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## Estimating the contribution of organic bases from microalgae to the titration alkalinity in coastal seawaters

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### Abstract

This study reports the potential contribution of organic bases to the alkalinity of seawater samples. The concentration of organic bases in these samples was inferred from the difference between the measured alkalinity and that calculated from a knowledge of pH and concentrations of the various inorganic acid-bases species such as total carbon, total boron, and so on. Significant concentrations of such organic bases were measured in cultures of the marine microalgae *Rhodomonas* sp. (800  $\mu\text{mol kg}^{-1}$ ) and *Isochrysis* aff. *Galbana* (400  $\mu\text{mol kg}^{-1}$ ), as well as in three marine environments (northern gulf of California, México; San Quintín Bay, B.C., Mexico; and San Diego Bay). These three sites are characterized by significant biological activity and restricted mixing, and the organic bases were found at concentrations greater than 50  $\mu\text{mol kg}^{-1}$  in each of these three locations.

Dickson (1992) points out that a complete understanding of the alkalinity of seawater requires not only a reliable analytical procedure (typically a titration of some form) but also a chemical model for seawater in which all the various acid-base species are identified, and their total concentrations and acidity constants known. As it is difficult to separate the one from the other, a reasonable model is required for an accurate titration.

The total alkalinity ( $A_T$ ) of a seawater sample is defined as the number of moles of hydrogen ion equivalent to the excess of proton acceptors (bases formed from weak acids with thermodynamic dissociation constant  $K > 10^{-4.5}$  at 25°C) over proton donors (acids with  $K > 10^{-4.5}$ ) in 1 kg sample (Dickson 1981):

$$A_T = [\text{HCO}_3^-] + 2[\text{CO}_3^{2-}] + [\text{B}(\text{OH})_4^-] + [\text{OH}^-] + [\text{HPO}_4^{2-}] + 2[\text{PO}_4^{3-}] + [\text{SiO}(\text{OH})_3^-] + [\text{NH}_3] + [\text{HS}^-] - [\text{H}^+]_F - [\text{HSO}_4^-] - [\text{HF}] - [\text{H}_3\text{PO}_4] + [\text{minor bases} - \text{minor acids}] \quad (1)$$

The brackets represent total concentrations of these constituents in solution,  $[\text{H}^+]_F$  represent the free hydrogen ion

concentration. The final term of Eq. 1 is an explicit (though perhaps unhelpful) recognition that the chemical model for seawater is certainly incomplete, and that possible components remain unidentified as yet.

In practice, minor organic acids and bases are usually omitted from such a model, although Bradshaw and Brewer (1988) inferred their presence in seawater by noting a discrepancy between the total inorganic carbon estimated from titration data and that from an extraction procedure. Although subsequent work (Dickson et al. 1991, Millero et al. 1993a) suggested that non-Nernstian behavior of the glass electrode used in the alkalinity titration might be responsible for this discrepancy, it is likely that such organic acids exist in seawater, and that their concentrations might become significant in environments with substantial organic input and restricted mixing.

Cai et al. (1998) have shown that humic materials can contribute significantly to the alkalinity of estuarine waters. They assessed the contribution of such organic materials to the alkalinity of a number of estuaries in Georgia (USA) by contrasting the alkalinity measured in the normal manner with a subsequent determination on the same sample after the  $\text{CO}_2$  had been removed and the sample returned to its initial pH (allowance was made for borate, etc.). Another—typically unrecognized—contribution may result from the titration of surface sites on phytoplankton and bacteria in unfiltered samples (Hyun-Cheol et al. 2006). These have been found to contribute up to 5  $\mu\text{mol kg}^{-1}$  to the measured alkalinity.

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Here we identify further examples of environments in which there is clear evidence of organic bases contributing to the measured alkalinity. We also show that similar effects may be found in culture media, implying that phytoplankton exudates may well be a possible source of such bases.

## Materials and procedures

**Measurement of  $C_T$ ,  $A_T$ , and pH**— $C_T$ ,  $A_T$ , and pH were determined using the derivative technique described by Hernández-Ayón et al. (1999). The apparatus consists of three principal parts: a closed acrylic cell, an electrode to measure pH with an exit to a port, and a programmable electronic syringe (Kloen). The electrode was calibrated against the standard seawater buffers aminopyridine and tris(hydroxymethyl)aminomethane, "tris" (DOE 1994, Del Valls and Dickson 1998). Standard 0.1 M HCl was made up in NaCl to bring the ionic strength to 0.7 M. The acid was calibrated against a seawater reference material (CRM) certified for  $A_T$  (obtained from Dr. Andrew Dickson). The magnitude of the error involved in using a salinity 35 buffer for most oceanic measurements (i.e., in the range 33–37) is probably less than 0.005 in pH (DOE 1994). After a titration, the data are processed in a MATLAB program that calculates the derivative (by difference) of the electrode response (mV) against volume of HCl in milliliters.  $C_T$  and  $A_T$  are then estimated from the positions of the maxima in this plot.

**Microalgal cultures**—The marine microalgae *Rhodomonas* sp. and *I. galbana* (Clone T-ISO) were obtained from the Instituto de Investigaciones Oceanológicas of the Universidad Autónoma de Baja California, México. A culture medium was prepared with initial  $\text{NO}_3^-$  and  $\text{PO}_4^{3-}$  concentrations of 1323 and 58.5  $\mu\text{mol L}^{-1}$ , respectively. Cultures were incubated for 7 d in duplicate 18 L glass bottles at  $20 \pm 1^\circ\text{C}$  in a continuous regime of light. The cells were continuously aerated at a rate of 4.5  $\text{L min}^{-1}$ . Cell concentrations were determined daily, in duplicate, under microscope, with a Neubauer chamber of 0.1 mm in depth. The initial cellular densities were *Rhodomonas* sp.  $5.6 \times 10^4$  cells  $\text{mL}^{-1}$  and *I. galbana*  $3.58 \times 10^5$  cells  $\text{mL}^{-1}$ . Samples analyzed for pH,  $C_T$ , and  $A_T$  were obtained from the 18 L culture bottles using 60 mL syringes, which prevented mixing with the atmosphere during analysis. Each day, duplicate volumes of  $\sim 40$  mL were obtained as samples, one in the morning and one in the afternoon.

### Samples from coastal lagoons

Northern Gulf of California. Samples for this work were collected at stations along the Gulf of California during late summer 15–27 September 1996, aboard the R/V *Francisco de Ulloa*. In this paper, we will discuss the data taken at 14 locations at depths between the surface and 100 m. The samples were taken from Niskin bottles mounted on a CTD/Rosette frame. The samples were collected using 50 mL syringes, and the  $A_T$ ,  $C_T$  and pH measurements were made immediately in the ship's laboratory.

San Diego Bay. A multi-disciplinary marine survey was performed in San Diego Bay in September 2001 aboard the R/V *ECOS* equipped with the Navy's Marine Environmental Survey

Capability (MESC) system. The survey consisted of a transect from the mouth to the head of the bay while collecting samples from surface in 27 predefined sampling "boxes" (Blake et al. 2004). The samples were collected using 50 mL stopped syringes, and the  $A_T$ ,  $C_T$ , and pH measurements were made on the vessel immediately after collection.

San Quintín Bay. A survey was performed in the San Quintín Bay, a coastal lagoon approximately 100 km south of Ensenada, B.C., during June 2002. A transect was made from the mouth of the bay to the inner of two arms, while collecting samples from the surface at 19 predefined sampling stations. The samples were again collected using 50 mL capped syringes, but the measurements were made on land at the end of the survey.

**Thermodynamic calculations**—Explicit and detailed descriptions of the equations that relate  $C_T$ ,  $A_T$ , pH, and  $\text{pCO}_2$ , and various equilibrium constants are found in DOE (1994) and Lewis and Wallace (1998). In the present work,  $A_T$  was calculated from the couple ( $C_T$ -pH) using the program developed by Lewis and Wallace (1998) using the carbonate constants from Mehrbach et al. (1973), as refit by Dickson and Millero (1987).

**Model experiments**—We did model titrations using the Haltafall program (Ingri et al. 1967) using the matrix reported by Dickson (1981). The goal was to simulate the titrations to obtain approximate equilibrium constants for the organic bases. First, we evaluated the effect of such bases on the  $C_T$  determinations for several pK values, and second, we simulated titrations of the culture media and included different concentrations of bases, with a specific pK.

## Results

### Analytical and thermodynamic accuracy

**Analytical.** The derivative technique of Hernández-Ayón et al. (1999) produces two peaks (corresponding to the titration of carbonate/borate and bicarbonate) and the maximum of each peak (corresponding to the titration inflection points) is used to determine  $C_T$  and  $A_T$  values. Although the inflection points are obtained empirically, the difference between the inflection points is equivalent to  $C_T$  within 4  $\mu\text{mol}$ . This is in good agreement with the observations of McIntyre (1978) who demonstrated that a small, systematic difference exists between the titration end points (the inflection points) and the stoichiometric carbonate and bicarbonate equivalence points.

**Precision and accuracy.** The results of the titrations of samples of CRM and the  $A_T$  calculated from the  $C_T$ -pH couple are presented in Table 1. The measured precision of pH is 0.0015 pH units, the error in  $A_T$  is 0.21%, and the error in the  $C_T$  measurement is 0.1%, all at one standard deviation. The measured mean value of  $C_T$  is 1991  $\mu\text{mol kg}^{-1}$ , within 3  $\mu\text{mol kg}^{-1}$  of the CRM value of 1988  $\mu\text{mol kg}^{-1}$ . The certified  $A_T$  value for batch 43 is 2202.86  $\mu\text{mol kg}^{-1}$  and our mean value was 2005  $\mu\text{mol kg}^{-1}$ . This difference is again within 3  $\mu\text{mol/kg}$  of our measured value. The use of a CRM to calibrate the acid has certain advantages. First, it limits errors in standardization of the acid.

**Table 1.** Measurements of  $A_T$  and  $C_T$  of CRMs

$A_T$ $\mu\text{mol kg}^{-1}$	$C_T$ $\mu\text{mol kg}^{-1}$	pH <sub>(T)</sub>	$A_T$ (pH- $C_T$ ) $\mu\text{mol kg}^{-1}$	$A_T - A_T$ (pH- $C_T$ ) $\mu\text{mol kg}^{-1}$
CRM Batch 43—S = 33.598; $A_T = 2202.86 \mu\text{mol kg}^{-1}$ ; $C_T = 1988.64 \mu\text{mol kg}^{-1}$				
2209	1990	7.905	2201.4	7.6
2209	1990	7.905	2201.4	7.6
2201	1994	7.908	2207.2	-6.2
2201	1990	7.907	2202.4	-1.4
CRM Batch 62—S = 35.224; $A_T = 2338.20 \mu\text{mol kg}^{-1}$ ; $C_T = 2126.46 \mu\text{mol kg}^{-1}$				
2338.2*	2126.0*	7.870*	2338.6	-0.4

\*Data measured at Scripps:  $C_T$  by coulometry, pH, and  $A_T$  by potentiometry.

Second, it compensates for small shifts in the position of the peaks on the x-axis that may be caused by using different titration rates. Finally, it also compensates for the small difference between the end points and the equivalence points.

Table 1 also show the  $A_T$  values calculated from the measured pH- $C_T$  values. The differences between measured and calculated values for  $A_T$  using samples of certified reference material (CRM) had a standard deviation of  $\sim 7 \mu\text{mol kg}^{-1}$  values with no clear sign of a bias. Table 1 also shows the small differences between measured and calculated values for  $A_T$ , using  $C_T$  and pH data measured by coulometry and potentiometry in the Dickson laboratory for a CRM from Batch 62.

**Effect of weak acids on  $C_T$  determinations using the derivative technique: Model analysis**—We did model simulations using concentrations of 20 and 200  $\mu\text{mol}$  of “organic substances” with pKs of 8, 7.5, 7.1, 7, 6, and 5 (Fig. 1). The results indicate an effect from organic bases on the  $C_T$  determination when their pKs are between 5 and 7. However, with pKs > 7 the effect was minimal. The large effect observed in the plot around pK = 6 is to be expected because this is the same as pK1 for carbonic acid, and thus the organic acid cannot be distinguished mathematically from bicarbonate ion.

#### Microalgae cultures

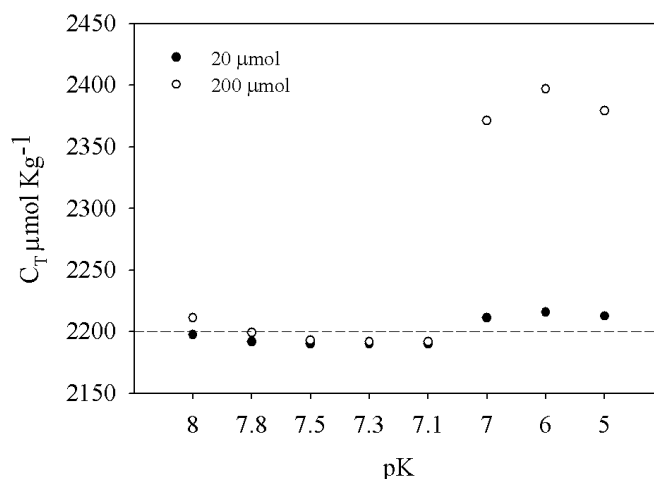
**Changes in  $A_T$ ,  $C_T$ , and pH.** The changes in  $C_T$  over time were apparent in both cultures (Fig. 2b).  $C_T$  values showed a clear decrease from an initial value of 1933 to about 1010  $\mu\text{mol kg}^{-1}$  during the first 4 d. By day six, the  $C_T$  for *Rhodomonas* sp. had decreased to  $\sim 585 \mu\text{mol kg}^{-1}$ , then  $C_T$  increased again to 1176  $\mu\text{mol kg}^{-1}$  on the last day (Fig. 2b). On the other hand, such an increase of  $C_T$  was not clearly observed in *I. galbana*, and a  $C_T$  concentration of  $1077 \pm 76 \mu\text{mol kg}^{-1}$  was maintained for the final 3 d. The general decrease of  $C_T$  was 25% higher for *Rhodomonas* sp. over *I. galbana*.

Before inoculation, the *Rhodomonas* sp. culture had a pH of 8.06 and then pH values increased up to 10.17 during the sixth day of incubation and then decreased to 9.50 during the last 2 d (Fig. 2c). In contrast, pH values for the *I. galbana* cul-

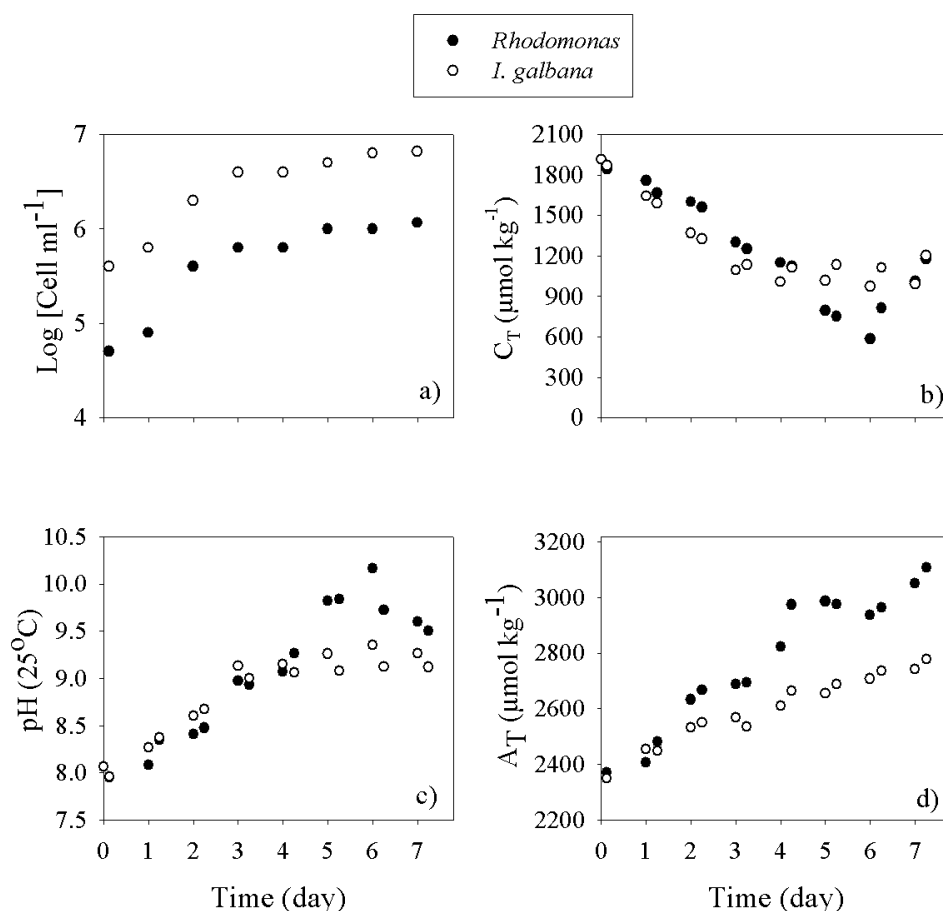
ture increased to 9.13 by the third day, and then fluctuated between 9.00 and 9.35 for the remainder of the experiment.

The initial  $A_T$  was 2189  $\mu\text{mol kg}^{-1}$ , and reached a maximum in both cultures on the last day (Fig. 2d). The maximum values measured were 3106  $\mu\text{mol kg}^{-1}$  for *Rhodomonas* sp. and 2776  $\mu\text{mol kg}^{-1}$  for *I. galbana*. After the fourth day,  $A_T$  in both cultures maintained a value of approximately 3000 to 3100  $\mu\text{mol kg}^{-1}$  for *Rhodomonas* sp. and 2700  $\mu\text{mol kg}^{-1}$  for *I. galbana*.

$A_T$  measured versus  $A_T$  calculated. In our experiments,  $A_T$  was higher in the culture of *Rhodomonas* sp. than in that of *I. galbana*. In Fig. 3a-b measured  $A_T$  is compared with  $A_T$  calculated ( $A_{TC}$ ) using the pH- $C_T$  combination for both cultures. When the experiment started, neither alga showed differences between  $A_T$  and  $A_{TC}$ . After the experiment began, the presence of organic bases increased in both cultures, but to a greater extent in *Rhodomonas* sp. (Fig. 3 and 4). The concentration of organic bases was estimated by subtracting the  $A_{TC}$  from  $A_T$  (Fig. 4). The maximum amount for *Rhodomonas* sp was  $\sim 800 \mu\text{mol}$



**Fig. 1.** Model determination of the sensitivity from organic bases on  $C_T$  determination using the derivative technique with pKs between 7 and 5



**Fig. 2.** Growth and changes in the concentration of pH,  $C_T$ , and  $A_T$  in cultures of *I. galbana* and *Rhodomonas* sp.

kg<sup>-1</sup> on the fifth day, and this gradually decreased to ~500 μmol kg<sup>-1</sup>. This decrease is attributed to remineralization processes occurring in the culture flask. *I. galbana* had maximum values of ~500 μmol kg<sup>-1</sup> on the third day and increased to ~600 μmol kg<sup>-1</sup> after the sixth day (Fig. 4). As can be seen from Fig. 2, although *I. galbana* had the higher cell count throughout the experiment, *Rhodomonas* sp. produced higher amount of organic bases suggesting that there may be a species dependence in such behavior.

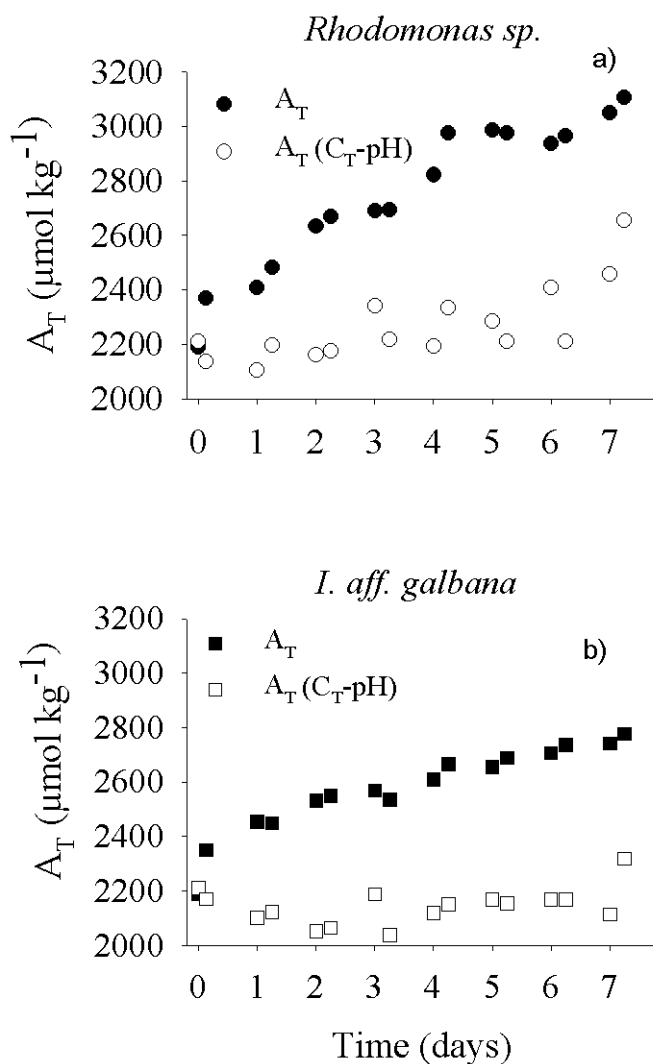
**Field data**—For all the seawater samples from the different surveys,  $A_T$  was measured directly as well as calculated from  $C_T$ -pH. Table 1 shows a standard deviation of ~7 μmol kg<sup>-1</sup> for ( $A_T - A_{TC}$ ) using measurements on CRMs. For coastal water, we will thus consider three times that value as indicative of the presence of organic bases.

**Northern Gulf of California.** The differences between  $A_T$  measured and  $A_{TC}$  from the Gulf of California are shown in Fig. 5a where the station 1 represents the northern part of the Gulf and station 14 is located south of Isla Tiburon in the central Gulf of California. In general, a clear gradient was found, with larger differences in the north (Fig. 5a). The first 5 stations

show the largest differences with an average of 82 μmol kg<sup>-1</sup>. For the rest of the stations, the estimated amount of organic bases decreased gradually toward more oceanic conditions. The highest difference was 109 μmol kg<sup>-1</sup> at station 1 and station 14 had the lowest values.

**San Diego Bay.** This survey took place shortly after an extensive red tide was seen in San Diego coastal water with high chlorophyll concentrations inside the entire bay. Station 1 is closest to the mouth of the Bay (oceanic) and the estimated amount of organic bases increases gradually toward the inner part of the bay. The lowest values found in San Diego Bay were similar to the highest values found in the northern Gulf of California. In general, high amounts of organic bases were found at all stations (Fig. 5b). The data show a tendency to increase away from the mouth of the bay, with the values of around 100 μmol kg<sup>-1</sup> to a maximum value of ~190 μmol kg<sup>-1</sup> at the head of the bay.

**San Quintín Bay.** San Quintín Bay lies on the Pacific and its physical mixing is strongly controlled by the California Current System (Ibarra-Obando et al. 2001). Stations 1 through 21 represent one arm, starting in the station nearest to the mouth

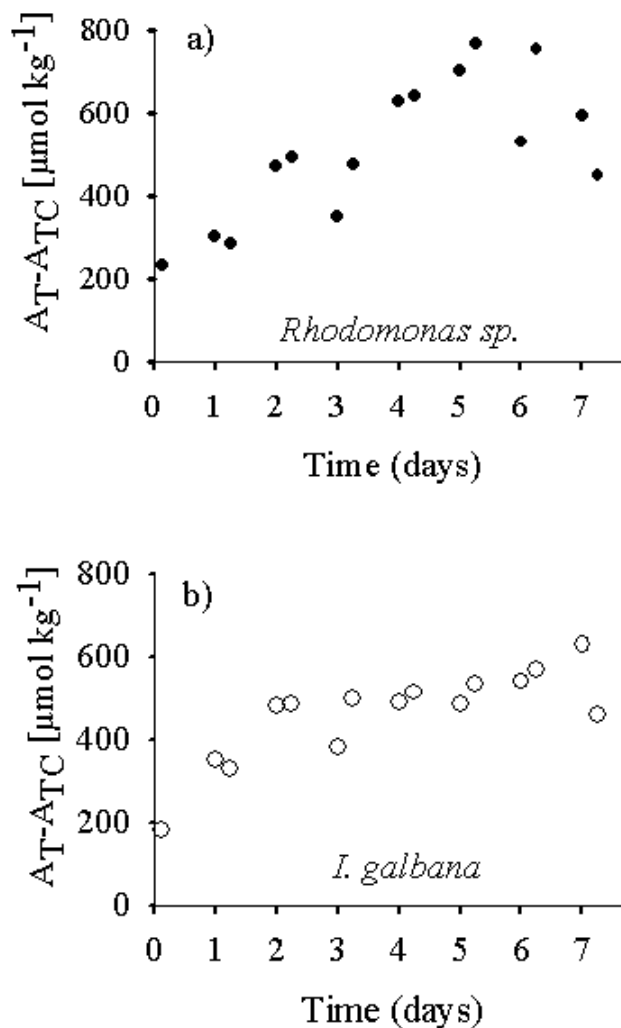


**Fig. 3.** Comparison of  $A_T$  measured and  $A_T$  calculated ( $pH - C_T$ ) for *Rhodomonas* sp. and *I. galbana*

(oceanic). The other arm includes stations 22 through 30. In general, the amounts of organic bases found in this bay were 20–75  $\mu\text{mol kg}^{-1}$  (Fig. 5c). The highest values were found in the stations located in the center of the bay with values above 30  $\mu\text{mol kg}^{-1}$ .

### Discussion

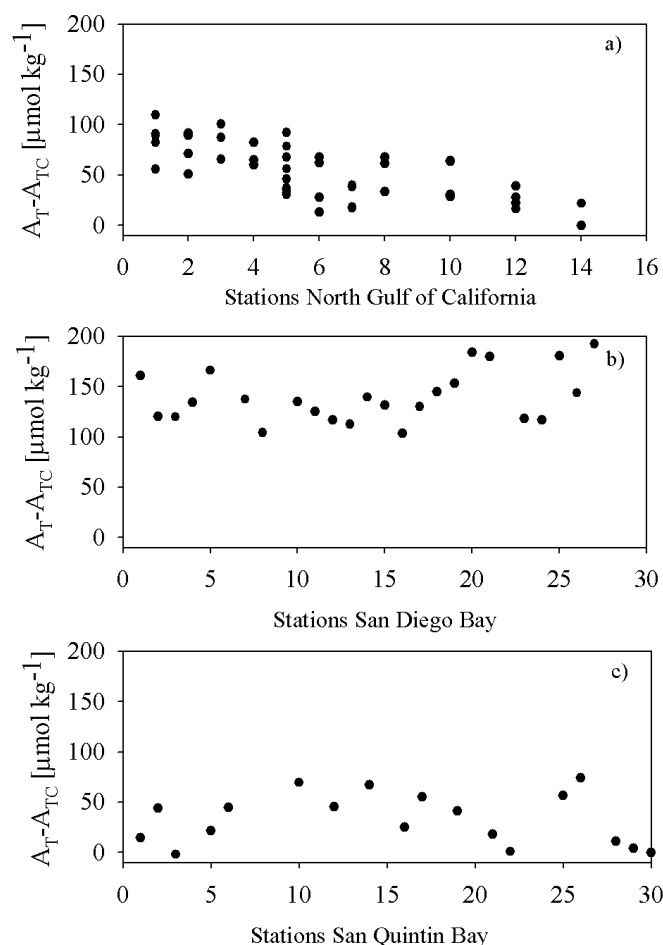
In the open ocean, in general, the  $A_T$  does not change appreciably with photosynthesis and respiration processes (Morel and Hering 1993). However, in cultures, significant  $A_T$  changes can result from the use of nitrogen species present in the medium. For example,  $A_T$  increases with the depletion of  $\text{NO}_3^-$  and  $\text{NO}_2^-$  and diminishes with the depletion of  $\text{NH}_4^+$  (Brewer and Goldman 1976, Goldman and Brewer 1980, Geider and Osborne 1992, Morel and Hering 1993). In our cultures, the medium was prepared with  $\text{NO}_3^-$ , and as a result, the  $A_T$  would be expected to increase as the phytoplankton grows.



**Fig. 4.** The concentration of organic bases estimated by subtracting the  $A_{TC}$  calculated from the  $A_T$  measured for *Rhodomonas* sp. (a) and *I. galbana* (b)

Our culture studies indeed showed this increase in  $A_T$  (Fig. 2) and also an increase in what we have assumed are organic bases—detected as the difference  $A_T - A_{TC}$  (Fig. 3). At the start of the culture experiment, this difference was essentially zero, and increased with time. Other culture experiments with marine phytoplankton (Biddanda and Benner 1997) have found a close link between  $C_T$  uptakes and dissolved organic carbon (DOC) production. Furthermore, the DOC produced consists of all types of biochemical products including organic acids such as glycollate, tricarboxylic, and hydroxamate (Zhang and Wang 2004), and such weak organic acids will be measured during the titrimetric determination of  $A_T$ . In our cultures, we found that the final values of  $A_T$  and DOC were higher in *Rhodomonas* sp. than in *I. galbana* cultures, possibly indicating an organism-dependent effect.



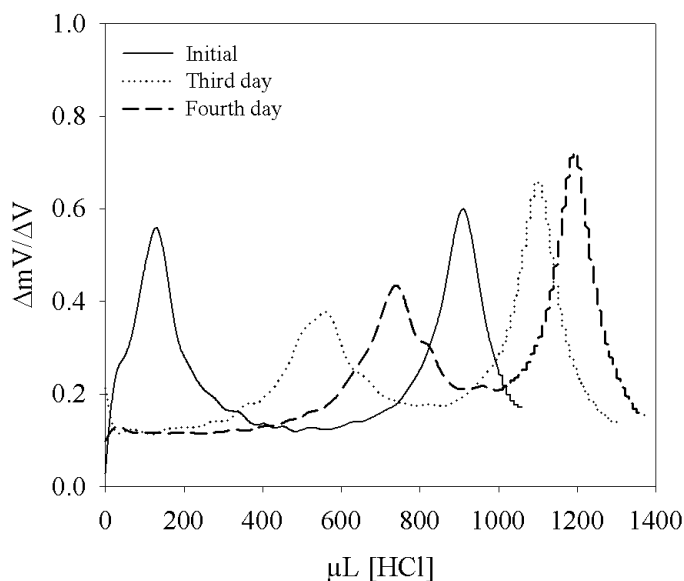


**Fig. 5.** Concentration of organic bases estimated for samples in a transect for (a) the north Gulf of California, (b) San Diego Bay, and (c) San Quintín Bay

Two lines of evidence indicate the presence of unidentified organic bases, both in our microalgal cultures and in the various coastal seawaters we studied. These are as follows:

(1) The deformation of the shape in the derivative titration curve as an indicator of the presence of additional bases.

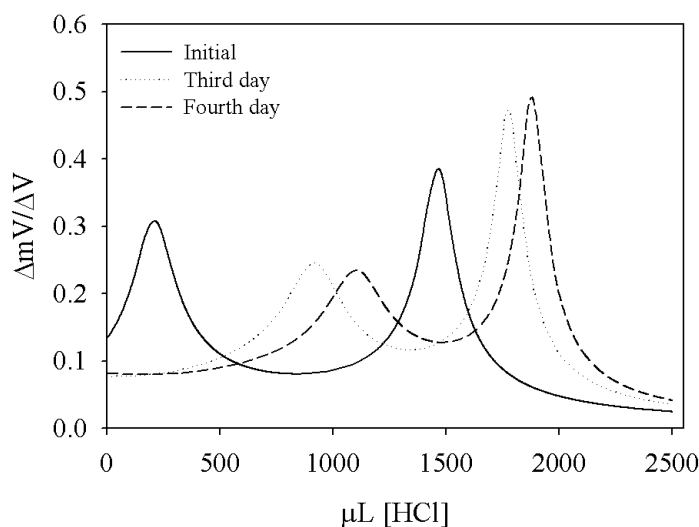
Peak height and peak shape in the derivative plot from the titrations can be indicators for the presence of additional bases apart from borate and carbonate (Hernández-Ayón et al. 1999). Unlike peak position, peak shape is sensitive to interferences. Hernández-Ayón et al. (1999) tested the sensitivity of peak height to organic matter using a multicomponent model by titrating with mixtures of three model organic compounds, “acetate,” “malonate,” and “catechol” in various proportions, in a humic substance-like matrix. The results indicated that “catechol” with effective  $pK_1$  of 9.4 and  $pK_2$  of 12.6 mostly depresses peak 1. Fig. 6 shows changes in the derivative curve as a function of incubation time in the *Rhodomonas* sp. experiments. The first peak decreases and the second peak increases during the first few days of culture. The simulations of Hernández-Ayón et al. (1999) indicate that the shrinking of the first peak suggests the presence of a ligand with  $pK > 7$ .



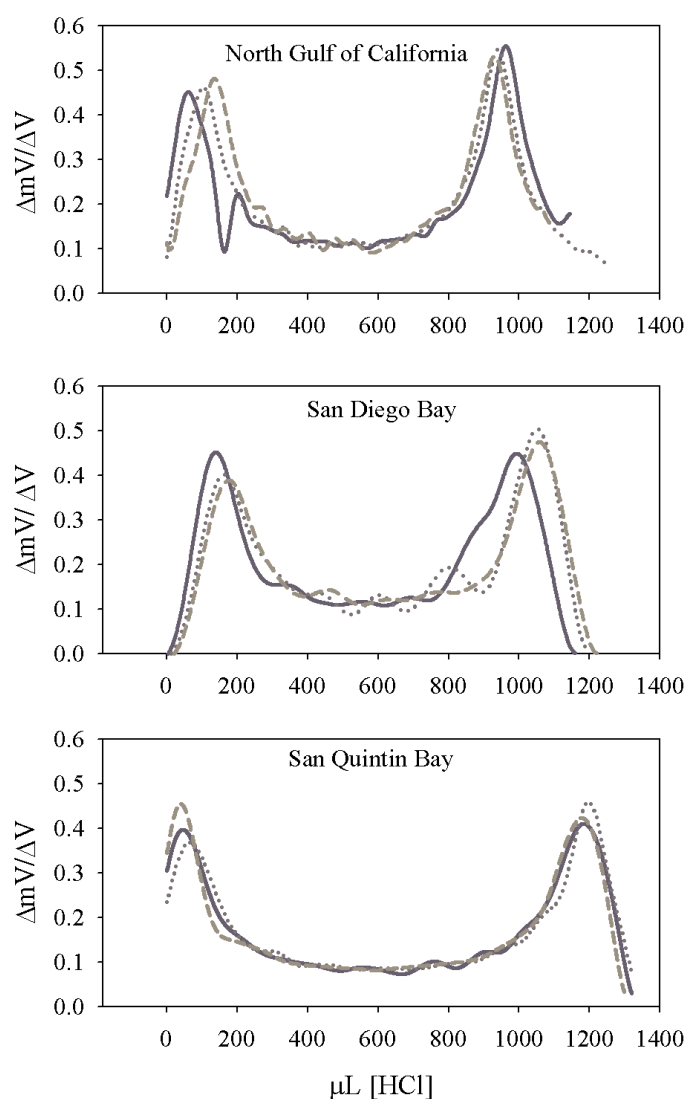
**Fig. 6.** Peak depression as a result increase in the concentration of organic bases in *Rhodomonas* sp. culture

We also simulated such titrations using a model where the  $C_T$  corresponded to the measured values, and included an organic base with a  $pK$  equal to 8 and a concentration ( $A_T - A_{TC}$ ) equivalent to that found in the *Rhodomonas* sp. experiments. The simulated titration curves (Fig. 7) show very similar shapes to those observed (Fig. 6), thus supporting our hypothesis that organic bases have been released into the solution during our culture experiments. A similar (though much smaller) effect can be seen in the titration curves from our coastal samples (Fig. 8).

(2) The failure of a simple inorganic model to get agreement between  $pH$ ,  $A_T$  and  $C_T$ .



**Fig. 7.** Model curves adjusted to the same  $C_T$  and organic base concentration as the curves from Fig. 7 using a simulated organic acid with a  $pK$  of 8



**Fig. 8.** Peak depression as a result of organic bases in seawater from coastal environments

In principle, if we know the total concentrations and acidity constants of the various acid-base pairs in seawater, we can calculate the alkalinity corresponding to a particular pH. For open ocean seawater, this can be done successfully with a model comprising water, total inorganic carbon, total phosphate, and total boron (calculated from salinity). If there are additional bases and acids present, the sum of their contributions to measured alkalinity can be estimated as the difference between measured alkalinity and alkalinity calculated from pH and  $C_T$  measurements with the simple inorganic model outlined above. This procedure works very well for our microalgal experiments where the final value of such organic bases was  $\sim 800 \mu\text{mol kg}^{-1}$  for *Rhodomonas* and  $\sim 400 \mu\text{mol kg}^{-1}$  for *I. galbana* (although the cell  $\text{mL}^{-1}$  was higher for *I. galbana*).

In the open ocean, the simple inorganic model typically works well (see e.g., Millero et al. 1993b). However, we have

shown that it fails in areas with restricted circulation and high DOC (Fig. 5), and we suggest that the difference is probably due to organic bases produced by phytoplankton. The accuracy of the estimated concentrations of organic bases is harder to determine. It depends, ultimately on the accuracy of our measurements of  $A_T$  and  $C_T$ . We have shown that they are reasonably accurate in open-ocean water (the CRMs), and that if the pK of the organic acids are above 7 (as the measurements on cultures suggest), then we expect the estimates of  $A_T$  and  $C_T$  to be unbiased even with significant amounts of organic bases present. However, if—for example—the pK of the organic acid is in the vicinity of 6, then the total carbon values obtained would be biased high (Fig. 1) and, consequently, the concentration of organic bases would be underestimated by this technique. Another possible concern might be the contribution of surfaces of bacteria and algae (as was described by Hyun-Cheol et al. 2006). However, in our work, we compared the measured alkalinity of an *Isochrysis galbana* culture with and without filtration and found no significant difference within  $5 \mu\text{mol kg}^{-1}$ .

In the measurements we present here, we used simple techniques: a potentiometric pH measurement, combined with  $A_T$  and  $C_T$  estimated from a closed-cell titration with a simple derivative technique. As a result, the sensitivity of our analyses is not optimal, and we consider the limit of detection for organic bases using these techniques to be about  $20 \mu\text{mol kg}^{-1}$ . Although this was sufficient sensitivity for the environments we studied, if high-quality oceanic techniques: spectrophotometric pH (DOE 1994), open-cell alkalinity titration (Dickson et al. 2003), and extraction/coulometry (DOE 1994) were to be used it should be practical to detect such organic bases at significantly lower concentrations. In addition, the potential problem with bias noted above would not be a factor. It remains to be seen just how prevalent such organic bases are in other environments.

Our one final potential concern is that if the presence of such organic bases goes unrecognized, then the calculation of  $p(\text{CO}_2)$  from another pair of carbonate systems parameters such as pH and  $A_T$  or  $A_T$  and  $C_T$  is compromised and any  $\text{CO}_2$  flux estimates will be similarly in error.

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