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# Marine crenarchaeotal membrane lipids in decapods: Implications for the $TEX_{86}$ paleothermometer

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[1] Pelagic Crenarchaeota produce glycerol dibiphytanyl glycerol tetraethers (GDGTs) as membrane lipids, and the GDGT composition changes according to growth temperature. This forms the basis of the $TEX_{86}$ paleotemperature proxy. This ratio correlates with sea surface temperature (SST) despite the fact that Crenarchaeota are distributed through the water column. Therefore there must be mechanisms that transport the surface signal to sediments such as repackaging in fecal pellets, marine snow, mass falls after phytoplankton blooms, or daily migration. To study GDGT transport, we analyzed stomachs and intestines of Atlantic and Mediterranean decapods as they are one of the major megafaunal groups, are easy to sample, and occur in both pelagic and benthic environments. GDGTs were found in most decapods' guts. GDGT abundances are significantly lower in intestines, but $TEX_{86}$-derived temperatures are not significantly different between stomachs and intestines (<1°C), suggesting that $TEX_{86}$ values are not altered during gut transit. Atlantic decapods show no difference in $TEX_{86}$ values between benthic detritivors and pelagic predators. However, Mediterranean decapods show a substantial difference between macroplankton feeders and bentho-pelagic predators. This is probably related to the freshness of the material consumed. $TEX_{86}$-derived temperatures in Atlantic decapods are close to the SST around the time of sampling, in agreement with stomach content analysis that shows fresh organic matter being ingested. For Mediterranean decapods, $TEX_{86}$ temperatures are significantly higher than SST around the time of sampling. This can be partly attributed to the large variability between decapod specimens and the low amounts of fresh material found in their stomachs.

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**Keywords:** $TEX_{86}$; sea surface temperature; Crenarchaeota; GDGT transport; decapods.

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## 1. Introduction

[2] Sea surface temperature (SST) is one of the most important parameters associated with past climate. Since the 1950s, several temperature proxies have been developed, the most common being $\delta^{18}O$ and Mg/Ca ratios of planktic foraminifera [*Chave*, 1954; *Emiliani*, 1955; *Nurnberg et al.*, 1996] and the $U^{k'}_{37}$ index of long-chain unsaturated ketones synthesized by haptophyte algae [*Brassell et al.*, 1986]. A new SST proxy was recently introduced, the TetraEther indeX of lipids with 86 carbons ($TEX_{86}$) [*Schouten et al.*, 2002], which may be a useful complementary paleotemperature tool. The $TEX_{86}$ proxy is based on temperature-related changes that occur in the composition of archaeal membrane lipids.

[3] Archaea were once thought to be extremophilic bacteria, but genetic studies showed that archaea constitute a different domain of life [*Woese et al.*, 1990]. Furthermore, molecular ecological studies revealed that archaea are ubiquitous and abundant in non-extreme settings such as the marine environment [*Hoefs et al.*, 1997; *Massana et al.*, 2000; *Karner et al.*, 2001]. One archaeal subgroup, the Crenarchaeota, is genetically related to (hyper)-thermophilic archaea and has adapted to the relatively cold marine waters [*Kuypers et al.*, 2001]. Archaeal membranes are composed of glycerol dibiphytanyl glycerol tetraethers (GDGTs) [*Sinninghe Damsté et al.*, 2002] and changes in the relative abundance of these lipids allow them to adjust their membrane fluidity as a response to fluctuations in water temperature. The relative abundance of GDGTs is quantified with the $TEX_{86}$ ratio [*Schouten et al.*, 2002]. An empirical correlation between $TEX_{86}$ values of sediment core tops from a wide variety of geographical locations and annual mean SST is used to calibrate the $TEX_{86}$ proxy [*Schouten et al.*, 2002]. Experiments with Crenarchaeota enrichment cultures and particulate organic matter studies have shown that this new proxy is strongly correlated with temperature and is not dependent on salinity or nutrient concentration [*Wuchter et al.*, 2004, 2005]. There is, however, a gap in knowledge that needs to be addressed in order to understand the origin of the $TEX_{86}$ signal. Although several studies have established that Crenarchaeota are distributed throughout the water column [*Murray et al.*, 1999; *Karner et al.*, 2001], $TEX_{86}$ values of particulate organic matter, sediment traps and sediment core tops are better correlated with SST (depth < 100 m) than with deeper water temperatures [*Schouten et al.*, 2002; *Wuchter et al.*, 2005; C. Wuchter et al., Archaeal tetraether membrane lipid fluxes in the northeastern Pacific and Arabian Sea: Implications for $TEX_{86}$ paleothermometry, submitted to *Paleoceanography*, 2006 (hereinafter referred to as Wuchter et al., submitted manuscript, 2006)]. Thus the signal in the deeper water layers and surface sediments seems to be primarily derived from the upper 100 meters of the ocean. Crenarchaeotal cells are too small (<1 $\mu$m [*Margot et al.*, 2002]) to sink to the sediment after cell death and this would prevent the $TEX_{86}$ signal from reaching the sediment surface. Thus there have to be mechanisms that allow GDGTs to reach the sediment. Transport of organic matter to the seafloor can be a passive or an active process, though the latter pathway is usually considered to be insignificant. The active pathway requires the vertical migration of surface organisms such as macroplankton, euphausiids or decapods which are located close to the bottom during daylight periods, where they are consumed by benthopelagic and benthic species [e.g., *Cartes*, 1993; *Cartes and Abelló*, 1992]. The passive pathways include the main transport mechanisms such as marine snow, fecal pellets and massive falls of phytoplankton material after surface blooms which are believed to agglomerate water-surface particles and enable them to sink quickly to the bottom of the ocean where they fuel benthic food webs. The formation of particle aggregates is higher in surface waters, where most food webs are active, which could explain why GDGTs produced in the surface of the ocean, carrying a SST $TEX_{86}$ signal, reach the seafloor. Further understanding of the transport mechanisms and deposition of the crenarchaeotal GDGTs is thus required to apply this new proxy. Once the GDGTs reach the sediment floor, benthic

activity is also likely to have an effect with unknown consequences for the $TEX_{86}$ signal.

[4] To address these issues we investigated the presence of GDGTs in decapod guts. Decapods are a key element of most pelagic food webs. Decapod crustaceans are widely diversified in bathyal marine environments from mid and subtropical latitudes and constitute the most abundant megafaunal group in deep water environments such as the deep Mediterranean Sea [*Cartes and Abelló*, 1992; *Maynou and Cartes*, 2000]. Although they are not the main consumers of plankton in the upper 100 m or the main fecal pellet producers, they are involved at different levels of the food web and can serve as a model system for other organisms such as copepods and microzooplankton. In contrast to copepods they are more easily collected and larger amounts of biomass can be obtained. Pelagic decapods could actively or passively consume lipids and participate in the transport of GDGTs from the surface ocean to the sediment, while benthic species potentially could alter the $TEX_{86}$ signal deposited in the sediment. The aim of this study was to assess if Crenarchaeota, and thus their GDGTs, are taken up by decapods and if the $TEX_{86}$ temperature signal may be partly transported or altered through decapod feeding activity. We selected species with different feeding habits, i.e., trophic resources both associated with bottom sediments (benthos) and/or to the water column (suprabenthos, zooplankton), and from two distinct settings, the oligotrophic Mediterranean Sea and the productive North Atlantic coast in two different seasons (Table 1). Potential bias of the $TEX_{86}$ ratio due to preferential degradation or differential retention time during passage through the gut system and possible differences caused by diverse decapod feeding strategies were also investigated.

## 2. Material and Methods

### 2.1. Study Area

[5] Samples were collected from two different areas: (1) the Mediterranean, around the Balearic island (cruise IDEA, 39°N, 2°39′E) at two sites situated northwest and southwest of Mallorca Island in February 2004, and (2) the North Atlantic coast of Spain (off Gijon) taken 20 km off the north coast of the Iberian Peninsula in April 2004 (ECOMARG03, 44°N, 4°29′W) (Figure 1). The two sites in the Mediterranean are rarely affected by wind mixing and a strong summer thermocline is often present. Monthly averaged SSTs varied between 28.4°C in August 2003 and 14.3°C in February 2004 around time of sampling (Table 2). The Mediterranean Sea is characterized by an almost constant water temperature of 13°C below 200 m, high salinity (37–39) and very low nutrient concentrations [*Comas et al.*, 1996]. The North Atlantic coast study area, known as Le Danois bank, is a marine mount-shape relief orientated E-W parallel to the northern coast of the Iberian Peninsula coast. SST in this area varied between 24°C in August 2003 and 11.9°C in February 2004 (Table 2), but in contrast to the Mediterranean, temperatures below 200 m in the water column decrease with increasing depth. Conductivity Temperature Depth (CTD) profile data collected in April 2004 during the ECOMARG03 indicated temperatures ~10°C below 30 m depth. The Atlantic has lower salinity values (35.7–35.8) and higher nutrient concentrations than the Mediterranean.

### 2.2. Samples

[6] We selected five decapod species with different feeding strategies and habitats. Two species, *Aristeus antennatus* (Risso, 1816) and *Plesionika martia* (A. Milne-Edwards, 1883) were collected at the Mediterranean sites in February 2004. The other three species, *Acanthephyra pelagica* (Risso, 1816), *Munida tenuimana* (Sars, 1872) and *Parapagurus pilosimanus* (Smith, 1879) were recovered in the North Atlantic (off Gijon) in April 2004 (Figure 1).

[7] Around the Balearic Islands, 11 specimens of *A. antennatus* and 9 specimens of *P. martia* were obtained at 775 and 675 m depth, respectively (Table 1; Figure 1). Eleven specimens of the bathypelagic shrimp *A. pelagica*, 12 of the squat lobster *M. tenuimana* and 11 of the hermit crab *P. pilosimanus* were collected in the north Atlantic at 1024 m depth (Table 1; Figure 1). A total of 54 specimens were analyzed and their stomachs and intestines were removed under a stereomicroscope (×10, ×40). Once stomachs and intestines were dissected, contents were collected with a spatula or, if they were liquid, pipetted into a tube, and subsequently freeze dried. Livers and gonads of 10 specimens from the Mediterranean were also dissected and portions were freeze dried and analyzed.

### 2.3. GDGT Analysis

[8] Freeze-dried stomach and intestine contents, and liver and gonad samples were weighed and extracted by sonication first with methanol, then a

**Table 1.** Characteristics of the Five Species Used in the Study[a]

| Species | *n* | Time | Location | Sampling Depth, m | Depth Interval, m | Habitat | Diet |
|---|---|---|---|---|---|---|---|
| *Plesionika martia* | 9 | 7/2/2004 | Mediterranean (39°N, 2°39′E) | 675 | 576–749 | necktobenthic | macroplankton |
| *Aristeus antennatus* | 11 | 7/2/2004 | Mediterranean (39°55′N, 2°36′E) | 775 | 576–775 | necktobenthic | epibenthos-benthos and pelagic prey |
| *Munida tenuimana* | 12 | 20/4/2004 | Atlantic (44°N, 4°29′W) | 1024 | 540–1024 | benthic | scavenging-detritivor and pelagic prey; phytoplankton detritus |
| *Parapagurus pilosimanus* | 11 | 20/4/2004 | Atlantic (44°N, 4°29′W) | 1024 | 820–1024 | benthic | benthic prey-scavenger; phytoplankton detritus |
| *Acanthephyra pelagica* | 11 | 20/4/2004 | Atlantic (44°N, 4°29′W) | 1024 | 550–1024 | bathypelagic | macroplankton predator; megabenthic remains |

[a] Included are time of sampling, location of fishing habitat, depth of samples, depth interval inhabited by species during the IDEA and ECOMARG surveys, and a summary of their main prey (diets). n, number of specimens analyzed.

mixture (1:1; v/v) of methanol (MeOH) and dichloromethane (DCM) and Last with pure DCM. After sonication, samples were centrifuged at 3500 rpm to remove particles. The supernatants were collected and after evaporating the solvents, the extracts were dried with a small pipette filled with anhydrous sodium sulphate. For the liver and gonad samples the total lipid extract was divided into apolar and polar fractions using a small column filled with activated alumina and using hexane/DCM (9:1; v/v) and DCM/MeOH (1:1; v/v) as eluents, respectively.

[9] The total extracts (stomachs and intestines) and polar fractions (livers and gonads) were redis-

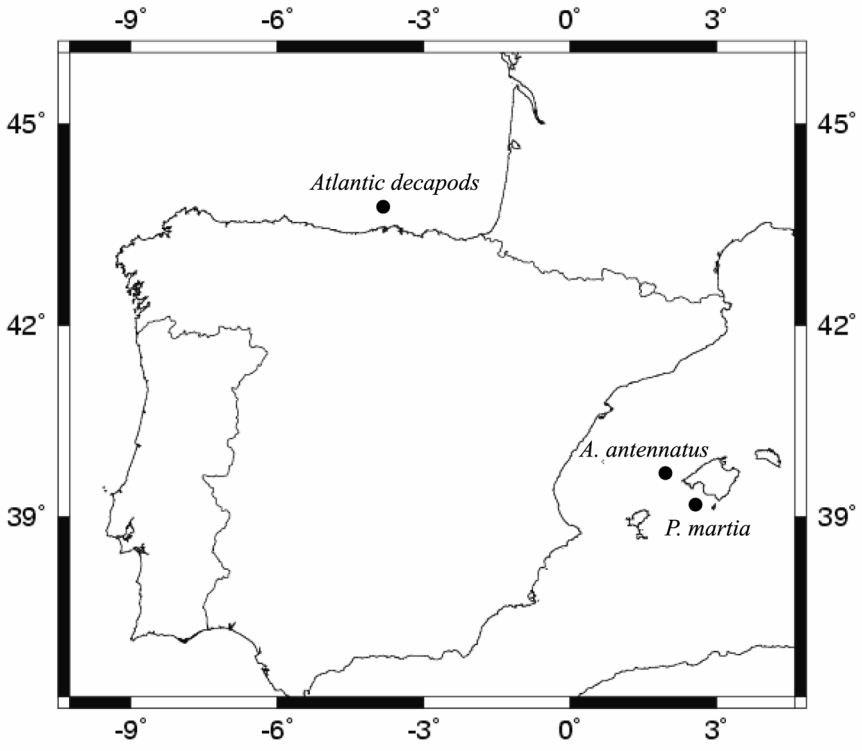


**Figure 1.** Map of the study area showing sample locations (circles).

**Table 2.** Mean Monthly Sea Surface Temperature Data From August 2003 to July 2004[a]

| Month | Species | Mediterranean SST, °C | Species | Atlantic SST, °C |
|---|---|---|---|---|
| March | | 14.8 | | |
| April | | 15.2 | | |
| May | | 18.7 | | 15.2 |
| June | | 23.0 | | 19.2 |
| July | | 26.5 | | 21.3 |
| August | | 28.4 | | 24.0 |
| September | | 26.2 | | 21.6 |
| October | | 23.2 | | 19.0 |
| November | | 19.0 | | 15.3 |
| December | | 16.0 | | 14.0 |
| January | | 14.7 | | 12.6 |
| February | | **14.3** | | 12.4 |
| March | | | | 11.9 |
| April | | | | **12.8** |
| **Annual mean** | | 20.0 | | 16.6 |
| $TEX_{86}$ | *A. antennatus* | 17.2 ± 2.2 | *A. pelagica* | 14.7 ± 2.0 |
| | *P. martia* | 21.9 ± 2.0 | *M. tenuimana* | 13.9 ± 0.7 |
| | | | *P. pilosimanus* | 14.5 ± 1.0 |

[a] Corresponding to sampling sites in the Mediterranean (39°N, 2°E) and Atlantic (44°N, 4°W) from the Advanced Very High Resolution Radiometer (AVHRR) data set at http://podaac.jpl.nasa.gov. Annual mean SST and average stomach and intestine $TEX_{86}$ temperatures for each decapod species are indicated. SST values at the time of sampling for Mediterranean decapods (February) and Atlantic decapods (April) are highlighted in bold.

solved in hexane/propanol (99:1; v/v) and filtered through 0.45 $\mu$m PTFE filters. An HP (Palo Alto, CA, USA) 1100 series LC-MS equipped with an auto-injector and Chemstation chromatography manager software was used to analyze the samples. Separation was attained on a Prevail Cyano column (2.1 × 150 mm, 3 $\mu$m; Alltech, Deerfield, Illinois, USA), maintained at 30°C. The GDGTs were eluted using a changing mixture of hexane and propanol. Detection was achieved using atmospheric pressure chemical ionization-mass spectrometry of the eluent [*Hopmans et al.*, 2000]. Single Ion Monitoring (SIM) was used instead of full mass scanning because SIM increases the signal-to-noise ratio and thus improves reproducibility. SIM was set to scan 5 $[M^+]$ + H ions of the GDGTs with a dwell time of 237 ms for each ion. Concentrations of GDGTs were used to calculate $TEX_{86}$ values using equation (1) [*Schouten et al.*, 2002]:

$$TEX_{86} = \frac{(GDGT_2 + GDGT_3 + Crenarchaeol - isomer)}{(GDGT_1 + GDGT_2 + GDGT_3 + Crenarchaeol - isomer)} \quad (1)$$

[10] The numbers refer to the number of cyclopentane moieties in the GDGT molecule; the crenarchaeol-isomer has 4 cyclopentane moieties and a cyclohexane moiety. $TEX_{86}$ temperatures were calculated with the following core top calibration [*Schouten et al.*, 2002]:

$$TEX_{86} = 0.015\ T + 0.28, \text{ with T = temperature } (^\circ C) \quad (2)$$

Absolute GDGT abundances were determined by comparison with an external standard curve obtained with a range between 10 and 200 ng of $GDGT_0$ (GDGT with no cyclopentane moieties).

## 3. Results

### 3.1. GDGTS in Decapods

[11] GDGTs were present in most decapod stomachs and intestines from both Atlantic and Mediterranean species (Tables 3 and 4). In contrast, analysis of both gonads and livers of decapods did not reveal the presence of GDGTs. The lowest average GDGT concentration (12 ± 3.7 $\mu g \cdot g^{-1}$gut content) was found in *P. pilosimanus* intestines (Table 3; Figure 2). The stomachs of *A. antennatus* contained the most GDGTs with on average 906 ± 428 $\mu g \cdot g^{-1}$gut (Table 4; Figure 2). Although average abundances of GDGTs were always higher in stomachs than in intestines (Figure 2), there were large variations between individual specimens (Tables 3 and 4). This resulted in substantial standard deviations for the average GDGT concentrations (Tables 3 and 4). However, a t-test revealed that intestine concentrations are overall lower than

**Table 3.** $TEX_{86}$ Calculated Temperatures and GDGT Concentrations for Stomachs and Intestines in Atlantic Decapods[a]

| | Stomach | | | Intestine | | |
|---|---|---|---|---|---|---|
| Species | n | $TEX_{86}$ T, °C | GDGT, mg · $g^{-1}$gut content | n | $TEX_{86}$ T, °C | GDGT, mg · $g^{-1}$gut content |
| *Acanthephyra pelagica* | *3* | 13.7 ± 1.4 | 10 | | *nd* | *nd* |
| | *3* | 14.6 ± 0.7 | 88 | *3* | 14.7 ± 0.6 | 90 |
| | *3* | 15.5 ± 0.9 | 22 | *3* | 17.3 ± 1.2 | 15 |
| | *1* | 12.8 | 3 | *2* | 13.9 ± 2.5 | 5 |
| | *2* | 15.5 ± 0.5 | 14 | *3* | 18.8 ± 3.1 | 4 |
| | *3* | 16.1 ± 0.9 | 17 | | *nd* | *nd* |
| | *3* | 15.3 ± 1.0 | 13 | *3* | 16.2 ± 1.5 | 11 |
| | *3* | 17.3 ± 2.5 | 20 | *3* | 15.3 ± 2.0 | 40 |
| | *3* | 12.9 ± 0.8 | 6 | *3* | 15.0 ± 1.2 | 10 |
| | *3* | 13.6 ± 0.3 | 95 | | *nd* | *nd* |
| | *3* | 12.8 ± 0.6 | 34 | *2* | 10.6 ± 0.2 | 2 |
| **Mean** | | **14.3** | **29** | | **15.2** | **22** |
| **Error** | | **1.6** | **32** | | **2.5** | **30** |
| *Munida tenuimana* | *3* | 13.1 ± 0.9 | 17 | *3* | 14.3 ± 0.9 | 16 |
| | *3* | 14.8 ± 1.6 | 10 | *3* | 13.2 ± 0.8 | 20 |
| | *3* | 14.1 ± 0.6 | 32 | *3* | 14.6 ± 0.5 | 29 |
| | *3* | 15.2 ± 0.3 | 32 | *3* | 14.2 ± 1.0 | 30 |
| | *2* | 13.5 ± 0.2 | 16 | *3* | 13.6 ± 1.5 | 20 |
| | *3* | 13.5 ± 0.3 | 33 | *3* | 13.2 ± 0.1 | 30 |
| | *3* | 13.8 ± 0.9 | 32 | *2* | 12.7 ± 0.2 | 25 |
| | *2* | 13.9 ± 0.5 | 78 | *3* | 13.7 ± 0.5 | 16 |
| | *3* | 14.5 ± 0.7 | 56 | *3* | 13.7 ± 1.4 | 24 |
| | *2* | 15.5 ± 0.4 | 49 | | *nd* | *nd* |
| **Mean** | | **14.2** | **35** | | **15.2** | **23** |
| **Error** | | **0.8** | **21** | | **2.5** | **6** |
| *Parapagurus pilosimanus* | *3* | 14.7 ± 0.3 | 37 | *1* | 16.0 | 17 |
| | *3* | 15.2 ± 0.7 | 17 | *3* | 15.1 ± 1.0 | 7 |
| | *3* | 12.9 ± 1.0 | 66 | *3* | 13.7 ± 1.7 | 13 |
| | *3* | 15.7 ± 1.4 | 5 | *3* | 14.0 ± 0.8 | 10 |
| | *3* | 15.9 ± 0.6 | 11 | *2* | 14.5 ± 0 | 10 |
| | *3* | 15.0 ± 0.7 | 49 | *2* | 12.8 ± 0.1 | 8 |
| | *3* | 13.3 ± 08 | 74 | *3* | 14.5 ± 0.7 | 14 |
| | *3* | 14.5 ± 0.6 | 5 | | *nd* | *nd* |
| | *2* | 16.1 ± 0.1 | 16 | *2* | 13.2 ± 0.7 | 14 |
| | *3* | 13.7 ± 0.4 | 49 | *3* | 14.4 ± 0.2 | 17 |
| **Mean** | | **14.7** | **33** | | **14.2** | **12** |
| **Error** | | **1.1** | **26** | | **1.0** | **4** |

[a]The number of measurements (n) is indicated. The standard deviations of the species-averaged temperatures are shown. The GDGT concentration for each specimen is indicated, and samples with GDGT concentrations below detection limit are labeled *nd*.

stomach values with a 99.9% confidence level. *P. martia* showed the largest difference, with an average of 332 ± 465 $\mu g \cdot g^{-1}$gut content in stomachs, but only 16 ± 9 $\mu g \cdot g^{-1}$gut content in intestines (Figure 2). This difference may be explained because only intestines of three specimens could be analyzed (Table 4) and this may not be representative of the total population. Species in the Mediterranean revealed average GDGT concentrations in stomachs and intestines that were up to 20 times higher than those observed in Atlantic decapods (Table 4; Figure 2).

## 3.2. $TEX_{86}$ Values

[12] $TEX_{86}$ values for the whole data set ranged from 0.50 to 0.63 and when converted to temperature with equation (2), these values correspond to 14.2 and 22.3°C respectively. Most decapods from the Atlantic coast were measured in triplicate

**Table 4.** $TEX_{86}$ Calculated Temperatures and GDGT Concentrations for Stomachs and Intestines in Mediterranean Decapods[a]

| | Stomach | | | Intestine | | |
|---|---|---|---|---|---|---|
| Species | n | $TEX_{86}$ T, °C | GDGT, mg · $g^{-1}$gut content | n | $TEX_{86}$ T, °C | GDGT, mg · $g^{-1}$gut content |
| *Aristeus antenatus* | *2* | 18.3 ± 1.4 | 1417 | *1* | 20.7 | 498 |
| | *2* | 13.2 ± 0.3 | 540 | *1* | 14.3 | 43 |
| | *2* | 18.1 ± 1.0 | 1416 | *2* | 18.4 ± 1.0 | 317 |
| | *2* | 15.5 ± 0.4 | 603 | *2* | 17.4 ± 0.2 | 424 |
| | | *nd* | *nd* | *1* | 16.1 | 75 |
| | *2* | 20.6 ± 0.2 | 1316 | *2* | 19.8 ± 1.6 | 772 |
| | | *nd* | *nd* | *1* | 13.4 | 71 |
| | *1* | 16.8 | 540 | *1* | 17.9 | 270 |
| | *2* | 14.5 ± 0.2 | 1247 | *2* | 14.5 ± 1.0 | 671 |
| | *1* | 18.7 | 423 | *1* | 17.4 | 215 |
| | *1* | 19.2 | 650 | *1* | 19.0 | 1217 |
| **Mean** | | **17.2** | **906** | | **17.2** | **416** |
| **Error** | | **2.4** | **428** | | **2.4** | **359** |
| *Plesionika martia* | *2* | 23.5 ± 0.5 | 1222 | | *nd* | *nd* |
| | *1* | 21.9 | 442 | | *nd* | *nd* |
| | *2* | 21.8 ± 0.2 | 996 | *1* | 17.8 | 29 |
| | *1* | 23.2 | 33 | *1* | 15.9 | 1 |
| | *2* | 24.3 ± 0.4 | 191 | *1* | 25.7 | 16 |
| | *1* | 19.5 | 80 | | *nd* | *nd* |
| | *1* | 23.7 | 10 | | *nd* | *nd* |
| | *1* | 21.3 | 5 | | *nd* | *nd* |
| | *2* | 22.7 ± 2 | 7 | | *nd* | *nd* |
| **Mean** | | **22.4** | **332** | | 19.8 | **16** |
| **Error** | | **1.5** | **465** | | 5.2 | **9** |

[a] The number of measurements (n) is indicated. The standard deviations of the specimen-averaged temperatures are shown. The GDGT concentration for each specimen is indicated, and samples with GDGT concentrations below detection limit are labeled *nd*.

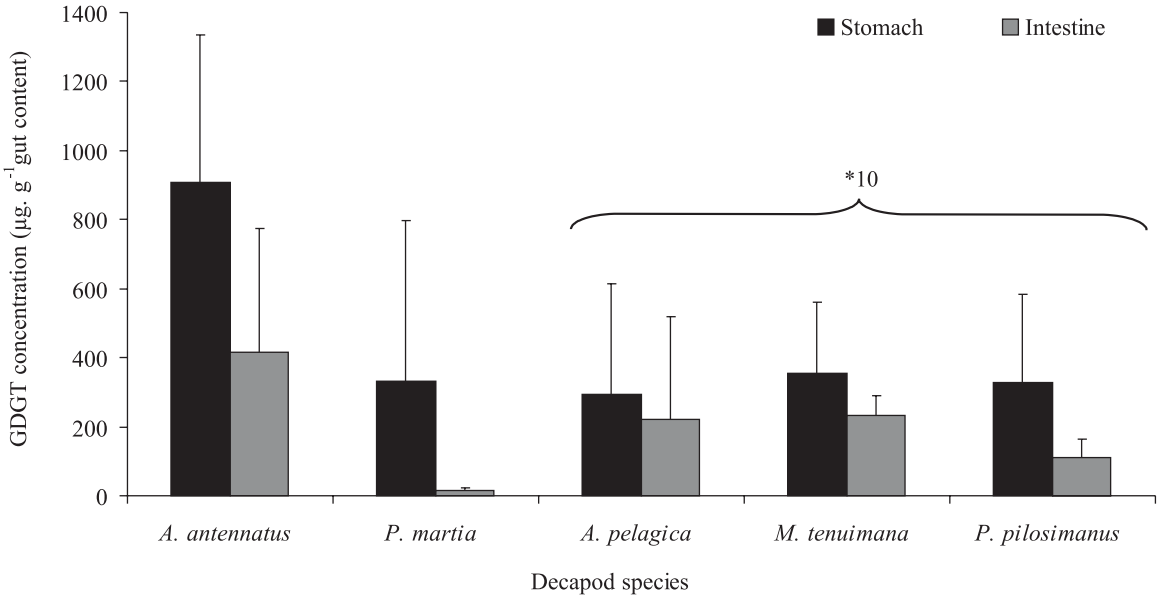


**Figure 2.** Average abundance of GDGTs in stomachs (black) and intestines (gray). The abundances in the Atlantic species (*A. pelagica, M. tenuimana*, and *P. pilosimanus*) have been magnified by a factor of 10 to enable a better comparison.

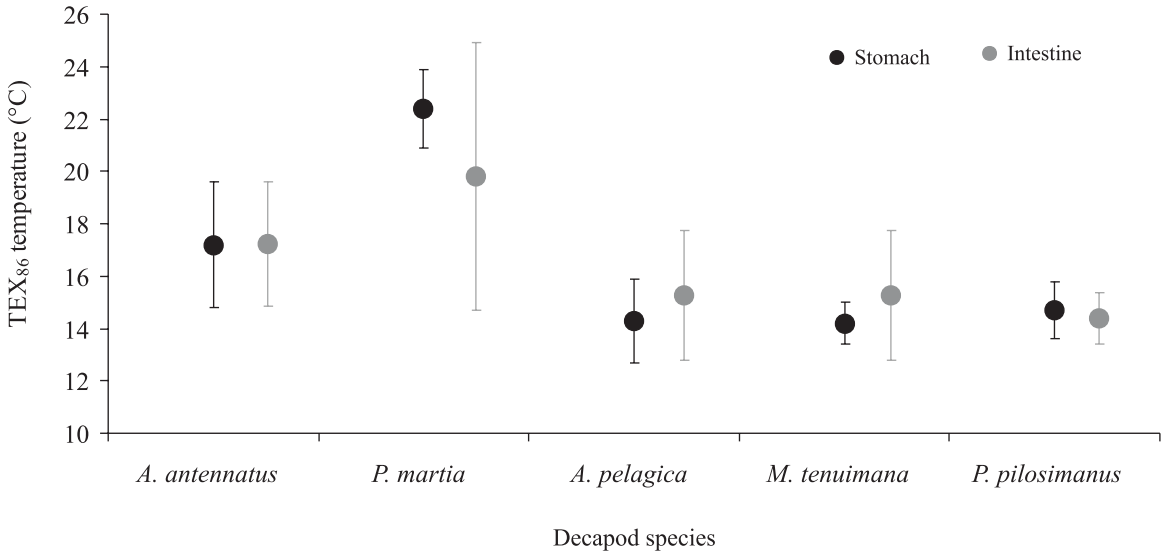


**Figure 3.** $TEX_{86}$ temperatures calculated from GDGTs present in stomachs (black) and intestines (gray).

(Table 3), with standard deviations ranging from 0.1 to 2.5°C for $TEX_{86}$-derived temperatures. Sample amounts from Mediterranean decapods were smaller, and thus could only be measured at best in duplicate and the standard deviations ranged from 0.2 to 1.6°C (Table 4). For species-averaged $TEX_{86}$-derived temperature standard deviations ranged from 0.8°C for *M. tenuimana* stomachs to 5.2°C for *P. martia* intestines (Tables 3 and 4; Figure 3).

[13] $TEX_{86}$-derived temperatures in Atlantic decapods are all very similar between specimens for all species, suggesting a relatively homogenous distribution over the total population (Table 3). The Atlantic decapods showed identical values for stomach $TEX_{86}$ temperatures of 14.3 ± 1.6°C (*A. pelagica*), 14.2 ± 0.8°C (*M. tenuimana*) and 14.7 ± 1.1°C (*P. pilosimanus*) (Table 3; Figure 3). $TEX_{86}$-derived temperatures for the intestine samples were also very similar ranging from 15.2 to 14.3°C (Table 3; Figure 3). Thus temperature values calculated with $TEX_{86}$ for stomachs and intestines are not significantly different in Atlantic species (Figure 3).

[14] $TEX_{86}$-derived temperatures calculated for Mediterranean decapods showed larger variability between specimens than Atlantic decapods, especially for *A. antennatus* (Table 4). Also, in contrast with Atlantic decapods, there was a significant difference between the two Mediterranean species, i.e., stomachs of *A. antennatus* have $TEX_{86}$ temperatures of 17.2 ± 2.4°C, while stomachs of *P. martia* have $TEX_{86}$ temperatures of 22.4 ± 1.5°C (Table 4 and Figure 3). However, both stomach and intestines of *A. antennatus* have similar $TEX_{86}$ temperature values of 17.2 ± 2.4°C. For *P. martia* this difference with $TEX_{86}$ temperatures is larger, i.e., 22.4 ± 1.5°C for stomachs and 19.8 ± 5.2°C for intestines. This difference is not significant considering the large standard deviation (Table 4, Figure 3).

## 4. Discussion

### 4.1. GDGTS in Decapod Guts

[15] Most stomachs and intestines analyzed in this study contain GDGTs, suggesting that Crenarchaeota may be consumed by decapods. Molecular ecological studies have previously shown the presence of marine archaea in guts of fish [*Van der Maarel et al.*, 1998] and holothurians [*McInerney et al.*, 1995], but in both cases it was not determined whether the marine archaea found in the gut were symbiotic members or had been ingested. Decapod guts are very basic structures compared to those of fish and holothurians, thus in our case the existence of gut symbionts seems unlikely. Moreover, the $TEX_{86}$-calculated temperatures and stomach content analysis (see below) indicate a surface origin of the GDGT signal while the decapod species were all collected below 500 m (Table 1). Last, some specimens did not contain GDGTs, which would contradict the idea of a symbiotic relationship. Thus it seems likely that GDGTs are taken up in the food web and incorporated in decapod fecal pellets. Even though there is evidence of GDGT consumption by decapods, we cannot discern if this is an active or a passive process. But, the scale of the mouth sorting struc-

ture of the decapod group does not allow selecting for particles of the size such as those of archaea (<1 $\mu$m [*Margot et al.*, 2002]). Therefore they must be accidentally ingested during feeding, either dissolved in the water, associated with food particles or already present in guts of prey organisms. Stomach content studies of decapod species showed that detritus, i.e., amorphous organic material, never represented more than 20% of the diet in weight, even in detritivors such as *Parapagurus pilosimanus* (J. E. Cartes et al., unpublished results, 2005). This further indicates that an active selection of archaea as a food source by decapods is unlikely.

[16] As the study included species with different feeding strategies (Table 1), some of the mechanisms suggested for surface water material transport to the sediments can be assessed. The passive pathway (marine snow, fecal pellets and phytoplankton massive falls) is confirmed by the presence of GDGTs in the guts of benthos feeders or scavengers-detritus feeders such as *A. antennatus*, *M. tenuimana* and *P. pagurus*. The active transport, by organism migration, is suggested by the other two decapod species feeding on migratory macroplankton (Table 1). The presence of GDGTs in decapod guts also suggests that surface organisms such as migrating macroplankton are ingesting GDGTs themselves. Thus heterotrophic organisms such as the very abundant copepods, which are closely related to the decapods, are likely to play an important role in producing fecal pellets in surface waters that will carry GDGTs to the sediment. In addition, our results show that once the GDGTs are deposited in the sediment benthic reworking of these compounds continues.

## 4.2. $TEX_{86}$ Signal Preservation

[17] The stomachs and intestines analyzed correspond to separate feeding episodes. However, most bathyal decapods feed continuously through the daily cycle without feeding peaks [*Maynou and Cartes*, 1998]. Thus the stomach and intestine contents can be considered as a snapshot of the general digestion process, and no major changes in abundance or food source are expected between the two. With only a few exceptions, GDGT abundances in the intestines are consistently lower than in the stomachs in all specimens studied (Tables 3 and 4; Figure 2) and this difference is statistically significant. The lower intestine abundances and absence of GDGTs in gonad and liver tissues suggest a partial degradation of GDGTs during stomach transit. Alternatively, the residence time for stomach contents might be higher than for intestines. However, this is unlikely as the feeding is a continuous process. Lower intestine than stomach contents can also be explained by a change of food source. However, this is unlikely as very few specimens show a higher intestine than stomach abundance (Tables 3 and 4) and benthic species such as *P. pilosimanus* or *M. tenuimana*, that usually cover a small area, also show lower intestine abundances. Thus it seems that a partial degradation is the most likely explanation for the lower intestine abundance of GDGTs. This could be problematic for the $TEX_{86}$ proxy if some of the GDGT isomers were degraded at different rates than others as this would alter the $TEX_{86}$ ratio. However, there is no significant difference between species averaged $TEX_{86}$ temperatures in stomachs and intestines (Figure 3). Thus it seems that all the GDGT molecules are equally affected by degradation. Therefore the $TEX_{86}$ signal which is taken up by decapods is likely unaffected by passage through the gut and repackaged in fecal pellets. This suggests that fecal pellets from pelagic species will help transport sea surface temperature signal to the bottom, but also that benthic decapods will not alter sedimentary signal settling or already deposited in the sediment.

## 4.3. Comparison of $TEX_{86}$ With in Situ Temperatures

### 4.3.1. Atlantic Decapods

[18] $TEX_{86}$-derived temperatures are similar in all the Atlantic decapods. $TEX_{86}$ temperatures are significantly higher than the 10°C deep-water (<30 m) signal recorded by the CTD, so we can assume a surface origin of the GDGT signal. At the time of sampling SST in the study area was 12.8°C, while the average $TEX_{86}$ value for the three species is 14.4 ± 1.3°C. Thus temperatures obtained from decapod guts compare quite well with local SST, being only 1.6°C higher (Table 2). This relatively small difference may be due to several factors including uncertainties in the calibration of the $TEX_{86}$ with temperature. Indeed, sediment trap studies in the Arabian Sea showed that $TEX_{86}$ values may overestimate surface water temperatures by 1–3°C (Wuchter et al., submitted manuscript, 2006). Alternatively, the decapods may have ingested GDGTs accumulated over an annual cycle (see below), thereby increasing $TEX_{86}$ values as annual mean SST (16.6°C) is higher than the SST around time of sampling (12.8°C).

[19] The three Atlantic decapod species studied are known to have different feeding strategies (Table 1), i.e., *M. tenuimana* and *P. pilosimanus* are mainly benthic scavengers while *A pelagica* is a macroplankton predator. However, we could not find any correlation between feeding mode and $TEX_{86}$ temperatures. This is likely because all the Atlantic decapods fed on food from the same origin (i.e., the upper water column) as they were sampled right after a peak in primary production. Even though *A. pelagica* and *M. tenuimana* usually feed on macroplanktonic organisms, with important scavenging activity particularly in the last species (Table 1), this does not seem to be the case in our study. No information is available on the diet of *P. pilosimanus*, but pagurids are often considered as benthic-detritus feeders and scavengers [*Lagardère*, 1977]. However, in our study it seems that the Atlantic decapods' signal is derived from surface waters and is either ingested though macroplankton predation (*A. pelagica*) or transported down by a flux of phytoplankton detritus and ingested in the benthos (*M. tenuimana* and *P. pilosimanus*). In fact, stable carbon and nitrogen isotope studies showed that marine snow can be the main food source for deep water decapods inhabiting the deep slope around the Balearic Island [*Polunin*, 2002]. Indeed, stomach content analysis of *M. tenuimana* and *P. pilosimanus* showed high contents of pigments derived from phytoplankton, suggesting that in this particular case these species fed on fresh phytoplanktonic detritus (J. E. Cartes et al., unpublished results, 2005). Thus the transport mechanism of the GDGTs carrying the $TEX_{86}$ signal may be linked to the flux of phytoplankton material after the spring bloom in the sampling area. This confirms one of the passive pathways proposed for quick transport of GDGTs from surface waters to the sediment, and it might be especially important in certain areas such as upwelling regions after periods of high primary production in the photic zone.

### 4.3.2. Mediterranean Decapods

[20] $TEX_{86}$ temperatures calculated for stomachs of the Mediterranean decapods studied are significantly different. *A. antennatus* indicates a temperature of ~17°C, while *P. martia* shows significantly higher values at ~22°C. This difference may be partly explained by the slightly different locations where the decapod species were sampled (Figure 1). This could explain warmer temperatures for *P. martia* as the specimens were obtained in a sheltered coastal area. However, this cannot be the only factor, as this would result in a relatively small temperature difference.

[21] Since Mediterranean temperatures are consistently <13°C below 200 m [*Comas et al.*, 1996] and $TEX_{86}$ values are considerably higher, they must correspond to SST. However, average $TEX_{86}$ temperatures from Mediterranean decapods are also significantly higher than SST around the time of sampling (14.3°C; Table 2). The two Mediterranean species consume different prey (Table 1) and may therefore be showing a signal obtained from distinctive food sources. *A. antennatus* is basically a benthic feeder while *P. martia* has been reported as an active predator of macrozooplankton [*Cartes*, 1993; *Fanelli and Cartes*, 2004]. Thus the feeding strategy may have affected the $TEX_{86}$ signal in Mediterranean decapods. As SST changes more than 14°C during the annual cycle (Table 2), the freshness of the organic matter consumed is important. We would expect a pelagic feeder like *P. martia* to ingest GDGTs present in the guts of surface water macroplankton organisms that migrate down the water column daily, and thus $TEX_{86}$ signal should be tied to recent SST. Benthic feeders, such as *A. antennatus*, could be ingesting secondary material such as an already packaged GDGT signal from marine snow and fecal pellets from the same or surrounding areas; this would probably result in a dilution of the fresh $TEX_{86}$ signal. Indeed, this could explain why the $TEX_{86}$ derived temperature in *A. antennatus* is an intermediate value between annual mean SST (20.0°C) and SST at the time of sampling. Notably, there is large variability in $TEX_{86}$ temperatures for individual specimens of *A. antennatus* with some values close to the SST around time of sampling and others close to annual mean SST. However, feeding strategy cannot explain the large offset between $TEX_{86}$ inferred SST for *P. martia* and SST at the time of sampling as this organism is supposed to feed on migratory macrozooplankton and the variability between specimens is relatively small. For reasons presently unclear, the $TEX_{86}$-derived temperatures for *P. martia* are closer to annual mean SST, suggesting a diet of older material. In this respect it is interesting to note that, in contrast to Atlantic decapods, stomach contents of both Mediterranean decapod species contained only low abundances of pigments (J. E. Cartes et al., unpublished results, 2005), suggesting that both decapods did not have fresh phytoplankton material in their diet. This may explain the offset between $TEX_{86}$ temper-

atures in Mediterranean decapods and that of SST around time of sampling.

## 5. Conclusion

[22] Crenarchaeota lipids have been found both in stomachs and intestines of decapods and are most likely accidentally ingested with water, organic particles or with prey organisms. Despite this uncertainty, we were able to demonstrate both passive and active transport mechanism that can explain, at least in part, the transport of GDGTs from surface waters to the sediment. Even though the GDGTs concentration decreases from stomachs to intestines we did not find evidence of incorporation of GDGTs in liver or gonad tissues of decapods. Most importantly, $TEX_{86}$ values do not show significant differences between stomach and intestines, and thus GDGT composition is not modified during the passage through the guts before it is transported to the sediment in fecal pellets. This holds true for both GDGTs collected from the water column or reworked in benthic settings. Furthermore, we observed that the fresh GDGT signal can be transported by massive falls of organic matter after peaks in surface production. Different feeding modes were investigated and while no differences in $TEX_{86}$ values were found in the Atlantic site, the Mediterranean decapods showed a significant difference probably related to the freshness of the material consumed. Finally, $TEX_{86}$ calculated with the GDGTs taken up by decapods shows a relatively good correspondence with SST at time of sampling for Atlantic decapods but represents a mix of annual mean SST and SST at time of sampling for Mediterranean decapods. We also suggest that other heterotrophic organisms closely related to decapods, such as the abundant copepods, are likely to play an important role producing fecal pellets that will carry GDGTs from surface waters to the sediment.

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