MEMORIA DE PROYECTO DEL INVESTIGACIÓN

STABLE ISOTOPIC SIGNATURES OF DIC IN THE WESTERN MEDITERRANEAN BASIN

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ABSTRACT

Evolution on isotopic composition of dissolved organic carbon (DIC) affected only by biological processes was studied at laboratory. Strong relations for isotopic carbon of DIC (δ^{13}C DIC) and concentration of nutrients were observed, especially for phosphates. This experimental study helped to understand processes affecting isotopic composition of DIC across the Western Mediterranean Sea. For this reason, carbon isotopic composition was measured in samples collected during Thresholds 2006 cruise, from Majorca to the Strait of Sicily. Two depths were sampled, surface seawater (δ^{13}C DIC=0.69±0.04‰) and deep chlorophyll maximum (δ^{13}C DIC=0.53±0.03‰); the different processes involving changes in carbon isotopic composition, isotope effects, were studied. From analysis of δ^{13}C values and biological variables was observed that biological activity predominantly influences in the vertical distribution of δ^{13}C values. These variables, e. g. AOU, chlorophyll and concentration of phosphates, all showed good relations when plotted against isotopic composition, and explained the dominance of biological activity. ^{13}C Suess effect was calculated by comparing our data from that obtained by Pierre (1999)in 1988, δ^{13}C DIC value has decreased -0.036 ± 0.004‰ yr^{-1} during the last two decades in the Western Mediterranean Sea. When the result obtained for Suess effect is compared with results for other oceanic regions, it seems that Western Mediterranean Suess effect is higher but it is not possible to assure due to seasonality and possible analytical errors. The result of Western Mediterranean Sea implies an anthropogenic CO2 sink role for this region since carbon isotopic composition of DIC has experiment a decrease over the years.

Keywords: Western Mediterranean Sea, dissolved inorganic carbon, carbon isotopic composition, biological activity, Suess effect.

INTRODUCTION

Since the Industrial Revolution, two hundred years ago, human activities have caused an increment in CO_2 atmospheric concentration from 280 ppm in 1750 to 380 ppm in 2005 (Solomon et al., 2007). The rise of atmospheric CO_2 due to these activities can be explained by the change in atmospheric carbon isotopic composition ($\delta^{13}C$) towards values more negative, demonstrating fossil fuel presence in the atmosphere. Even though, these values represent only 50% of the anthropogenic emissions, the other 50% has been taken up by the ocean and the land biosphere. The natural processes occurring in the carbon cycle result in massive exchanges between these three carbon reservoirs.

Oceans are probably the most significant carbon reservoirs since they are responsible of 30% of the emission uptake (Sabine et al. 2004), and dissolved inorganic carbon (DIC) in seawater is approximately 50 times that of the atmosphere (Falkowski et al., 2000). When CO_2 enters the ocean, forms a weak carbonic acid which reacts with carbonate ions to form bicarbonate ions; this buffer reaction changes dissolved CO_2 concentration and enables high levels of oceanic uptake of CO_2, absorbing part of the anthropogenic emissions. This absorption is possible due to a chemical disequilibrium between ocean and atmosphere, which is only reached after several hundred years (Sarmiento and Gruber, 2006).

Oceanic DIC reservoir can be divided in three different parts according to water column depth; surface seawater, intermediate and deep water and, finally surface sediments. The largest reservoirs are intermediate and deep waters which contain approximately 37,000Gt C compared to surface seawater with 900Gt C (Sabine et al., 2004). Although, values in surface are not significant compared to intermediate waters, their importance is related to processes taking place in these waters, air-sea exchange and strong biological activities. These two processes along with anthropogenic emissions produce fluxes between atmosphere surface seawater and intermediate and deep water; where DIC can be storage over hundreds of years. During photosynthesis of phytoplankton, CO_2 is fixed and dissolved organic carbon (DOC) is released and sunk; where it is consumed by bacterial respiration and transformed to DIC in deep waters or sediments (Ducklow et al., 2001). Air-sea exchange is also very important since atmospheric CO_2 enters the ocean by this process; the direction of the net air-sea exchange is set by differences in partial pressure of CO_2 (pCO_2) (Siegenthaler and Sarmiento, 1993). Then,

slightly basic pH and biological activity in temporal geochemical sceneries with low pCO_2 relative to that of the atmosphere, will act as a sink of atmospheric CO_2, whereas temporal episodes and/or regions with high pCO_2 will act as a source of CO_2 to the atmosphere. In this process, solubility is also important; CO_2 is more soluble in waters characterized by low temperatures and high levels of salinity; when atmospheric CO_2 enters the oceans, it tends to sink with cold and dense water forming deep water which will capture atmospheric CO_2 (Falkowski et al., 2000). Finally, it is expected that as atmospheric CO_2 concentration increases, oceanic sink role weakens due to a decrease in the concentration of carbonate ion which enables CO_2 oceanic uptake (Le Quéré et al., 2009).

The factors controlling variations on the $^{13}C/^{12}C$ ratio of DIC, also named fractionation factors due to the discrimination against the heavier isotope during the process; are those which control CO_2 uptake. During C3 plants photosynthesis, ^{12}C is preferred by the rubisco enzyme to synthesize organic matter, causing a relative increase in $\delta^{13}C$ values of DIC; isotopic values of aquatic plants (-19‰ vs. V-PDB) is less negative than in terrestrial plants (about -27‰) (Oleary, 1981) due to algae utilization of ^{13}C enriched HCO_3^- (Hoefs 2009). On the other side, remineralization of organic matter, which is depleted in ^{13}C, causes a decrease in $\delta^{13}C$ values of DIC (Lynch-Stieglitz et al., 1995).

The isotopic effects of the other two processes which affect CO_2 fluxes and hence, $\delta^{13}C$ distribution of DIC, are extremely related. Atmospheric CO_2 from fossil fuel emissions has a very negative $\delta^{13}C$ value of -27‰ (Hoefs, 2009) due to discrimination against the heavier carbon isotope during photosynthesis of plants forming fossil fuels (Oleary, 1981). For this reason, atmospheric $\delta^{13}C$ value of CO_2 has decreased from -6.3‰ (V-PDB) in pre-industrial levels to -7.8‰ at 1994 (Keeling et al., 1995), and -8.4 ‰ at present, this effect was first described by Suess (1953). Since $\delta^{13}C$ values in the atmospheric CO2 decreases, due to the fossil fuel burning, a negative signature is transmitted to the oceans resulting in a decrease of $\delta^{13}C$ value of DIC. $\delta^{13}C$ value of DIC will experiment an increase in oceanic regions where the net air-sea flux leads to the CO_2 release to the atmosphere, (Kroopnick 1985).

By studying the Suess effect, which is observed in every reservoir of the carbon cycle, it can be confirmed whether an ocean region is acting as a net sink of anthropogenic CO_2 (Bacastow et al., 1996; Key and Schlosser, 1999). Ocean will act as a net source when

the calculated value for Suess effect is positive, whereas negative values of this effect will mean a net sink role of the ocean region. If the time series analysed is large enough, it is possible to search for temporal trends on this effect, if the value rises up with time the ocean role of CO_2 source or sink is being weakened; on the contrary, a decrease in this value when compared in time shows a progressive enhancement of the ocean role. The Suess effect has been studied in multiple ocean regions as Bermuda (Bacastow et al., 1996), North Pacific Ocean (Tanaka et al., 2003) or Nordic Seas (Olsen et al., 2006).

The aim of this study is to characterise carbon isotopic composition of DIC in surface seawaters (SSW) of the Western Mediterranean Sea in order to determine its distribution towards the deep maximum chlorophyll (DCM). For this purpose, a laboratory experiment is carried out to analyze the evolution of the isotopic composition of DIC when biological activity is maximal in Mediterranean waters. Afterwards, $\delta^{13}C$ values of DIC of Western Mediterranean waters are compared with biological variables as apparent oxygen utilization (AOU) and phosphates, since biological processes affect considerably carbon isotopic composition. After describing $\delta^{13}C$ values for Western Mediterranean Sea, a comparison between results obtained in 1988 by Pierre (1999) and our results will be performed, in order to determine the Suess effect for Western Mediterranean Sea.

The Mediterranean Sea is a semi-enclosed oligotrophic sea considered as a net sink for atmospheric CO_2 (Bethoux, 1999). The Western basin is connected to the Atlantic Ocean by the Strait of Gibraltar (300m deep) and to the Eastern basin through the Strait of Sicily (400m deep). There is an Atlantic inflow in the upper part and a Mediterranean outflow in the lower part of the Strait of Gibraltar. This process along with an exceeding evaporation, compared to low precipitation, and river input, characterize the Mediterranean Sea by high evaporation and salinity values. The Mediterranean Sea is also considered a region of deep water formation in the oceans, occurring in the Gulf of Lions for the western basin. This process is influenced by cold and dry winds at winter which cause the mixing and sinking of cold and salty water mass (Zavatarelli and Mellor, 1995). The formation of deep water is crucial for the CO_2 sink role assigned to the oceans as it was explained before; therefore, the importance of understanding all processes involved in air-sea flux of CO_2 and formation of deep waters need to be highlighted as the dynamics of carbon cycle is changing.

METHODS

In order to measure variations in the carbon isotopic composition due to biological processes, we measured unfiltered seawater from Port Des Canonge, Mallorca (39° 41' 57.88"N, 2° 32' 52.20"E). Nutrient solutions were added to 3L of this seawater to enhance phytoplanktonic activity; after mixing, it was placed in a closed system, sealed bags which are impermeable to gas, with no air bubbles and a sampling port to avoid interactions between seawater and atmospheric air during sampling. Three different replicates were set and placed in an illuminated incubator at 27°C, which is the temperature of Mediterranean surface seawater at this time of the year. Samples were collected at the start of the experiment, 48, 120, 168, 216, and 288 and 336 hours after; measurements of carbon isotopic composition, concentration of nutrients and chlorophyll were made. Chlorophyll concentration was determined by fluorometric determination with a Turner Designs Trilogy fluorometer. For this purpose, samples of 200mL were collected, filtered through Wathmann GF/F filters and frozen. After that, 9mL of 90% acetone was added for 24h and centrifuged to extract chlorophyll. For nutrient concentration, samples were collected and analyzed in a Bran Luebe AA3 autoanalyzer.

Samples from Western Mediterranean Sea were collected between June and July 2006 during Thresholds 2006 cruise (O/V García del Cid), at 9 different stations from Balearic Islands to the Strait of Sicily, figure 1. Two samples were taken at each station, one at surface depth (5m) and other one at DCM. Each one was filtered through a 0.45 µm cellulose acetate filter and poisoned, immediately after collection, with mercuric chloride ($HgCl_2$) to avoid biological activity, which would cause isotopic fractionation. At the same time samples were collected, other parameters were measured in the seawater for each sample depth, such as temperature, salinity, dissolved oxygen and concentrations of nutrients.
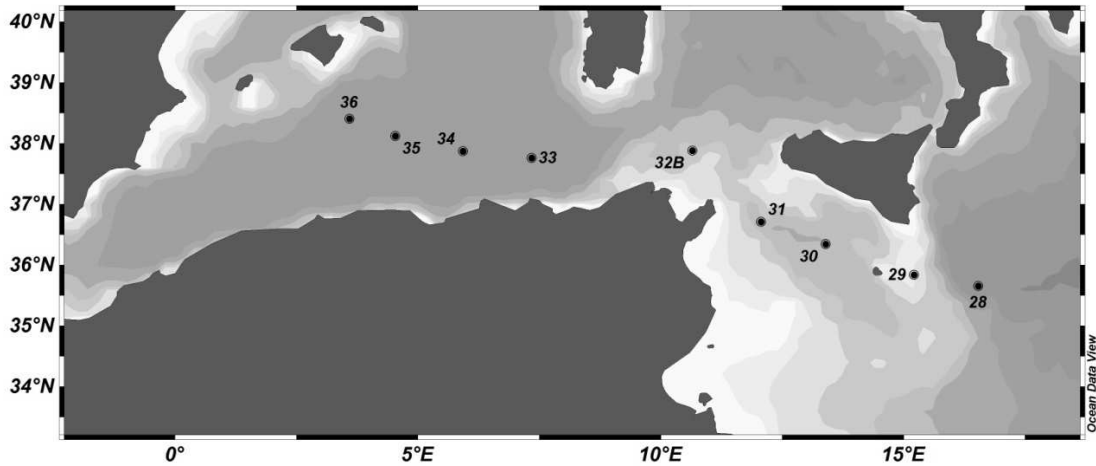
Figure 1. *Location of the 9 stations where samples were collected during Thresholds 2006.*

For measurements of $\delta^{13}C$ value of DIC of seawater, in both experimental and field samples, three aliquots of each sample were reacted in a closed system with 100% pure phosphoric acid to acidify the sample and to react all chemical forms of DIC contained in the water and to measure them as gaseous CO_2. Therefore, chemical forms of DIC are transformed to CO_2, as a consequence of a shift in chemical equilibrium; this CO_2 is easily removed from seawater by gas exchange in the headspace. The reaction took place in a 12 ml tube totally closed which has been filled with 99.99% pure helium and all possible atmospheric CO_2 is removed from the tubes. Samples (0.5 ml) were agitated and injected for reaction with the acid and immediately stirred again. The sample was reacting with phosphoric acid during 24 hours at constant temperature of 25ºC. This is the time needed for the reaction of all chemical forms of DIC with the acid preventing discrimination from exchange between phases.

After reaction, samples were measured in a Finnigan GasBench II attached to a Finnigan DeltaPlusXP isotope ratio mass spectrometer, CO_2 (99,995%) is needed as a reference gas. Data obtained is reported as $\delta^{13}C$ value relative to PDB standard, which is a Cretaceous belemnite from the PeeDee formation of South Carolina, United States:

$$\delta^{13}C = ((R_x - R_{std})/R_{std}) * 1000$$

Where R is the ratio of abundance of the heavy to the light isotope, in this case R is $^{13}C/^{12}C$, x denotes the sample and, std is the reference standard. Therefore, a negative value means that the sample is depleted in ^{13}C respective to the standard gas (Craig, 1957). The analytical precision is 0.05‰ and reproducibility of measurements is <0.1‰.

For the experimental Suess effect in the Mediterranean Sea, differences on values obtained in this study and those of Pierre (1999) were calculated. Sues effect is given as ‰ yr^{-1}.

RESULTS

Laboratory work. Regarding to fractionation in carbon isotopic composition of DIC due to only biological activity, we found an increase in $\delta^{13}C$ values after inducing phytoplanktonic bloom (r = 0.96, p < 0.5), as it is observed in figures 2 and 3. The relation between amplitude obtained during measurements with the spectrometer and the time elapsed is shown in figure 4. This relation is quadratic, and we observe a decrease of amplitude with time; amplitudes are relevant since this parameter is proportional to concentration of DIC; it is observed negative relation between $\delta^{13}C$ DIC values and amplitude, i.e. when amplitudes decay, it is assumed that concentration of DIC decreases. Regarding to concentrations of nutrients, it is noticed good relations with phosphates and with nitrites and nitrates, although no clear relation was found for SiO2 (silica) and for chlorophyll (Figures 5 and 6)

Field work. Samples in Thresholds 2006 cruise were considered as paired samples, since they were collected at different depths but at the same station, after performing a Wilconxon sign-rank test (p=0.006) implying no equals means. $\delta^{13}C$ value range in SSW (1-10m) is 0.50-0.84‰ (average = 0.69 ± 0.04‰) and 0.40-0.61‰ (0.53 ± 0.03‰) for DIC in the DCM. Results obtained for this cruise are shown in table 1; all station depths are considered within the mixed layer, except station 36 which is considered within the deep layer.

It is observed a decrease in the carbon isotopic composition of DIC from surface to DCM as shown in figure 7. This decrease in $\delta^{13}C$ values is also confirmed when temperature decrease at higher depths, as it was expected. In general, samples collected at surface with a temperature of $25^{o}C$ have a range of 0.50-0.81‰, whereas samples collected in the DCM at $15^{o}C$ have a range of 0.40-0.61‰.

Table 1. *$\delta^{13}C$ DIC values measured for Thresholds 2006 samples. Temperature, AOU, concentration of chlorophyll a, DOC and phosphates were also measured. Indirect values of AOU were calculated as the difference between oxygen concentration and oxygen saturation for measured values of temperature, salinity and pressure.*

ST.	DEPTH (m)	$\delta^{13}C$ DIC (‰)V-PDB	TEMP (°C)	AOU (µmol/kg)	CHL A (mg/m²)	DOC(µmolC/L)	PO$_4^-$ (µmol/kg)
28	SSW (5.16)	0.63	24.71	24.62	0.21	154.81	0.44
	DCM (74.07)	0.42	14.76	48.25	1.08	84.04	4.76
29	SSW (5.40)	0.74	23.34	36.16		78.75	0.56
	DCM (90.66)	0.58	14.59	60.62		62.89	0.80
30	SSW (4.78)	0.83	24.84	35.34	0.21	81.47	
	DCM (84.27)	0.57	14.39	56.01	0.90	62.92	0.46
31	SSW (4.52)	0.74	23.52	37.96	0.23	85.41	0.37
	DCM (76.18)	0.61	14.56	58.85	1.13	82.53	0.55
32	SSW (5.22)	0.50	23.33	38.45	0.21	61.43	0.42
	DCM (68.25)	0.55	13.86	55.07	1.39	64.65	0.44
33	SSW (5.03)	0.75	22.98	42.19	0.22	70.81	0.32
	DCM (73.48)	0.52	14.92	68.49	1.09	61.63	0.52
34	SSW (5.28)	0.74	24.44	41.89	0.29	100.44	0.55
	DCM (68.11)	0.59	14.42	55.46	1.35	49.80	0.66
35	SSW (4.83)	0.65	23.67	83.42	0.28	68.74	0.30
	DCM (85.28)	0.63	13.07	58.59			
36	166.53	0.84	13.29	94.27			
	1500.24	0.56	14.22	76.86	1.03	58.43	0.50

All correlations made between $\delta^{13}C$ values and biological characteristics have low correlation factors ($R^2 < 0.5$) for the mixed layer, even though qualitative tendencies can be noticed from these correlations. By comparing carbon isotope composition against AOU, it seems that samples with low $\delta^{13}C$ values have a higher AOU within the mixed layer, whereas samples with high $\delta^{13}C$ values have lower AOU values (Figure 8). After noticing this relation, $\delta^{13}C$ value was plotted against values of chlorophyll, DOC and concentration of phosphates, for better characterization of isotope effects related to biological processes. Referring to chlorophyll (Figure 9), it seems that surface waters with low concentrations of chlorophyll present a wider range of $\delta^{13}C$ values than that of DCM.

The relation between $\delta^{13}C$ values and DOC within the mixed layer can be interpreted similarly to that of chlorophyll; both SSW and DCM seems to have a wide range of DOC concentration for a given $\delta^{13}C$ value range. It is also observed that DOC concentration is higher in SSW where $\delta^{13}C$ values of DIC are also more positive (Table 1), except station 32 which have lower values of DOC concentration and $\delta^{13}C$ values in SSW. Concentration of nutrients, specifically phosphates, presents a negative relation with carbon isotopic composition; i.e. when the concentration of phosphates in water rises (Figure 13) for mixed layer depths, $\delta^{13}C$ values decreases. This effect can be explained by the degradation of the organic matter by respiration, so phosphates and negative CO_2 are generate at the same time.

Finally, experimental ^{13}C Suess Effect was calculated as a general average for DCM and SSW, and separately for both depths. There are not significant differences between the three results, shown in table 2. The average for ^{13}C Suess effect is -0.036 ± 0.004‰ yr^{-1}.

Table 2. *Suess effect in the Western Mediterranean Sea. It is noticed that Mediterranean Sea is acting as net sink of anthropogenic CO2 since the value for this effect is negative.*

| Surface Suess Effect	DCM Suess Effect	Suess Effect Average
$-0,033 \pm 0,003$ ‰ yr^{-1}	$-0,039 \pm 0,002$ ‰ yr^{-1}	$-0,036 \pm 0,004$ ‰ yr^{-1}

DISCUSSION

Laboratory work. When biological activity was evaluated, a temporal distribution was found, in which $\delta^{13}C$ values increased after inducing phytoplanktonic bloom. Three replicates were studied during a period of 336 hours (figure 2); we note that replicate S-3, behaves differently from the other two ($R^2 = 0.87$) by observing figure 2. A. where all measurements are plotted. If this replicate is not considered, we observe a straight $\delta^{13}C$ DIC evolution with time (Figure 2. B). The result obtained from the third replicate is probably due to analytical errors when settling down the experiment. Anyhow, the first replicate is the best adjusted to this relation (Figure 3. A).
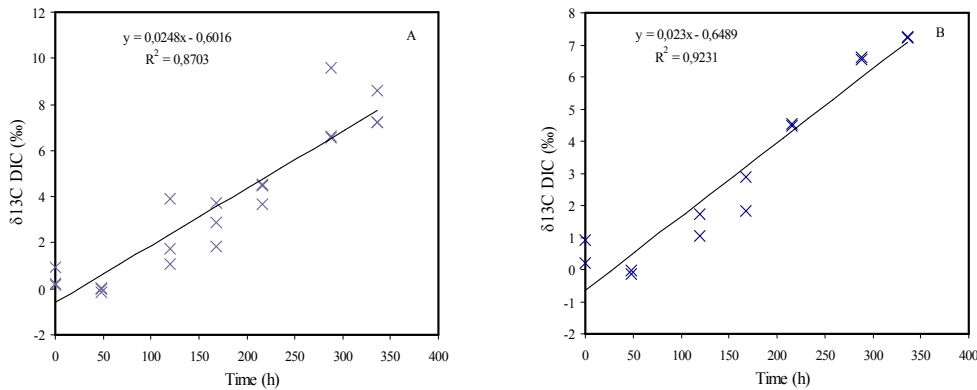
Figure 2. *$\delta^{13}C$ DIC (‰) versus time (h) when only biological processes occur. Fig. 2. A. shows the correlation for all measurements in the three replicates; fig. 2. B. shows the same correlation when replicate 3 is omitted. Perfect correlation of $\delta^{13}C$ DIC (‰) and time (h) is noticed.*
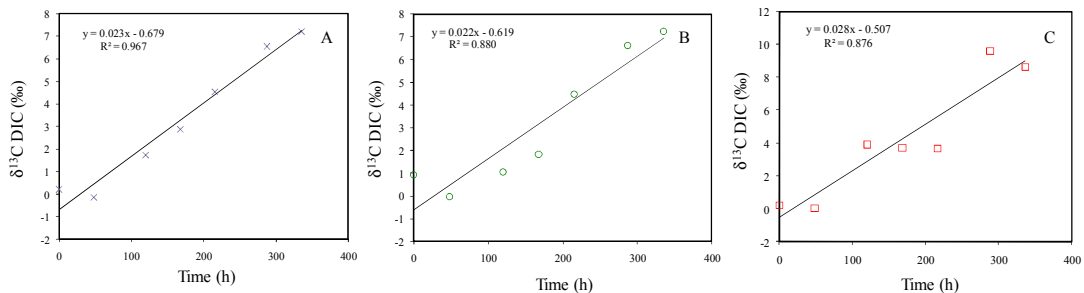

Figure 3. *$\delta 13C$ DIC (‰) versus time (h) when only biological processes occur. Fig. 3. A. shows the results for replicate 1, fig 3. B. for replicate 2 and fig 3.C. for replicate 3. Although, replicate 1 seems to have the highest correlation with time, it can be state a positive relation between $\delta 13C$ DIC (‰) and time (h) when only biological activities are taken place.*

The negative relation between amplitudes obtained during measurements with the spectrometer and the time elapsed is shown in figure 4. A.; negative relations between δ^{13}C DIC values and amplitudes are observed in figure 4. B. These amplitudes are directly proportional to concentration of DIC in seawater; meaning that as time passes, DIC of water decreases and δ^{13}C DIC values increases. Therefore, it can be concluded that relation of δ^{13}C DIC and concentrations of DIC is a Rayleigh type relation (Rayleigh, 1896), where lighter isotopic carbon is preferentially enriched in phytoplankton, and seawater progressively becomes more depleted in the lighter isotope, as it occurs in raindrops vs. the cloud, and in other geochemistry systems (Hoefs, 2009). We expected these results since a negative relation between concentration of DIC and δ^{13}C DIC values was observed before for North Atlantic Ocean (Kroopnick, 1974) due to the utilization of DIC during photosynthesis.
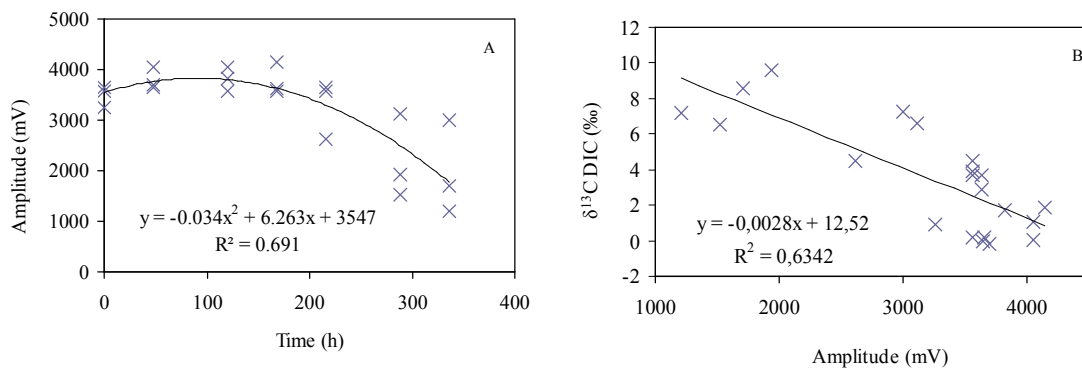

Figure 4. *Fig 4. A. shows the relation amplitude (mV) versus time (h) of measurements from samples when only biological processes occur. Fig. 4. B. shows the correlation for δ^{13}C DIC (‰) and amplitude for the same measurements; as amplitude increases values of δ^{13}C DIC (‰) becomes higher.*
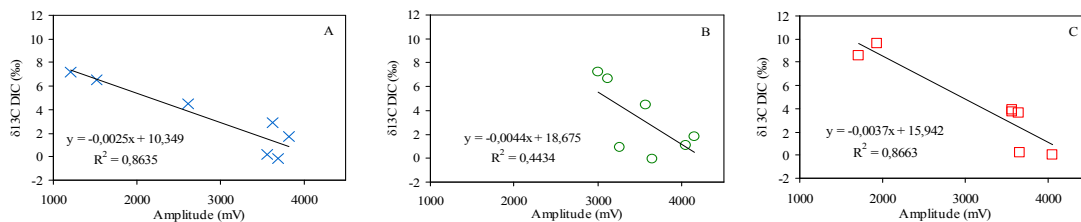

Figure 5. *Relation between δ^{13}C DIC (‰) and amplitude (mV) for replicate 1 (Fig 5. A.), for replicate 2 (Fig 5. B.), and for replicate 3 (Fig 5. C.)*

In figure 6, the evolution of nutrients and chlorophyll during the experiment are plotted. Concentrations of nutrients experiment a decrease during the evolution of the experiment as it was presumed. Chlorophyll appears to increase at the start of the experiment, after 216 hours it decrease again, probably due to low concentrations of nutrients.
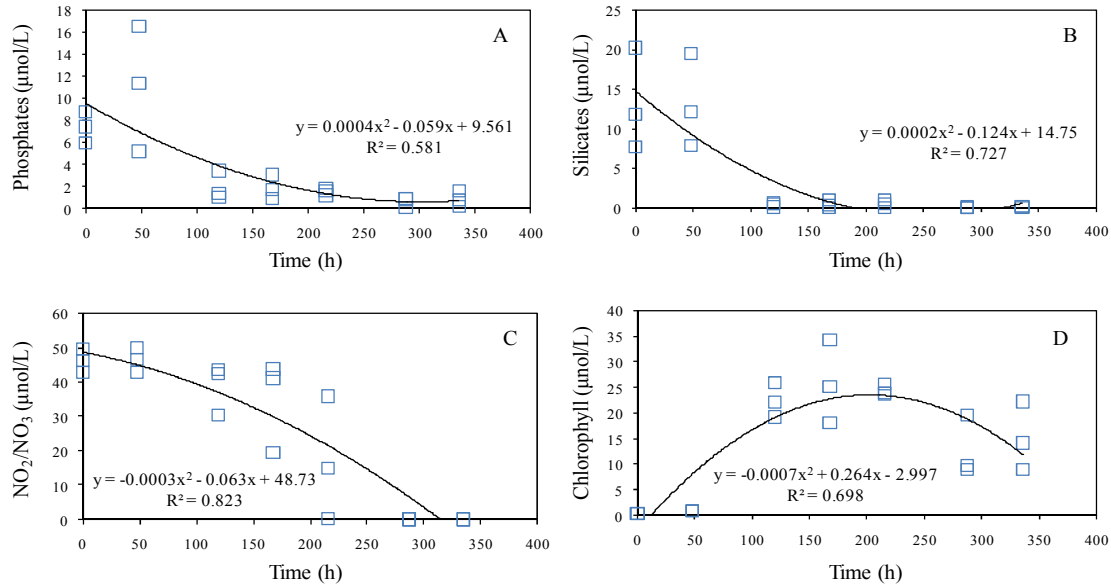

Figure 6. *Evolution of nutrients and chlorophyll during the experiment. Fig 6. A. shows evolution of phosphates (μmol L⁻¹), fig 6. B. silicates (μmol L⁻¹), fig 6. C. nitrates (μmol L⁻¹) and fig 6. D. shows chlorophyll (μmol L⁻¹) evolution.*

Relations of δ^{13}C DIC with concentrations of nutrients are very strong for phosphates and for nitrites and nitrates, although it is not possible to confirm this relation for silica (Figure 7). When concentrations of phosphates which is the limiting nutrient in the Mediterranean Sea (Thingstad et al. 1998, Marty et al., 2002) is high, photosynthesis occurs, thus δ^{13}C DIC and chlorophyll increase; but if phosphates are insufficient, chlorophyll growth diminishes and concentrations decay, although it is not yet reflected in δ^{13}C DIC values. From figure 7. A., it is found another Rayleigh relation for δ^{13}C DIC and phosphates, where a decrease in concentration of phosphates rises up rapidly δ^{13}C DIC, since phosphates are limiting photosynthesis and hence, rapidly removed from the system.
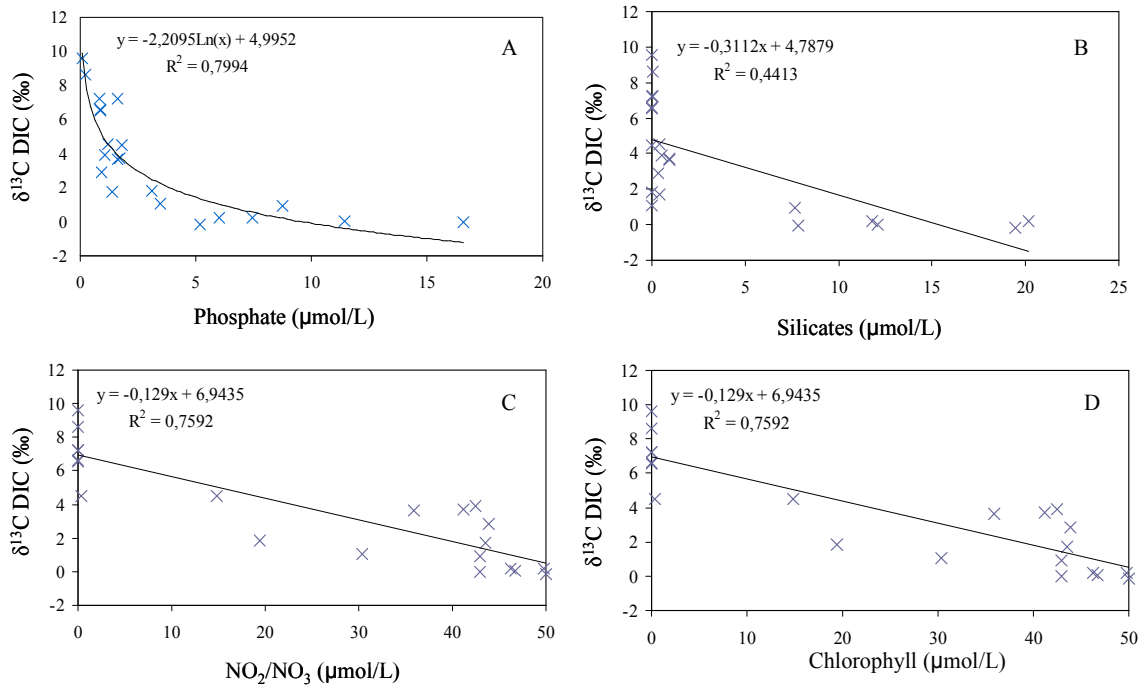
Figure 7. *Relations between $\delta^{13}C$ DIC (‰) and concentrations of nutrients Fig 7. A. shows relation with phosphates (µmol L^{-1}), fig 12. B. silicates (µmol L^{-1}), fig 12. C. nitrates (µmol L^{-1}) and fig 12. D. shows relation with chlorophyll (µmol L^{-1}).*

Field work. Our samples were collected at the surface of Western Mediterranean Sea and in the DCM, SSW are characterized by lower concentrations of nutrients (Figure 9); DCM has high levels of biomass, concentrations of nutrients and organic materials as it can be noticed by the results in table 1. At this depth, planktonic primary production will be enhanced by nutrient input; and organic matter imported to these waters causing planktonic respiration and hence, a balance between both biological processes (Regaudie-de-Gioux et al., 2009). Observing figure 8, it is possible to state that isotopic composition of DIC in the Western Mediterranean Sea decreases with depth and, in consequence, with temperature as it is shown in figure 10. These results demonstrate the predominance of biological activity over air-sea exchange.
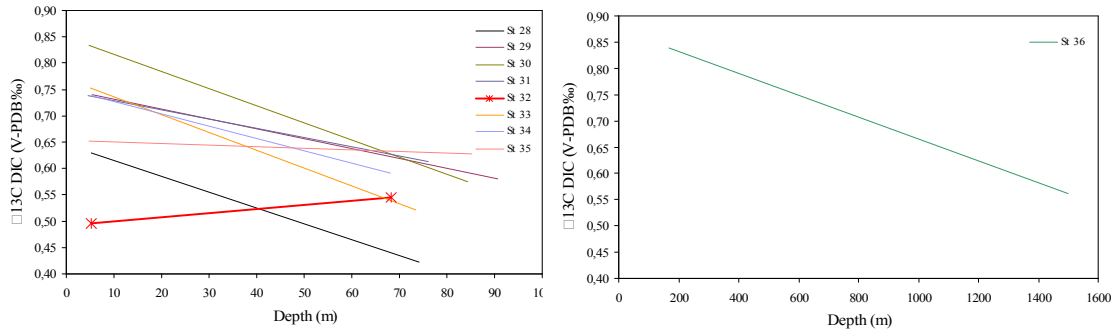
Figure 8. *δ¹³C DIC (‰) vs sample depth (m). A general decrease in δ¹³C is observed when increasing depth, except for st. 32 located at the Strait of Sicily. St 39 was collected at 160m and 1500 m deep.*
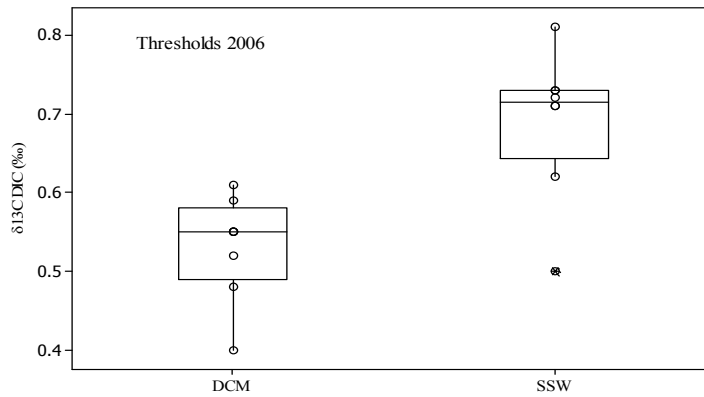
**Figure 9.** *Vertical distribution of δ¹³C DIC values (‰) for Thresholds 2006.*

During photosynthesis, phytoplankton preferentially incorporates ^{12}C *vs.* ^{13}C, it discriminates against ^{13}C, therefore; it is assumed higher $\delta^{13}C$ values for the residual DIC. Decreasing values with depth means that photosynthesis governs in surface waters whereas respiration and photosynthesis are balanced in DCM due to the higher concentration of chlorophyll.

Temperatures in DCM are more stable than at surface, where biological productivity is stronger; from comparison of the results obtained for both depths, increments of $\delta^{13}C$ DIC (‰) are observed when temperature ascends. These differences in temperature are considerable during summer when water is vertically stratified but; high vertical mixing occurs as a result of very dry and cold winds during winter (Zavatarelli and Mellor, 1995). This water mixing homogenise the mixed layer and differences in temperature will not be very high, as it is observed from Pierre (1999) results of samples collected

in February-March 1988 (Figure 15). Consequently, primary production will decrease and δ^{13}C DIC (‰) values will also decrease respective to those obtained during summer.
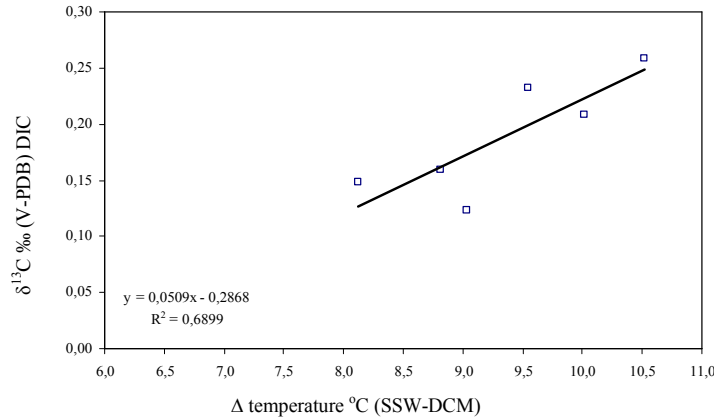

Figure 10. *Increment of δ^{13}C DIC (‰) vs. temperature differences (^{o}C).*

These results are confirmed by the correlation between AOU and δ^{13}C DIC values (r = -0.54, p = 0.032), which was described first by Kroopnick (1985); and it is represented in figure 11. The AOU is defined as an estimation of the dissolved oxygen utilized by biogeochemical processes since the last time the water mass equilibrated with atmosphere (NOAA, 2006).

$$AOU = O_{2(sat)}-O_{2(meas)}$$

Therefore, high values for AOU means that oxygen is being consumed and respiration occurs, whereas low values of AOU means oxygen production and photosynthesis predominance. Thus, correlation between δ^{13}C DIC values and AOU should be negative, i.e. δ^{13}C DIC values decrease as AOU increases.
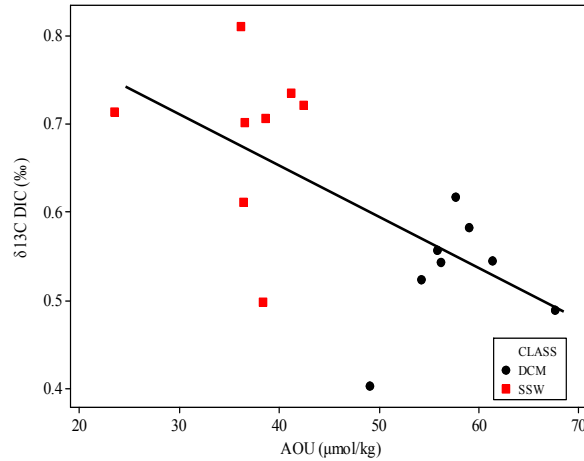
Figure 11. *$\delta^{13}C$ DIC (‰) versus AOU (µmol/kg). A general decrease in $\delta^{13}C$ is observed when AOU increases, higher AOU values are found at DCM.*

Regarding to other biological properties as chlorophyll or DOC, similar tendencies are recognized which confirmed de predominance of biological processes along the column of water. In figure 12, negative relation between chlorophyll and $\delta^{13}C$ DIC values is observed (r = -0.61, p = 0.019).
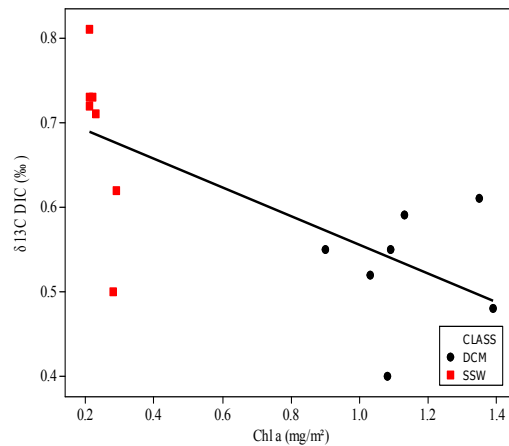

Figure 12. *$\delta^{13}C$ DIC (‰) versus chlorophyll (mg m^{-2}). A general decrease in $\delta^{13}C$ is observed when concentration of chlorophyll increases. As chlorophyll increases, biomass experiments also an increment; therefore, photosynthesis will be balanced with respiration a remineralization of organic material.*

DCM is formed due to the high input of nutrients at this depth, which cause an increase in biomass values and thus, in chlorophyll concentration. At this depth, values for photosynthesis are high but, remineralization of organic matter and planktonic respiration takes places at high levels too which liberates more negative CO_2. In

consequence, $\delta^{13}C$ DIC values in DCM will be slightly lower than in the surface seawater; since photosynthesis discriminates against ^{13}C and causes an increase in $\delta^{13}C$ DIC values, whereas remineralization and respiration of plankton causes the opposite effect, decreasing $\delta^{13}C$ DIC values.

By studying correlations with DOC, it is not possible to find a clear tendency; although DOC has lower values in DCM respective to surface seawater (Table 1), following $\delta^{13}C$ DIC pattern; except for station 32 where DOC concentrations are higher at DCM. These lower levels of DOC in DCM are possibly caused by the remineralization of organic matter, which gets its maximum levels at 1000m deep. As this process occurs, DIC should increase since CO_2 is the final product, derived from organic matter which isotopic composition is very negative; therefore, $\delta^{13}C$ DIC values should decrease as CO_2 is produced (Kroopnick, 1985). There is two main reasons for not noticing this decrease with DOC; first, our samples are in the mixed layers where other processes occur, i.e. air-sea exchange and photosynthesis, which alter concentration of DOC by adding atmospheric CO_2 and subtracting CO_2 for photosynthesis; hence, it is not possible to find linear variations of $\delta^{13}C$ DIC values due to removal of DOC of the seawater. The second reason is related to anthropogenic activities and the increment on organic matter input to the ocean due to these activities.

Nutrients have an important role in the DCM, since their higher concentrations at this depth cause an increase in biomass. Thus, a correlation between $\delta^{13}C$ DIC values and phosphates (Figure 13) has been calculated (r = -0.56, p = 0.019). Results obtained from western Mediterranean samples confirm the results obtained in the laboratory (figure7.A); meaning that isotopic composition of DIC in Western Mediterranean Sea is strongly related to biological processes; since low values of phosphates in seawater implies high photosynthetic activity and high $\delta^{13}C$ values of DIC.
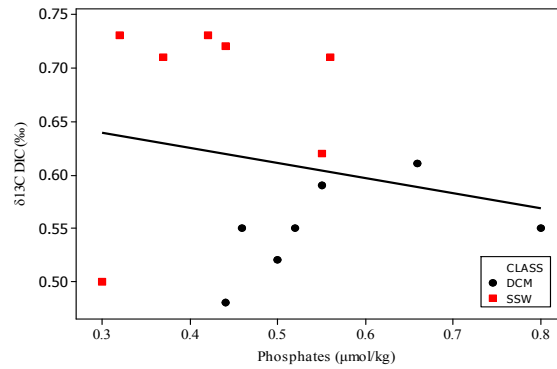
Figure 13. *Relation between δ13C DIC (‰) and phosphates (μmol kg⁻¹) for surface seawater and DCM. Higher concentrations of nutrients are found in DCM; as concentration of phosphates increase δ13C DIC decreases.*

Finally, we compared our results from Thresholds 2006 cruise from those obtained by Pierre (1999) who demonstrated a geographical distribution for δ^{13}C DIC values for Mediterranean Sea decreasing δ^{13}C DIC values when moving eastwards from data obtained in 1988. By looking at our results, we could sustain it surface waters but it is not possible for DCM. Anyhow, we understand that the lack of stations reduces the possibilities to demonstrate any well documented geographical distribution (Figure 14), since we only sampled from Majorca to the Strait of Sicily. At surface, the δ^{13}C DIC values suffer more dispersion than at DCM; in station 32, there are no variations between SSW and DCM, probably due to the cyclonic gyre occurring in this area (Zavatarelli and Mellor 1995) and the consequent upwelling which rise deeper waters with lower δ^{13}C DIC values to the surface. Carbon isotopic composition increases again when crossing the Strait of Sicily to drop again after passed over the strait. Even though, more stations are needed in order to indicate a geographical distribution as Pierre has done, we also suggest a strong sampling along the Strait of Sicily due to its hydrodynamic properties.
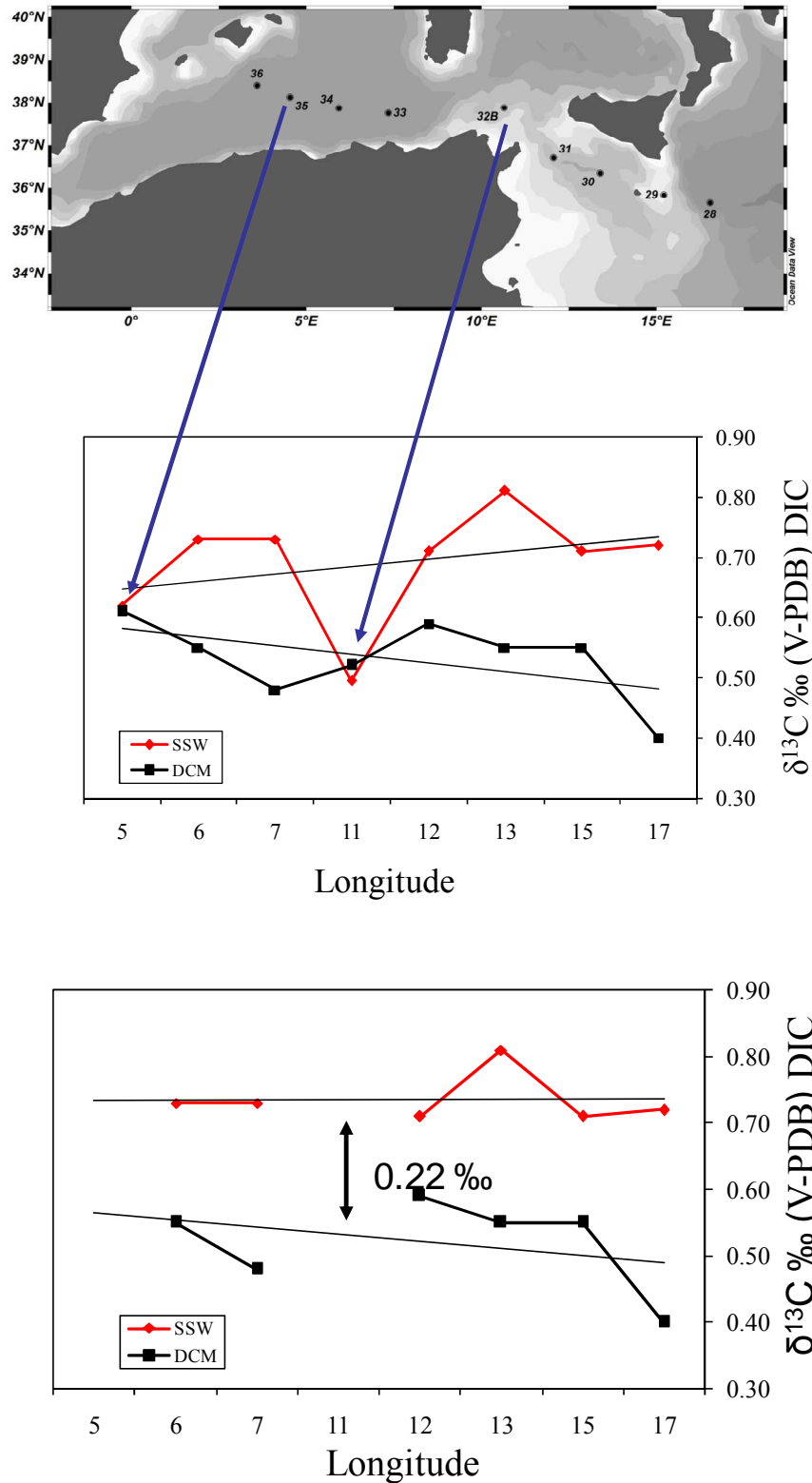
Figure 14. *δ¹³C DIC of SSW vs. DCM. If station 32 and 35 are considered anomalous areas and eliminated, DCM presents an average of 0.22‰ lower that SSW. These stations seem to have locations where water is homogenized.*

When results from Pierre and our results are compared, it is noticed that $\delta^{13}C$ DIC values have decreased over time (Figure 15).
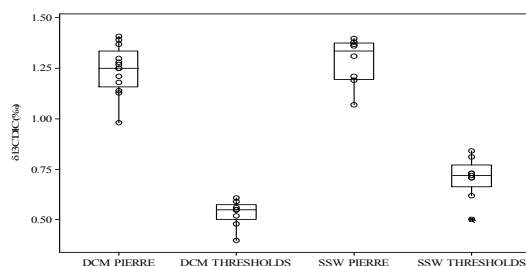
**Figure 15.** *Comparison of Pierre (1999) and Thresholds 2006 results. Outlier is found for St. 32.*

Pierre (1999) obtains a δ^{13}C DIC value of 1.29 ± 0.04 ‰ for surface seawater in the Western Mediterranean Sea and 1.24 ± 0.03‰ for DCM (Table 3). Samples studied by Pierre (1999) were collected during February-March 1988 when seawater temperature is lower and slight differences between SSW and DCM are noticed. By comparing results a decrease of 0.65 ± 0.08‰ on average in the last 18 years, is obtained; although, if only data from DCM is compared, δ^{13}C DIC values has decreased 0.71±0.03‰.

Table 3. *Data obtained by Pierre (1999) and this study.*

Year	Depth	δ13C DIC	Standard Standard error	Difference SSW-DCM	Average
1988-89	Surface Sea Water (0-10m)	1.29	0.04	0.05± 0.05	1.27 ± 0.05
(Pierre et al.)	Deep Maximum of Chlorophyll (50-100m)	1.24	0.03		
2006 (This study)	Surface Sea Water (0-10m)	0.69	0,04	0.16 ±0.05	0.61 ± 0.05
	Deep Maximum of Chlorophyll (50-100m)	0.53	0,03		

This is possibly due to the Suess effect in the Mediterranean Sea (Figure 16). The role considered for the Western Mediterranean Sea is to be a net sink of atmospheric CO_2 over the last few decades (Louanchi et al., 2009); as depleted ^{13}C anthropogenic CO_2 enters the ocean, the carbon isotopic composition of seawater becomes higher, since the carbon entering is ^{12}C enriched and hence, ^{13}C/^{12}C ratio disminishes. Fossil fuels have very low δ^{13}C values and are being liberating to the atmosphere constantly, in form of

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CO_2 which enters oceans with atmospheric CO_2 decreasing oceanic $\delta^{13}C$ DIC values, just as it occurs in the Western Mediterranean Sea.
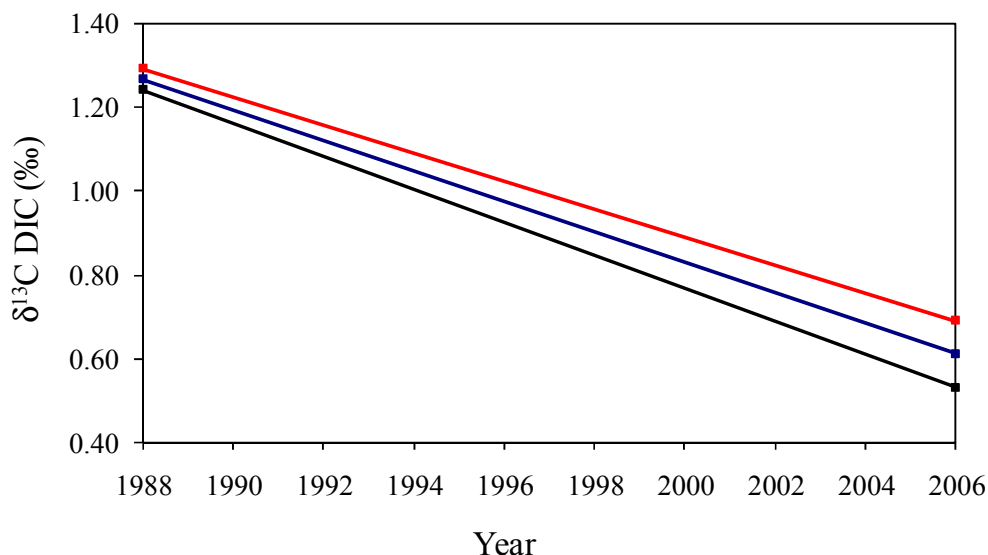
Figure 16. *^{13}C Suess effect for Western Mediterranean Sea calculated from 1988-2006.*

The average Suess effect measured in Western Mediterranean Sea is $-0.036\pm0.004\text{‰yr}^{-1}$ (-0.039 ± 0.002‰ if only DCM values are considered). This results leads to the conclusion of Mediterranean Sea being a sink for anthropogenic CO_2 since DIC values are decreasing with time. The Suess effect has never been calculated for Mediterranean Sea, although for other concrete sites of the ocean (Table 4).

Table 4. *^{13}C Suess effect for different region of the ocean.*

Location	Year	Sues effect	References
Sargasso Sea	1984-1993	$-0.025\pm0.002\text{‰ yr}^{-1}$	Bacastow et al. 1996
North Pacific Subpolar region	1997-2001	$-0.012\pm0.01\text{‰ yr}^{-1}$	Tanaka, 2003
Oahu (Hawaii)	1988-1996	$-0.030\pm0.003\text{‰ yr}^{-1}$	(Gruber et al. 1999)
Central Tropical Pacific	1980-1994	$-0.015\pm0.006\text{‰ yr}^{-1}$	
Indian Ocean	1978-1995	$-0.020\pm0.004\text{‰ yr}^{-1}$	
Lofoten Basin	1981-2003	$-0.018\pm0.002\text{‰ yr}^{-1}$	Olsen et al. 2006
Norwegian Basin	1981-2003	$-0.024\pm0.002\text{‰ yr}^{-1}$	
Western Mediterranean Sea	1988-2006	$-0.036\pm0.04\text{‰ yr}^{-1}$	This study

By comparing our results with those from other sites of the ocean, it appears to be a higher Suess effect in the Western Mediterranean Sea; even though, we cannot assure this is correct if we consider standard errors; our value is very approximate to the one obtained for other regions (e.g. Ohau). Another reason for not to assert this conclusion is the lack of a long time series which corroborates this increment for the Western Mediterranean Sea. Although it is obvious that $\delta^{13}C$ DIC values have decreased along these 18 years, we must consider the option of seasonality influence. Data from Pierre (1999) was collected during February-March whereas Thresholds 2006 data was collected during June-July. Thus, processes of stratification and winter vertical mixing could have biased the results. But, the perimediterranean region is very industrialized and it probably suffers higher inputs of anthropogenic CO_2 than other regions were industrial activities did not rise as much as in Europe. There are other processes involved which distort the results obtained, i. e. analytical effects related to sample conservation and the different methodologies used to measure samples. Seasonal variability and marine hydrodynamics influence in the results; vertical mixing occurs during winter and it homogenizes water, so differences will not be very significant but, during summer water column stratifies and higher differences can be noticed. Algerian current flows from Alboran Sea towards East Mediterranean Sea forming temporal eddies in the Algerian basin (Millot et al., 1990); these eddies last weeks or months, therefore they can influence on the stability of the column and thus, on the differences between SSW and DCM as it probably occurs in station 35.

CONCLUSSIONS

Mediterranean Sea has been very studied from different disciplines but, there is very poor knowledge on carbon isotopic composition of DIC. We performed this analysis with the objective to understand biogeochemical processes taking place in the Western Mediterranean Sea, although our data is not sufficient to state quantitative patterns, it gives us comprehension the importance of the different isotope effects which change carbon isotopic composition for this part of the ocean.

By studying only biological processes influencing carbon isotopic composition of DIC, it was possible to observe the strong relation between these values and concentrations of nutrients; i.e. when nutrients decreased, $\delta^{13}C$ values increased as biological activity occurs. Chlorophyll also increased at the first episodes of the experiment; but a direct relation between chlorophyll and $\delta^{13}C$ values was not clear, since chlorophyll and thus, biomass decreased when nutrient started to limit biological activity. It is expected to experiment an increase in $\delta^{13}C$ values when concentration of chlorophyll rises up, but when this concentration decreases, isotopic composition should follow it. The time of experiment was not sufficient to notice this relation; therefore, we suggest an increment of time, in order to observe this temporal trend. From the same experiment, we observed very large increments of $\delta^{13}C$ values suggesting a Rayleigh fractionation type for biological processes involving changes in carbon isotopic composition.

It is possible to confirm from our results that Western Mediterranean Sea is dominated by biological processes, although air-sea exchange is considerable since deep water formation occurs in three different sites of the whole Mediterranean Sea. Regarding to photosynthesis, it seems that it is the predominant process which alters $\delta^{13}C$ values in surface waters, since these values are higher in SSW than in DCM, where photosynthesis balanced with respiration and remineralization of organic matter. In any case, differences between SSW and DCM are more accentuated during summer, when differences in temperature are higher.

Results from the calculation of ^{13}C Suess effect are very interesting due to the lack of information for Mediterranean Sea, although our results seems to be higher when compared with other regions of the ocean. The problems involved from ^{13}C Suess effect calculation is primarily, the lack of a good time series which correct seasonal variability

in $\delta^{13}C$ values of DIC, since the data used from Pierre (1999) was collected during winter of 1989 and our samples were collected in early summer of 2006, after 18 years. This could result in variability due to winter mixing and stratification processes. Anyhow, we estimate that our results are good enough for a first approach in the Western Mediterranean Sea. But we also encouraged to proportionate a longer time series of carbon isotopic composition which involve seasonality corrections since Suess effect estimates the role of oceans as net source and sink of anthropogenic CO2 and, the consequent evolution of this role.

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