculated from TA and pH using the package seacarb 3.08 for R (<https://cran.r-project.org/web/packages/seacarb/>). Calculations were based on a set of constants, K1 and K2, taken from Lueker *et al.* (2000).

2.4. CHLOROPHYLL A CONCENTRATION

Chlorophyll a and phaeopigment concentrations were estimated with a Turner Designs Trilogy fluorometer equipped with an acidification method Chl a module and calculations used the equations from Strickland and Parsons (1972). For each sample, a 1000 ml subsample was filtered on a Millipore polycarbonate 2 μm filter to retain the fraction of phytoplankton cells greater than 2 μm , and another 1000 ml subsample was filtered on a Whattmann GF/F glass microfiber filter to retain the total phytoplankton population. Pigments were extracted in 90% acetone for 24 h in the dark at 4°C. The picoplankton pigment concentration was calculated as the difference between the total and 2 μm fractions.

2.5. PRIMARY PRODUCTION

Primary production was measured using the ^{14}C method of Steeman-Nielsen (1952) using clear and dark polycarbonate bottles. 4 μCi of ^{14}C in bicarbonate form were added to each bottle prior to incubation for 4 to 5 hours. After incubation, each bottle was filtered sequentially through filters with pore sizes 2 and 0.7 μm (Whattmann GF/F glass microfiber filters). Filters were dried, fumed overnight with HCl and placed in scintillation vials with 8 ml of Optiphase Hi-Safe scintillation cocktail and measured in a liquid scintillation counter.

2.6. MACROALGAE PRODUCTIVITY

Padina pavonica was selected to assess its primary productivity, as it was the more abundant species in all of the intertidal pools, making the bulk of the total biomass. Net macroalgae primary productivity and respiration were measured by oxygen production and oxygen depletion after an incubation time. Samples of a wet mass of about 0.300-0.500 g of *P. pavonica* were selected and placed into a transparent plastic beaker with 100 ml of water taken from the studied pool. Initial O_2 concentration was measured with an O_2 sensor (VWR OX 4000 H portable) and initial pH_{NBS} with a pH meter (Metrohm mobile meter with a Primatrode NTC IP pH electrode and temperature sensor), before introducing the sample into the beaker. The beakers were then sealed and placed in the pools for *in situ* incubations. Beakers for dark respiration measurements were covered with aluminum foil and an extra beaker with no samples was incubated as a blank. Incubations lasted for 2 hours. After the incubation time the samples were removed from the beaker, and final



O₂ concentration and pH_{NBS} were measured. Several incubations were performed during the day with the same samples, replacing the water from the incubation vessel. The algae pieces were stored for dry mass determination, drying the samples in the oven at 60°C for 48 hours. Dry mass was measured using a precision balance. Net primary production and respiration were calculated as mg O₂/g dry mass hour.

3. RESULTS

The studied intertidal pools showed a diurnal pH oscillation, which translated into a *p*CO₂ variation of equivalent magnitude. Daily minimum pH_{NBS} levels were obtained early in the morning, at dawn, while maximum pH_{NBS} occurred in the afternoon, near the sunset (figure 1). The higher daily variation was detected in «Punta del Hidalgo», where pH_{NBS} values oscillated within a range of 0.23 pH units in PH1 and 0.26 pH units in PH3. In this site, the pH sensors were deployed for two days, allowing the detection of two diurnal cycles. In PH1, the afternoon higher pH levels measured pH_{NBS} 8.31 and pH_{NBS} 8.25, while the daily morning minimum levels were pH_{NBS} 8.08 and pH_{NBS} 8.09. In PH3, pH varied from pH_{NBS} 8.33-8.38 as the afternoon values to pH_{NBS} 8.11-8.13 as the minimum values of the morning. In the other intertidal site, «Finca El Apio», the pH sensors were deployed for one daily cycle. At this site, the pH variation range was shorter than in the first site's intertidal pools, probably due to the higher water dynamics present during the sensor deployment. In one of the pools (FA1, figure 1), pH_{NBS} varied by only 0.07 pH units during the day, with the afternoon maximum at pH_{NBS} 8.22 and the early morning minimum at pH_{NBS} 8.16. The other pool (FA2) produced a higher oscillation, from pH_{NBS} 8.07 to pH_{NBS} 8.21.

In pools PH1 and PH2 at the site located in «Punta del Hidalgo», the increase of pH detected during the day suffered a perturbation at about 11:00 (fig. 1): the rising tendency of pH is paused for about 4 hours, coinciding with the high tide reaching the pools, producing the mixing of the pool water with that from the subtidal zone. After this, the rising tendency of pH during the daytime hours continues till reaching its maximum at the end of the day.

A proportional but inverse variation occurs with *p*CO₂: high values in the morning and low values in the late afternoon, with the pools at the site in «Punta del Hidalgo» showing more variation than the ones at «Finca El Apio». At PH1, the lower *p*CO₂ numbers were of 192 and 230 μatm during the studied two days' cycle. The higher, early in the day, *p*CO₂ levels were of 369 μatm (a variation of 177 μatm) and 373 μatm (a variation of 143 μatm). PH3 showed a higher *p*CO₂ oscillation, similar to that observed in the pH measurements.

Oxygen concentration showed a daily cycle with lower measurements in the morning and higher O₂ concentration at sunset. O₂ concentration of PH1 and PH3 at sunrise were 7.17 mgO₂/l, and 6.94 mgO₂/l, respectively. At sunset O₂ concentration had reached 7.81 mgO₂/l and 8.01 mgO₂/l. At PH1, however, maximum O₂ concentration occurred at 10:00 (7.88 mgO₂/l), followed with a reduction of O₂ concentration to 7.57 mgO₂/l, and a further increase at 17:20. This O₂ reduction



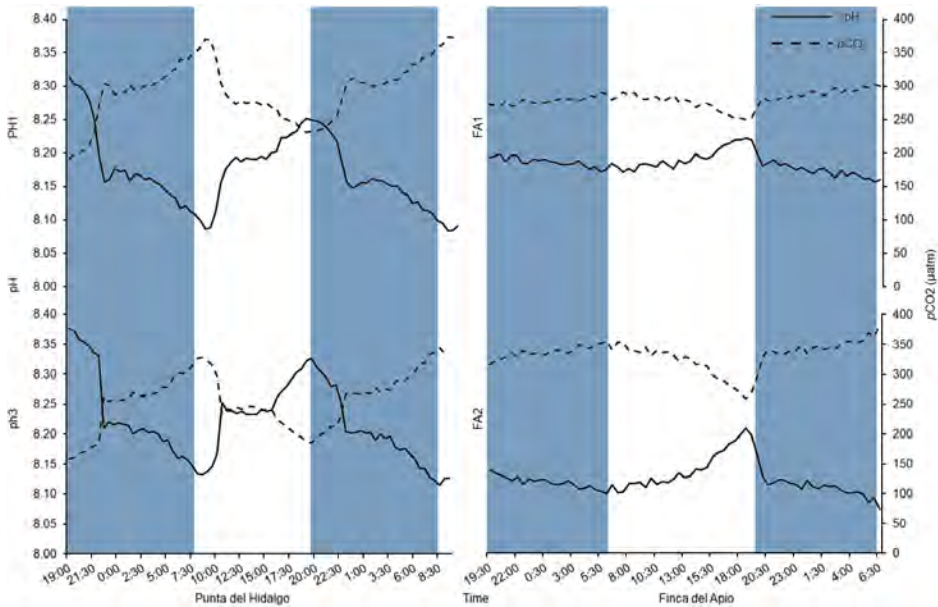


Figure 1. Daily pH and $p\text{CO}_2$ oscillation at the studied intertidal pools. The shaded area represents night time.

coincides with the entry of water to the pool due to the high tide. At the «Finca El Apio» site, the morning low O_2 concentration data were more elevated than at the intertidal pools of «Punta del Hidalgo» (figure 2), with a maximum data of $7.77 \text{ mgO}_2/\text{l}$ at FA1 and 8.03 at FA2.

3.1. WATER TEMPERATURE

Temperature measurements obtained from sensors located at the pools followed an expected daily pattern, more evident at the smaller pools: lowest temperature at night, which increases after sunrise to a maximum in the afternoon, at 16:00-17:00 (figs. 3-4). This is easily observed at PH2 and PH4, with a daily temperature range of 4.67°C and 3.31°C respectively, compared with a daily range of 0.74°C and 1.17°C at pools PH1 and PH3. Shorter daily temperature ranges were observed at the intertidal pools of «Finca El Apio», 3 with daily variations of 0.31 and 0.38°C (figure 4).



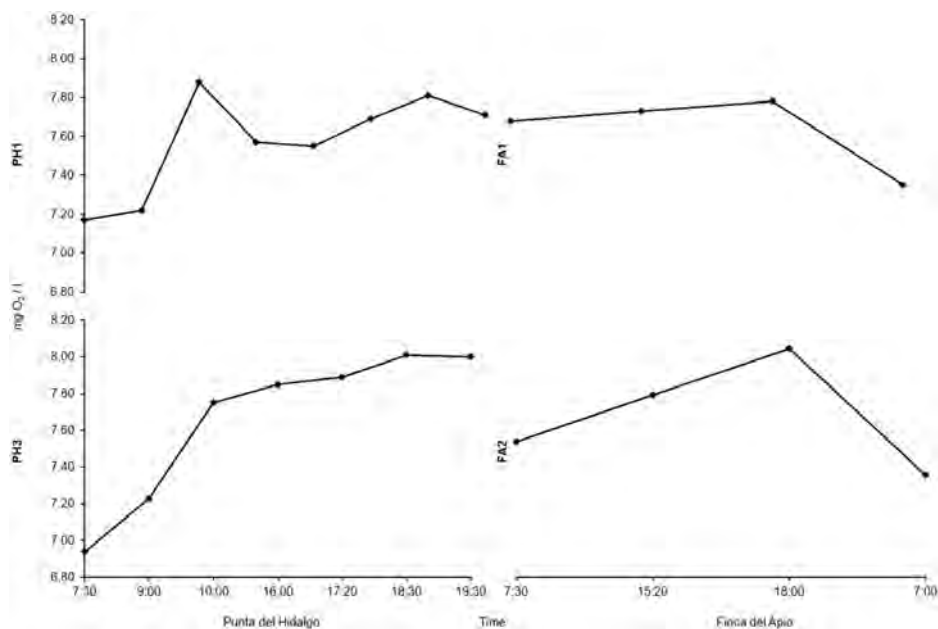


Figure 2. Dissolved oxygen variation at the studied pools.

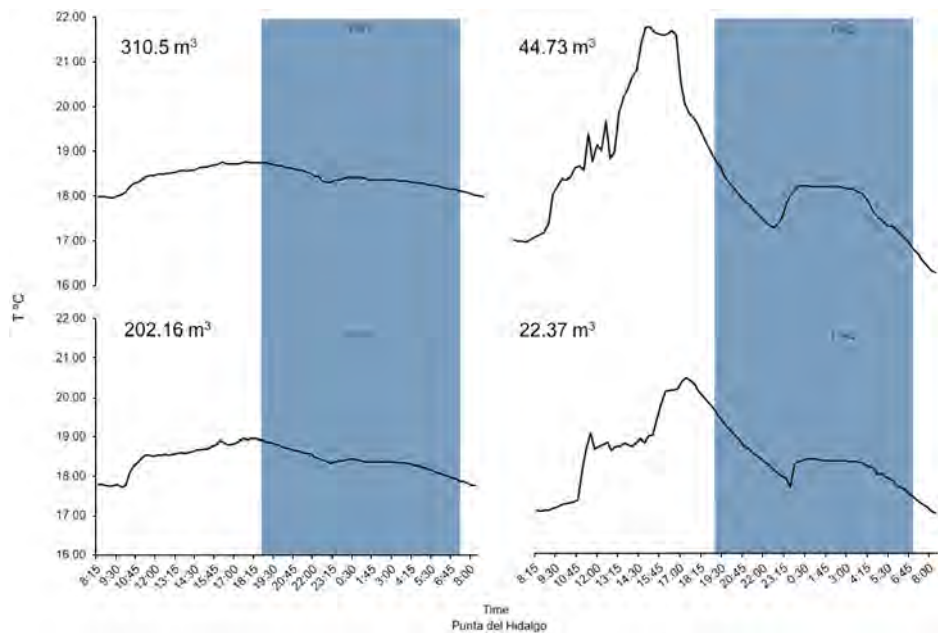


Figure 3. Daily temperature variation of the intertidal pools at location «Punta del Hidalgo».

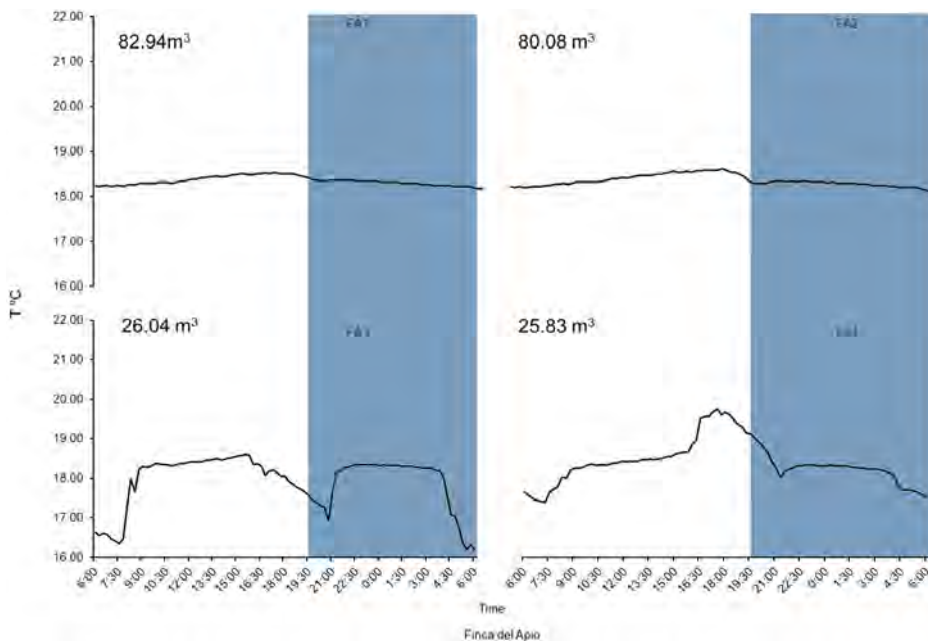


Figure 4. Daily temperature variation of the intertidal pools at location «Finca El Apio».

3.2. PHYTOPLANKTON BIOMASS AND PRODUCTION

Phytoplankton biomass was estimated using Chla concentration as a proxy. It was higher at the «Finca El Apio» site in both studied fractions of phytoplankton (cells larger than $2\ \mu\text{m}$ and cells smaller than $2\ \mu\text{m}$) (figure 5), where larger cells dominated with $[\text{Chla}]>2$ higher than $1.3\ \text{mg}/\text{m}^3$ and $[\text{Chla}]<2$ in the range of $0.20\ \text{mg}/\text{m}^3$. At «Punta del Hidalgo», $[\text{Chla}]>2$ was $0.40 \pm 0.02\ \text{mg}/\text{m}^3$ at PH1 and $0.24 \pm 0.02\ \text{mg}/\text{m}^3$ at PH3. $[\text{Chla}]<2$ was $0.18 \pm 0.03\ \text{mg}/\text{m}^3$ at PH1 and $0.13 \pm 0.01\ \text{mg}/\text{m}^3$ at PH3.

Phytoplankton production was higher at the pools located at «Finca El Apio» (figure 5), where the larger cells were more productive than the smaller than $2\ \mu\text{m}$ ones. The size distribution of the phytoplankton production from the pools located at «Punta del Hidalgo» showed that the cells smaller than $2\ \mu\text{m}$ were being more productive at both studied pools (figure 5).

3.3. MACROALGAE PRIMARY PRODUCTION

Padina pavonica was selected to assess its primary productivity, as it was the more abundant species in all of the intertidal pools. This species' mean O_2 production was $2.03 \pm 0.39\ \text{mgO}_2/\text{g dry mass h}$. No significant differences were observed between



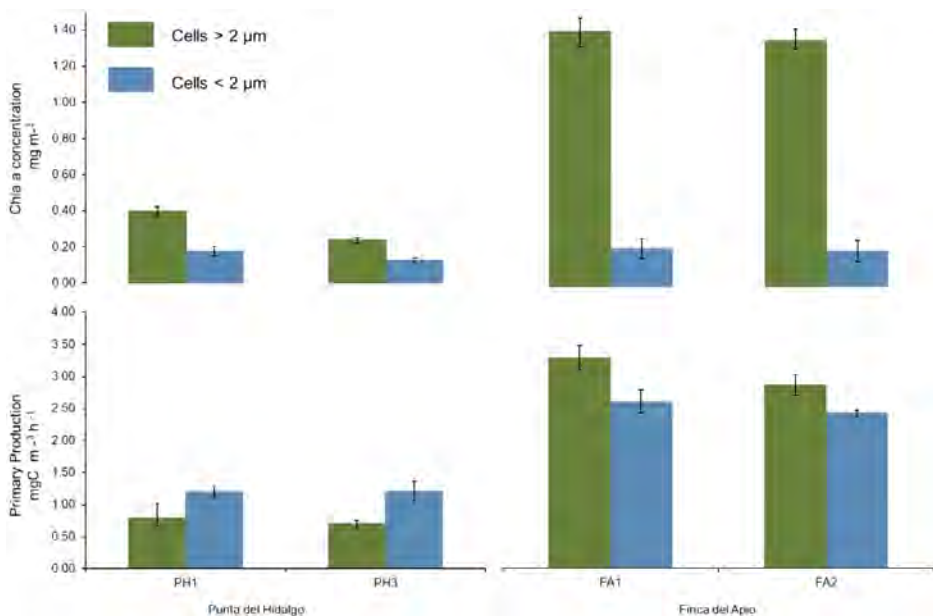


Figure 5. Fractionated phytoplankton chlorophyll a concentration and primary production at the studied pools.

sites (fig. 6). Different incubations were performed in the morning, mid-morning and afternoon, but overall no significant differences were obtained between them. Only at PH3 of «Punta del Hidalgo» oxygen production from *P. pavonica* was significantly lower at 8:00, the first incubation of the day (1.25 ± 0.04 mgO₂/g dry mass h).

4. DISCUSSION

The results show the carbon system parameters and O₂ variability in four large intertidal pools located at two different locations at the North of Tenerife, Canary Islands. A clear diel pH was recorded by our sensors, driven by the biological activity of the pool's biota, especially primary producers. Typically, intertidal environments suffer constant change: during high tide, seawater floods these areas, but they become isolated when the tide recedes. Metabolic processes play a fundamental role in driving pH variability: during the day hours, primary producers rise oxygen concentration in seawater through their photosynthetic activity, rising pH at the same time through its associated inorganic carbon uptake. The sensor measurements reveal that pH tops at the end of the light hours, when light intensity is not enough to maintain a photosynthetic rate above respiration. Then seawater pH decreases during night, when CO₂ is respired with no carbon uptake occurring. Minimum levels are reached before dawn. The observed range of daily pH measured at these

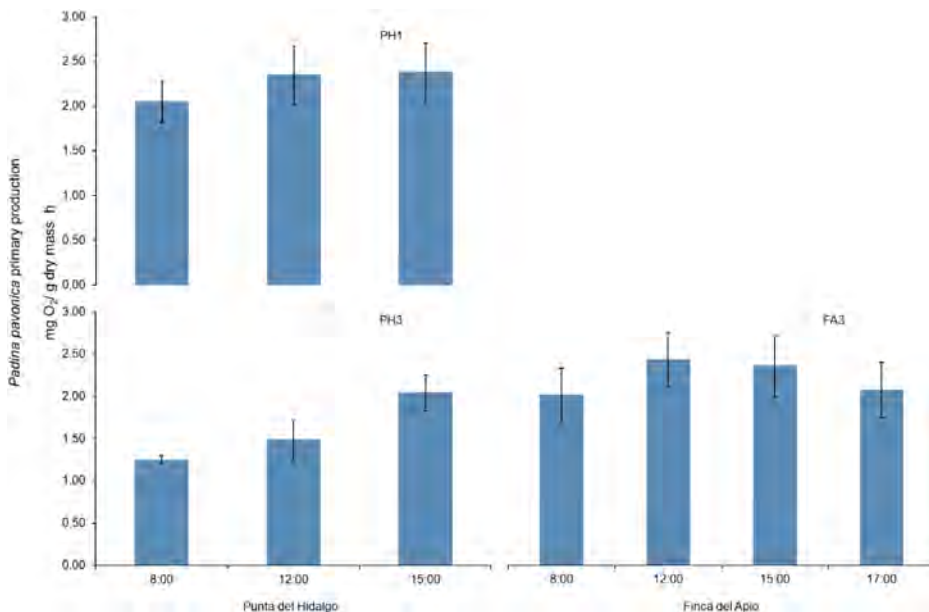


Figure 6. Oxygen production of *Padina pavonica* at the different studied pools.

intertidal pools encompasses maximums that exceed average ocean pH before the Industrial Revolution (Jacobson 2005) (8.35 before dusk) and minimums below 8.10, comparable to actual mean ocean pH (Kleypas 2006). These diurnal pH fluctuations were comparable to previous observations at subtidal areas of Canary Islands (Hernández *et al.* 2015), but Variability of pH and carbon parameters in rockpools communities of Canary Islands had not previously been reported. Other diurnal pH fluctuations measured at other latitudes were 0.06-0.24 units in shallow seagrass meadow at the Mediterranean (Hendriks *et al.* 2014), 0.1 pH units/day in spring in the Bay of Calvi in the Mediterranean (Frankignoulle and Bouquegneau 1990) and 0.15 pH units/day in the Bay of Bengal in the Indian Ocean (Subramanian and Mahadevan 1999). At smaller or more productive (denser seaweeds canopy) pools, pH variability can be much higher. An extreme example, in a subarctic eutrophic area, in small rockpools algal production caused a pHt of 9.0 during the day and pHt of 7.4 at night (Krause-Jensen *et al.* 2015).

Oxygen concentration rose during the daylight hours driven by photosynthesis, from minimum values before dawn to maximum data before sunset, showing a linear relationship with pH change. The highest O₂ concentration variation range was of 1.07 mg/l at PH3 at «Punta del Hidalgo», while at FA1 at «Finca El Apio» which was more connected to the subtidal zones, presented a much shorter variation range (0.1 mg/l). Seawater dynamics were much stronger during the study at this site, keeping the studied pools flooded most of the time which probably caused the



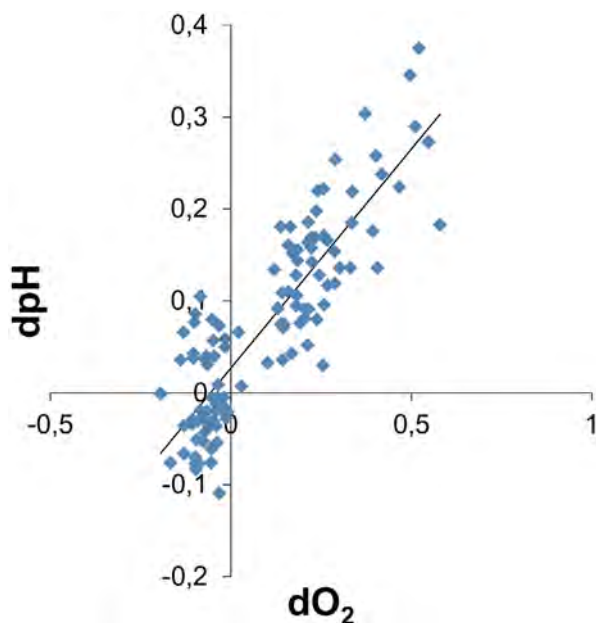


Figure 7. Scatter plot showing oxygen vs pH variation in the incubation vessels for *Padina pavonica*.

shorter pH, temperature and oxygen oscillations observed when compared to those at «Punta del Hidalgo». This adds another variation factor at these intertidal ecosystems.

Pool size, primary producers' biomass and water dynamics are the main factors affecting the carbon system dynamics at intertidal pools. A slowdown in O_2 concentration and pH increase was detected at both of the studied pools at «Punta del Hidalgo» (see figures 1 and 2) corresponding to the entry of seawater caused by the high tide. This new water entry affected the temperature cycle as well; being this apparent in the smaller pools (figs. 3-4, PH2 and PH4). The pools with less water volume suffered a wider fluctuation of pH and temperature. A strong correlation between O_2 and pH variation during incubations for the measurement of macroalgal productivity was detected ($R^2= 0.74$, figure 7), showing that pH increased with increasing O_2 due to photosynthesis and its associated carbon uptake, and that respiration produced the release of carbon, lowering pH.

Phytoplankton did not seem to have an important role in pH and O_2 change during our study. Phytoplankton production was rather high when the «Finca El Apio» area was studied, with primary production data in the range of the highest annual peaks present in oligotrophic areas as the Canary Islands (De Leon and Braun 1973). No significant differences were detected between algal production at both sites, even though a phytoplankton spring bloom was occurring when the sensors were deployed at «Finca El Apio» pools. Diel pH variability was lower at this site's

pools probably because water dynamics were stronger during measurements at this site, keeping the pool flooded longer, mixing its water with that from the adjacent subtidal zone thus mitigating the concentration of O₂ and pH rise.

This study adds more data about the complexity of carbon chemistry at coastal regions, specifically at intertidal pools. Complexity here is higher than at subtidal zones, as water dynamics, pool size, autotrophic and heterotrophic biomass, distance to the coastline and light irradiance are factors that have important influence over pH variations. The results of our study suggest that local carbon chemistry should be measured and taken into consideration when designing ocean acidification experiments in preference to the use of regional averages and mimic these natural changes. In order to obtain a diel pH cycle in laboratory studies for OA, the use of macroalgae in conjunction with light/dark cycle could be viable as a source of pH variability during experiments.

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6. AUTHORS' CONTRIBUTION

Conceptualization: CH, JCH.

Methodology and field work: CH, JCH.

Data analysis: CH, JCH.

Original draft: CH.

Review and edition of the final draft: CH, JCH.



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