

The CO₂ system

(M. Álvarez, H. San León Bartolomé, T. Tanhua)

Along the HotMix cruise water column samples were taken and analyzed on board for pH, alkalinity and the concentration of carbonate ion (CO₃²⁻) on every station and depth. Scattered water column samples for Dissolved Inorganic Carbon (DIC) were taken and stored to be analyzed in the GEOMAR CO₂ lab (Kiel, Germany). Surface partial pressure CO₂ (pCO₂) data were automatically measured on board with the pCO₂ General Oceanics system facility on board the Sarmiento de Gamboa.

pH determination

Spectrophotometric pH in seawater was measured following Clayton and Byrne (1993) at every station and depth along the HotMix cruise and reported at 25°C and on the Total scale, hereinafter pH_{25T}.

Sampling

pH was sampled after Dissolved Inorganic Carbon (DIC), which was also immediately taken after dissolved oxygen and the tracers, when sampled. Samples were collected in cylindrical optical glass 10-cm pathlength cells, which were filled to overflowing and immediately stoppered. After sampling the cells are immediately stabilised at 25°C.

Analytical method.

Seawater pH was measured using a double-wavelength spectrophotometric procedure (Byrne, 1987). The indicator was a solution of m-cresol purple (Sigma Aldrich) prepared in seawater (2 mM). The indicator was kept in a blood bag out of contact with the air and light.

All the absorbance measurements were obtained in the thermostatted (25±0.2 °C) cell compartment of a SHIMADZU UV-2600 double beam spectrophotometer. The temperature was controlled with a POLYSCIENCE (12L) thermostatic bath.

After blanking with the sampled seawater without dye, 50 µl of the dye solution were added to each sample using an adjustable repeater pipette (Eppendorf Multipette plus). The absorbance was measured at three different fixed wavelengths (434, 487.6, 578 and 730 nm), pH, on the total hydrogen ion concentration scale, is calculated using the following formula (Clayton and Byrne, 1993):

$$\text{pH}_T = 1245.69/T + 3.8275 + (2.11 \cdot 10^{-3})(35-S) + \log((R-0.0069)/(2.222-R \cdot 0.133))$$

where R is the ratio of the absorbances (A) of the acidic and basic forms of the indicator corrected for baseline absorbance at 730 nm ($R = (578A - 730A) / (434A - 730A)$), T is temperature in Kelvin scale and S is salinity. Measurements at the isosbestic point 487.6 nm are used to control the amount of dye added.

As the injection of the indicator into the seawater perturbs the sample pH slightly, the absorbance ratios measured in the seawater samples (R_m) should be corrected to the R values that would have been observed in an unperturbed analysis (R_{real}). In order to do this, we obtain the correction in the absorbance ratio of every sample as a function of the absorbance ratio measured (R_m). This linear function was calculated from second additions of the indicator over samples with a wide range of pH:

$$R_{real} = R_m - (-0.0048 \pm 0.0013 \cdot R_m + 0.0033 \pm 0.0022); \quad r^2 = 0.10, n = 135$$

This function also corrects for deviations in the linear relationship between absorbance and the indicator concentration; i.e., deviations from the Beer Law in the spectrophotometer. Figure 1 shows the relationship between the first addition ratio (R_1) and the ΔR ($R_2 - R_1$) for the set of double additions over a range of R , equivalent to pH.

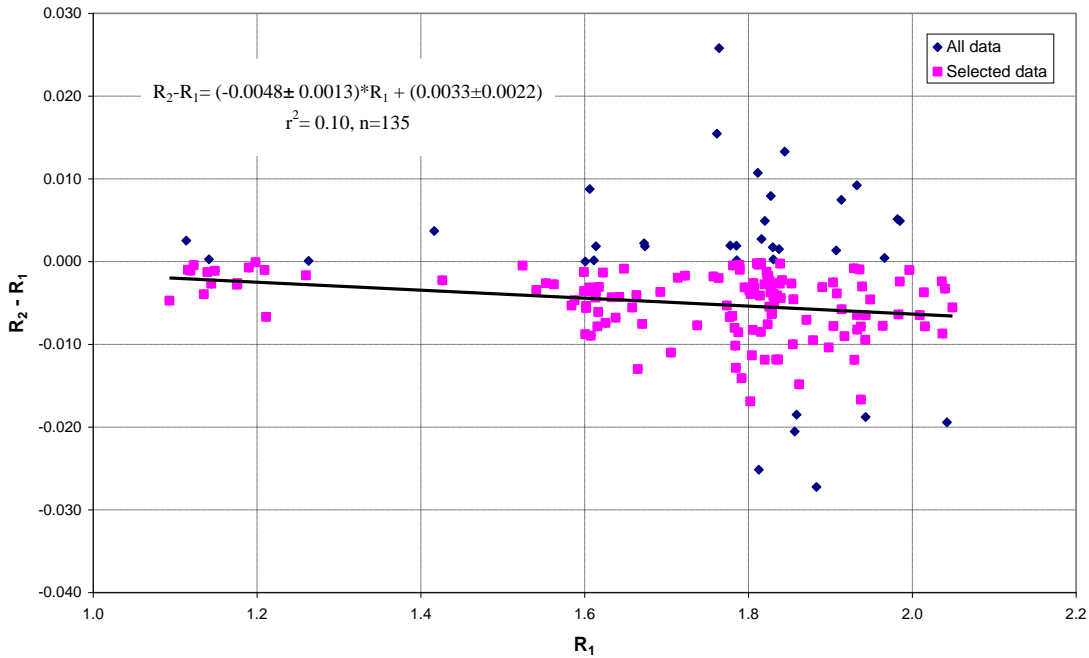


Figure 1. Perturbation of sample pH induced by addition of indicator, expressed as ΔR ($=R_2 - R_1$) as a function of R_1 . R_1 is the first addition and R_2 the double addition. R is the ratio between absorbances $((578A - 730A)/(434A - 730A))$.

All the pH measurements are referred to 25°C and corrected for the addition of the indicator using the former formula. The magnitude of that correction over our range of pH is small ranging from 0.0008 to 0.0016 pH units, which are added to the uncorrected pH values.

Accuracy.

In order to check the precision of the pH measurements, samples of CO₂ reference material (CRM, batch 117, distributed by A.G. Dickson from the Scripps Institution of Oceanography, SIO) were analyzed during the cruise. Ten to fifteen samples from a CRM bottle (batch 117, certified chemical characteristics for salinity, 33.503; silicate, 1.7 $\mu\text{mol kg}^{-1}$; nitrate, 1.14 $\mu\text{mol kg}^{-1}$; nitrite, 0.00 $\mu\text{mol kg}^{-1}$; phosphate, 0.37 $\mu\text{mol kg}^{-1}$; total alkalinity, 2239.18 \pm 0.64 $\mu\text{mol kg}^{-1}$; and total inorganic carbon, 2009.99 \pm 0.52 $\mu\text{mol kg}^{-1}$) were drawn carefully to avoid bubbles and analysed for pH using the spectrophotometric method. The corresponding theoretical pH_{25T} value for this batch using the dissociation constants from Mehrbach *et al.* (1973) refitted by Dickson & Millero (1987) is 7.9424.

The series of CRMs measurements analysed for pH are shown in Table 1. Our pH measurements are lower than the theoretical value. This issue will be commented when the complete set of CO₂ measurements are measured and reported, and the corresponding internal consistency analysis is done.

Table 1. Mean and standard deviation (STD) values for the CRM batch 117 determinations on pH. N stands for the number of measurements and pH diff is the difference with the theoretical value using the Mehrbach *et al.* (1973) refitted by Dickson and Millero (1987) CO₂ constants.

pH _{25T}	STD	N	pH diff
7.9325	0.0016	11	-0.0099

Reproducibility

Regarding the reproducibility of our measurements, two times along the cruise when enough water and time was available, we analysed several samples collected from the same Niskin bottle (Table 2). The mean of the STD is \pm 0.0014 which could be considered as the reproducibility of pH measurements during the cruise.

Table 2. Characteristics of the replicate samples taken along the cruise, the mean and standard deviation (STD) and number of cells collected from each bottle for the pH analysis.

Station_Cast	Niskin	Pressure (dbar)	Salinity	pH _{25T}	STD	N
24_1	8	551	38.510	7.8710	0.0007	8
20_1	24	1000	38.497	7.9036	0.0020	5

Alkalinity determination

Total Alkalinity (TA) along the HotMix cruise was analysed following a double end point potentiometric technique by Pérez y Fraga (1987) further improved in Pérez et al. (2002). This technique is faster than the whole curve titration but comparable (Mintrop et al., 2000).

Sampling.

Seawater samples for TA were collected after pH samples, in 600 ml borosilicate bottles and stored in the laboratory until analysis, usually no later than 2 days. Samples were filled to overflowing and immediately stopped.

Analytical method

TA was measured using an automatic potentiometric titrator "Titrand 909 Metrohm", with a Metrohm Aquatrode Plus 6.302.6150 combination glass electrode and a Pt-1000 probe to check the temperature. The system is coupled with a 5 mL exchangeable unit. Potentiometric titrations were carried out with hydrochloric acid ($[HCl] = 0.1N$) to a final pH of 4.40 (Pérez and Fraga, 1987). The electrodes were standardised using an ftatalate buffer of pH 4.42 made in CO₂ free seawater (Pérez *et al.*, 2002). Concentrations are given in $\mu\text{mol kg}^{-1}$. Table 3 shows the value of the asymmetrical pH (pHas), which is the value of the electrode pH after its calibration. The 0.1N hydrochloric acid was prepared mixing 0.5 mol (18.231 g) of commercially HCl supplied by Riedel-deHaën® (Fixanal 38285) with distilled water into a graduated 5-L beaker at controlled temperature conditions. The HCl normality is exactly refereed to 20°C. The variation of salinity after the titration is lower than 0.1 units, which is taken into account in the final TA calculation.

Accuracy

CRM analyses were performed in order to control the accuracy of our TA measurements (Figure 2). Accordingly, the final pH of every batch of analyses was corrected to obtain the closest mean TA on the CRM analyses to the certified value. Table 3 shows the pH (ΔpH) correction applied to each batch and the mean value of the CRM determinations after applying the former correction.

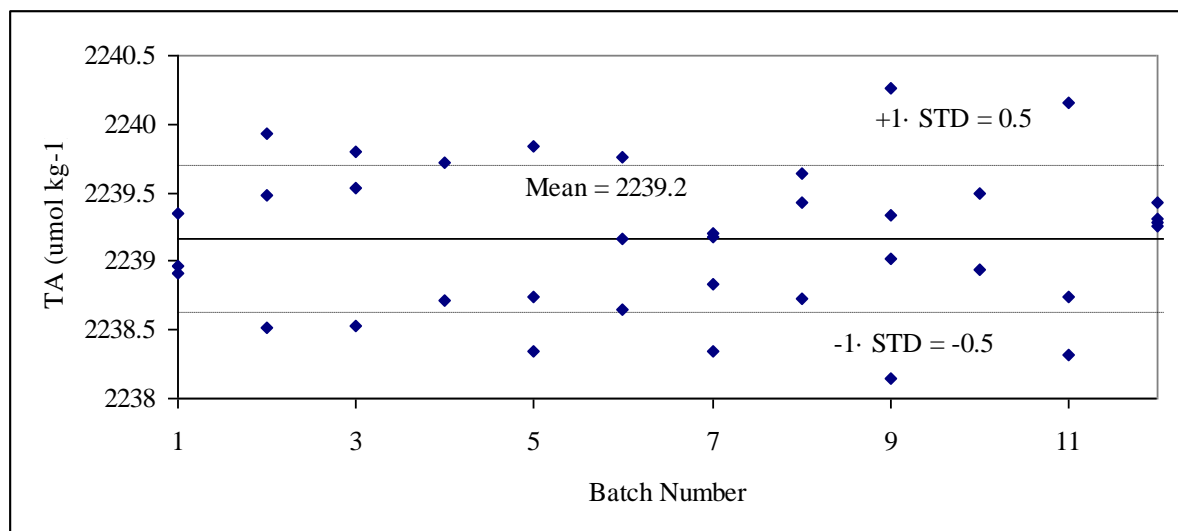


Figure 2. Alkalinity ($\mu\text{mol kg}^{-1}$) measurements on the CRM batch 108 during the cruise against station number. The final mean and standard deviation (STD) for the 65 determinations was $2239.2 \pm 0.5 \mu\text{mol kg}^{-1}$.

Usually, each sample is analysed twice for alkalinity. Table 3 shows the average standard deviation of the replicates analysed during each batch of analysis. This difference was about $1.0 \mu\text{mol kg}^{-1}$. In order to check the precision of the TA measurements, surface seawater was used as a “quasi-steady” seawater substandard (SB). It consists in surface seawater taken from the non-toxic supply and stored in the dark into a large container (35 L) during 2 days before use. This substandard seawater was analyzed at the beginning and at the end of each batch of analyses to control the drift in the analyses for each batch. The estimated drift for each day was very low.

Table 3. Alkalinity analysis supplementary information for each batch of analysis: N_{HCl} is the normality referred to 20°C of the hydrochloric solution used; ΔpH is the pH correction applied to refer the TA determinations on the CRM to the corresponding nominal value (batch 117 with a certified TA of $2239.18 \pm 0.64 \mu\text{mol kg}^{-1}$). The mean value of the TA measurements on the CRM samples is also shown (Fitted TA \pm standard deviation (number of analysis)). The average of the difference (Av. Dif.) in the duplicate's analyses is shown.

Batch	April-May 2014	Stations	N_{HCl}	ΔpH	Fitted TA	Av. Dif.
1	30	1-2	0.099959	0.027	$2239.08 \pm 0.24(3)$	0.6
2	02	3-4	0.099959	0.020	$2239.31 \pm 0.72(3)$	1.0
3	04	5-6	0.099959	0.020	$2239.29 \pm 0.67(3)$	0.7
4	06	7-8	0.099959	0.017	$2239.21 \pm 0.71(2)$	0.8
5	08	9-10	0.099959	0.025	$2238.97 \pm 0.77(3)$	1.0
6	11	11-12-13	0.099959	0.026	$2239.19 \pm 0.56(3)$	0.7
7	14	14-15-16	0.099959	0.026	$2238.89 \pm 0.4(4)$	0.7
8	17	17-18-19	0.099959	0.040	$2239.27 \pm 0.48(3)$	0.6
9	20	20-21-22	0.099959	0.028	$2239.19 \pm 0.87(4)$	0.6
10	23	23-24	0.100156	0.026	$2239.21 \pm 0.39(2)$	0.7
11	25	25-26	0.100156	0.032	$2239.07 \pm 0.44(3)$	0.5
12	28	27-28-29	0.100156	0.030	$2239.32 \pm 0.07(4)$	0.7

Reproducibility

We analysed several samples collected from the same Niskin bottle (Table 4), the obtained STD value of $\pm 0.8 \mu\text{mol kg}^{-1}$ could be considered as the reproducibility of TA measurements during the cruise.

Table 4. Characteristics of the replicate samples taken along the cruise, the mean and standard deviation (STD) and number of analysis performed from each bottle for the TA analysis.

Station_Cast	Niskin	Pressure (dbar)	Salinity	TA $\mu\text{mol kg}^{-1}$	STD	N
19_1	13	2001	38.479	2583.4	0.8	11

Dissolved Inorganic Carbon sampling and storage

Samples for DIC were taken at selected depths during the cruise and properly stored to be finally measured at the GEOMAR lab using a coulometric technique, SOMMA unit.

Samples were taken in either 250 or 500 ml borosilicate bottles, which are filled after the oxygen samples and immediately poisoned with saturated HgCl_2 (100 or 200 μL , respectively). A small headspace is allowed in the DIC bottle for the water to expand. The stoppers were greased with Apiezon L and sealed with a rubber band and collar.

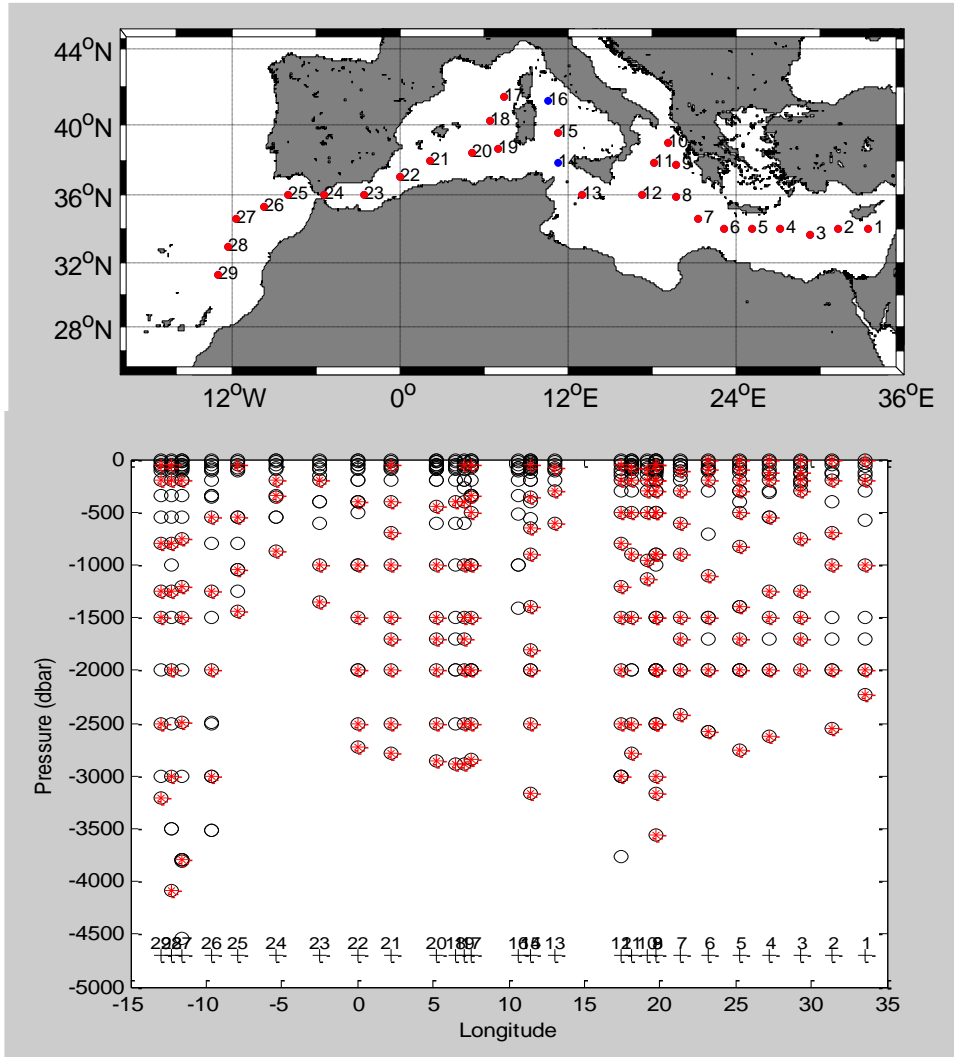


Figure 3. Longitudinal and vertical distribution of the DIC sampling.

Carbonate ion concentration

The carbonate ion concentration (CO_3^{2-}) was determined spectrophotometrically following the recent method first proposed by Yao and Byrne (2008) and further reformulated by Easley et al. (2013).

Sampling.

CO_3^{2-} was sampled after pH. Samples were collected in cylindrical optical quartz 10-cm pathlength cuvettes, which were filled to overflowing and immediately stoppered. After sampling the cells are immediately stabilised at 25°C.

Analytical method

The concentration of CO_3^{2-} in seawater was measured in every sample using the method proposed by Yao and Byrne (2008). A solution of 1.1 mM of the titrant PbCl_2 (Sigma Aldrich, 99.999% purity dissolved in Milli-Q water) is added to the seawater sample, the complex PbCO_3 formed afterwards is detected spectrophotometrically in the UV spectra. All the absorbance measurements were obtained in the thermostatted ($25 \pm 0.2^\circ\text{C}$) cell compartment of a SHIMADZU UV-2600 double beam spectrophotometer. The temperature was controlled with a POLYSCIENCE (12L) thermostatic bath.

After blanking with the sampled seawater without the PbCl_2 solution, 225 μl of the dye solution were added to each sample using an adjustable repeater pipette (Eppendorf Multipette plus). The absorbance was measured at three different three wavelengths (234, 250 and 350 nm), 234 nm is the isosbestic point of PbCO_3 , 250 nm is the mean value of the wavelengths presenting high absorbance variation and 350 nm is a non-absorbing wavelength to monitor PbCO_3 .

Total carbonate ion concentration is given by:

$$-\log([\text{CO}_3^{2-}]_T) = \log_{\text{CO}_3}\beta_1 + \log((R - e_1)/(e_2 - R \cdot e_3))$$

where R is the ratio of the absorbances (A) ($R = (250A - 350A) / (234A - 350A)$), $\text{CO}_3\beta_1$ is the PbCO_3 formation constant, and e_i are the molar absorptivity ratios dependent on salinity. The fitting parameters are taken from Easley et al. (2013). Small deviations from fitting temperature 25°C (maximum 1.5°C) are corrected using the equation from Fajar (2013) where T is temperature in °C:

$$[\text{CO}_3^{2-}]_T / [\text{CO}_3^{2-}]_{25} = -(0.033 \pm 0.001) \cdot (T - 25) + (1.007 \pm 0.002)$$

As determined in Easley et al. (2013) no perturbations are taken into account due to the complexation of free CO_3^{2-} by Pb(II) from the PbCl_2 titrant.

Reproducibility

Regarding the reproducibility of our measurements, once along the cruise when enough water and time was available, we analysed several samples collected from the same Niskin bottle (Table 3). The STD is $\pm 2.2 \mu\text{mol/kg}$ (1.13% precision) which could be considered as the reproducibility of CO_3^{2-} measurements during the cruise.

Table 3. Characteristics of the replicate samples taken, the mean and standard deviation (STD) and number of cells collected from each bottle for the CO_3^{2-} analysis, concentrations in $\mu\text{mol/kg}$.

Station_Cast	Niskin	Pressure (dbar)	Salinity	CO_3^{2-}	STD	N
20_1	24	1000	38.497	194.2	2.2	6

First Quality Control – flags

- QC flags = 3 for pH

Station	Cast	Bottle	Pressure
1	1	7	2000.33
2	1	8	1700.71
5	2	21	3.62
5	3	21	201.17
12	2	3	151.3
15	1	4	3164.72
21	2	5	59.96

- QC flags = 3 for CO_3^{2-}

Station	Cast	Bottle	Pressure
2	1	13	1000.53
3	1	9	1501.47
5	2	21	3.62
6	1	7	2000.7
7	3	7	301.33
8	2	21	4.18
8	2	5	100.53
8	2	3	119.75
9	1	18	500.61
11	2	21	4.55
11	3	11	201.15
12	1	20	1200.95

12	1	4	3770.93
13	1	22	3.97
14	1	11	40.77
14	1	6	200.91
15	1	7	2500.63
19	3	8	400.95
20	2	5	64.53
25	2	2	108.83
28	2	3	110.17
29	2	21	4.05
29	2	1	111.18

- QC flags = 3 for TA

Station	Cast	Bottle	Pressure
8	1	14	2500.42
12	3	22	800.29
23	2	8	50.28

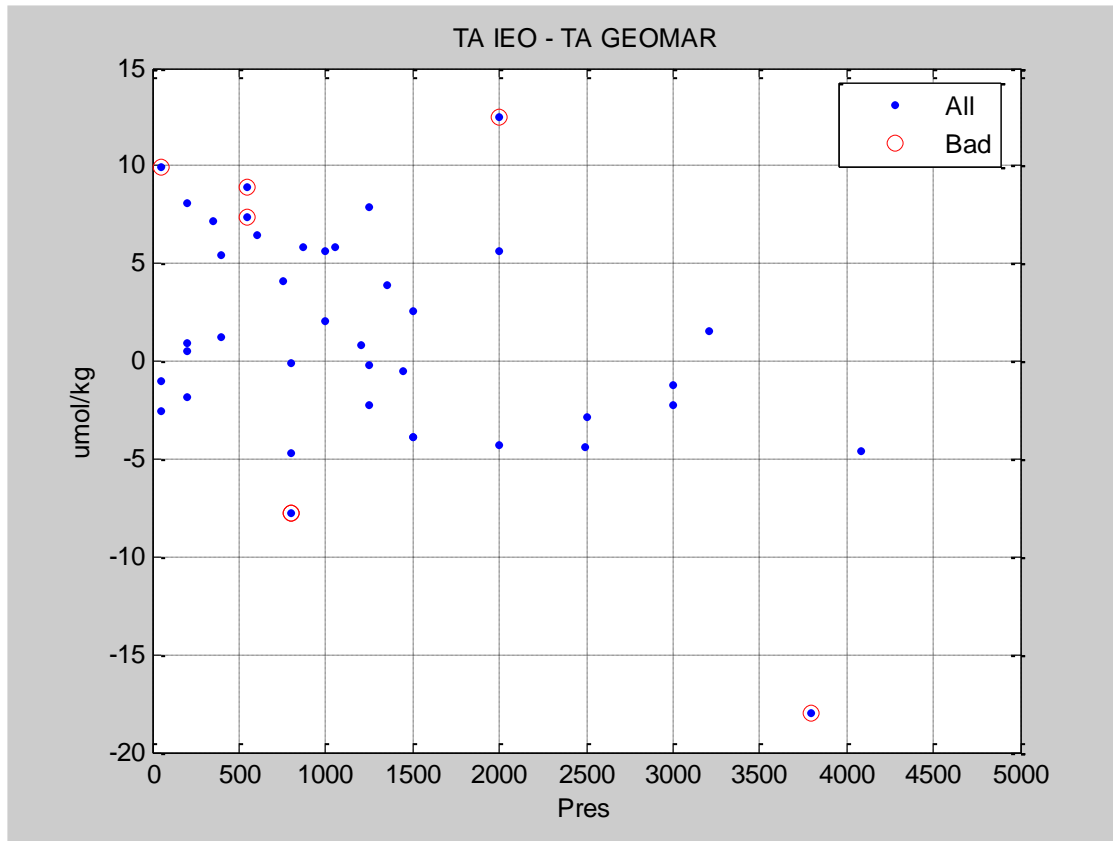
AFTER RECEIVING THE GEOMAR MEASUREMENTS

1- Comparison between IEO and Geomar TA measurements.

The figure below shows the difference between measured TA on board (IEO) and at lab (Geomar), after taking out the IEO or Geomar data considered wrong (red circles), the mean and STD of the difference is $1 \pm 4 \mu\text{mol/kg}$, N=34.

Note that due to different bottle sizes used, only TA_Geomar samples were analyzed after station 22.

According to Toste's excel, a positive correction was applied to the measured samples (3.3 or 6.5 $\mu\text{mol/kg}$) according to a CRM correction (batches used 108 & 135).



2- DIC comparison: IEO (calculated from on board measurements) and Geomar (lab) DIC.

The table below shows the mean and STD of the differences between lab (Geomar) and calculated DIC from measurements (TA and pH25T) on board (IEO) according to different CO₂ constants and two options for the total borate concentration and the sulphate constant. CO₂SYN was used. The table shows only the mean and STD of the DIC residuals after taking out the IEO or Geomar data considered wrong. DIC Geomar was taken all along the HotMix cruise track (see corresponding map above). I consider the Geomar DIC data from stations 1 to 6 bad, as they have a very high CRM correction (>40 μM).

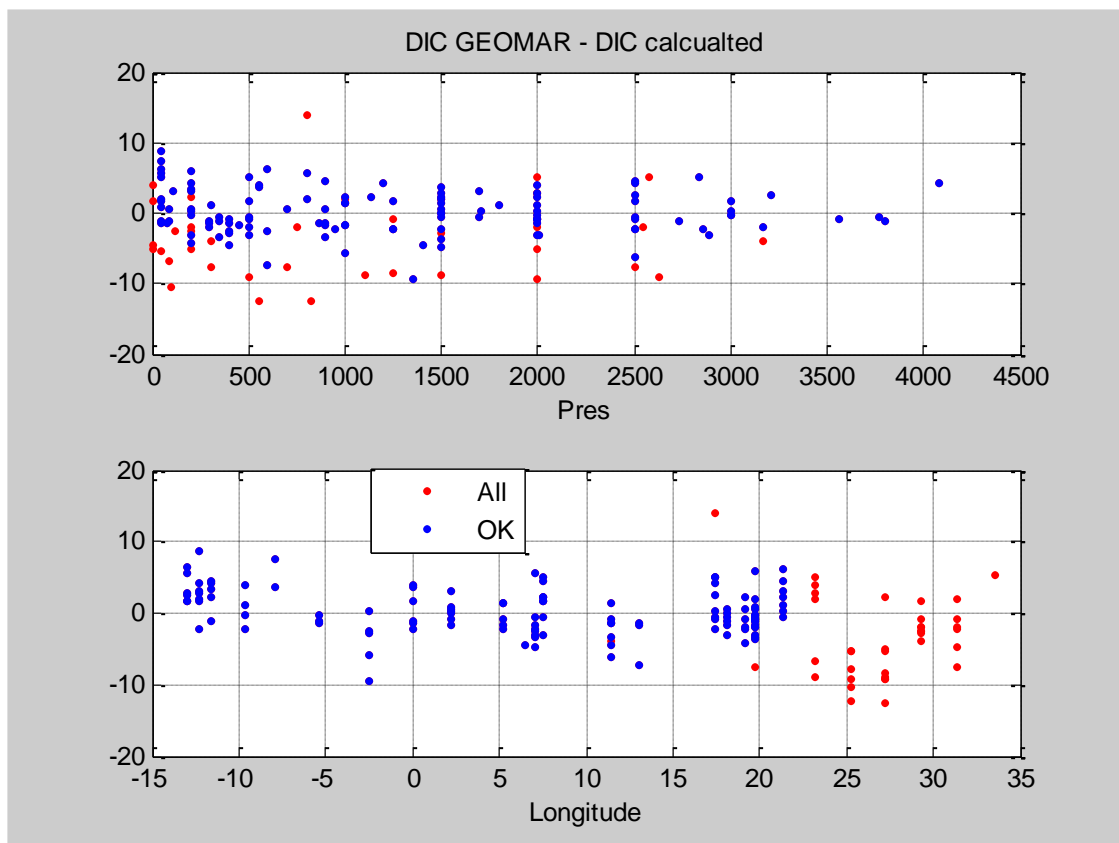
Table 1. Mean values and standard deviation of the residuals (measured minus calculated) for Dissolved Inorganic Carbon (DIC) calculated with different thermodynamic constants for CO₂ according to the CO2SYS program k1k2 options and two options for the sulphate constant and total borate concentration. N= 121 samples. In red the minima.

CO ₂ Constants	kBkHSO ₄ =1	kBkHSO ₄ =3
1	14 ± 3.4	17 ± 3.6
2	9.9 ± 3.2	13 ± 3.4
3	4.6 ± 3.1	7.6 ± 3.2
4	-0.2 ± 3.1	3.2 ± 3.1
5	3.5 ± 3.1	6.5 ± 3.1
6	-5.6 ± 3.1	-5.6 ± 3.0
7	3.5 ± 2.9	3.5 ± 2.9
9	-14.6 ± 5.1	-11.5 ± 4.8
10	-0.3 ± 3.1	2.8 ± 3.1
11	9.9 ± 2.9	6.9 ± 3.1
12	-2.2 ± 3.3	11.8 ± 3.6
13	-1.1 ± 3.1	0.8 ± 3.0
14	0.0 ± 3.1	1.9 ± 3.0

1- Roy (1993); 2- Goyet and Poisson (1989); 3- Hansson (1973a,b) refit by Dickson and Millero (1987); 4- Mehrbach et al. (1973) refit by Dickson and Millero (1987); 5- Hansson (1973a,b) and Mehrbach et al. (1973) refit by Dickson and Millero (1987); 6- GEOSECS choice (Takahashi et al., 1982); 7- Peng et al. (1987); 9- Cai and Wang (1998); 10- Lueker et al. (2000); 11- Mojica-Prieto and Millero (2002); 12- Millero et al. (2002); 13- Millero et al. (2006); 14- Millero et al. (2010).

The results during HotMix (2014) are in agreement with those obtained during the M84/3 (2011) cruise where the best sets of constants were either k1k2=4 & kBkHSO₄=1 or k1k2=13 & kBkHSO₄=3.

The figure below shows DIC measured minus calculated for the HotMix (2014) cruise [k1k2=4 & kBkHSO₄=1, N=121, -0.2 ± 3.1].



The figure below shows DIC measured minus calculated for the HotMix (2014) cruise [k1k2=4 & kBkHSO4=1, N=121, 0.2 ± 3.1] and those during the M84/3 (2011) cruise [k1k2=4 & kBkHSO4=1, N=751, -0.1 ± 3.0].

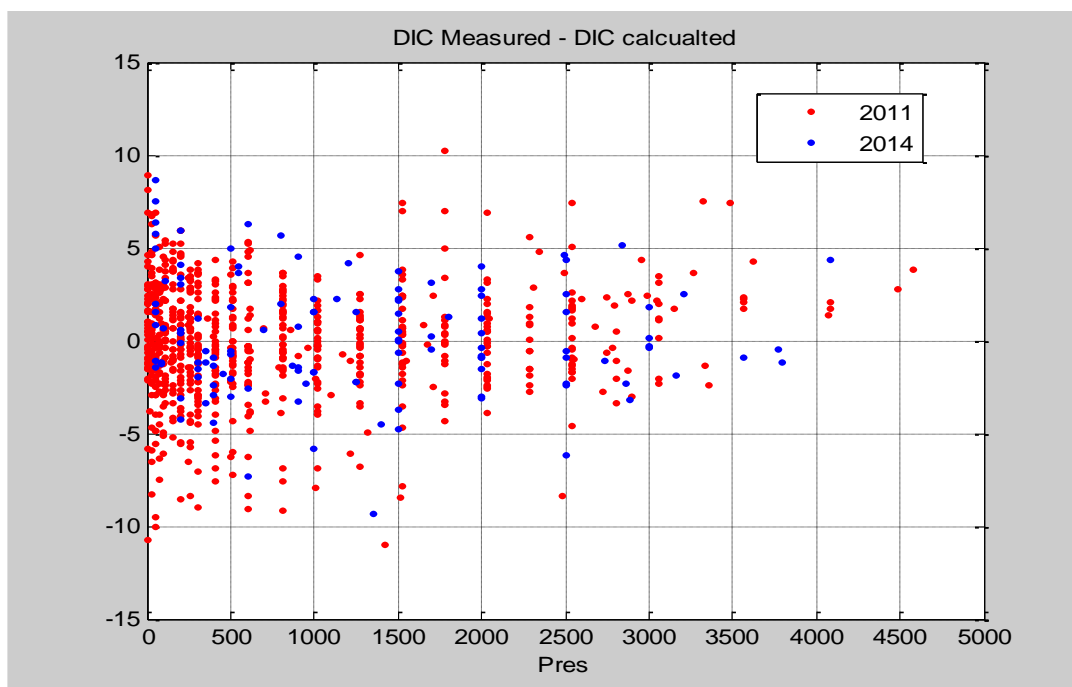
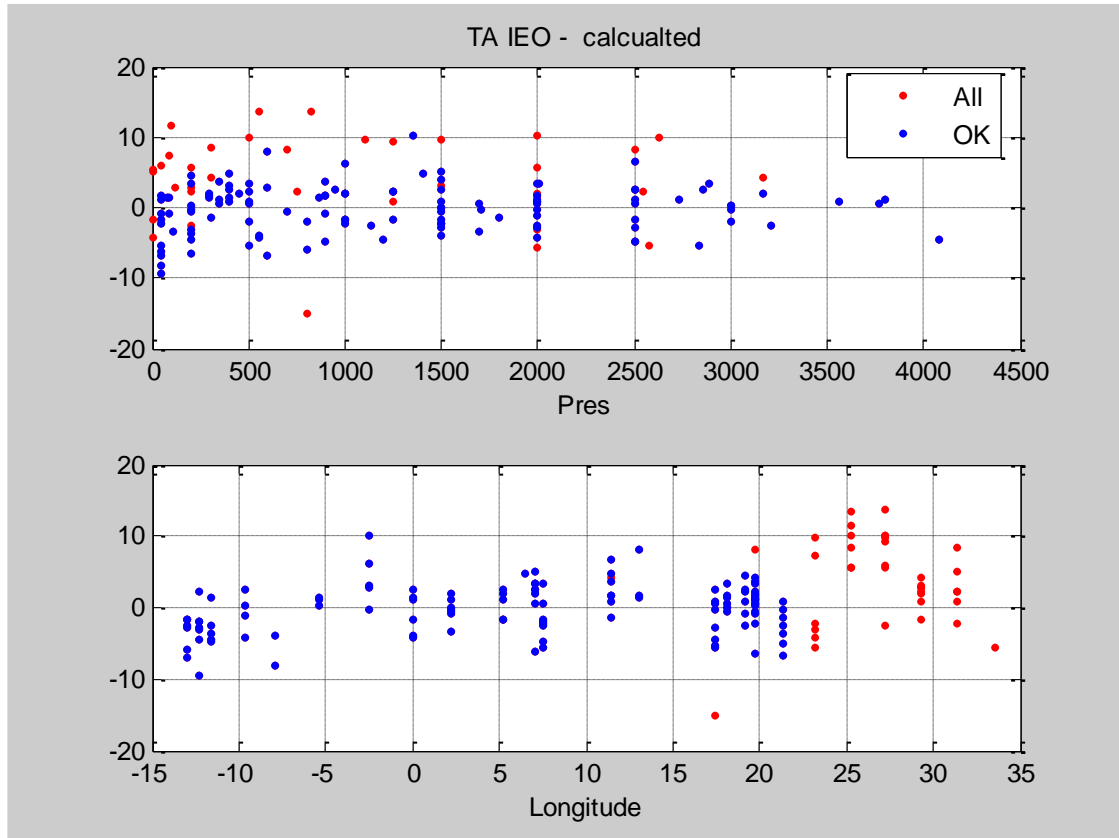


Table 2. Mean values and standard deviation of the residuals (measured minus calculated) for TA calculated with different thermodynamic constants for CO₂ according to the CO2SYS program k1k2 options and two options for the sulphate constant and total borate concentration. N= 121 samples. In red the minima. Only considered good data for DIC Geomar, pH y TA were included.

CO ₂ Constants	kBkHSO4=1	kBkHSO4=3
1	-15.5±3.8	-18.8±4
2	-10.8±3.6	-14.1±3.8
3	-5±3.4	-8.2±3.5
4	-0.2±3.4	-3.5±3.3
5	-3.8±3.3	-7.1±3.4
6	6±3.3	6±3.3
7	-3.8±3.2	-3.8±3.2
9	15.7±5.6	12.4±5.3
10	0.3±3.4	-3±3.4
11	-4.2±3.2	-7.5±3.4
12	-9.5±3.7	-12.8±4
13	2.4±3.3	-0.9±3.3
14	1.2±3.3	-2.1±3.3

The figure below shows TA measured minus calculated for the HotMix (2014) cruise [k1k2=4 & kBkHSO4=1, N=121, -0.2 ± 3.4].



3- $[\text{CO}_3^{2-}]_T$ comparison: calculated indirectly (f-pH&TA, f-pH&DIC_Geomar) and directly measured.

In the calculation of $[\text{CO}_3^{2-}]_T$ the $k_b k_{\text{HSO}_4}$ used does not matter.

Within CO2SYS $[\text{CO}_3^{2-}]_T$ is calculated within the

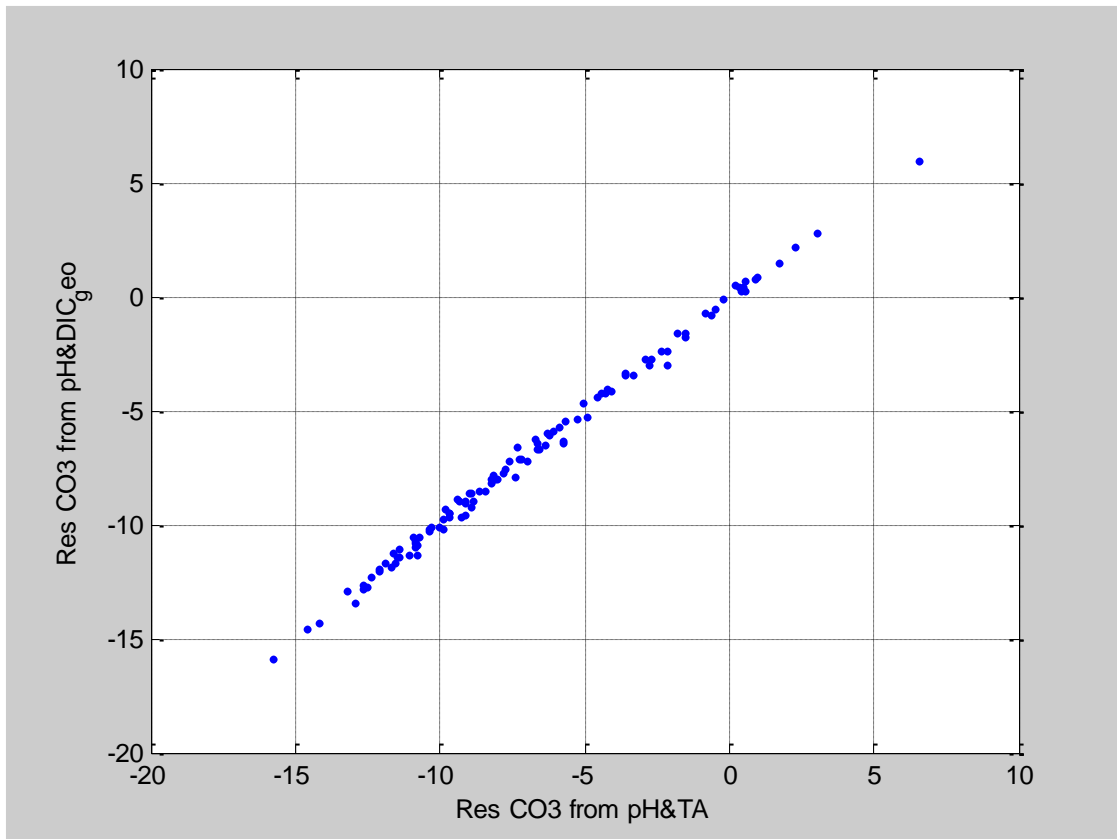
function varargout=CalculateAlkParts(pHx, TCx) where

$$\text{CO}_3 = \text{TCx} \cdot K_1 \cdot K_2 / (K_1 \cdot H + H \cdot H + K_1 \cdot K_2);$$

So from the pair pH&TA, DIC (=TCx) is first calculated, from the pair pH&DIC, carbonate is directly calculated with the above formula.

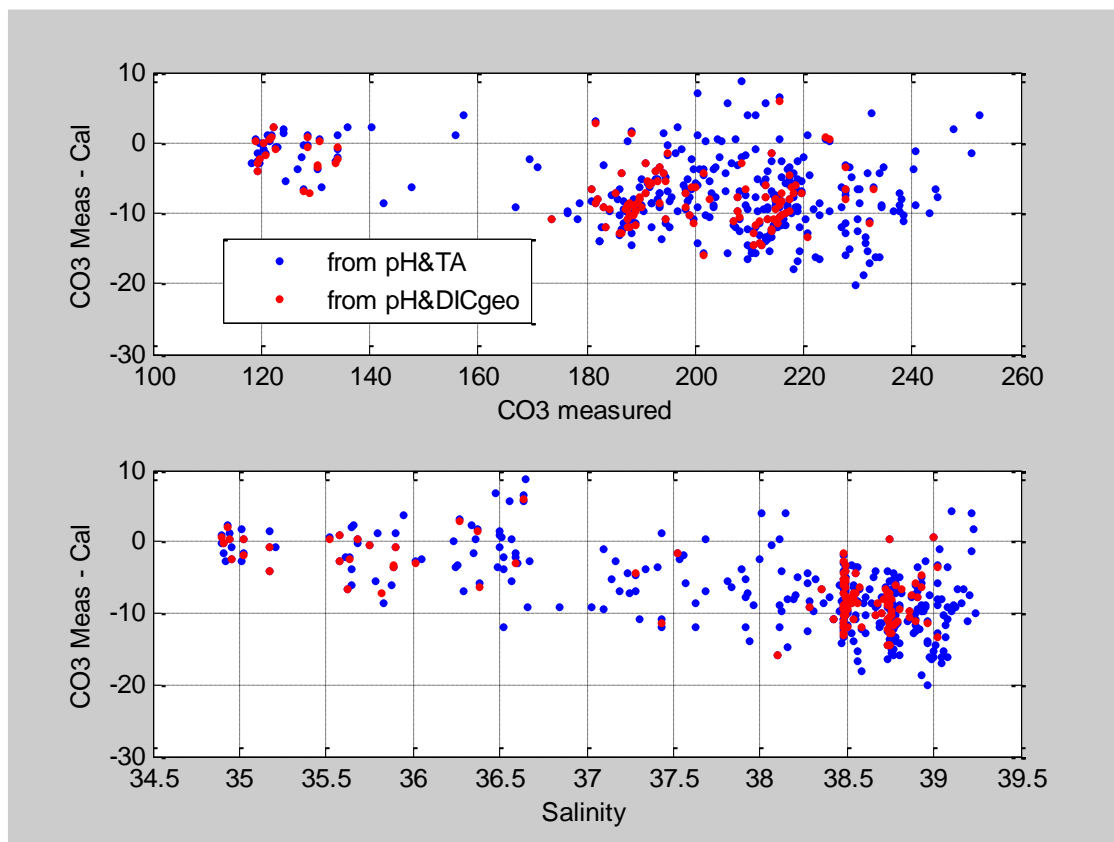
In the case of the HotMix data, the residuals of measured and calculated carbonate either from pH&TA or pH&DIC_geomar are practically equal with a regression line of practically 1:1 (see figure below).

$$\text{ResCO}_3\text{pH\&DIC} = 0.988 \cdot \text{ResCO}_3\text{pH\&TA} - 0.1 \quad (R^2=0.9967, N=118)$$



The figure below shows the distribution of the CO_3^{2-} residuals (measured – calculated) with the spectrophotometrically measured CO_3^{2-} and with salinity. Option k1k2=4 &

kBkHSO4=1 was used. Only CO_3^{2-} , pH, TA (N=345) and DIC_geo (N=118) data good were considered in this analysis.



The salinity dependence of the CO_3^{2-} residuals is clear in the figure above and the table below, which is not detected in the DIC residuals. Option k1k2=4 & kBkHSO4=1 was used.

	All data	Sal<36	Sal>=36
DIC	-0.2 ± 3.1 (N=121)	1.9 ± 2.2 (N=18)	--0.1 ± 3.1 (N=103)
CO3 from pH&TA	-7.2 ± 5.1 (N=345)	-1.2 ± 2.8 (N=39)	-7.9 ± 4.9 (N=306)
CO3 from pH&DICgeo	-6.9 ± 4.4 (N=118)	-1.6 ± 2.5 (N=20)	-8 ± 3.9 (N=98)

References

- Byrne R. H. (1987). Standardization of standard buffers by visible spectrometry. *Analytical Chemistry*, 59, 1479-1481.
- Byrne, R. H. and Yao, W. (2008). Procedures for measurement of carbonate ion concentrations in seawater by direct spectrophotometric observations of Pb(II) complexation, *Marine Chemistry*, 112(1-2), 128–135, doi:10.1016/j.marchem.2008.07.009.
- Clayton and Byrne (1993). Spectrophotometric seawater pH measurements: total hydrogen ion concentration scale concentration scale calibration of m-cresol purple and at-sea results. *Deep-sea Research I*, Vol. 40, 10, 2115-2129.
- Dickson, A.G., Millero, F.J. (1987). A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep-Sea Research* 34, 1733–1743.
- Easley, R.A., Patsavas, M.C., Byrne, R. H., Liu, X., Feely, R.A., Mathis, J.T. (2013). Spectrophotometric measurement of calcium carbonate saturation states in seawater. *Environ. Sci. Technol.*, 47 (3), 1468–1477.
- Merzbach, C., Culberson, C.H., Hawley, J.E., Pytkowicz, R.M. (1973). Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnology and Oceanography*, 18, 897–907.
- Mintrop, L., F. Fernández-Pérez, M. González Dávila, A. Körtzinger and J.M. Santana Casiano (2000). Alkalinity determination by potentiometry- intercalibration using three different methods. *Ciencias Marinas* , 26 , 23-37.
- Pérez, F.F. and F. Fraga (1987). A precise and rapid analytical procedure for alkalinity determination. *Marine Chemistry*, 21, 169-182
- Pérez, F.F., A.F. Ríos, T. Rellán and M. Álvarez (2000). Improvements in a fast potentiometric seawater alkalinity determination. *Ciencias Marinas*, 26, 463-478.