1 Spectrophotometric measurements of the carbonate
2 ion concentration: aragonite saturation states in the
3 Mediterranean Sea and Atlantic Ocean

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10 **ABSTRACT**

11 Measurements of ocean pH, alkalinity and carbonate ion concentrations ($[\text{CO}_3^{2-}]$) during three
12 cruises in the Atlantic Ocean and one in the Mediterranean Sea were used to assess the reliability
13 of the recent spectrophotometric $[\text{CO}_3^{2-}]$ methodology and to determine aragonite saturation
14 states. Measurements of $[\text{CO}_3^{2-}]$ along the Atlantic Ocean showed high consistency with the
15 $[\text{CO}_3^{2-}]$ values calculated from pH and alkalinity, with negligible biases ($0.4 \pm 3.4 \mu\text{mol}\cdot\text{kg}^{-1}$). In
16 the warm, salty, high alkalinity and high pH Mediterranean waters, the spectrophotometric
17 $[\text{CO}_3^{2-}]$ methodology underestimates the measured $[\text{CO}_3^{2-}]$ ($4.0 \pm 5.0 \mu\text{mol}\cdot\text{kg}^{-1}$), with anomalies
18 positively correlated to salinity. These waters also exhibited high *in situ* $[\text{CO}_3^{2-}]$ compared to the
19 expected aragonite saturation. The very high buffering capacity allows the Mediterranean Sea
20 waters to remain over the saturation level of aragonite for long periods of time. Conversely, the
21 relatively thick layer of undersaturated waters between 500-1000 m depths in the Tropical
22 Atlantic is expected to progress to even more negative undersaturation values. Moreover, the
23 northern North Atlantic presents $[\text{CO}_3^{2-}]$ slightly above the level of aragonite saturation, and the
24 expected anthropogenic acidification could result in reductions of the aragonite saturation levels
25 during future decades, acting as a stressor for the large population of cold-water-coral
26 communities.

27 **TOC graphic**

TEXT

INTRODUCTION

Approximately one-third of the total anthropogenic CO_2 (C_{ant}) emitted since the onset of industrialization has been absorbed by the global ocean,¹ leading to rapid changes in the carbonate chemistry of the upper layers of the ocean, as revealed by a decrease of ~ 0.11 in pH and $\sim 13\%$ in carbonate ion concentration ($[\text{CO}_3^{2-}]$). Natural dynamic processes such as the Meridional Overturning Circulation (MOC) in the Atlantic Ocean and the double closed active overturning cells observed in the Mediterranean Sea^{2,3} convey heat, salt and oxygen to the deep-ocean.^{4,5} They also promote strong accumulations of C_{ant} in the intermediate and deep waters of the Atlantic Ocean⁶⁻⁸ and the Mediterranean Sea,⁹ favoring a rapid decrease in pH of the intermediate waters due to the low buffering intensity of the Atlantic Ocean^{10,11} and despite the high buffering capacity of the Mediterranean Sea.¹² If the current rate of CO_2 emissions is maintained, pH reductions exceeding 0.2-0.3 units are expected by the year 2100 in approximately 23% of the North Atlantic deep-sea canyons and 8% of the seamounts.¹³ These

areas are of special interest because they are inhabited by cold-water corals (CWC) with calcareous skeletons (aragonite), such as *Lophelia pertusa* or *Madrepora oculata*.^{14,15} *Lophelia pertusa* reefs and deep-water coral carbonate mounds are important hotspots of biodiversity in the Atlantic Ocean and the Mediterranean Sea located between 700 and 1200 m depth.¹⁶ Many of these sites have been proposed as marine protected areas. The average global reduction in $[\text{CO}_3^{2-}]$ of 56% projected by the year 2100¹⁷ may be critical for the existence of CWC reefs because 95% of these reefs are located above the aragonite saturation horizon.¹⁸ For instance, in the intermediate and deep waters of the Iceland Basin (1200 ± 300 m depth), the observed decrease in pH of 0.0008-0.0013 units per year during the last three decades^{10,19} permits an estimation that these waters would be undersaturated when the CO_2 concentration in the atmosphere is greater than 530 ± 30 ppm (which will be reached between ~2035 to 2060). These changes are likely to affect the structure and functioning of marine ecosystems, including reduced growth and net erosion of coral reefs.¹⁷

CaCO_3 saturation states in seawater are chiefly determined by $[\text{CO}_3^{2-}]$ because the calcium concentration ($[\text{Ca}^{2+}]$) is conservative, and, to a very good approximation, depends only on salinity ($0.01028 \times S/35 \text{ mol} \cdot \text{kg}^{-1}$).²⁰ Prior to 2008, $[\text{CO}_3^{2-}]$ could only be estimated using the thermodynamic equations of the carbonate system in seawater,²¹ and two of the main measurable variables of the system in seawater (pH, total alkalinity $-A_T-$, total inorganic carbon $-C_T-$ and fugacity CO_2). In 2008, Byrne and Yao²² published the first shipboard routine technique to directly measure $[\text{CO}_3^{2-}]$ in seawater via spectrophotometric measurements of Pb(II) complexed with CO_3^{2-} . Recently, Easley et al.²³ applied this technique for the first time during field measurements in the North Pacific and Arctic Ocean, also improving the method by refining the molar absorbance ratios previously reported by Byrne and Yao²² by comparing directly measured

[CO₃²⁻] in seawater with those indirectly estimated by conventional means (i.e., calculated from the pairings pH-C_T or pH-A_T).

The new parameterization by Easley et al.²³ resulted in [CO₃²⁻] measurements consistent with the thermodynamics of the relatively low-salinity seawater from the Pacific and Arctic coastal waters. In the present study, we increase the range of salinity, pH and A_T used by Easley et al.²³ by compiling [CO₃²⁻] measurements from three cruises in the Atlantic Ocean and one in the Mediterranean Sea. The ranges of salinity, pH and A_T in the presented database practically cover those found in the global ocean. Spectrophotometric [CO₃²⁻], as directly measured at sea, is compared with that indirectly estimated by conventional means (i.e., calculated from the pH-A_T pair). The spatial variability of the *in situ* aragonite saturation state derived from measured carbonate and A_T is also discussed.

MATERIALS AND METHODS

Discrete seawater samples for [CO₃²⁻], pH and A_T were collected and analyzed on board (see Supporting Information, hereafter *SI*) during three oceanographic cruises in the Atlantic Ocean and one in the Mediterranean Sea (Fig. 1). The CO₃²⁻ samples were analyzed spectrophotometrically at 25°C according to the method established by Byrne and Yao²² and further reformulated by Easley et al.²³ This method consists of the addition of a PbCl₂ solution to a seawater sample so that Pb(II) reacts with the dissolved CO₃²⁻, forming the complex PbCO₃. The [CO₃²⁻] is calculated in terms of the UV absorbance ratio (R) using equation 1.²²

$$-\log[\text{CO}_3^{2-}] = \log_{\text{CO}_3} \beta_1 + \log\left(\frac{R - e_1}{e_2 - R \cdot e_3}\right) \quad (1)$$

where $R = \left(\frac{A_{\lambda_2} - A_{\lambda_3}}{A_{\lambda_1} - A_{\lambda_3}}\right)$, in which A denotes the absorbance at wavelengths λ_1 (234 nm, isosbestic point of PbCO₃), λ_2 (250 nm, mean value of the wavelength with high absorbance variation) and

λ_3 (350 nm, non-absorbing wavelength to correct the absorbance due to sample manipulation). The Pb(II) UV absorbance spectrum is directly dependent on salinity; therefore, the formation constant β_1 and the coefficients e_1 , e_2 and e_3 were determined as second-order polynomial functions of salinity.^{22,23} We used the most recent parameterizations of Easley et al.²³ to compute e_1 , e_2 and β_1 . The resulting $[\text{CO}_3^{2-}]$ (reported at 25°C) has an uncertainty of 2.4 $\mu\text{mol}\cdot\text{kg}^{-1}$ at $[\text{CO}_3^{2-}]$ of 210 $\mu\text{mol}\cdot\text{kg}^{-1}$ (precision 1.1%).

The **pH** samples were measured using the spectrophotometric method described by Clayton and Byrne²⁴ (see *SI*). The pH values are reported at 25°C and on the total scale (pH_{T25}). The reproducibility of the pH_{T25} measurements was less than 0.001, with an accuracy of 0.0055.²⁵ The effect of impurities in the dye used in the shipboard pH measurements was also taken into account (see *SI*).

The **A_T** samples were measured by potentiometric titration and determined by a double endpoint method.²⁶⁻²⁸ **A_T** measurements of Certified Reference Materials (CRMs) of CO_2 were also performed following the same procedure. The uncertainty of this potentiometric method was less than 2 $\mu\text{mol}\cdot\text{kg}^{-1}$.²¹

RESULTS AND DISCUSSION

CO_3^{2-} variability. Importantly, because the parameterization of equation 1 is salinity-dependent, the salinity range of the samples studied here differs from that of the data used by Easley et al.²³ (Fig. 2). The salinity range in our sample collection (34.5-39.4; Figs. 1, 2) is wider and tends toward higher values compared to the salinity range in the data set compiled by Easley et al.²³ from the Pacific and Arctic Oceans (26.6-34.9; Fig. 2). Moreover, the bulk of the samples with high $[\text{CO}_3^{2-}]$ reported by Easley et al.²³ were obtained from the low-salinity and cold waters of the Arctic Ocean, whereas our samples with high $[\text{CO}_3^{2-}]$ are extremely saline and warm,

collected primarily from the Mediterranean Sea and from the Surface Atlantic Water (SAW). However, the samples with low $[\text{CO}_3^{2-}]$ exhibit similar temperature/salinity ranges in both sets of samples: approximately 2-8°C and 34-34.7 of salinity in the Pacific Ocean, which is associated with the intermediate and deep waters, and approximately 4-10°C and 34.3-35 of salinity in the Atlantic Ocean, which is associated with Antarctic Intermediate Water (AAIW) and South Atlantic Central Water (SACW). Therefore, the samples analyzed here are good benchmarks for validating the parameterizations of Easley et al.²³

Calculated versus measured CO_3^{2-} . We used $\text{pH}_{\text{T}25}$ and A_{T} as input measurements to calculate $[\text{CO}_3^{2-}]$ from the thermodynamic equations of the carbonate system combined with the dissociation acid constants reported by Mehrbach et al.²⁹ and refitted by Dickson and Millero.³⁰ The correlation between the spectrophotometrically measured ($[\text{CO}_3^{2-}]_{\text{meas}}$) and estimated ($[\text{CO}_3^{2-}]_{\text{calc}}$) carbonate concentrations (both reported at 25°C) is very high ($r^2 = 0.992$), with a mean and standard deviation of the differences (μ_{d} , σ_{d}) of $0.1 \pm 4.5 \mu\text{mol}\cdot\text{kg}^{-1}$ (Fig. 3A, B). All of the Atlantic cruise data showed slopes very close to $Y = X$, with intercepts indistinguishable from zero ($p\text{-level} < 0.001$) and high r^2 (≥ 0.992). However, in the Mediterranean Sea (HOTMIX cruise) data, the correlation between $[\text{CO}_3^{2-}]_{\text{calc}}$ and $[\text{CO}_3^{2-}]_{\text{meas}}$ exhibited a slope < 1 (0.963 ± 0.008 ; $p\text{-level} < 0.01$) with a positive intercept. According to the typical uncertainties in $\text{pH}_{\text{T}25}$ (± 0.005) and A_{T} (0.1%), $[\text{CO}_3^{2-}]_{\text{calc}}$ is much more sensitive to $\text{pH}_{\text{T}25}$ than to A_{T} . The effect of the uncertainty of $\text{pH}_{\text{T}25}$ in $[\text{CO}_3^{2-}]_{\text{calc}}$ is ten times greater than that derived from A_{T} . At high $\text{pH}_{\text{T}25}$ (~ 8.1), an error of 0.005 in $\text{pH}_{\text{T}25}$ results in an error of $2 \mu\text{mol}\cdot\text{kg}^{-1}$ in $[\text{CO}_3^{2-}]_{\text{calc}}$, whereas at low $\text{pH}_{\text{T}25}$ (~ 7.65) an error of 0.005 in $\text{pH}_{\text{T}25}$ only yield an error of $1 \mu\text{mol}\cdot\text{kg}^{-1}$ in $[\text{CO}_3^{2-}]_{\text{calc}}$. In the case of $[\text{CO}_3^{2-}]_{\text{meas}}$, the main source of the variability is also associated with $\text{pH}_{\text{T}25}$ (97%), with a minor influence of A_{T} (6%).

The distribution of $[\text{CO}_3^{2-}]$ differences (Fig. 3B-F) provides insights into the goodness of the model fit proposed by Easley et al.²³ within a wider salinity and pH range. The histograms of the differences (Fig. 3B) show a well-centered difference for the MOC2 and OVIDE cruises, with very low σ_d (3.0 and 3.3 $\mu\text{mol}\cdot\text{kg}^{-1}$, respectively) and with ~61% of the samples within 2 $\mu\text{mol}\cdot\text{kg}^{-1}$ of $[\text{CO}_3^{2-}]_{\text{calc}}$, which is comparable with the results of Easley et al.²³ (73%). The randomness of the spatial vertical distribution of the differences in the MOC2 and OVIDE cruises (Fig. 3D, E) corroborates the high consistency between $[\text{CO}_3^{2-}]_{\text{calc}}$ and $[\text{CO}_3^{2-}]_{\text{meas}}$. The other Atlantic cruise, CAIBOX, shows a small bias of μ_d (-2.9 $\mu\text{mol}\cdot\text{kg}^{-1}$) with a quite low σ_d (2.8 $\mu\text{mol}\cdot\text{kg}^{-1}$) (Fig. 3B). The spatial vertical distribution of the $[\text{CO}_3^{2-}]$ differences along this cruise (Fig. 3C) is random and almost 0 for the northern part of the CAIBOX cruise; however, the $[\text{CO}_3^{2-}]_{\text{meas}}$ is slightly higher than $[\text{CO}_3^{2-}]_{\text{calc}}$ in the southern part of the section, south of 33°N (Fig. 3C). These biases might be related to the fact that these measurements were the first shipboard spectrophotometric $[\text{CO}_3^{2-}]$ measurements performed in the Atlantic Ocean by our research group.

Different behavior was observed for the first $[\text{CO}_3^{2-}]$ measurements in the Mediterranean Sea during the HOTMIX cruise. They exhibit slightly high $[\text{CO}_3^{2-}]_{\text{calc}}$ compared to $[\text{CO}_3^{2-}]_{\text{meas}}$ ($4.0 \pm 5.0 \mu\text{mol}\cdot\text{kg}^{-1}$) (Fig. 3A, B). The spatial vertical distribution of the $[\text{CO}_3^{2-}]$ differences (Fig. 3F) is random and approximately 0 for the Atlantic part of the section (similar to the distributions in the MOC2 and OVIDE cruises), whereas inside the Mediterranean Sea, positive values occur, even reaching differences greater than 10 $\mu\text{mol}\cdot\text{kg}^{-1}$. These differences are related to the high salinity values within the HOTMIX cruise (Figs. 1, 2). In fact, in the Eastern and Western Mediterranean Basins, where the differences reach the highest values, the differences are well correlated with salinity ($\Delta[\text{CO}_3^{2-}] = [(1.7 \pm 0.2) \times (S - 35.66)]$; $p\text{-level} < 0.001$ with a standard

error of $4.5 \mu\text{mol}\cdot\text{kg}^{-1}$). Moreover, the high pH_{T25} and A_{T} values of the Mediterranean Sea
 samples (Figs. 1, S1) produced noisier $[\text{CO}_3^{2-}]_{\text{calc}}$ in the HOTMIX cruise. To double check the
 causes of the positive bias of $[\text{CO}_3^{2-}]_{\text{calc}}$, $[\text{CO}_3^{2-}]$ was also calculated using coulometric C_{T}
 measurements ($N = 154$) and A_{T} ($[\text{CO}_3^{2-}]_{\text{calc_CT}}$). The difference between $[\text{CO}_3^{2-}]_{\text{calc_CT}}$ and
 $[\text{CO}_3^{2-}]_{\text{meas}}$ shows the same spatial pattern and correlation versus salinity ($\Delta[\text{CO}_3^{2-}]_{\text{calc_CT}} = [(2.1$
 $\pm 0.3) \times (S - 34.5)]$; $p\text{-level} < 0.001$) as the differences resulting from the pair $\text{pH}_{\text{T25}}\text{-}A_{\text{T}}$. This
 similarity suggests that both calculated $[\text{CO}_3^{2-}]$ are consistent irrespective of which pair of
 carbonic variables is chosen and that the recent parameterizations of Easley et al.²³ produce low
 $[\text{CO}_3^{2-}]_{\text{meas}}$ values at salinity > 36 and $[\text{CO}_3^{2-}] > 150 \mu\text{mol}\cdot\text{kg}^{-1}$. In fact, Easley et al.²³ also
 reported low $[\text{CO}_3^{2-}]_{\text{meas}}$ values ($5\text{-}10 \mu\text{mol}\cdot\text{kg}^{-1}$ lower than the $[\text{CO}_3^{2-}]_{\text{calc}}$) at high $[\text{CO}_3^{2-}]$ values,
 which might indicate that the spectrophotometric model fit by Easley et al.²³ underestimates
 $[\text{CO}_3^{2-}]$ and, consequently, underestimates saturation states at high saturation levels of CaCO_3 in
 the ocean. The same conclusions are deduced when the set of CO_2 constants by Millero et al.³¹ or
 the new reported total borate concentration³² is used. The previous parameterization by Byrne
 and Yao²² produced slightly but significantly different results from those obtained using the
 parameterization by Easley et al.²³, depending strongly on pH_{T25} . The new parameterization
 corrects the deviation of the measured values from the calculated ones for $\text{pH}_{\text{T25}} < 7.9$ and pH_{T25}
 > 8.02 , leading to better agreement between $[\text{CO}_3^{2-}]_{\text{calc}}$ and $[\text{CO}_3^{2-}]_{\text{meas}}$. Recently, Patsavas et al.³³
 improved the methods of Byrne and Yao²² and Easley et al.²³ by using $\text{Pb}(\text{ClO}_4)_2$ as a titrant
 instead of PbCl_2 because $\text{Pb}(\text{ClO}_4)_2$ is more soluble, resulting in better signal-to-noise ratios.
 Their procedure improved the agreement between $[\text{CO}_3^{2-}]_{\text{calc}}$ and $[\text{CO}_3^{2-}]_{\text{meas}}$ for $[\text{CO}_3^{2-}] > 180$
 $\mu\text{mol}\cdot\text{kg}^{-1}$. However, we cannot test the parameterization of Patsavas et al.³³ because it was
 available after our measurements and they used a different the titrant solution.

Aragonite saturation. Typically, the *in situ* degree of aragonite saturation (Ω_A) is given by

$$\Omega_A = \frac{[\text{Ca}^{2+}][\text{CO}_3^{2-}]_{\text{is}}}{K_A} \quad (2)$$

where K_A is the CaCO_3 aragonite solubility product and subscript “is” denotes $[\text{CO}_3^{2-}]$ under *in situ* temperature and pressure conditions. The $[\text{Ca}^{2+}]$ behaves conservatively²⁰ and is obtained from $[\text{Ca}^{2+}] = 0.01028 \times S/35 \text{ mol} \cdot \text{kg}^{-1}$.

An often convenient measure of the CaCO_3 aragonite saturation state is simply the difference between the observed *in situ* $[\text{CO}_3^{2-}]$ ($[\text{CO}_3^{2-}]_{\text{is}}$) and the saturation $[\text{CO}_3^{2-}]$ ($[\text{CO}_3^{2-}]_{\text{sat}}$; $\Omega_A = 1$), i.e., the excess carbonate ion concentration ($[\text{CO}_3^{2-}]_{\text{xs}}$):

$$[\text{CO}_3^{2-}]_{\text{xs}} = [\text{CO}_3^{2-}]_{\text{is}} - [\text{CO}_3^{2-}]_{\text{sat}} \quad (3)$$

Positive (negative) $[\text{CO}_3^{2-}]_{\text{xs}}$ indicates the water is supersaturated (undersaturated) with respect to CaCO_3 . The absolute value of $[\text{CO}_3^{2-}]_{\text{xs}}$ is a measure of the tendency for the mineral CaCO_3 to precipitate/dissolve. The $[\text{CO}_3^{2-}]_{\text{xs}}$ has an advantage over Ω_A in that it is directly comparable to $[\text{CO}_3^{2-}]_{\text{is}}$ and, therefore, to $[\text{CO}_3^{2-}]_{\text{meas}}$. Before development of the spectrophotometric technique by Byrne’s group, $[\text{CO}_3^{2-}]_{\text{is}}$ was typically computed from pairs of two conventional measured carbonate system variables (pH- C_T or pH- A_T). Taking advantage of $[\text{CO}_3^{2-}]_{\text{meas}}$, we also determined $[\text{CO}_3^{2-}]_{\text{is}}$ using $[\text{CO}_3^{2-}]_{\text{meas}}$ and A_T . Both pairings (pH- A_T and CO_3^{2-} - A_T) produced similar results ($r^2 = 0.995$), with similar μ_d and σ_d values at 25°C and 1 atm ($0.4 \pm 4.5 \text{ } \mu\text{mol} \cdot \text{kg}^{-1}$). Easley et al.²³ described very similar behavior in the North Pacific in the carbonate equilibrium equations needed to compute $[\text{CO}_3^{2-}]_{\text{is}}$.

In Figure 4 (panels A, C, E, and G), we present the first results of directly measured $[\text{CO}_3^{2-}]$ in the Atlantic Ocean and the Mediterranean Sea. Our $[\text{CO}_3^{2-}]$ measurements were performed in oceanic waters within wide ranges of temperature, salinity, pH and A_T , whereas the previous studies of Easley et al.²³ and Patsavas et al.³³ were performed in relatively coastal areas. Our

[CO₃²⁻]_{xs} (computed from [CO₃²⁻]_{meas} and A_T) shows a large range of variability (Fig. 4B, D, F, and H): from -55 μmol·kg⁻¹ in the deep waters of the Atlantic Ocean to 196 μmol·kg⁻¹ in the surface waters of the Western Tropical Atlantic, with values greater than 150 μmol·kg⁻¹ in the surface waters of the Eastern Mediterranean.

The Mediterranean Sea data exhibits an eastward trend of decreasing A_T and increasing pH, in which the eastern basin presents higher A_T (2560-2644 μmol·kg⁻¹) and pH_{T25} (7.935-8.032) and less variable C_T (2247-2331 μmol·kg⁻¹) than the western basin (with A_T of 2388-2608 μmol·kg⁻¹, pH_{T25} of 7.861-7.988 and C_T of 2110-2336 μmol·kg⁻¹).¹² This trend leads to an eastward [CO₃²⁻] increase (Fig. 4A, B). The saline and warm Surface Mediterranean Water (SMW; Fig. 2) in the Eastern Basin of the Mediterranean Sea presents very high [CO₃²⁻]_{meas} (201-252 μmol·kg⁻¹; Fig. 4A) and [CO₃²⁻]_{xs} (> 150 μmol·kg⁻¹; Fig. 4B), which are closely associated with the high pH_{T25} (8.00-8.05; Fig. S1) caused by the near-equilibrium conditions between the atmosphere and water in this area. Below SMW, the Levantine Intermediate Water (LIW; 13.55 ± 0.06°C and 38.755 ± 0.015 salinity; Fig. 2) presents values of [CO₃²⁻]_{meas} (213 ± 3 μmol·kg⁻¹) and [CO₃²⁻]_{xs} (~125 μmol·kg⁻¹) similar to those of SMW. This similarity is due to the high buffering capacity (low Revelle factor)^{12,34} in these high-A_T (Fig. S1) and warm Mediterranean waters, which contributes to high CaCO₃ supersaturation on long timescales, even though the convergence circulation in this basin transports substantial amounts of C_{ant} to the deep waters.⁹ The transition from the Eastern to the Western Mediterranean Basin at the Strait of Sicilia leads to a clear decrease in [CO₃²⁻]_{meas} and [CO₃²⁻]_{xs}, mainly due to the decrease in salinity, pH_{T25} and A_T (Figs. 2, S1). In fact, the Mediterranean Deep Water (MDW; 12.92 ± 0.05°C and 38.489 ± 0.013 salinity; Fig. 2) presents average values of [CO₃²⁻]_{meas} (192 ± 4 μmol·kg⁻¹) and [CO₃²⁻]_{xs} (> 50 μmol·kg⁻¹) lower than those observed in the deep waters of the Eastern Basin. The transit from

the Mediterranean Sea to the Atlantic Ocean through the Strait of Gibraltar is marked by a decrease in $[\text{CO}_3^{2-}]_{\text{meas}}$ and $[\text{CO}_3^{2-}]_{\text{xs}}$ in the deep waters, which reflects their low pH_{T25} . The surface layer in the Atlantic part of the HOTMIX cruise has high $[\text{CO}_3^{2-}]_{\text{meas}}$ and $[\text{CO}_3^{2-}]_{\text{xs}}$ associated with high pH_{T25} , which results from close equilibrium with the atmosphere for these waters. The effect of pressure on aragonite saturation produces a strong vertical gradient of $[\text{CO}_3^{2-}]_{\text{xs}}$ below the upper layer (~500 m depth), with undersaturated waters below 2500 m depth. The vertical gradient of $[\text{CO}_3^{2-}]_{\text{meas}}$ is interrupted by a local minimum of $103 \pm 2 \mu\text{mol}\cdot\text{kg}^{-1}$ at approximately 800 m depth, close to the Canary Islands, associated with the vestiges of AAIW (weak salinity minimum of 35.25 in Fig. 2) originating from the South.

The CAIBOX cruise, which covers the Iberian Basin (30-45°N), also presents a strong vertical gradient of $[\text{CO}_3^{2-}]_{\text{xs}}$ (Fig. 4C), with supersaturated surface waters that present a northward decrease of $[\text{CO}_3^{2-}]_{\text{xs}}$ due to the northward decrease in temperature and undersaturated deep waters (North Atlantic Deep Water; NADW; $2.64 \pm 0.55^\circ\text{C}$ and 34.95 ± 0.05 salinity; Fig. 2). The vertical gradient is also interrupted by the weak minimum of $[\text{CO}_3^{2-}]_{\text{meas}}$ ($\sim 120 \pm 3 \mu\text{mol}\cdot\text{kg}^{-1}$; south of 32°N and at ~1000 m depth) because of the influence of AAIW, and also by a weak maximum of $[\text{CO}_3^{2-}]_{\text{meas}}$ ($\sim 135 \pm 3 \mu\text{mol}\cdot\text{kg}^{-1}$; 37-41°N at ~1000 m depth) associated with the eastward-moving Mediterranean Water (MW; $9.8 \pm 0.7^\circ\text{C}$ and 35.86 ± 0.08 salinity; Fig. 2) in the Atlantic Ocean.³⁵ In fact, this slight maximum is coincident with a core of maximum salinity values (> 35.8 ; red dashed line in Fig. 4D). The moderate values of pH_{T25} (7.771 ± 0.006 ; Fig. S1) for this water mass promote the downward displacement of the $50 \mu\text{mol}\cdot\text{kg}^{-1}$ isoline of $[\text{CO}_3^{2-}]_{\text{xs}}$. Interestingly, the levels of aragonite saturation of the deep waters shoal slightly northwards because of the deep penetration of C_{ant} derived from the North.^{36,37}

246 The $[\text{CO}_3^{2-}]_{\text{meas}}$ of the surface waters continuously decreases from the Iberian Peninsula to
 247 Greenland (OVIDE cruise; Figs. 1, 4E), which responds to the temperature decrease. The most
 248 striking feature of the OVIDE cruise is the core of minimum $[\text{CO}_3^{2-}]_{\text{meas}}$ ($114 \pm 3 \mu\text{mol}\cdot\text{kg}^{-1}$),
 249 located between 600 and 3000 m depth, denoting the presence of the recently high-ventilated
 250 Labrador Sea Water (LSW) from the Labrador Sea. The $[\text{CO}_3^{2-}]_{\text{xs}}$ of the core of LSW in the
 251 Iceland Basin (salinity < 34.93 ; dashed yellow line in Fig. 4F) ranges from 0 to $20 \mu\text{mol}\cdot\text{kg}^{-1}$.
 252 This rapid decrease of $[\text{CO}_3^{2-}]_{\text{xs}}$ is due to the effect of the high C_{ant} levels found here and the low
 253 buffering capacity of these waters.¹¹ In the pressure range of the LSW core (1000-1800 m depth),
 254 important ecosystems sustained by the presence of CWC will probably be in danger in future
 255 decades¹³ because of the rapid decrease in pH and the low $[\text{CO}_3^{2-}]_{\text{xs}}$.

256 The MOC2 cruise (7.5°N) presents a very narrow surface layer with the highest $[\text{CO}_3^{2-}]_{\text{meas}}$
 257 ($246 \pm 4 \mu\text{mol}\cdot\text{kg}^{-1}$) and very high $[\text{CO}_3^{2-}]_{\text{xs}}$ ($> 175 \mu\text{mol}\cdot\text{kg}^{-1}$) in the West Tropical Atlantic
 258 (Fig. 4G, H), which corresponds to SAW (Fig. 2). These values decrease very sharply with
 259 increasing depth to 500 m. Below this layer of high $[\text{CO}_3^{2-}]_{\text{meas}}$ and $[\text{CO}_3^{2-}]_{\text{xs}}$, a strong minimum
 260 of $[\text{CO}_3^{2-}]_{\text{meas}}$ is present along the entire section between ~250-1250 m depth; the minimum of
 261 $[\text{CO}_3^{2-}]_{\text{meas}}$ is associated with minimum values of $[\text{CO}_3^{2-}]_{\text{xs}}$. This layer of minimum $[\text{CO}_3^{2-}]_{\text{meas}}$ is
 262 derived from the sustained accumulation of C_T derived from the mineralization of biogenic
 263 matter during the long transit time of AAIW from its formation region (the sub-Antarctic
 264 convergence zone, 60-55°S).³⁸⁻⁴⁰ The AAIW layer, centered at 800 m depth (salinity < 34.68 ;
 265 yellow dashed line in Fig. 4H), presents an average $[\text{CO}_3^{2-}]_{\text{meas}}$ of $80 \pm 4 \mu\text{mol}\cdot\text{kg}^{-1}$ and is
 266 characterized by a salinity minimum⁴¹ (34.60 ± 0.06 ; Fig. 2) and low pH_{T25} (7.539 ± 0.021 ; Fig.
 267 S2). Although AAIW is more important in the western part of the MOC2 cruise (lower salinity
 268 values), the minimum of $[\text{CO}_3^{2-}]_{\text{meas}}$ ($70 \pm 1 \mu\text{mol}\cdot\text{kg}^{-1}$) occurs close to the African coast (~400-

269 800 m depth), where the layer of minimum $[\text{CO}_3^{2-}]_{\text{xs}}$ is thicker. This $[\text{CO}_3^{2-}]_{\text{meas}}$ minimum might
270 be related to the strong depletion in oxygen and to the increase in C_T in the Guinea Dome (East
271 of 22°W). This explanation is consistent with the strong carbon fixation in the photic layer
272 characteristic of eastern upwelling systems. This strong carbon fixation enhances the presence of
273 strongly mineralized subsurface waters with very low pH_{T25} and $[\text{CO}_3^{2-}]$ in the tropics, such as
274 those found during the MOC2 cruise in an oxygen minimum layer (centered at 400 m depth)
275 coinciding with SACW (Fig. 2). These levels of corrosive (aragonite undersaturated) old waters
276 have also been observed over the shelf of the California coast.^{23,42} These processes would be
277 enhanced in future global change scenarios reaching the surface layers close to the African
278 upwelling systems.⁴³

279 Another striking feature of the MOC2 cruise is the $[\text{CO}_3^{2-}]_{\text{xs}}$ maximum below the minimum
280 related to AAIW, which is associated with the upper component of NADW detected on the basis
281 of its relative maximum salinity (salinity > 34.95 ; red dashed line in Fig. 4H), which is related to
282 some contributions of MW⁴¹ that also slightly increase $[\text{CO}_3^{2-}]_{\text{xs}}$. NADW is transported
283 southwards by the Deep Western Boundary Current (DWBC),^{5,44} which ventilates the deep
284 ocean from the North Atlantic toward Antarctica all along the western basin (Fig. 1). A clear
285 signal of NADW was detected in the MOC2 cruise between 2000 and 4000 m depth, west of
286 40°W , by a relatively deep $[\text{CO}_3^{2-}]_{\text{meas}}$ maximum (Fig. 4D). The NADW core at this position
287 ($2.74 \pm 0.63^\circ\text{C}$ and 34.941 ± 0.017 salinity) presents relatively high $[\text{CO}_3^{2-}]_{\text{meas}}$ (120 ± 2
288 $\mu\text{mol}\cdot\text{kg}^{-1}$), which is slightly higher than in the northern North Atlantic (OVIDE) because of the
289 lower contribution of C_{ant} .

290 We conclude that the parameterizations of e_1 , e_2 and β_1 given by Easley et al.²³ provide better
291 agreement between the observed and calculated $[\text{CO}_3^{2-}]$ (from pH_{T25} and A_T) than the earlier

parameterizations from Byrne and Yao.²² The Atlantic Ocean cruises where $[\text{CO}_3^{2-}]$ ranged within the values of Easley et al.²³ ($< 250 \mu\text{mol}\cdot\text{kg}^{-1}$) provide very high consistency between measured and calculated $[\text{CO}_3^{2-}]$, with negligible biases. Approximately 61% of the samples in the Atlantic cruises fell within $2 \mu\text{mol}\cdot\text{kg}^{-1}$ of the calculated $[\text{CO}_3^{2-}]$, consistent with the results of Easley et al.²³ However, in the warm, salty, high- A_T and high-pH Mediterranean waters, the parameterization by Easley et al.²³ appears to underestimate the carbonate ion concentration and, consequently, the saturation state, thus suggesting a need to refine the proposed equations or to apply the new method of Patsavas et al.³³

Our CO_3^{2-} measurements allow us to describe a large variety of environments in terms of aragonite saturation states. The Mediterranean Sea has a high excess of $[\text{CO}_3^{2-}]$ despite a high C_{ant} inventory. This excess is related to the Mediterranean Sea's very high buffering capacity, which should allow Mediterranean Sea water to remain supersaturated well into the future. Therefore, the Mediterranean Sea is a favorable location for the development of CWC. In the opposite sense, the relatively thick layer of undersaturated waters between 500-1000 m depth in the Tropical Atlantic is expected to progress to even more negative $[\text{CO}_3^{2-}]_{\text{xs}}$. As Easley et al.²³ described in the California Upwelling System, these undersaturated subsurface waters are prone to advection to the upper twilight and shelf water layers, dramatically affecting ecosystems.⁴³ However, the already undersaturated Atlantic waters below 3000 m depth, which have very low concentrations of C_{ant} , are not expected to experience large changes in future decades.

The northern North Atlantic ($> 45^\circ\text{N}$) presents slightly positive $[\text{CO}_3^{2-}]_{\text{xs}}$ in a large fraction of its waters. However, the expected increase of the C_{ant} content in the intermediate waters and the subsequent acidification will result in a reduction of their aragonite saturation levels, and possibly attainment of undersaturation in subsequent decades. Most of the CWC communities

already occur in aragonite-saturated waters¹⁸ and are very abundant in the North Atlantic below 1200 m depth.^{16,45,46} The long-term monitoring of *in situ* $[\text{CO}_3^{2-}]$ using automated spectrophotometric techniques based on the Pb(II) method may help to monitor these expected future changes and should be promoted given the results reported here.

FIGURES

Figure 1. Hydrographic stations of the four cruises where carbonate ion concentration ($[\text{CO}_3^{2-}]$), pH and total alkalinity (A_T) measurements were performed. The table shows the ranges of the

323 main variables: Salinity, $\text{pH}_{\text{T}25}$ (pH on the total scale at 25°C), A_{T} in $\mu\text{mol}\cdot\text{kg}^{-1}$ and $[\text{CO}_3^{2-}]$
 324 reported at 25°C in $\mu\text{mol}\cdot\text{kg}^{-1}$. SG = Strait of Gibraltar, SS = Strait of Sicily, DWBC = Deep
 325 Western Boundary Current.

326
 327 **Figure 2.** Potential temperature/salinity diagram showing $[\text{CO}_3^{2-}]$ variability from our collected
 328 data in the Atlantic Ocean (CAIBOX, MOC2 and OVIDE) and the Mediterranean Sea (Med.
 329 Sea; HOTMIX), and the North Pacific and Arctic Ocean data presented by Easley et al.²³, all

330 reported at 25°C. SMW = Surface Mediterranean Water, SAW = Surface Atlantic Water, NACW
 331 = North Atlantic Central Water, SACW = South Atlantic Central Water, LIW = Levantine
 332 Intermediate Water, AAIW = Antarctic Intermediate Water, MW = Mediterranean Water, LSW
 333 = Labrador Sea Water, MDW = Mediterranean Deep Waters, NADW = North Atlantic Deep
 334 Water.

335 **Figure 3.** A) Calculated ($[\text{CO}_3^{2-}]_{\text{calc}}$) versus measured ($[\text{CO}_3^{2-}]_{\text{meas}}$) carbonate ion concentration
 336 at 25°C. B) Histogram of differences between $[\text{CO}_3^{2-}]_{\text{calc}}$ and $[\text{CO}_3^{2-}]_{\text{meas}}$ at 25°C. C-F) Vertical
 337 distribution with longitude or latitude of these differences along the cruise tracks. The Y-axis
 338 (depth in meters) is expanded in the upper 2000 m. Units are $\mu\text{mol}\cdot\text{kg}^{-1}$.
 339

Figure 4. Left panels (A, C, E, G): measured carbonate ion concentration reported at 25°C ($[\text{CO}_3^{2-}]_{\text{meas}}$). Right panels (B, D, F, H): excess carbonate ion concentration over aragonite saturation under *in situ* conditions ($[\text{CO}_3^{2-}]_{\text{xs}}$). All panels are vertical distributions with longitude or latitude according to the cruise. The Y-axis (depth in meters) is expanded in the upper 2000 m. Units are $\mu\text{mol}\cdot\text{kg}^{-1}$. Red (yellow) dashed lines indicate salinity maximum (minimum) values.

ASSOCIATED CONTENT

SUPPORTING INFORMATION (SI)

Details regarding sampling and measurements of CO_3^{2-} , pH and A_T . Additional figures are also included to show measured pH and A_T distributions along the section tracks, and excess of the $[\text{CO}_3^{2-}]$ over the aragonite saturation along the Equatorial section of MOC2 cruise. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

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ABBREVIATIONS

AAIW, Antarctic Intermediate Water; A_T , total alkalinity; C_{ant} , anthropogenic CO_2 ; CRM, Certified Reference Material; C_T , total inorganic carbon; CWC, cold-water corals; DWBC, Deep Western Boundary Current; LIW, Levantine Intermediate Water; LSW, Labrador Sea Water; MDW, Mediterranean Deep Waters; MOC, Meridional Overturning Circulation; MW, Mediterranean Water; NADW, North Atlantic Deep Water; pH_{T25} , pH on the total scale at 25°C; SACW, South Atlantic Central Water; SAW, Surface Atlantic Water; SMW, Surface Mediterranean Water;

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SUPPORTING INFORMATION FOR

Spectrophotometric measurements of the carbonate ion concentration: aragonite saturation states in the Mediterranean Sea and Atlantic Ocean

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METHODS

CO₃²⁻ sampling and measurements: Unfiltered seawater samples were directly taken from Niskin bottles and transferred into cylindrical quartz Perkin Elmer cells with a volume of 28 mL and a path length of 100 mm. These cells were carefully stored in a thermostatic bath at 25°C for at least 1 h before the analysis. Then, absorbance measurements were performed using double-beam spectrophotometers (Table S1). First, the absorbance measurements of the samples were performed directly, and then 225 µL of stock solution of PbCl₂ (1.1 mM) was added to the samples and the absorbance measurements were performed again. To test the precision of the spectrophotometer, six CO₃²⁻ samples of open ocean surface water were analyzed, resulting in a standard deviation of 1.9 µmol·kg⁻¹ (precision 0.9%) for the Perkin Elmer spectrophotometer when [CO₃²⁻] = 210 µmol·kg⁻¹, 2.9 µmol·kg⁻¹ for the Shimadzu UV 2401 spectrophotometer when [CO₃²⁻] = 230 µmol·kg⁻¹ (precision 1.3%), and 2.2 µmol·kg⁻¹ for the Shimadzu UV-2600 spectrophotometer when [CO₃²⁻] = 194 µmol·kg⁻¹ (precision 1.13%).

pH sampling and measurements: Unfiltered seawater samples were directly taken from Niskin bottles and transferred into special optical glass spectrophotometric Hellma cells with a path length of 100 mm. These cells were carefully stored in a thermostatic bath at 25°C approximately 1 h before the analysis. Absorbance measurements were then performed using double-beam spectrophotometers (Table S1). The methodology consisted of measuring the absorbance values at two wavelengths (λ = 434 and 578 nm) before and after the addition of 75 µL of m-cresol purple (Sigma-Aldrich mCP; ~0.2 mM) to the seawater sample. Parameterization of the molar absorptivity ratios was required to determine the pH from absorbance ratio (R); this parameterization was completed using a Kodak mCP in the study by Clayton and Byrne.¹ The

small effect of the change in R due to the dye was evaluated for each cruise.^{1,2} Recently, Yao et al.³ demonstrated that the impurities in the indicator dyes of different manufacturers cause uncertainties in the measured pH values. Thus, the equation provided by Yao et al.³ was also applied to harmonize the spectrophotometric pH measurements (pH_{meas}) using the Clayton and Byrne¹ parameterizations. Equation SI.1 shows the pH values used in this work:

$$\text{pH} = \text{pH}_R - [0.0010 + 0.0008 \times (\text{pH}_{\text{meas}} - 7.2) + 0.0042 \times (\text{pH}_{\text{meas}} - 7.2)^2] \quad (\text{SI.1})$$

A more recent parameterization⁴ using purified mCP allowed us to estimate the effect of impurities in the Sigma-Aldrich mCP used for the shipboard pH measurements. The pH measured using the Sigma-Aldrich mCP were, on average, 0.004 ± 0.002 (N = 8) higher than those using a purified mCP and the parameterizations reported by Lui et al.⁴ in the pH range from 7.5 to 8.15, which is within our uncertainty estimate of the pH. This bias leads to a small effect ($+1.2 \mu\text{mol} \cdot \text{kg}^{-1}$) in the $[\text{CO}_3^{2-}]$ values computed from pH and A_T .

A_T sampling and measurements: Unfiltered seawater samples were directly taken from Niskin bottles and transferred to 600 mL borosilicate glass bottles.⁵ We washed the sampling bottles twice with the sample before filling each bottle from the bottom using a silicone pipe, overflowing half the equivalent volume of the bottle, and immediately stoppering. The samples were stored for at least 24 hours before the analyses. Measurements of A_T were performed using the one-endpoint method⁶⁻⁸, and A_T was measured using an automatic potentiometric titrator. A gravimetrically calibrated Knudsen pipette was used to transfer the seawater samples from the borosilicate glass bottles to an open Erlenmeyer flask in which the potentiometric titration was performed to a final pH of 4.40 using HCl (0.1 M) as the titrant.⁶ To estimate the accuracy of the A_T method, Certified Reference Material (CRM; distributed by A.G. Dickson from the Scripps Institution of Oceanography; batches 84, 99, 108, 118 in CAIBOX, MOC2, HOTMIX and

OVIDE, respectively) analysis was also performed. In addition, an additional calibration (substandard) was performed using a closed container of 50 L of filtered and nutrient-exhausted open ocean surface water in each cruise. This substandard was analyzed to assess possible daily drifts of the electrode and to double check the CRM single analysis measurements. Two replicates of each A_T sample, CRM and the substandard were measured, resulting in a standard deviation no greater than $2 \mu\text{mol}\cdot\text{kg}^{-1}$ between replicates.

Perturbation CO_3^{2-} measures: During the $[\text{CO}_3^{2-}]$ measurements, small perturbation in $[\text{CO}_3^{2-}]$ of the sample occur. Byrne and Yao⁹ and Easley et al.¹⁰ observed no discernible perturbations. However, Fajar¹¹ detected a small acidification of the samples (~ 0.01 pH units), which could imply a change in $[\text{CO}_3^{2-}]$ between 1.8 and 4 (0.4%) depending on the sample pH. The parameterizations of Easley et al.¹⁰ include the effect of this perturbation because they fit the parameters of equation (1) using the absorbance measurements and $[\text{CO}_3^{2-}]_{\text{calc}}$ from pH and A_T . Any further changes in the set of equations and constants used to compute $[\text{CO}_3^{2-}]_{\text{calc}}$ from pH and A_T should require reformulation of the parameters of equation (1). In fact, the universal ratio of boron to salinity has recently increased by 4%, which implies changes in $[\text{CO}_3^{2-}]_{\text{calc}}$ of less than $1 \mu\text{mol}\cdot\text{kg}^{-1}$. Moreover, the use of the new equilibrium constants for carbonic acid, as reported by Millero et al.,¹² would lead to slight changes (0.4%) in $[\text{CO}_3^{2-}]_{\text{calc}}$.

Table S1. Double-beam spectrophotometers and their precision.

Cruise	CO ₃ ²⁻ Spectrophotometer	Precision (μmol·kg ⁻¹)	Cruise	pH Spectrophotometer
CAIBOX/OVIDE	Perkin Elmer λ 800	1.9	CAIBOX/MOC2	Shimadzu UV 2401
MOC2	Shimadzu UV 2401	2.9	OVIDE	Perkin Elmer λ 800
HOTMIX	Shimadzu UV-2600	2.2	HOTMIX	Shimadzu UV-2600

Figure S1. Vertical distributions (Y-axis is depth in meters, X-axis is longitude or latitude depending on the cruise) for measured pH on the total scale at 25°C (A, C, E, G) and measured total alkalinity in $\mu\text{mol}\cdot\text{kg}^{-1}$ (B, D, F, H) for the HOTMIX, CAIBOX, OVIDE and MOC2 cruises.

Figure S2. Excess of the $[\text{CO}_3^{2-}]$ (in $\mu\text{mol}\cdot\text{kg}^{-1}$) over the aragonite saturation under *in situ* conditions along the equatorial transect of the MOC2 cruise (X-axis is longitude).

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