Spatio-temporal changes in totally and enzymatically hydrolyzable amino acids of superficial sediments from three contrasted areas

Antoine Grémare a,*, Dimitri Gutiérrez b, Pierre Anschutz c, Jean Michel Amouroux a, Bruno Deflandre d, Gilles Vétion a

a Observatoire Océanologique de Banyuls, Laboratoire d'Océanographie Biologique, UMR CNRS 7621, Université Pierre et Marie Curie, BP 44, F66651 Banyuls-sur-Mer, France
b Direction of Oceanographic Research, Peruvian Institute of Marine Research (IMARPE), P.O. Box 22, Callao, Peru
c Département de Géologie et Océanographie, UMR CNRS 5805, Université de Bordeaux 1, F33405 Talence Cedex, France
d Laboratoire de Géochimie des Eaux, UMR CNRS 7047 Physico-chimie des Fluides Géologiques, Case 7052, Université Denis Diderot – Paris 7 & IPGP, 2 place Jussieu, F75251 Paris Cedex 05, France

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Abstract

Spatio-temporal changes in totally and enzymatically hydrolyzable amino acids (THAA and EHHA) and EHAA/THAA ratios of superficial sediments were assessed during 1997–1999 in three areas (i.e., the Gulf of Lions, the Bay of Biscay, and Central Chile) differing in their primary productivity. In all three areas, and even off Central Chile where a strong El Niño event took place during 1997–1998, spatial changes were always much greater than temporal ones. The factors affecting the spatial distributions of amino acid concentrations differed among areas. In the Gulf of Lions, sediment granulometry was apparently the most important driving force of THAA, EHAA, and EHAA/THAA, and there was no marked difference between stations located on the open slope and those in submarine canyons. Conversely, in the Bay of Biscay, there were clear differences between the stations located off Cap-Breton, on the open slope, and those in the Cap-Ferret canyon; the latter two featuring lower EHAA and THAA but higher EHAA/THAA. This pattern is likely to result from the predominance of different sources of organic matter and especially from the importance of continental inputs to the Cap-Breton canyon. Off Central Chile, amino acid concentrations and ratios were both maximal around 100 m depth, probably reflecting the interaction between the primary productivity gradient and the presence of an oxygen minimum zone (OMZ) reducing the degradation of sedimentary organics. When comparing the average values collected in the three areas studied, THAA and EHAA were highest in Central Chile, intermediate in the Bay of Biscay and lowest in the Gulf of Lions. EHAA/THAA ratios were also highest in Central Chile but were lowest in the Bay of Biscay. Differences between the Gulf of Lions and the Bay of Biscay could have been affected by sampling design. In

* Corresponding author. Tel.: +33 4 68 88 73 59; fax: +33 4 68 88 73 95.
E-mail address: gremare@obs-banyuls.fr (A. Grémare).

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Central Chile, the use of labile organic carbon to total organic carbon (C-LOM/TOC) and EHAA/THAA as indices of organic matter lability led to very similar results. This was not the case in the Bay of Biscay. It is therefore argued that the use of C-LOM/TOC should be restricted to highly productive areas.

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Keywords: Sedimentary organics; Amino acids; Spatio-temporal changes; Gulf of Lions; Bay of Biscay; Central Chile

1. Introduction

It is currently believed that benthic food chains are food limited (Lopez & Levinton, 1987), and that they mostly rely on external input of organic matter (Graf, 1992). Benthic/pelagic coupling has been extensively documented both in shallow and deep ecosystems (Graf, 1987; Graf, 1989; Graf, 1992; Graf, Schultz, Peinert, & Meyer-Reil, 1983; Lampitt, 1985; Lampitt & Antia, 1997), and many studies have been devoted to the assessment of the relationship between sedimentary organics and both the numerical abundance and the biomass of benthic fauna (Grémare et al., 2002; Neira, Sellanes, Levin, & Arntz, 2001a; Neira, Sellanes, Soto, Gutierrez, & Gallardo, 2001b; Soltwedel & Thiel, 1995). This coupling differs between shallow and deep environments, since: (1) inputs originating from the water column are likely to be the only source of organic matter in deep-sea benthic ecosystems (Smith et al., 1996), (2) organic matter degradation during sedimentation increases with depth, and (3) there is feedback between benthic and pelagic ecosystems through nutrient regeneration in shallow environments (Cloern, 1982).

The dependence of benthic systems upon external inputs of organic matter has important consequences for benthic food chains. The primary consumers feed on an especially heterogeneous food source both in the origin and the level of available particulate organic matter (Tenore, Cammen, Findlay, & Phillips, 1981; Tenore & Hanson, 1980). There are important discrepancies in the biochemical composition of individual components of sedimentary organics at the micronutrient level, such that the availability of essential micronutrients may potentially limit benthic macrofauna (Marsh, Grémare, & Tenore, 1989; Phillips, 1984). Furthermore, an important component of the sedimentary organics is either refractory by nature (i.e., phanerogam detritus) or extensively degraded and thus cannot be absorbed by benthic fauna (Plante & Jumars, 1992; Plante & Mayer, 1994; Plante & Shriver, 1998a; Plante & Shriver, 1998b). Thus, the assessment of the relationship between sedimentary organics and benthic fauna cannot only rely on the use of global descriptors of organic matter but must consider specific biochemical parameters accounting both for POM digestibility and potential limitations of benthic fauna by micronutrient availability.

One attempt to overcome this problem is the biomimetic, kinetic-based approach proposed by (Mayer et al., 1995; Mayer, Schick, & Setchell, 1986) for the quantification of enzymatically hydrolyzable amino acids (EHAA) in marine sediments. In this approach, digestion is mimicked through incubation in the presence of a non-autohydrolyzable enzyme, and potential limitations by nitrogenous compounds are assessed by quantification of individual essential amino acids using high-performance liquid chromatography (HPLC). Based on a 4-year time series of sediment trap collected material, Medernach, Grémare, Amourous, Colomines, and Vétion (2001), and then Grémare et al. (2003) concluded that EHAA spectra were rather constant, and that digestibility was thus probably more important than micronutrient availability to benthic fauna in the Gulf of Lions. Moreover, bioassays tended to support the use of available proteins (an equivalent of EHAA) as an index of POM nutritional value (Grémare et al., 1997).

In spite of such potential interest, field surveys assessing the EHAA contents of marine sediments are still scarce. Moreover, such studies have often considered stations only sampled once (Dauwe & Middelburg, 1998; Dauwe, Middelburg, Herman, & Heip, 1999a; Dauwe et al., 1999b; Grémare et al., 2002), with
the exception of a study by Medernach et al. (2001). Therefore, little sound information is currently available on the kinetics and on the possible causes of temporal changes in amino acid concentrations and spectra. This complicates the assessment and the interpretation of spatial changes in EHAA, total hydrolyzable amino acids (THAA), and EHAA/THAA. During the present study, we tackled the problem of the interaction between temporal and spatial changes in THAA, EHAA, and EHAA/THAA by assessing spatio-temporal changes in three areas characterized by important differences in pelagic primary productivity, namely the Gulf of Lions (NW Mediterranean), the Bay of Biscay (N Atlantic) and Central Chile (S Pacific). Our primary aims were: (i) to assess the relative importance of temporal and spatial changes, (ii) to identify the main factors controlling the amino acid concentrations and ratios within these three areas, and (iii) to compare the average values of THAA, EHAA, and EHAA/THAA ratios among these three areas.

2. Materials and methods

2.1. Stations and sampling areas

A total of 38 stations were sampled during the present study (Fig. 1). Their geographical coordinates are provided in Table 1 together with main characteristics and sampling dates.

The Gulf of Lions is composed of four hydrological provinces (Lefèvre et al., 1997): (1) the Gulf of Marseilles which is a typical coastal oligotrophic system with a primary productivity of 88 gC m$^{-2}$ year$^{-1}$, (2) the mouth and the plume of the Rhône river which are restricted to a limited area of a few km$^2$ and show no striking seasonal variation in chlorophyll $a$ concentrations (about 1 mg m$^{-3}$), (3) the dilution area west of the Rhône river that has a late-winter early spring diatom bloom and smaller winter blooms, and (4) the Southern area including the frontal zone associated with the Liguro-Provençal current that shows a strong seasonality and a high primary production. Overall, primary production in the Gulf of Lions is between 78 and 142 gC m$^{-2}$ year$^{-1}$ (Lefèvre et al., 1997). The January 1999 cruise was carried out just before the spring bloom, whereas the June 1998 one took place during the transition between the period of higher primary production and summer, which is characterized by pronounced oligotrophy.

The Bay of Biscay has a wide continental shelf oriented NW–SE off the French, and EW off the Spanish coast. The dominant circulation is cyclonic and characterized by a weak northward current, which follows the shelf and intensifies during winter. The direction of this current sometimes reverses depending on tides and wind fields. Primary production data in the southern part of the Bay of Biscay are relatively scarce. Based on field measurements, Laborde, Urrutia, and Valencia (1999) computed that primary production is between 145 and 170 gC m$^{-2}$ year$^{-1}$. The spring bloom is the strongest seasonal pulse of primary production in the Bay of Biscay. Although its precise timing is still debated, it is thought to begin in March and to end in May (Treguer, Le Corre, & Grall, 1979). Laborde et al. (1999) reported mean maximal values of 1 mg Chl $a$ m$^{-3}$ for surface waters during May 1990 and May 1991. Coccolithophorid blooms may also occur in summer, as a consequence of upwelling cells developing along the shelf under NE winds (Heaps, 1980). According to Laborde et al. (1999), primary production is almost nil during winter.

Due to the Peru–Chile Current that generates persistent coastal upwelling, the coastal waters off Central Chile are highly productive (i.e., up to 3500 gC m$^{-2}$ year$^{-1}$) according to Gallardo (1977). An important component of the POM produced sinks to the bottom, accounting for higher sediment organic content than in most other coastal marine areas (Neira et al., 2001b). These waters exhibit a complex hydrography depending on short-term seasonal and inter-annual events. At intervals of 2–7 years, El Niño events result in considerable warming and improved oxygenation of surface waters, leading to a decline of primary
productivity. Most of the stations we studied were sampled between May 1997 (i.e., just after onset of the 1997/98 El Niño) and November 1998 (i.e., well after the end of the 1997/98 El Niño). In Northern Chile, this particular event, reduced chlorophyll $a$ concentrations in superficial waters from 5.0 (January 1977) to 1.6 mg m$^{-3}$ (July 1997) (Gonzalez, Ortiz, & Sobarzo, 2000). It should be pointed out that according to Strutton and Chavez (2000), this El Niño was in some aspects the strongest ever observed.
2.2. Collection of sediment samples

2.2.1. Gulf of Lions

Twenty-one stations were sampled during the Moogli II and III cruises, aboard the R/V Suroît during June 1998 and January 1999, respectively (Fig. 1). Average bottom water temperature was between 13 and...
13.7 °C, median grain size of surface sediment between 6.1 and 224.3 μm and organic contents of surface sediments between 0.19 and 1.01%DW (Table 1). At each station, sediment cores (9 cm in diameter) were taken using a Bowers and Connolly® midicorer. There were two successful drops per station. Three cores with intact sediment–water interface and clear overlying waters were randomly selected from the two drops and used for biochemical assays. The overlying water was immediately removed and the first centimeter of each core was cut, centrifuged (4500 rpm during 3 min) briefly rinsed in distilled water, centrifuged again (4500 rpm during 3 min) and then frozen (−20 °C). Back at the laboratory, sediment samples were freeze-dried, crushed, sieved on a 200 μm mesh, and stored at −20 °C before analysis.

2.2.2. Bay of Biscay
Nine stations were sampled during the OXYBENT 1–7 cruises which took place between October 1997 and January 1999 aboard the R/V Côte d’Aquitaine (Fig. 1). Average bottom water temperatures were between 4 and 12.3 °C (Table 1). Median diameter was between 7.3 and 12.9 μm and organic carbon contents were between 0.80 and 2.23%DW. Sediment samples were collected using a Barnett multicorer. At each station, a single core (10 cm in diameter) with intact sediment–water interface and clear overlying water was used for biochemical assays. The top of this core was sliced in two 0.5 cm horizontal sections. A sub-sample of each slice was frozen under nitrogen. Back at the laboratory, those sub-samples were freeze-dried and stored at −20 °C. They were crushed and sieved (200 μm mesh) prior to analysis. Biochemical assays were carried out on the two 0.5 cm sections and then averaged for comparison with the Gulf of Lions and Central Chile data.

2.2.3. Central Chile
Eight stations were sampled off Central Chile (Fig. 1). Stations 4, 7, 14, 18, and 26 were located along a depth transect off the Bay of Concepcion (ca. 36°S). Stations 4 and 7 were located in and at the mouth of the eutrophic Bay of Concepcion. Stations 14, 18, and 26 were, respectively, categorized as inner-shelf, mid-shelf, and outer-shelf by Gutiérrez et al. (2000) and then by Neira et al. (2001b). These five stations were sampled during four seasonal cruises (i.e., May and November 1997, May and November 1998) from the R/V Kay Kay. Stations 4 and 26 were also sampled during April 1999, together with stations 3, 4C, and 5D, which were located deeper on the continental slope. The timing of these samplings relative to the 1997–1998 El Niño event is presented in Fig. 2. The first survey (May 1997) took place shortly after the onset of El Niño and the November 1998 survey shortly after it ended. Average bottom water temperature was between 3.7 and 13.9 °C, mean median grain size between 5.8 and 6.1 μm and organic carbon contents between 1.13 and 4.76%DW (Table 1). Samples were taken using either a gravity Rumohr corer (May 1998 cruise) or a minimsample corer (Barnett, Watson, & Connelly, 1984). Here again, care was taken to select one core with intact sediment water interface and clear overlying waters. This was especially true for the Rumohr corer, which proved efficient in collecting Thioploca mats, which are strictly restricted to the sediment surface (Gutiérrez, personal observation). The first centimeter of the core was sliced, deep frozen, and then processed as described for the Gulf of Lions survey.

2.3. Biochemical assays
To assess THAA, 15 mg DW of sediment were submitted to a strong acid hydrolysis (500 μl of 6 N HCl, 100 °C, 24 h, under vacuum). Sub-samples of the hydrolyzates (0.4 ml) were neutralized with 0.4 ml of 6 N NaOH and buffered with 0.8 ml of H3BO3 (0.4 M, pH 8). Fluorescent derivatives were obtained by adding 6 μl of an orthophtaldialdehyde solution (125 mg in 2.5 ml of methanol and 0.125 ml of mercaptoethanol) and 400 μl of H3BO3 to 100 μl of those samples. THAA quantification was directly based on fluorescence measurements compared to a standard mixture of 19 amino acids. Excitation wavelength was 335 nm and emission wavelength was 450 nm.

Enzymatically hydrolysable amino acids (EHAA) were extracted following the biomimetic approach proposed by (Mayer et al., 1995). Hundred milligrams DW of sediment was poisoned with 1 ml of a solution
containing two inhibitors of bacterial active transport systems (0.1 M sodium arsenate and 0.1 mM pentachlorophenol within a pH 8 sodium phosphate buffer). This mixture was incubated for 1 h at room temperature. Then, 100 μl of proteinase K solution (1 mg ml⁻¹) were added, and the samples were incubated for 6 h at 37 °C. After centrifuging to discard remaining particulate material, 75 μl of pure TCA were added to 750 μl of supernatant to precipitate macromolecules, which are considered to be unsuitable for absorption. After more centrifuging, 750 μl of the supernatant were hydrolyzed and processed as described for THAA. A blank accounting for possible degradation of the enzyme was carried out. EHAA were quantified using the same procedure as for THAA.

All biochemical assays were run on triplicates. For both THAA and EHAA, analytical variability was close to 5%.

EHAA/THAA ratios expressed in % were computed for each combination of station and sampling date.

3. Results

3.1. Gulf of Lions

Analytical variability was 4.4% and 5.1% for THAA and EHAA, respectively (Table 2). Variation coefficients for within-site variability were much lower than those for between sites variability (9.9 vs 34.6 and 10.4 vs 44.4 for THAA and EHAA, respectively).

In the Gulf of Lions, THAA were between 4.93 (Station O, January 1999) and 28.04 nmol mg DW⁻¹ (station H, January 1999) (Table 3). EHAA ranged from 0.78 (Station O, January 1999) and 6.78 nmol mg DW⁻¹ (station H, January 1999). EHAA/THAA ratios were between 15.8% (station O, January 1999) and 33.0% (station D, January 1999). For all these parameters, there were no significant differences between June 1998 and January 1999 (Signed rank tests, \( p = 0.182 \), \( p = 0.767 \), and \( p = 0.142 \), for THAA, EHAA, and EHAA/THAA, respectively). Lack of significant difference was mostly due to the high variability at the shallow stations (Fig. 3).
Analysis of the relationships linking amino acid concentrations and ratios with depth (Fig. 4) confirmed that concentrations were higher within the 200–1000 m range (i.e., the upper continental slope) during June 1998.

Overall, THAA, EHAA, and EHAA/THAA did not correlate negatively with depth ($p_{D} = 0.298$, $p_{D} = 0.778$, and $p_{D} = 0.249$, respectively). There was no unique pattern of change over the whole range of depth (Fig. 4). During both cruises, both THAA and EHAA clearly decreased with depth between 0 and 200 m, then tended to increase between 200 and 1200 m. The pattern at greater depth was difficult to interpret due to the small number of sampled stations. The same trend was also apparent, although less clearly, for EHAA/THAA. During the June 1998 cruise, there was no marked difference in THAA, EHAA or EHAA/THAA between station I on the open slope, and station U in the Lacaze-Duthiers canyon (Table 3).

### 3.2. Bay of Biscay

In the Bay of Biscay, THAA was between 20.03 (station F, January 1998) and 70.07 nmol mgDW$^{-1}$ (station D, January 1999) (Table 4), EHAA ranged from 4.82 (station G, October 1998) to 12.77 nmol mgDW$^{-1}$

#### Table 2

**Gulf of Lions**: Analytical, within site and between-site variability of THAA and EHAA

<table>
<thead>
<tr>
<th></th>
<th>Analytical variability</th>
<th>Within site variability</th>
<th>Between sites variability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Within site, within core</td>
<td>Within site, between cores</td>
<td>Between sites</td>
</tr>
<tr>
<td>THAA</td>
<td>4.4</td>
<td>9.9</td>
<td>34.6</td>
</tr>
<tr>
<td>EHAA</td>
<td>5.1</td>
<td>10.4</td>
<td>44.4</td>
</tr>
</tbody>
</table>

#### Table 3

**Gulf of Lions**: Average THAA and EHAA concentrations and EHAA/THAA in the superficial sediments of the stations sampled during the June 1988 and/or the January 1999 cruises

<table>
<thead>
<tr>
<th>Stations</th>
<th>Depth (m)</th>
<th>June 1998</th>
<th>January 1999</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>THAA (nmol mgDW$^{-1}$)</td>
<td>EHAA (nmol mgDW$^{-1}$)</td>
<td>EHAA/THAA (%)</td>
</tr>
<tr>
<td>A</td>
<td>1640</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>B</td>
<td>1200</td>
<td>23.96</td>
<td>5.35</td>
</tr>
<tr>
<td>C</td>
<td>157</td>
<td>7.46</td>
<td>1.65</td>
</tr>
<tr>
<td>D</td>
<td>78</td>
<td>13.52</td>
<td>2.28</td>
</tr>
<tr>
<td>E</td>
<td>1380</td>
<td>16.15</td>
<td>3.82</td>
</tr>
<tr>
<td>F</td>
<td>240</td>
<td>14.82</td>
<td>2.81</td>
</tr>
<tr>
<td>G</td>
<td>95</td>
<td>10.05</td>
<td>1.99</td>
</tr>
<tr>
<td>H</td>
<td>75</td>
<td>24.13</td>
<td>5.88</td>
</tr>
<tr>
<td>I</td>
<td>830</td>
<td>18.40</td>
<td>4.82</td>
</tr>
<tr>
<td>J</td>
<td>340</td>
<td>12.47</td>
<td>3.03</td>
</tr>
<tr>
<td>K</td>
<td>94</td>
<td>13.65</td>
<td>3.36</td>
</tr>
<tr>
<td>L</td>
<td>87</td>
<td>23.09</td>
<td>4.83</td>
</tr>
<tr>
<td>M</td>
<td>1240</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>N</td>
<td>380</td>
<td>14.11</td>
<td>2.51</td>
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<tr>
<td>O</td>
<td>92</td>
<td>10.04</td>
<td>2.06</td>
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<td>P</td>
<td>91</td>
<td>17.71</td>
<td>4.86</td>
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<td>R</td>
<td>175</td>
<td>6.18</td>
<td>1.40</td>
</tr>
<tr>
<td>S</td>
<td>96</td>
<td>15.97</td>
<td>4.15</td>
</tr>
<tr>
<td>T</td>
<td>66</td>
<td>20.54</td>
<td>5.47</td>
</tr>
<tr>
<td>U</td>
<td>910</td>
<td>19.55</td>
<td>5.03</td>
</tr>
<tr>
<td>V</td>
<td>35</td>
<td>22.20</td>
<td>6.57</td>
</tr>
</tbody>
</table>
(station D, January 1999) and EHAA/THAA ratios were between 12.4 (station D, June 1998) and 30% (station A, January 1999). Temporal changes in THAA, EHAA, and EHAA/THAA at a given station were small. For THAA, coefficients of variation (CV) were between 6.2 (station B) and 24.3% (station G). For EHAA they ranged between 8.9 (station A) and 36.0% (station G). The range of CV of EHAA/THAA was even narrower, between 9.0 (station B) and 13.1% (station E). The high CV values of both THAA and EHAA at station G, were due to the high concentrations recorded in January 1998. The CV of EHAA at station D was also high, mostly due to the high concentration recorded during June 1998. Overall there was no clear seasonal pattern of change in amino acid concentrations or ratio. Median THAA, EHAA, and EHAA/THAA differed significantly among stations (Kruskal–Wallis ANOVA, $p < 0.007$ in all cases). Both THAA and EHAA average values at each station over all cruises correlated negatively with depth ($p < 0.0001$ and $p < 0.03$, respectively), and EHAA/THAA correlated positively with depth ($p < 0.0001$). More specifically, THAA and EHAA concentrations recorded on the continental shelf, in the Cap-Breton canyon and on the open slope tended to decrease with depth down to 1000 m and then tended to increase at the deepest, open-slope station (Fig. 5). There were clearly two groups of stations in respect to EHAA/THAA ratios. The five shallowest stations all had ratios lower than 18%, whereas the four deepest all featured ratios between 24% and 28%. At comparable depths, there was no marked difference in the amino acid concentrations or ratio between the Cap-Breton canyon and the open slope stations.

### 3.3. Central Chile

Off Central Chile, THAA was between 45.39 (station 3, April 1999) and 135.28 nmol mgDW$^{-1}$ (station 4, May 1997) (Table 5), EHAA ranged from 13.21 (station 4C, April 1999) to 53.16 nmol mgDW$^{-1}$ (station 4, April 1999) and EHAA/THAA ratios were between 16.2 (station 14, November 1998) and 59.3% (station 4, April 1999). The CV over the five stations (i.e., 4, 7, 14, 18, and 26), which were repeatedly sampled were between 6.4% and 23.7% for THAA, 5.0% and 33.5% for EHAA, and 6.0% and 36.1% EHAA/THAA. Stations 4 and 7 did not show any clear pattern of temporal change in either THAA or EHAA between May
Fig. 4. *Gulf of Lions*. Relationships between THAA, EHAA, and EHAA/THAA and depth. Stations I (open slope) and U (Lacaze-Duthiers canyon) are indicated by the corresponding letters.
1997 and May 1998 (Fig. 6). Station 14 showed slight decreases in THAA and EHAA between May 1997 and May 1998. Station 18 showed a continuous decrease in both THAA and EHAA between May 1997 and May 1998. Station 26 also showed a decrease in both THAA and EHAA during the same period. However, this decrease was much less pronounced than at station 14.

During April 1999, THAA, EHAA, and EHAA/THAA were lower at the three open slope stations (i.e., stations 3, 4C, and 5) than at stations 4 (inner Bay) and 26 (shelf break). Unlike EHAA and EHAA/THAA, the THAA recorded at station 4 on the April 1999 cruise was lower than those recorded during 1997 and 1998. When pooling the results of all cruises, average THAA, EHAA, and EHAA/THAA all significantly differed among stations (Kruskal–Wallis non parametric ANOVAs, $p = 0.011$, $p = 0.006$, and $p = 0.023$, respectively). THAA tended to decrease with depth between 28 and 88 m (i.e., between stations 4 and 18) (Fig. 7), showed a secondary peak at 120 m (station 26), then was much lower at greater depths. This pattern was almost identical for EHAA and EHAA/THAA, except that: (1) the initial decrease occurred between 28 and 64 m (i.e., stations 4 and 14), and (2) that station 18 ($z = 88$ m) corresponded to an absolute maximum in EHAA/THAA.

### 3.4. Comparison between areas

Average THAA were 15.36, 43.60, and 86.11 nmol mgDW$^{-1}$ in the Gulf of Lions, The Bay of Biscay, and Central Chile, respectively (Fig. 8), differing significantly among those regions (Kruskal–Wallis
Fig. 5. Bay of Biscay. Relationships between THAA, EHAA, and EHAA/THAA and depth. Vertical bars are standard deviations computed at each station based on seasonal sampling.
non-parametric ANOVA, \( p < 0.0001 \). Average EHAA were 3.59, 8.14, and 3.52 nmol mgDW\(^{-1}\) in the Gulf of Lions, The Bay of Biscay, and Central Chile, respectively, and differed significantly among areas (Kruskal–Wallis non-parametric ANOVA, \( p < 0.0001 \)). Average EHAA/THAA ratios differed significantly among areas as well (Kruskal–Wallis non-parametric ANOVA, \( p < 0.0001 \)). Mean values of the ratio were 23.1%, 19.6%, and 35.6%, in the Gulf of Lions, the Bay of Biscay, and Central Chile, respectively. Thus, the average EHAA/THAA ratio tended to be lower in the Bay of Biscay than in the Gulf of Lions, unlike THAA and EHAA.

Overall, OC contents correlated positively with THAA, EHAA, and to a lesser extent with EHAA/THAA (\( N = 38, r^2 = 0.837, r^2 = 0.850, \) and \( r^2 = 0.382, p < 0.0001 \) in all cases, Fig. 9). The best fits were obtained using a linear model (THAA) and an exponential model (EHAA and EHAA/THAA). Correlations between OC and both THAA and EHAA remained significant when considering the three areas separately, except for THAA in Central Chile. The linear regression linking OC and THAA did not significantly differ between the Gulf of Lions and the Bay of Biscay (ANCOVA, \( p = 0.523 \) and \( p = 0.120 \) for intercepts and slopes, respectively). The linear regressions linking OC and EHAA significantly differed among areas (ANCOVA, \( p = 0.026 \) for intercepts and \( p = 0.228 \) for slopes). EHAA/THAA did not correlate significantly with OC in the Gulf of Lions and in Central Chile. EHAA/THAA ratios correlated negatively with OC in the Bay of Biscay. Overall both THAA and EHAA tended to decrease with median grain size (data not shown). However, the relationships between these two variables and sediment character differed between areas mostly due to differences in the range of granulometry of the sampled sediments. These relationships were significant only in the Gulf of Lions (\( N = 18, r^2 = 0.712, \) and \( r^2 = 0.719 \) for THAA and EHAA, respectively, \( p < 0.0001 \) in both cases). EHAA/THAA ratios did not correlate significantly with median grain size.

### Table 5
**Central Chile: Average THAA and EHAA concentrations and EHAA/THAA in the superficial sediments of the stations sampled during the different cruises**

<table>
<thead>
<tr>
<th>Cruises</th>
<th>Stations</th>
<th>Depth (m)</th>
<th>THAA (nmol mgDW(^{-1}))</th>
<th>EHAA (nmol mgDW(^{-1}))</th>
<th>EHAA/THAA (%)</th>
</tr>
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Fig. 6. Central Chile. Temporal changes in THAA, EHAA, and EHAA/THAA at the five stations, which were seasonally sampled.
4. Discussion

4.1. Gulf of Lions

Grémare et al. (2003) reported THAA between 12.3 and 22.8 nmol mgDW\(^{-1}\) along a depth gradient (35–915 m) off Banyuls-sur-Mer sampled seasonally between February 1997 and December 1998. Corresponding EHAA were between 2.1 and 4.9 nmol mgDW\(^{-1}\), and EHAA/THAA between 16% and 32%. All these values are in good agreement with the results of the present study.

The spatial distributions of THAA, EHAA, and EHAA/THAA in the Gulf of Lions have already been described by Grémare et al. (2002) based on the sole results of the June 1998 cruise. The spatial distribution recorded during January 1999 is similar and characterized by a sharp decline in THAA and EHAA with depth on the continental shelf, followed by an increase with depth on the upper slope and a decrease at higher depths. Our results confirm the existence of such a pattern in the Gulf of Lions. According to Grémare et al. (2002), it mostly reflects spatial changes in sediment granulometry (i.e., the occurrence of coarser sediments near the edge of the continental shelf due to the Liguro-Provençal current). This interpretation is further confirmed by the existence of negative correlations between sediment median grain size and both THAA and EHAA.

In most of the Gulf of Lions, primary productivity shows a typical seasonality with higher values during the spring and lower ones during summer and early winter (Lefèvre et al., 1997). THAA and EHAA did not differ significantly between the two cruises carried out during the present study. This mostly resulted from high variability at shallow stations since both THAA and EHAA were always higher in June 1998 than in January 1999 at the stations located on the continental slope. Based on a seasonal survey carried out along a depth gradient, Grémare et al. (2003) also concluded that temporal changes were rather limited relative to spatial ones. Among the five stations they sampled, two were located on the continental shelf and three on the slope. These last three did not systematically exhibit higher concentrations of THAA and EHAA during spring than during winter. Moreover, the magnitude of temporal changes in THAA and EHAA tended to decrease with depth. Seasonal changes in THAA and EHAA are thus probably minor in the Gulf of Lions. However, further studies are still clearly needed to better unravel temporal and spatial changes in sedimentary organic composition within this area.
Fig. 8. Comparison between areas. Overall average of THAA (A), EHAA (B), and EHAA/THAA (C) in the three studied areas. Vertical bars are 95% standard errors.
Fig. 9. Comparisons between areas. Relationships between THAA (A), EHAA (B), and EHAA/THAA (C) and OC.
4.2. Bay of Biscay

To our knowledge, these are the first reports of THAA, EHAA, and EHAA/THAA in the Bay of Biscay. As in the Gulf of Lions, all three parameters exhibited restricted seasonal changes. Etcheber et al. (1999) already assessed spatio-temporal changes in the quality of sedimentary organics in the Bay of Biscay based on an intensive sampling carried out in and near the Cap-Ferret Canyon. These authors as well did not observe any significant seasonal variations of organic matter at the surface of the sediment. They attributed this pattern to a quick mineralization of sedimented labile organic matter.

Highest THAA and EHAA were recorded at station D on the continental shelf. THAA and EHAA tended to decrease with depth down to 1000 m with no marked difference between the Cap-Breton canyon and the open slope. These observations are consistent with the organic carbon concentrations of superficial sediments measured by Hyacinthe, Anschutz, Carbonel, Jouanneau, and Jorissen (2001) at stations A, B, C, D, E, and I during January 1999. Unlike THAA and EHAA, EHAA/THAA tended to increase with depth. They were minimal on the continental shelf and in the Cap-Breton canyon and maximal at deep open slope stations. These spatial distributions mainly reflect the predominance of different sources of organic matter. Due to its proximity to the coast, the Cap-Breton canyon can be fed directly by continental inputs. Conversely, the Cap-Ferret Canyon is located too far away from the continent to be directly fed by riverine sedimentary discharge (Castaing et al., 1999; Ruch, Mirmand, Jouanneau, & Latouche, 1993). Lateral along-slope advection appears to be the dominant particle transport mechanism in the Cap-Ferret canyon (De Madron, Castaing, Nyffeler, & Courp, 1999; Heussner et al., 1999) whereas sedimentary remobilization by gravity, mass or turbidity flows is believed to be limited (Cremer, Weber, & Jouanneau, 1999). Suspended particles feeding the canyon apparently preferentially originate from two sources located: (i) on the shelf and upper slope (<380 m depth), and (ii) deeper (<1000 m depth) south of the Cap-Ferret Canyon (Heussner et al., 1999; Radakovich & Heussner, 1999). These mechanisms may account for the occurrence of high concentrations of mostly refractory organic matter of terrestrial origin in the Cap-Breton canyon and for the apparent homogeneity of EHAA/THAA on the open slope and in the Cap-Ferret canyon. This pattern also accounts for the negative correlation between OC and both EHAA/THAA, which was only found in the Bay of Biscay.

4.3. Central Chile

Our THAA and EHAA were a little lower than those between 130 and 470 nmol mgDW$^{-1}$ reported by Henrichs, Farrington, and Lee (1984) off Peru. This may result from the more permanent and more intense oxygen deficiency impinging on the Peruvian margin (Arntz, Tarazona, Gallardo, Flores, & Salzwedel, 1991), enabling a better preservation of labile compounds than in the more seasonal and milder oxygen regime that characterizes the Central Chile margin.

The hydrography of our study area is characterized by the occurrence of a marked oxygen minimum zone (OMZ) intercepting the continental shelf between 100 and 300 m depth, but which can reach much shallower depths during upwelling events (Gallardo, Carrasco, Roa, & Canete, 1995). This whole system is subjected to ENSO variability. El Niño events generally result in a decrease of primary productivity and an increase in oxygen availability. Both of these factors affect benthic standing crops and activities (Gutiérrez et al., 2000; Levin et al., 2002; Neira et al., 2001a; Neira et al., 2001b). Oxygen availability seems to play the major role in controlling the quantitative characteristics of macrofauna (Levin et al., 2002) and meiofauna (Neira et al., 2001a). Our results suggest that oxygen availability also controls the quantitative and qualitative characteristics of the sediment like THAA, EHAA, and EHAA/THAA, since all were maximal around 100 m depth (i.e., within the OMZ). Only a few studies have assessed changes in both the quantitative and the qualitative characteristics of sedimentary organics during El Niño events. Neira et al. (2001b) studied these consequences by comparing the concentrations of organic carbon, proteins, lipids and carbohydrates
at a subset of our stations (i.e., stations 4, 7, 14, 18, and 26) during the 1997–1998 El Niño. They concluded that the five stations were not affected in the same way, with a clear decrease in sedimentary organic concentrations at station 4, no significant changes at stations 7 or 14, and a decrease (although less important than at station 4) at stations 18 and 26. Our own results confirm the occurrence of a slight decrease in THAA and EHAA at stations 18 and 26. They also confirm that station 7 was not affected. They, however, do not support the occurrence of a strong drop in THAA and EHAA concentrations at station 4 between May 1997 and May 1998, although it should be underlined that the April 1999 cruise showed a clear increase in EHAA and EHAA/THAA at station 4.

Neira et al. (2001b) used the ratio of the sum of proteins carbohydrates and lipids to total organic carbon as an index of the lability of sedimentary organics. The spatial pattern of this index was similar with that of EHAA/THAA, with high values within the Bay and at the shelf break and lower ones in the inner Bay. The index used by Neira et al. (2001b) strongly decreased between May 1997 and May 1998 at station 4, remained almost unchanged at stations 7 and 14 and only slightly decreased at stations 18 and 26. Here again, our results do not support the occurrence of a sharp decrease in the lability of sedimentary organics at station 4 between May 1997 and May 1998.

4.4. Comparison between areas

THAA, EHAA, and EHAA/THAA were higher in Central Chile than in the Gulf of Lions and in the Bay of Biscay. Moreover, THAA, EHAA, and EHAA/THAA in the sediments off the Central Chile continental margin are higher than most of those currently available in the literature (Dauwe et al., 1999b; Grémare et al., 2002 and Grémare et al., 2003). This result is of no surprise since: (i) our study area probably constitutes one of the most intense upwelling centers along the Chilean coast, with primary production rates up to 3600 gC m−² year⁻¹ during upwelling events (Fossing et al., 1995), and (ii) a large proportion of this primary production is directly channeled to the sediment resulting in high OC (Neira et al., 2001a). This correlation linking primary productivity and amino acid data tends to support the observations already made at a smaller spatial scale along a productivity gradient in the North Sea (Dauwe et al., 1999b).

THAA and EHAA tended to be lowest in the Gulf of Lions and intermediate in the Bay of Biscay, whereas EHAA/THAA tended to be lowest in the Bay of Biscay and intermediate in the Gulf of Lions. This suggests that primary productivity is not the only factor controlling the characteristics of sedimentary organics and pinpoints the importance of sampling design when comparing different areas. In the Bay of Biscay, EHAA/THAA ratios were lower on the continental shelf and in the Cap-Breton Canyon than on the open slope and in the Cape-Ferret canyon. Conversely, both THAA and EHAA were higher on the continental shelf and in the Cap-Breton Canyon than on the open slope and in the Cape-Ferret Canyon. As stated above, this pattern was interpreted as reflecting the accumulation of more refractory organic carbon, probably of terrestrial origin, on the continental shelf and in the Cap-Breton Canyon. Average EHAA/THAA on the continental shelf and in the Cap-Breton Canyon was 17.2% versus 24.3% on the open slope and in the Cape-Ferret Canyon. The occurrence of lower values of EHAA/THAA in the Bay of Biscay than in the Gulf of Lions (23.1%) is thus depending on the sampling design (i.e., the proportions of continental shelf/Cap-Breton Canyon versus open slope use during the present study).

Overall THAA, EHAA, and EHAA/THAA correlated positively with OC. However, there were clear differences in these relationships among areas. In the Gulf of Lions and in the Bay of Biscay, THAA correlated positively with OC and there was no significant difference in the relationships linking these two parameters within these two areas. This suggests that only little qualitative information is gained on sedimentary organics when assessing THAA instead of OC in the Gulf of Lions and the Bay of Biscay. In Central Chile, the lack of significance of the correlation between OC and THAA is mostly due to high variability. Among the eight sampled sites, stations 3, 4, 7, and 14 seem to follow the general relationship characterizing the other two areas, station 5D featured higher THAA than expected based on OC, and stations 4C, 18, and 26
featured lower THAA than expected based on OC. Unexpected differences in THAA contents thus do not appear to be linked with the bathymetry or the location of the stations. The reason for such discrepancy between Central Chile and the other two areas is not clear. It also exists for EHAA, and it would certainly prove interesting to assess both THAA and EHAA in Central Chile using HPLC (Medernach et al., 2001; Grémare et al., 2002) instead of bulk fluorimetric measurements to check for possible interference due to the high abundance of organic matter.

Overall, the best fits between EHAA and OC was obtained using exponential regression models, because EHAA tends to be higher in Central Chile than expected based on the comparison of its OC relative to those in the Gulf of Lions and in the Bay of Biscay. When the three areas were considered separately, the best fits between OC and EHAA were obtained using simple linear regression models. Unlike for THAA, these relationships differed significantly between areas and especially between the Gulf of Lions and the Bay of Biscay, reflecting differences in the pathways of organic matter transfers and especially the occurrence of a high concentration of more refractory carbon on the continental shelf of the Bay of Biscay and in the Cap-Breton Canyon (see above).

Overall EHAA/THAA correlated positively with OC. As for THAA, this was mostly due to the occurrence of high EHAA/THAA in Central Chile. However, when considering each area separately, the only significant correlation between EHAA/THAA and OC was negative (Bay of Biscay). Here again this reflects the pattern observed on the continental shelf and in the Cap-Breton canyon. In Central Chile, EHAA/THAA tended to increase (although not significantly) with OC. More measurements are clearly needed to establish this pattern better.

Organic matter lability can be defined relative to a large variety of biogeochemical processes. When focusing on the relationship between sedimentary organics and benthic fauna, it refers to the portion of sedimentary organics that can readily be absorbed by benthic primary consumers due to the characteristics of their digestive systems (Plante & Jumars, 1992; Plante & Mayer, 1994; Plante & Shriver, 1998a; Plante & Shriver, 1998b). Most lability indices are computed as the ratio between measures of labile and total organic matter. The EHAA/THAA is no exception. EHAA accounts for the portion of sedimentary organics that is hydrolyzed during digestion and can thus be absorbed by benthic primary consumers, whereas THAA refers to total organic matter. The ratio of the sum of protein, carbohydrate and lipid carbon (which is often called labile organic matter carbon or LOM-C) to total organic carbon (TOC) is based on the same approach and has already been measured both in the Bay of Biscay (Etcheber et al., 1999) and in Central Chile (Neira et al., 2001b). Average LOM-C/TOC in the Bay of Biscay was between 8% and 10%, which is much lower than the average EHAA/THAA (19.6%) recorded during the present study. In Central Chile, LOM-C/TOC was between 16.2% and 45.3%, respectively, which is much closer to the range of EHAA/THAA recorded during the present study (i.e., between 19.6% and 59.3%). Carbohydrates are usually associated with a more refractory component of sedimentary organics than lipids and proteins (David, 2003; Grémare et al., 2003). In fact, carbohydrates can be either digestible or refractory depending on their structural complexity. The similarity between LOM-C/TOC and EHAA/THAA in a highly productive area such as Central Chile suggests that carbohydrates originate quickly from primary production and mostly consist of simple and digestible forms. In areas where the lability of sedimentary organics is lower such as the Bay of Biscay, the proportion of complex and refractory carbohydrates increases, which results in a higher discrepancy between LOM-C/TOC and EHAA/THAA. Our results thus suggest that the use of C-LOM/TOC as a lability index is much more appropriate in productive than in more oligotrophic areas.

5. General conclusions

In all three areas studied, spatial changes in amino acid concentrations and ratios appeared to be much more important than temporal ones. This was not surprising for areas such as the Gulf of Lions and the Bay
of Biscay where primary productivity is rather low. It was much less expected for Central Chile where: (i) primary production is extremely high, (ii) sedimented organic matter is potentially preserved from degradation due to low oxygen availability, and (iii) both primary productivity and oxygen availability show important temporal changes due to ENSO.

The main environmental factors apparently involved in the control of the spatial distributions differed between areas. Sediment granulometry and continental inputs were especially important in the Gulf of Lions and in the Bay of Biscay, respectively.

Amino acid concentrations and EHAA/THAA were higher off Central Chile than in the Gulf of Lions or in the Bay of Biscay. Unlike THAA and EHAA, EHAA/THAA tended to be higher in the Gulf of Lions than in the Bay of Biscay. However, it should be underlined that these differences partly resulted from: (1) differences in the ranges of granulometry of the sampled sediments, and (2) the high proportion of stations sampled within the Cap Breton Canyon and on the continental shelf of the Bay of Biscay.

Overall, there were significant correlations between amino acid concentrations and EHAA/THAA and OC. However, there were also significant differences in these relationships between areas. These discrepancies were more marked for EHAA and EHAA/THAA than for THAA, reflecting differences in the qualitative characteristics of sedimentary organics. Unlike in the Bay of Biscay, there was good agreement between the overall values and the spatio-temporal changes in C-LOM/TOC and EHAA/THAA off Central Chile. This is linked with the higher lability of sedimentary organics off Central Chile, and is probably due to the nature of the (mostly labile) carbohydrates originating from pelagic primary production. It is thus suggested that the use of C-LOM/TOC as an index of the lability of sedimentary organics should be restricted to highly productive areas.

Acknowledgments

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References


