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BENTHIC FORAMINIFERA RESPONSE TO METHANE RELEASE IN AN ADRIATIC SEA POCKMARK

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Key words. Benthic foraminifera, methane release (pockmark), $\delta^{13}C$, foraminiferal taxonomy, Bonaccia field, Adriatic Sea, Italy.

Abstract. The presence of methane-bearing shallow sediments in the Adriatic Sea has been known since the fifties, but little is known about the benthic foraminiferal assemblages associated with them. One seep and two control cores were collected in the Bonaccia field (central Adriatic Sea) at a water depth of 80 m to investigate the possible influence of the release of gas from marine reservoirs on these foraminiferal assemblages. The seep core was drilled inside a pockmark in the vicinity of an active mud-volcano. Two control cores were collected in nearby areas unaffected by presence of methane. Benthic foraminiferal assemblages from the seep core are comparable to those from the seep-free cores and are composed of species common in the central Adriatic Sea. However, foraminiferal density in the seep-core is remarkably lower than in the control ones cores. Besides, calcitic foraminiferal tests from the seep core revealed unusual trends in stable carbon isotope composition. Even though not within the same range, a similar trend was observed throughout the seep core for the $\delta^{13}C$ values of *Gavelinopsis lobatulus*, *Cassidulina carinata*, and *Bulimina marginata*. In particular, negative carbon isotope values were recorded for *Gavelinopsis lobatulus* at the top of the core where methane seeps were detected and deep inside the core sediments (50 cm). These findings seem to point to temporal variations in seep activity, proving that the $\delta^{13}C$ values of foraminiferal tests reflect hydrocarbon release and may hence be used to reconstruct seep activity history.

Riassunto. La presenza di metano nei sedimenti di alcune aree del mare Adriatico è nota da tempo, ma i suoi effetti sulle associazioni a foraminiferi bentonici sono ancora poco conosciuti. A questo scopo sono state studiate, sia dal punto di vista micropaleontologico sia isotopico, tre carote prelevate nell'Adriatico centrale ad una profondità di 80 m, una in prossimità di una zona influenzata dalla risalita di metano e due in un'area dove esso è assente. I risultati di questa ricerca hanno messo in evidenza che nella carota interessata dalla risalita di metano, la densità microfaunistica è fortemente ridotta e i valori degli isotopi stabili del carbonio nei foraminiferi bentonici (in particolare *Gavelinopsis lobatulus*) registrano risalita di metano. I segnali isotopici dei foraminiferi possono quindi essere utilizzati per ricostruire la storia dell'attività delle fuoriuscite di idrocarburi.

Introduction

Shallow marine sediments containing gas have been reported from several areas of the Adriatic Sea. The Bonaccia field (central Adriatic Sea, Italy) represents one of the most important of these areas, with a significant amount of gas-related features such as seeps and pockmarks (Fig. 1). Oil companies have often performed hydrocarbon-related studies in the area. Literature on the subject is however still scant. The survey carried out during this study in 1995 revealed the presence of pockmarks, mud volcanoes, abundant amounts of authigenic carbonates (crusts, hard grounds and mounds) (Fig. 1), and methane presence in sub-bottom sediments, in accordance with previous observations by Curzi & Veggiani (1985), Mazzotti et al. (1987), Colantoni et al. (1997), and Taviani et al. (1997).

As clearly emerged from the discussion of several participants at the "Third International Congress on Environmental Micropaleontology, Microbiology and Meiobenthology" held in Vienna (Austria) in September 2002, there is great interest in the response of benthic foraminifera to various pollution sources (heavy metal and/or hydrocarbon) in stress environments. Given that benthic foraminifera represent one of the most useful tools for paleoenvironmental and paleoceanographic study (Gooday 1994; Van der Zwaan et al. 1999 among others), investigators have recently also begun to explore the ecological role of these protists in hydrocarbon-based environments. Findings, however, have so far not been univocal. In order to have a reliable record of the response of fossil and recent benthic foraminiferal assemblages from methane rich sediments, three cores

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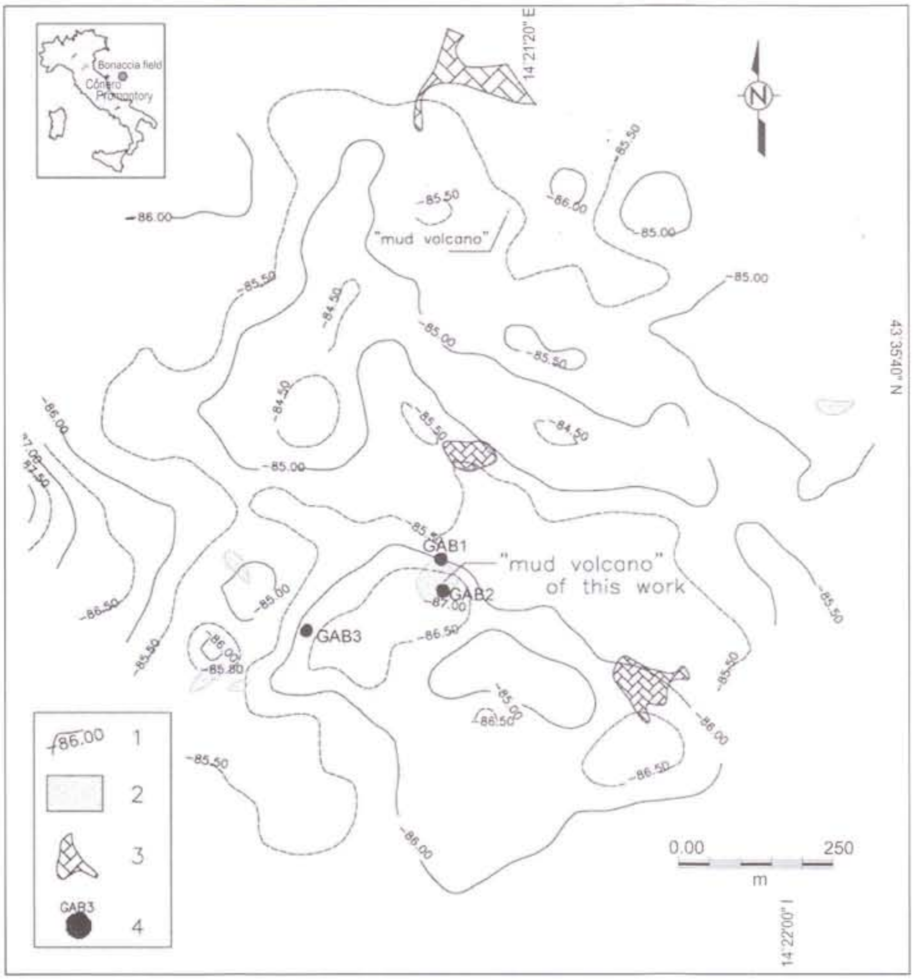

Fig. 1 - Location of the cores in the Bonaccia field (central Adriatic Sea). Captions: 1) Isobaths (0.5 m interval); 2) Coarse biogenic sediments; 3) Hard bottom, carbonates biogenic concretions; 4) Gravity cores location.

were investigated. One was taken from a pockmark depression in the vicinity of a mud-volcano in the presence of methane release source, while the other two from gas-free areas in the Bonaccia field in the central Adriatic Sea. The top 10 cm of the cores contained sandy mud with grey mud below, down to the bottom.

The study set out to determine: 1) possible variations in the benthic foraminiferal taxonomy; 2) the relationship between methane seep and stable isotopic composition of benthic foraminifera; 3) the viability of using the isotopic signature of benthic foraminifera to reconstruct the history of seep activity.

Study area

The hydrocarbon seep considered in the present study is located in the Bonaccia gas-field in the central Adriatic Sea about 35 km off the Conero Promontory, at a water depth of 80 m (Fig. 1). Gas-fields in the area are present in Plio-Pleistocene turbidite sequences (Mattavelli et al. 1991). In this part of the Adriatic basin, thin sandy deposits called relict sands were deposited during the Versilian Transgression and Holocene mud belts (Van Straaten 1970; Colantoni et al. 1979) were deposited as a high-stand system tract (Trincardi et al. 1994). The Holocene mud belt in this area is diffusely impregnated by biogenic gas, mainly methane (Gabbianelli, pers. comm.).

Seismic surveys performed during this study exhibit characteristics that may be interpreted as relating to natural gas seeping from the Plio-Pleistocene turbidite into Holocene sequences. Sediments diffusely charged with locally rising gas were observed in the sub-bottom profile of the site from which core GAB2 was taken (Fig. 2). The seismic profile exhibited a 2-5 m-thick layer of sandy silt sediment lying over a peat and clay lens of continental or transitional environments. The fine-grained gas charged sediment pores were observed to be partially filled with pore water and free gas (Gabbianelli, pers. comm.). Gas quantity was presumed to be relatively low, being contained in the sediment as a millimetre scale bubble so as to rule out any significant reworking. Geochemical analyses (Mattavelli et al. 1991) proved the biogenic origin of the methane in the area.

Materials and methods

Three Kullenberg cores (with an inner diameter of 10 cm) drilled in the vicinity of the pockmark of the study area (Fig. 1) were analysed. Core GAB1 (lat 43 35' 26"/long 14 21' 35") was drilled at

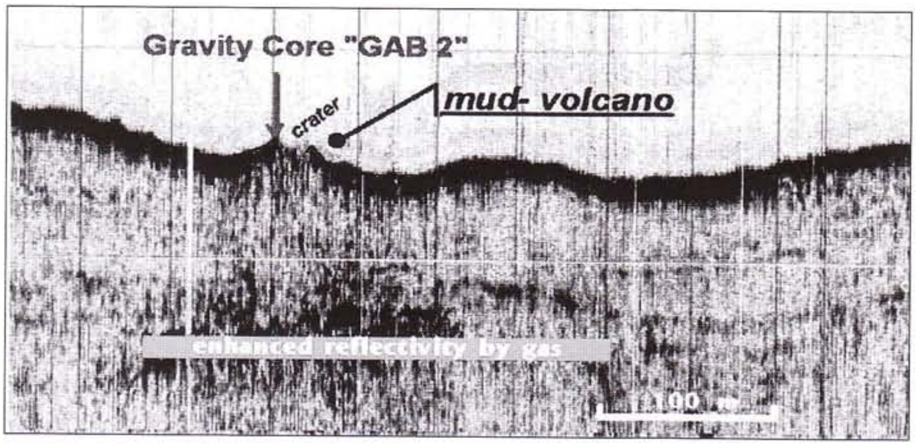

Fig. 2 - Details of a 3.5 kHz sub-bottom profiles showing the volcano with riding gas detected and the GAB2 position in the Bonaccia field. See location in Figure 1.

the base of the pockmark, core GAB2 (lat 43 35' 25"/long 14 21' 35") was taken close to the mud volcano at the centre of the pockmark, and core GAB3 (lat 43 35' 23"/long 14 21' 25") in the nearby area unaffected by methane seeps.

One-centimetre-thick sediment samples taken at 10 cm intervals along the entire cores were used for foraminiferal and geochemical analyses. After drying and weighing, unconsolidated samples were soaked in water for 1-2 days. Each sample was washed through a 63μm mesh sieve, dried, and then the residues were split into aliquots containing about 300 benthic foraminifera. Benthic foraminifera were identified and counted (Appendix). Simple species percentages over the total assemblage (see **the Online Background Dataset. http://www.gp.terra.unimi.it/rivista/109n3.htm**) and micropaleontological density expressed as number of specimens per gram dry weight were determined on the basis of benthic foraminiferal specimen counts (Appendix).

To avoid the erroneous estimation due to the occurrence or absence of rare specimens, diversity was not determined on the basis of the number of species in the sample (S) but on the number of species and their frequency according to the Shannon-Wiener formula, $H(S) = -\Sigma_{i=1}^{S} p_i \ln p_i$, where p_i is the proportion of the nth species (Appendix). Statistical analysis was conducted so as to gain more detailed information on environmental stability.

Foraminiferal specimens for isotopic analyses were sampled from core GAB2. Different species of benthic foraminifera (*Bulimina marginata*, *Cassidulina carinata*, and *Gavelinopsis lobatulus*) were selected. To minimize the possibility of contamination by calcite overgrowths or carbonate grains, each specimen underwent screening by binocular microscopy to detect any traces of carbonate incrustation on the tests.

Diffractometric (XRD) analyses were performed by means of a Philips PW3710 X-ray powder diffractometer on about 100 tests of *Gavelinopsis lobatulus* sampled from the GAB2 core. The scanning angular range was from 25° to 31° (2θ) with an acquisition time of 60 minutes for statistical meaning.

Benthic foraminifera as indicators of hydrocarbon release

The release of methane from marine reservoirs into overlying sediments is known to affect carbon isotope ratios at the sediment-water interface (Graber et al. 1990). For instance, $\delta^{13}C$ values of bicarbonate and dissolved inorganic carbon (DIC) may diminish (Roberts & Aharon 1994). Generally, the chemistry of benthic foraminifera is a reliable proxy for numerous environmental parameters as the tests are secreted in isotopic equilibrium with the sea-water (Grossman 1987). It has also been observed that the carbon isotopic signature of this group of Protista is more negative in hydrocarbon seeps than in hydrocarbon free-areas (Sen Gupta & Aharon 1994; Sen Gupta et al. 1997). Negative excursions in $\delta^{13}C$ values of fossil benthic foraminifera in Quaternary sediments (Cannariato & Kennett 1996; Kennett et al. 1996, 2000; Wefer et al. 1994) and near the Paleocene/Eocene boundary (Dickens et al. 1995, 1997) have been related to methane release caused by gas hydrate dissociation during cooling episodes. In particular, cooler episodes favour a drop in sea level and the subsequent decrease in hydrostatic pressure may allow the release of considerable quantities of methane from gas hydrate dissociations. Carbon data referred to benthic foraminifera from the Northern California margin suggest that methane release may be related to temporal variations in seep activity caused by temperature variations (Rathburn et al. 2000).

Results

Benthic foraminifera. The species found in the benthic foraminiferal assemblages of the three cores examined are well-documented in the literature on this area of the Adriatic Sea (Jorissen 1988). The results of each core examined are reported below.

GAB1 - Between 41 and 49 taxa per sample were identified in the GAB1 core (Appendix). The Shannon-Wiener index ranges from 3.39 to 3.19. After an increase from the base up to 50 cm, where the highest value was reached (5.39), a decrease was observed followed by a slow increase up-core (Fig. 3).

Microfaunal density values were found to increase from the base up-core reaching 6125 at the top of the core (Fig. 4).

The benthic foraminiferal assemblage (Fig. 5) is characterized by *Cassidulina carinata*, which was seen to slowly increase up-core reaching 14.6% at the top. *Bulimina marginata* exhibited a marked increase at 40 cm (14%) and two marked drops at 30 cm (ca 7%) and at the top of the core where the lowest value (4.4%) was

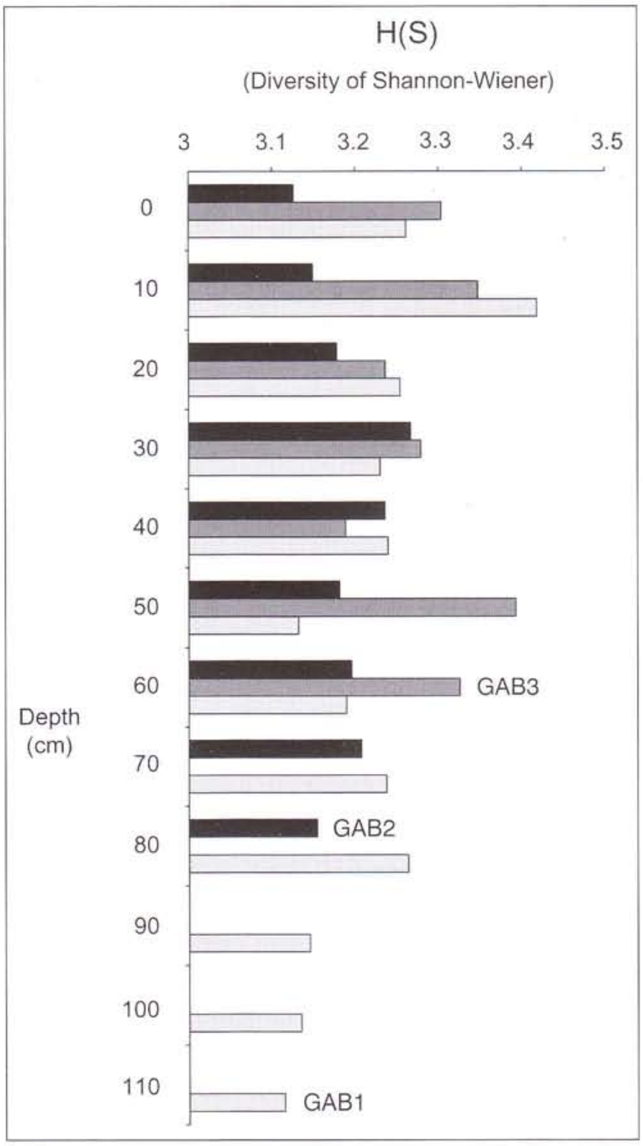

Fig. 3 - Stratigraphic variations in diversity H(S) Shannon-Wiener index in core GAB1, GAB2, and GAB3.

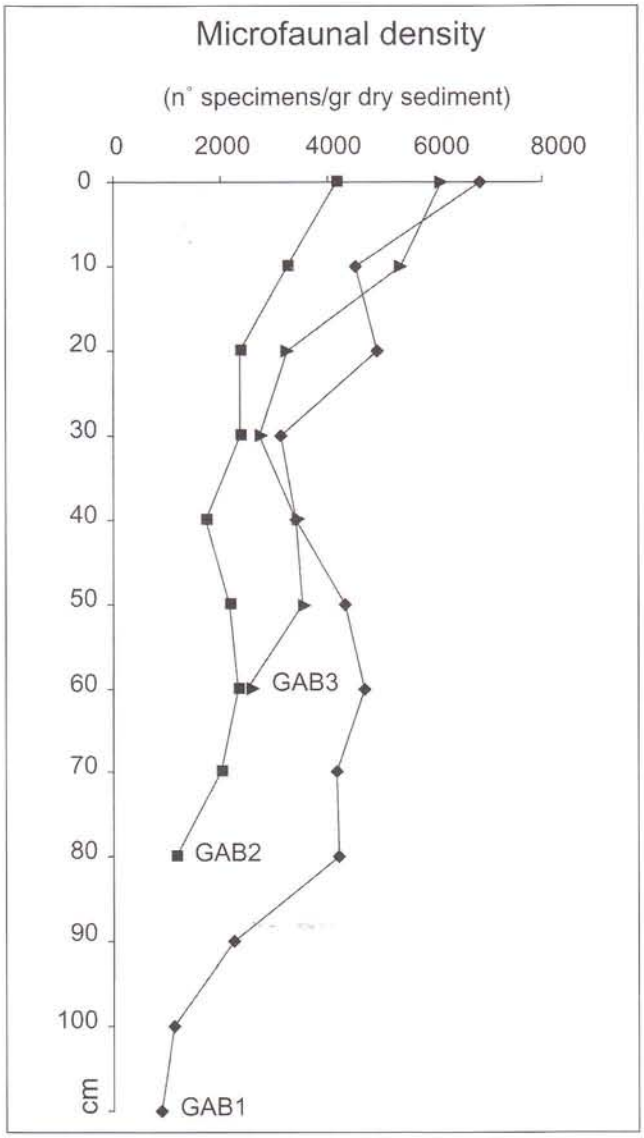

Fig. 4 - Stratigraphic variations in microfaunal density (number of specimens per 1 gram dry-weight) in core GAB1 (triangle), GAB2 (square) and GAB3 (rumble) from the Bonaccia field (central Adriatic Sea).

reached. *Cribroelphidium decipiens* rose throughout the core reaching ca 13% at the top, whilst *Cribroelphidium granosum* exhibited relatively stable occurrences (ca 7%). *Gavelinopsis lobatulus* increased from the base to 10 cm where a small peak (7.7%) was seen to be followed by a strong decrease up to the top (2.7%). *Globocassidulina subglobosa* was found to increase from the base up to 30 cm (ca 9%) and from 20 cm to the top where it reached 6.8%. An accessory species that was seen to decrease upcore was *Bigenerina nodosaria*.

GAB2 - In this core between 40 to 47 taxa were found per sample with the Shannon-Wiener index ranging from 3.13 to 3.27 (Appendix). The H(S) remained almost constant from the bottom up to 30 cm, although a small decrease at 50 cm was observed (3.18), where a small peak was followed by a marked decreasing trend up to the core top (Fig. 3). The lowest H(S) was in fact observed at the top (3.13), where the presence of methane was detected by seismic profile.

Microfaunal density values were at their lowest in the GAB2 core (Fig. 4). After slowly decreasing upcore throughout the core, the lowest value of 4166 was reached at the top.

Figure 5 shows the temporal distribution of the significant GAB2 core. Benthic foraminiferal assemblage was found to be dominated by the genera *Cassidulina* (*C. carinata* and *C. crassa*) and by *Bulimina marginata*. *Cassidulina carinata* increased from the core base reaching ca 14% at 50 cm and ca 15% at 20 cm. After the last peak it decreased up to the top. The quantity of *Cassidulina crassa* slowly diminished towards the core top. *Bulimina marginata* frequency diminished from the base to 40 cm (7.1%), and then increased up to 20 cm. A new drop at 10 cm was followed by a strong peak at the top of the core

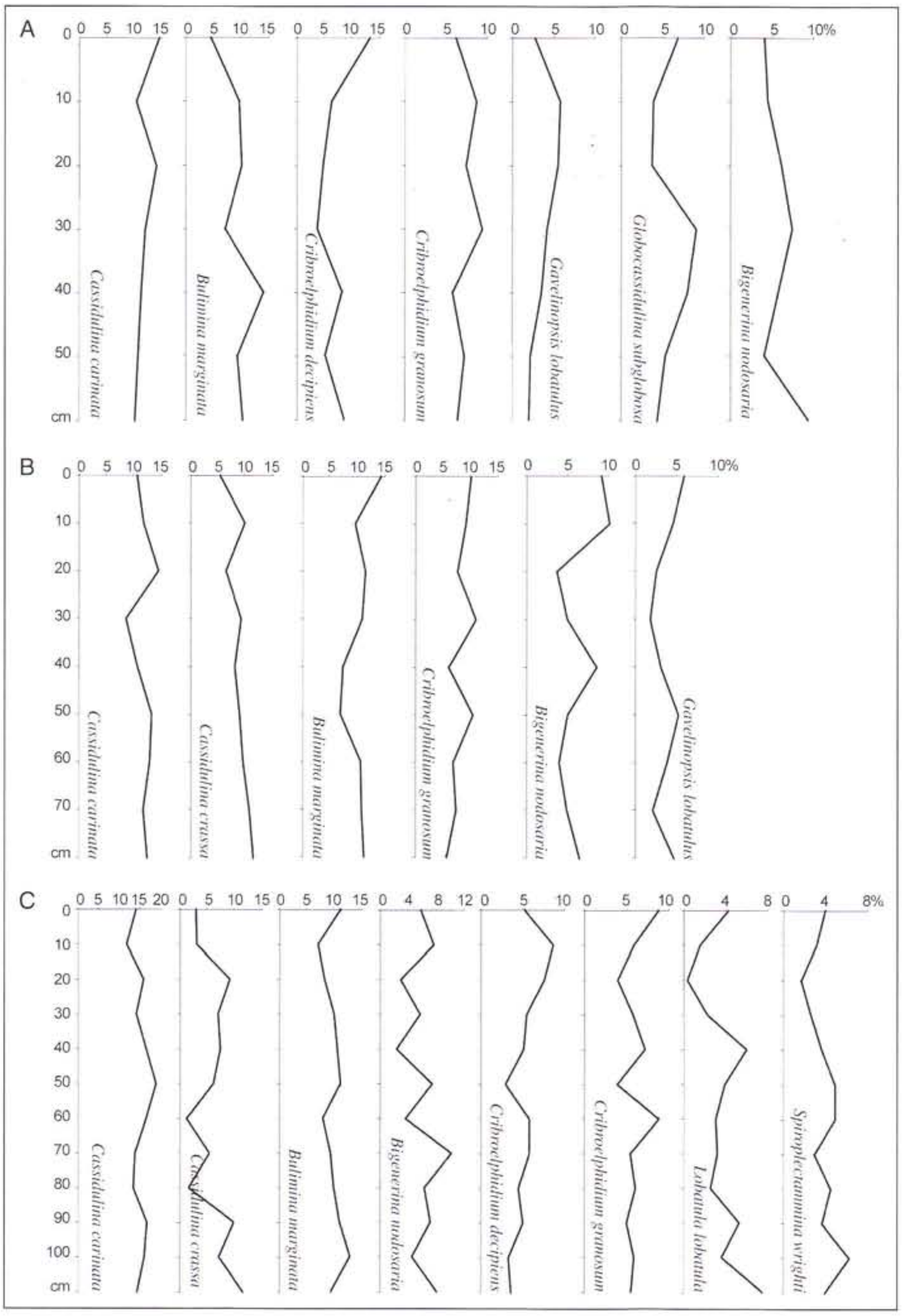

Fig. 5 - Summary diagram of benthic foraminifera frequency of some species (> 5%) plotted against the depth. 5A - Frequency in core GAB1. 5B - Frequency in core GAB2. 5C - Frequency in core GAB3.

where the species reached its highest value of 14%. *Cribroelphidium granosum*, which was seen to generally increase up-core, exhibited two peaks of 10.7 % and 11.3% at 50 and 30 cm, respectively. After decreasing up to 60 cm, *Bigenerina nodosaria* was found to increase up to 40 cm (8.4%), decrease up to 20 cm, and then increase again up to 10 cm, where it was most abundant of all (ca 10%). *Gavelinopsis lobatulus* showed marked peaks of 5.33 and 6% at 50 cm and at the core-top, respectively.

GAB3 - Taxa count values in the core ranged from 40 to 47 (Appendix). Strong variations were recorded in the Shannon-Wiener diversity index H(S). After increasing from the bottom to 80 cm, the index reached its lowest value at 50 cm (3.13), newly increasing to 10 cm where it reached 3.42, and finally dropping once more to 3.26 at the core top (Fig. 3).

Microfaunal density increased up-core, although a slight drop was observed between 50 and 30 cm (Fig. 4).

Foraminiferal assemblage showed (Fig. 5) *Cassidulina carinata* initially increasing and peaking at 50 cm (18.8%) before decreasing at the top. *Cassidulina crassa* was found to decrease from the core base to 60 cm and then to slowly increase to 20 cm where it reached 9.2%, before decreasing again up to the top. After a small peak at 100 cm (12.4%), *Bulimina marginata* was seen to decrease up to 60 cm (7.6%). At 50 cm another small peak was recorded followed by a drop up to 10 cm (6.8%), with a final increase at the top of the core (10.7%). Despite

Sediment depth (cm)	$\delta^{13}C$	$\delta^{18}O$	Identification
0-1	-0.74	1.54	*Bulimina marginata*
	-0.74	1.87	*Cassidulina carinata*
	-5.72	0.54	*Gavelinopsis lobatulus*
9-10	-0.66	0.75	*Bulimina marginata*
	-3.41	0.76	*Gavelinopsis lobatulus*
19-20	-0.08	2.09	*Bulimina marginata*
	0.56	1.71	*Cassidulina carinata*
	-0.51	0.69	*Gavelinopsis lobatulus*
29-30	-0.32	1.63	*Bulimina marginata*
	-0.95	-1.44	*Cassidulina carinata*
	-0.35	1.24	*Gavelinopsis lobatulus*
39-40	-0.03	0.52	*Bulimina marginata*
	-0.94	0.96	*Cassidulina carinata*
	0.70	0.60	*Gavelinopsis lobatulus*
49-50	-0.46	2.00	*Bulimina marginata*
	-0.47	-0.60	*Cassidulina carinata*
	-2.92	-0.14	*Gavelinopsis lobatulus*
59-60	0.07	1.35	*Bulimina marginata*
	0.24	1.37	*Cassidulina carinata*
69-70	-0.61	1.27	*Bulimina marginata*
	1.81		*Cassidulina carinata*
79-80	-0.51	0.98	*Bulimina marginata*
	0.05	2.37	*Cassidulina carinata*
	1.37	0.37	*Gavelinopsis lobatulus*

Tab. 1 - Stable isotopic values (‰ relative PDB) from GAB2 core taxa. Sample which generated variable isotope oxygen signals (standard deviation of >0.05‰) are not indicated.

Bigenerina nodosaria's odd up-and-down pattern cyclic increases and decreases, its occurrence was fairly stable at about 6%. *Cribroelphidium decipiens* increased up core with a peak of 8.6% at 10 cm, whilst *Cribroelphidium granosum*, quantitatively constant from the base up to 70 cm, peaked at 60 cm (8.8%) and at the core top (8.8%).

Accessory species that were seen to increase up core include *Miliolinella subrotunda* and *Rosalina globularis*, while the decreasing ones were found to be *Lobatula lobatula* and *Spiroplectammina wrighti*.

Stable isotopes. Carbon and oxygen isotopic analyses (Tab.1) were performed on three species of benthic foraminifera collected every 10 cm in the GAB2 core. *Bulimina marginata, Cassidulina carinata,* and *Gavelinopsis lobatulus* were selected because present in relatively quantities throughout.

Specimens found at the core top (0-1 cm) were considered as representing the modern sea floor and the present-day environment. Changes in $\delta^{13}C$ for each analysed highlighted a similar isotopic pattern throughout almost all the core (Fig. 6).

Bulimina marginata $\delta^{13}C$ values were seen to range from -0.74 to 0.07‰. A slight shift was observed in the $\delta^{13}C$ values of the species from positive (0.07%) at 60 cm to negative (-0.74%) ones at the core top, with a general decrease up-core. *Bulimina marginata* $\delta^{18}O$ values exhibited only a positive spread, becoming more positive from the base (0.98‰) up to 50 cm (2‰) (Tab. 1). A less positive $\delta^{18}O$ value was recorded at 40 cm followed by an increase up to the top, with peaks at 20 cm (2.09‰) and at the core top (1.54‰).

Carbon isotope signature values for *Cassidulina carinata* ranged from -0.95 to 1.81‰, initially increasing (1.81‰) up to 70 cm and then markedly dropping (-0.95‰) up to 30 cm. A positive peak at 20 cm (0.56‰) was followed by a new drop to negative values at the core top (-0.95). Further variations were observed in $\delta^{18}O$ values, with the most negative at 50 and 30 cm (-0.60‰ and -1.44‰, respectively).

Gavelinopsis lobatulus $\delta^{13}C$ values exhibited even greater variations, with the largest changes to more negative values being recorded at 50 cm (-2.92‰) and at the core top (-5.27‰). As isotope data was not obtainable at 60 and 70 cm, a diminishing trend from the core base up to 50 cm may be presumed - (Fig. 6). Oxygen isotope values for *Gavelinopsis lobatulus* are quantitatively constant (ca 0.6‰) along the core except a small peak (1.24‰) recorded at 30 cm.

Discussion

General faunal characteristics. Progress in assessing the effect of hydrocarbon release on benthic foraminiferal communities has been considerable over the last few years, even though the exact interaction affecting the taxonomic structure of this group of organisms is still under debate. The findings of the study on the three cores from the central Adriatic Sea are a further contribution for interpreting the effects of the methane seepage.

All the species identified in the cores are well-documented in the literature of the Adriatic Sea (Jorissen 1988). The findings of the study were hence in accordance with those of previous Authors (Sen Gupta et al. 1997; Bernhard et al. 2001), so that unlike what was observed for a group of megafaunal components by Sibuet & Olu (1998), the absence of endemic species in hydrocarbon-based environments was confirmed.

Benthic foraminiferal assemblage composition was found to be similar in all three cores. The lack of any true assemblage differences between the GAB2 methane-affected and the GAB1 and GAB3 control cores may be ascribed to insufficient and/or short/intermittent duration of methane seepages. This is probably why a distinctive methane-seep association was not observed, with differences in microfaunal density and H(S) values only being recorded for complex situations. Microfaunal density (number of benthic foraminiferal specimens in one gram of dry sediment) was found to be severely reduced in the GAB2 seep-core. At the same water depth and hence over the same interval of time the lowest values were in fact recorded in this core compared to the control ones. Ben-

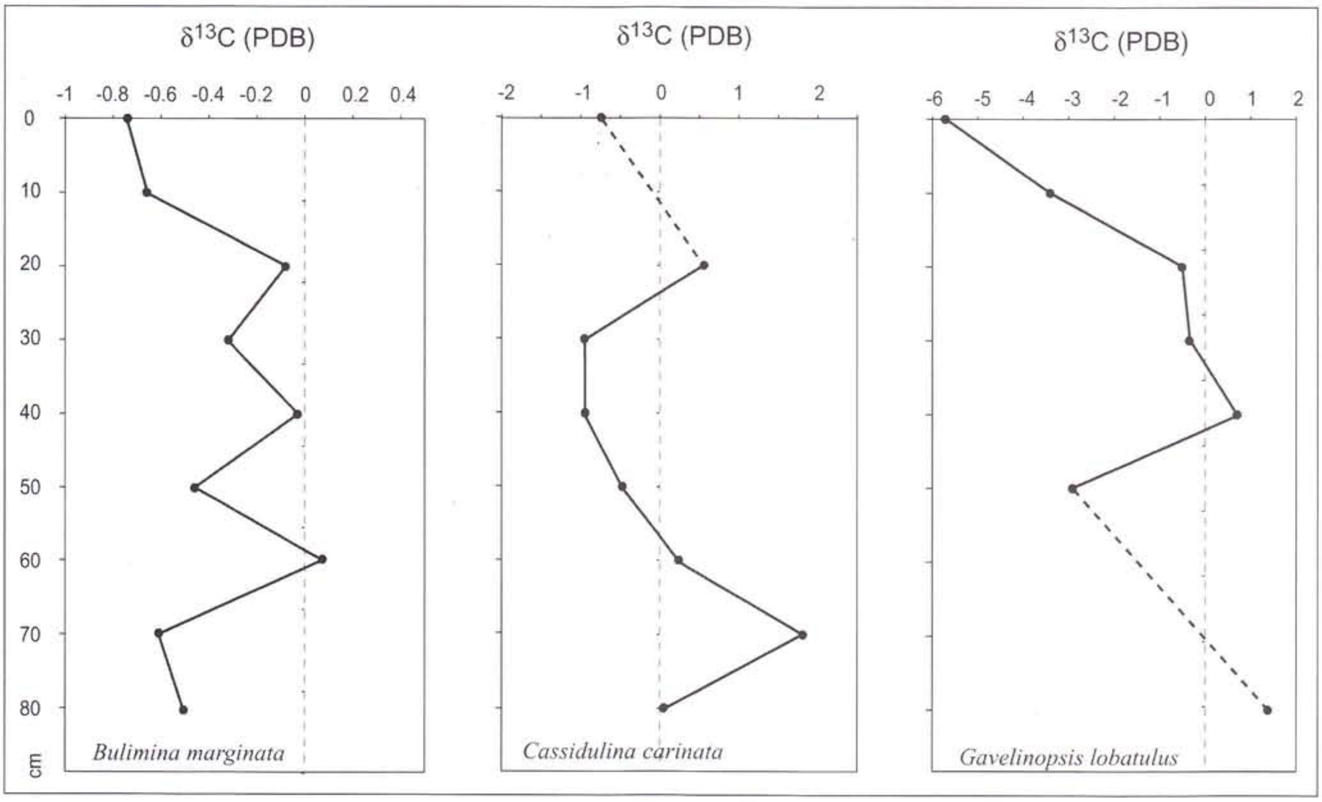

Fig. 6 - Downcore stable isotope ($\delta^{13}C$) records in GAB2 core obtained from *Bulimina marginata*, *Cassidulina carinata*, and *Gavelinopsis lobatulus*.

thic foraminiferal density has been observed to be linked to the trophic structure (i.e. food and oxygen levels) of the habitat. A number of studies have pointed out that the size of foraminiferal population is controlled by oxygen content at ocean floor level and in sediment pore waters (e.g. Lohmann 1978; Perez-Cruz & Machain-Castillo 1990; Sen Gupta & Machain-Castillo 1993; Loubere 1994), as well as by organic carbon availability (Althenbach & Sarnthein 1989; Herguera 1992; Jorissen et al. 1995; Corliss & Emerson 1990; Gooday 1996; Rathburn et al. 1996). The similar upward trend in microfaunal density observed in all analysed cores suggests a general improvement in the area. But the reduced microfaunal density reported for the GAB2 core, which appeared especially marked at the core-top where methane was detected during the survey (Fig. 2), suggests the presence of an unusual ecosystem ascribable to methane release and characterised by reduced oxygen content and high quantities of organic matter. These findings are in line with those recently reported by Yanko & Flexer (1991) for a study in the Odessa Bay area, and by Sen Gupta et al. (2002) for a study on cold seeps in the Gulf of Mexico based on a 24 cm-long push core taken at a site characterised by present-day hydrate dissociation and gas expulsion.

The H(S) Shannon-Wiener index permits an overall description of faunal diversity and hence affords a detailed account of environmental stability. Given that foraminifera population diversity distribution is a function of environmental dynamics and variability (Gibson 1966), the lowest values recorded in the GAB2 seep core may be reasonably presumed to depend on the seep-induced stress undergone by benthic foraminifera population. In fact, in all likelihood the seep makes for an unstable ecosystem as the very presence of seeping fluid and its variability due to changing fluid flow intensities favour reduced oxygen availability and organic compound build up. The decreasing up-core trend observed in the upper interval of GAB2, that terminates with the absolute lowest value, suggests seepage intensification in terms of quantity and/or duration at the core top. Similar results have been reported by Sen Gupta et al. (2002) according to whom the reduced diversity observed by them in a methane-hydrate affected area of the Gulf of Mexico was ascribable to seepage onset or intensification.

At specific level, *Bulimina marginata* belong to a genera usually considered capable of coping with high environmental stress, especially low oxygen content (e.g. Van der Zwaan 1982; Murray 1991; Sen Gupta & Machain-Castillo 1993), and their presence has been reported in most seep data sets (Akimoto et al. 1994; Sen Gupta et al. 1997; Rathburn et al. 2000; Bernhard et al. 2001). In the GAB2 core an up-core increase of the species was observed, with values at the top greater than those of the other cores. This enhanced frequency, that proved detrimental to other species, appears to be closely related to depleted oxygen content as the species does not seem to be at all adversely affected by dysoxic conditions, under which it is also capable of reproducing itself (Bernhard & Sen Gupta 1999). The species may hence be presumed to be pre-adapted to thrive in methane-saturated environments.

Gavelinopsis lobatulus, which, as suggested by its isotopic signatures and discussed below, is of considerable importance, peaks at 50 cm and at the core-top. Gupta (1999) reported this species in an assemblage from DSDP Site 214 in the East Indian Ocean characterised by high energy, good oxygenation, and probably low organic carbon content and ascribed these conditions to its presence. The conditions prevailing at the GAB2 core site were such that Gupta's assumption as to *Gavelinopsis lobatulus* environmental affinity cannot be confirmed. Furthermore, the relatively low frequency of the species and the prevalence of taxa (for instance, *Cassidulina* spp. and *Bulimina* spp.) marking environmental conditions that are the reverse of those reported by Gupta make for a different interpretation of the abundance peaks observed at 50 cm and at the core-top that would hence appear ascribable to other factors.

Stable isotopes. Being extremely useful as indicators of paleoenvironmental and paleoceanographic conditions the stable isotopic composition of benthic foraminifera has been recently utilized also in seep environments. According to the literature, hydrocarbons affect the carbon isotope values of benthic foraminifera at seep sites (Sen Gupta & Aharon 1994; Sen Gupta et al. 1997; Rathburn et al. 2000; Kennett et al. 2000; Wefer et al. 1994). In hydrocarbon release sites the increased levels of DIC (dissolved inorganic carbon) caused by microbial processes involving sulphate reduction and hydrocarbon oxidation are characterized by negative $\delta^{13}C$ values (Roberts & Aharon 1994). Calcite tests of foraminifera according to Sen Gupta & Aharon (1994) and Sen Gupta et al. (1997) appear reliable for yielding such results. Moreover, determining the time and extent of methane release on the basis of the isotopic records of methane-affected foraminifera caused by cold episodes occurring at different times in the climate cycle, also in case of methane release being caused by dissociated clathrates (Wefer et al. 1994; Kennett et al. 1996, 2000; Cannariato & Kennett 1996; Zachos et al. 1994; Dickens et al. 1995, 1997) has been deemed to be a viable method.

Despite the partial findings of the present study, some of the carbon isotopic values reported here strongly agree with those of the literature cited above. The isotopic results obtained for *Gavelinopsis lobatulus*, *Cassidulina carinata* and *Bulimina marginata* exhibit the same pattern throughout almost the full length of the GAB2 core even though with different magnitudes. Such inter species covariance suggests that the $\delta^{13}C$ values are a record of variations in environmental conditions that trigger changes in foraminiferal isotopic signature. Negative $\delta^{13}C$ values for *Gavelinopsis lobatulus* were recorded at the top, at 10, and at 50 cm of core depth. Similar results were also reported for *Cassidulina carinata* and *Bulimina marginata* (no value was recorded for *Cassidulina carinata* at 10 cm).

A salient finding of the present study is the very negative $\delta^{13}C$ values recorded for the well-known *Gavelinopsis lobatulus*. The oxidation of organic matter rich in ^{12}C may determine such negative $\delta^{13}C$ values in foraminiferal tests (McCorkle et al. 1985, 1990). The isotopic values recorded here for *Gavelinopsis lobatulus* may be ascribable to such a factor. This assumption cannot however be confirmed given the lack of other supporting evidence. The lack of information on species living in environments rich in decomposing organic matter is yet a further obstacle to making viable comparisons.

In a study by Rathburn et al. (1996) in the Sulu Sea relative PDB values for *Gavelinopsis lobatulus* were found to be negative, ranging from -2.96 to -3.57‰ .The finding was explained by the authors on the basis of the fact that "unexpectedly low" $\delta^{13}C$ values were yielded by the aragonitic test made on the species. Given the crucial importance to the present study of the isotopic values of species, diffractometric analyses were made to determine the nature of the test. Results pointed to a calcite test for *Gavelinopsis lobatulus*, so that the observed depletion must be accounted for by other causes.

Bearing in mind that $\delta^{13}C$ excursions of up to 5‰ have only been reported for specimens collected in methane-affected environments (Sen Gupta & Aharon 1994; Wefer et al. 1994; Kennett et al. 1996; Sen Gupta et al. 1997; Rathburn et al. 2000) and that the GAB2 site considered in the present study was very close to the seep, it may be presumed that the very low $\delta^{13}C$ values recorded for *Gavelinopsis lobatulus* in the initial centimetres of the core length are ascribable to the presence of methane. It would seem that the $\delta^{13}C$ values of the *Gavelinopsis lobatulus* tests record the high levels of dissolved inorganic carbon (DIC), the very negative values being attributable to methane oxidation. If this is so, then the stratigraphic interval at 50 cm, where *Bulimina marginata* and *Cassidulina carinata* carbon isotopic values were also seen to diminish, is further evidence of of yet further methane release.

It may be presumed that this temporal distribution of carbon isotopic composition reflects changes in the quantity of seeped methane. If so, the finding is in line with that of other studies that consider methane seepage to be a viable explanation of temporal changes recorded by shifts to more negative foraminiferal $\delta^{13}C$ values (Wefer et al. 1994; Kennett et al. 1996). On the other hand, according to Williams et al. (2002) in a study on live (rose Bengal stained) foraminifera associated with methane seeps in the California Margin off the Eel River and in Monterey Bay, the carbon isotopic signatures of benthic foraminifera were not in line with the very negative methane-influenced values of the pore water, while the presence of methane affected instead the carbon isotopic variability between seep foraminifera (2.9‰ measured for *Globobulimina pacifica* and ca 1.95‰ for *Uvigerina peregrina*). Be it as it may, the results of the present study agree with Sen Gupta et al. (1997) and Rathburn et al. (2000). According to these authors large excursions of 3 to 4‰ in isotopic signatures for the same species living in the same area over a short amount of time are evidence of methane effects.

Considering that the *Gavelinopsis lobatulus* calcite test record methane in sediments, it is difficult to explain why only this species shows negative carbon values. The interspecific differences among taxa in the same interval are difficult to explain. It is also interesting to note that *Gavelinopsis lobatulus* has an epifaunal habitat, while *Cassidulina carinata* and *Bulimina marginata* have a shallow infaunal one. According to McCorkle et al. (1990, 1997) the $\delta^{13}C$ values of epifaunal species (i.e., those species that live on or within the upper 1 cm in the sediments) reflect the $\delta^{13}C$ values of the bottom-water DIC, whereas those of infaunal species (i.e., those species capable of living deeper in sediment) reflect the more negative $\delta^{13}C$ values of pore waters. But the results of the present study did not confirm these conclusions at all. In fact, the more depleted carbon values were reported for the epifaunal species. As borne out by previous studies (Woodruff et al. 1980; Grossman 1984; McCorkle et al. 1990; Loubere et al. 1995; Rathburn et al. 1996), the differences in isotopic composition among benthic foraminifera taken from the same interval may be reasonably assumed to depend on a number of joint causes such as viability, microhabitat, food preference, and vertical movement within the sediment. Be it as it may, the relationship between the isotopic composition and ecological behaviour of foraminifera is still, however, an open question.

According to DeNiro & Epstein (1978) the $\delta^{13}C$ of foraminiferal calcite formed from metabolic CO_2 may be strongly influenced by the $\delta^{13}C$ of the food source. Zahn et al. (1986) concluded that the "habitat effect" might also strongly influence the carbon isotopic signature of benthic foraminifera, and seasonal movement has been observed for some species in the Adriatic Sea (Jorissen et al. 1992).

$\delta^{18}O$ values for *Gavelinopsis lobatulus*, *Bulimina marginata*, and *Cassidulina carinata* were found to be usually invariable throughout the core. The values are in line with the literature (Rathburn et al. 1996) also for the Adriatic Sea (Asioli 1996).

Comparison between carbon and oxygen isotope present data suggests that no relation can be determined and it is unlikely that the two isotope systems are controlled by independent factors.

Conclusions

The following conclusions may be drawn from this study.

(1) Several species of benthic foraminifera live in sediments in proximity to methane seep sites in the Adriatic Sea. The species are cosmopolitan and not endemic to these particular ecosystems.

(2) The chief response of the foraminifera to hydrocarbon presence in sediments of the analysed sites was a drastic drop in density and diversity H(S).

(3) Additional proof that foraminifera are capable of living and growing in hydrocarbon seep site sediments was the negative carbon-13 shifts recorded in the species examined, especially in *Gavelinopsis lobatulus*.

(4) Isotopic results showed that the carbon isotopic signatures of benthic foraminifera provide/afford a useful record of methane release events and hence of seep activity history.

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Appendix - Number, total number of benthic foraminifera counted, (S), H(S), and microfaunal density for benthic foraminiferal taxa in the ›63 micron size fractions from GAB1, GAB2 and GAB3 cores.

Number of benthic foraminifera from GAB1core samples

Depth (cm)	0	10	20	30	40	50	60
Ammonia beccarii Linnaeus		3	4		7	12	5
Ammonia perlucida (Heron-Allen & Earland)	1						
Ammonia tepida (Cushman)				1		1	
Amphicoryna scalaris (Batsch)	2		2	1	2	2	1
Angulogerina angulosa (Williamson)			2		5	4	
Arenaceous		1			1		6
Asterigerinata mamilla (Williamson)	9	12	11	7	6	12	8
Asterigerinata planorbis (d'Orbigny)		2	2		6	4	4
Bigenerina nodosaria d'Orbigny	12	14	20	23	18	13	28
Biloculinella globula (Bornemann)		1	2			1	
Biloculinella labiata (Schlumberger)	2						
Bolivina albatrossi Cushman		2	1				1
Bolivina pseudoplicata Heron-Allen & Earland				2			
Bolivina punctata d'Orbigny	1						
Bolivina subspinescens Cushman	2	1	2	3		1	1
Brizalina alata (Seguenza)	1						1
Brizalina dilatata (Reuss)	4	2	8	7	4	2	4
Brizalina spathulata (Williamson)		4	1		3	1	
Buccella granulata (di Napoli Alliata)	4	6	3	4	4	11	12
Bulimina elongata d'Orbigny							1
Bulimina etnea Seguenza	1	1		1			2
Bulimina marginata d'Orbigny	13	30	33	22	44	30	31
Bulimina sublimbata Panizza	4	5	11	1	5	4	2
Cassidulina carinata Silvestri	43	32	46	37	35	34	30
Cassidulina crassa d'Orbigny	4	6	7	4	10	8	15
Cornuspira involvens (Reuss)	3	5	1	2		1	1
Cribroelphidium articulatum (d'Orbigny)							1
Cribroelphidium decipiens (Costa)	38	19	15	11	25	16	25
Cribroelphidium granosum (d'Orbigny)	18	27	24	29	18	23	19
Elphidium advenum (Cushman)	1	5	3	2	5	3	4
Elphidium crispum (Linnaeus)	7	2	8	12	9	12	7
Elphidium macellum (Fichtel & Moll)	3	4		1		3	1
Epistominella exigua (Brady)	3	2	5	4	1	7	
Fissurina apiculata (Reuss)	1						
Fissurina castanea (Flint)	1						
Fissurina pseudorbignyana (Buchner)	1	3	1	1	1		1
Fissurina staphyllearia (Schwager)	1					1	
Fursenkoina schreibersiana (Czjzek)						1	
Gavelinopsis lobatulus (Parr)	8	24	18	13	11	7	6
Globocassidulina subglobosa (Brady)	20	12	12	28	25	17	13
Hanzawaia boueana (d'Orbigny)	2	2	2	3	4	4	4
Haynesina depressula (Walker & Jacob)	1	1	1	6	3	3	4
Haynesina germanica (Ehrenberg)			8				
Hyalinea balthica (Schroeter)	2			1	2	2	
Lagena striata (d'Orbigny)					1		
Lobatula lobatula (Walker & Jacob)	6	1	8	7	6	5	6
Melonis barleeanum (Williamson)	2	1	2	2	4		1
Miliolids	7	15	10	7	10	9	9
Miliolinella subrotunda (Montagu)	4	10	12	5	2	7	1
Neoconorbina terquemi (Rzehak)				3			
Nonionella turgida (Williamson)			2	4		2	2
Oolina hexagona (Williamson)	1	4	2		1		1
Planorbulina mediterranensis d'Orbigny		1		1			
Pullenia quadriloba Reuss							1
Pyrgo bulloides (d'Orbigny)		2					
Pyrgo oblonga (d'Orbigny)						1	
Quinqueloculina asperula Seguenza	1	1					1
Quinqueloculina bicarinata d'Orbigny				1			
Quinqueloculina inaequalis d'Orbigny	1	1					
Quinqueloculina padana Perconig	2	1		3	1		
Quinqueloculina seminulum (Linné)	6	1	11	13	8	13	6
Reussella spinulosa (Reuss)	13	5	10	5	7	9	10
Rosalina globularis d'Orbigny	2	7	2	10	2	2	3
Sigmoilinita tenuis (Czjzek)				1		1	1
Sigmoilopsis celata (Costa)	6		2	7	2	5	3
Sigmoilopsis schlumbergeri (Silvestri)	4	3			1	3	5
Siphotextularia affinis (Fornasini)		3	1	1	1	3	
Spiroloculina canaliculata d'Orbigny	2	3		1	3	1	
Spiroplectammina wrighti (Silvestri)	7	5	5	1	8	10	2
Spiropthalmidium acutimargo concava (Wiesner)		2		2	1		1
Textularia aciculata d'Orbigny	4	3	2		1		2
Textularia sagittula Defrance			1				2
Textularia soldanii Fornasini	7			3		1	3
Uvigerina mediterranea Hofker	4	1	2	1	1	5	2
Others	2	14	1	6		5	4
Total specimens counted	294	312	326	310	314	322	304
(S)	49	45	43	46	41	47	48
H(S)	3,30	3,35	3,24	3,28	3,19	3,39	3,33
Microfaunal density	6125	5379	3260	2719	3413,04	3500	2471

Number of benthic foraminifera from GAB2 core samples.

Depth (cm)	0	10	20	30	40	50	60	70	80
Alabaminella weddellensis (Earland)		1			1				
Ammonia beccarii Linnaeus		2	1	3	7		1	1	7
Ammonia perlucida (Heron-Allen & Earland)			1					1	
Ammonia tepida (Cushman)			1	1		2	2	1	
Amphicoryna scalaris (Batsch)	1		2	1	2		1	1	
Angulogerina angulosa (Williamson)		1		1				3	
Arenaceous	2			1	6			1	4
Asterigerinata mamilla (Williamson)	5	9	6	7	6	7	9	5	2
Asterigerinata planorbis (d'Orbigny)	1			1	5	2	2		1
Astrononion stelligerum (d'Orbigny)	1						1		1
Bigenerina nodosaria d'Orbigny	27	32	11	15	26	15	12	15	20
Biloculinella globula (Bornemann)	1								
Biloculinella labiata (Schlumberger)									1
Bolivina subspinescens Cushman			2	2			1	1	
Brizalina dilatata (Reuss)		3	2	5	2	1		1	
Brizalina spathulata (Williamson)	1	1	3	2	1		7	2	3
Buccella granulata (di Napoli Alliata)	7	10	1	13	17	6	8	9	12
Bulimina etnea Seguenza		3	1	1		2		3	1
Bulimina marginata d'Orbigny	42	30	33	32	22	20	31	32	34
Bulimina sublimbata (Panizza)	1	8	9	9	11	15	8	10	2
Cassidulina carinata Silvestri	32	38	43	26	33	40	39	36	39
Cassidulina crassa d'Orbigny	16	32	19	28	25	27	29	33	36
Cornuspira involvens (Reuss)	2		1	9	2		4	1	4
Cribroelphidium articulatum (d'Orbigny)		1				1			
Cribroelphidium decipiens (Costa)	18	15	21	3	21	19	20	23	18
Cribroelphidium granosum (d'Orbigny)	31	30	23	34	19	32	21	23	18
Elphidium advenum (Cushman)	1	6	13	17	8	11	12	8	11
Elphidium crispum (Linnaeus)	1	3	2	2	9	4	1		4
Elphidium macellum (Fichtel & Moll)		5	10	5	6	4	4	2	1
Epistominella exigua (Brady)	1							1	
Fissurina marginata (Montagu)						1			
Fissurina pseudorbignyana (Buchner)	3	3		3		2	1		
Fissurina staphyllearia (Schwager)	1								
Gavelinopsis lobatulus (Parr)	17	15	8	6	10	16	11	7	14
Globocassidulina oblonga (Reuss)									2
Globocassidulina subglobosa (Brady)	1								1
Gyroidinoides umbonatus (Silvestri)	1			1					
Hanzawaia boueana (d'Orbigny)	5	1	4	1	2	4	4	1	1
Haynesina depressula (Walker & Jacob)		2	2	4	5	1	2	6	4
Haynesina germanica (Ehrenberg)	1			3		1	1	2	
Hyalinea balthica (Schroeter)	1	1		1	2	1		3	3
Lagena sulcata (Walker & Jacob)				1					
Lobatula lobatula (Walker & Jacob)	11	5	11	2	4	4	3	3	10
Melonis barleeanum (Williamson)	7	4	3	3		2	1		
Miliolids	3	4	2	11	3	7	11	7	7
Miliolinella subrotunda (Montagu)	5	4	11	6		5	1	5	6
Nonionella turgida (Williamson)		1		3	1	2	1	1	3
Oolina hexagona (Williamson)	2	3	4	3			1		
Planorbulina mediterranensis d'Orbigny		1			1				
Pseudoclavulina crustata Cushman					1				
Pullenia quinqueloba (Reuss)	1								
Pyrgo bulloides (d'Orbigny)								1	
Pyrgo oblonga (d'Orbigny)								1	
Quinqueloculina asperula Seguenza	1	2	2	2		2	1	6	1
Quinqueloculina inaequalis d'Orbigny	1	1				2		1	
Quinqueloculina padana Perconig	1		1	1	3	1	3	2	1
Quinqueloculina seminulum (Linné)	5	1	3	3	1	3	4		2
Reussella spinulosa (Reuss)	6	9	7	14	15	8	3	11	3
Rosalina globularis d'Orbigny	4	5	2		4	6	7	3	3
Sigmoilina sigmoidea (Brady)			1			1	1		
Sigmoilinita tenuis (Czjzek)	3			1	1		2	1	
Sigmoilopsis celata (Costa)					1				
Sigmoilopsis schlumbergeri (Silvestri)	10	7	7	1	8	6	12	10	6
Siphotextularia affinis (Fornasini)	2	6	2	1		5	3	2	3
Spirillina vivipara Ehrenberg					1				
Spiroloculina canaliculata d'Orbigny	1	1	2	3	1	1	1	3	1
Spiroplectammina wrighti (Silvestri)	6	6	7	5	6	5	3	5	5
Spiropthalmidium acutimargo concava (Wiesner)	1						1		
Textularia aciculata d'Orbigny	4	3	5	2		3	2	6	8
Textularia sagittula Defrance			1		1				
Uvigerina mediterranea Hofker	2	3	2	2	3	1	1		3
Valvulineria bradyana (Fornasini)									1
Others	3	3	3	1	5	2	7	6	5
Total specimens counted	300	321	295	302	308	300	301	306	312
(S)	46	42	41	47	40	42	45	44	42
H(S)	3,13	3,15	3,18	3,27	3,24	3,18	3,20	3,21	3,15
Microfaunal density	4167	3242	2341	2378	1721	2174	2315	2000	1156

Number of benthic foraminifera from GAB3 core samples.

Depth (cm)	0	10	20	30	40	50	60	70	80	90	100	110
Ammonia beccarii Linnaeus	4	1	2	1	3				2		2	5
Ammonia parkinsoniana d'Orbigny										1		1
Ammonia perlucida (Heron-Allen & Earland)			1							1	2	1
Ammonia tepida (Cushman)					1			2	1	3	8	14
Amphicoryna scalaris (Batsch)	1					2		2	2	2		
Angulogerina angulosa (Williamson)	1	2	1		1						1	
Arenaceous	1	4	3	3	0	3	0	0	3	1	0	3
Asterigerinata mamilla (Williamson)	2	7	20	12	7	9	9	6		7	3	2
Asterigerinata planorbis (d'Orbigny)				1					1			1
Astrononion stelligerum (d'Orbigny)					1							
Bigenerina nodosaria d'Orbigny	18	25	9	18	8	23	12	32	20	26	14	25
Biloculinella globula (Bornemann)		2	4	1	3		3	1				
Bolivina albatrossi Cushman						2	2			1		
Bolivina subspinescens Cushman		1	2	1	1		2	1		2		
Brizalina dilatata (Reuss)	3	5	1	4	4	8	7	3	5	4	3	4
Brizalina spathulata (Williamson)		2	4	1	3	4		3			2	2
Buccella granulata (di Napoli Alliata)	3	2	1	2	4	7	2	5	9	5	7	4
Bulimina etnea Seguenza		2	1					2	1	1		
Bulimina marginata d'Orbigny	33	22	24	30	34	33	25	28	30	38	38	28
Bulimina sublimbata (Panizza)	10	11	9	16	4	5	13	9	6	10	6	5
Cassidulina carinata Silvestri	43	38	48	44	55	58	54	43	42	60	49	44
Cassidulina crassa d'Orbigny	9	10	28	22	25	19	4	17	5	36	22	36
Cornuspira involvens (Reuss)	2	9	1		3		3	1	1		3	3
Cribroelphidium decipiens (Costa)	16	28	23	17	17	9	19	18	14	18	10	11
Cribroelphidium granosum (d'Orbigny)	27	19	12	18	24	12	29	17	19	18	18	17
Elphidium advenum (Cushman)	3	5	8	4	3	3	7	2	3	4	6	8
Elphidium crispum (Linnaeus)	5	3	6	2	4	2	5	7	12	2	5	3
Elphidium macellum (Fichtel & Moll)	3	3		1	3	3	5	3	2	7	5	2
Elphidium sp.		4	4	2	2	4		1		4	2	2
Epistominella exigua (Brady)	1	4	3		1	1	4	1	4	2		
Fissurina apiculata (Reuss)			1						1			
Fissurina marginata (Montagu)	1		2				1					
Fissurina pseudorbignyana (Buchner)	3		4	1	1	1	3			1		
Fissurina staphyllearia (Schwager)					1		1					
Gavelinopsis lobatulus (Parr)	8	14	9	16	7	8	8	8	12	9	9	6
Globocassidulina subglobosa (Brady)	4		1		1	1	29	7	20			2
Gyroidinoides umbonatus (Silvestri)								1				
Hanzawaia boueana (d'Orbigny)	4	3	1	5	1		2	4	4	2	3	1
Haynesina depressula (Walker & Jacob)		2	3	2	2	6	1		5	1	1	
Haynesina germanica (Ehrenberg)					2	3	2	1		5	8	7
Hyalinea balthica (Schroeter)	1	1		1	1	1			3	1		
Lagena apiopleura Loeblich and Tappan			1									
Lagena striata (d'Orbigny)	1					2	1	1				
Lagena sulcata (Walker & Jacob)										1		
Lobatula lobatula (Walker & Jacob)	13	5	1	7	20	12	10	10	8	19	11	23
Melonis barleeanum (Williamson)	2	2	4	3	3	1	1	3	6	3		
Miliolids	3	9	15	11	9	14	8	13	8	10	7	4
Miliolinella subrotunda (Montagu)	13	10	9	5	8	3	9	8	8	7	1	2
Nonionella turgida (Williamson)		3	4		2	2	2		1	1	2	4
Oolina hexagona (Williamson)	1	1	2	1	2	3		1	1			
Planorbulina mediterranensis d'Orbigny	1			2					4			4
Pseudoclavulina crustata Cushman				2							1	
Pyrgo oblonga (d'Orbigny)												1
Quinqueloculina asperula Seguenza		1	1									
Quinqueloculina inaequalis d'Orbigny											1	1
Quinqueloculina oblonga (Montagu)	1			1				1				
Quinqueloculina padana Perconig	2	4		2	1					3	1	1
Quinqueloculina seminulum (Linné)	8	3	2	2	4	4	6	9	15	1	2	2
Reussella spinulosa (Reuss)	5	5	5	7	7	4	3	8	5	7	5	2
Rosalina globularis d'Orbigny	7	6	4	10	8	2	2	6	4	2	2	2
Sigmoilinita tenuis (Czjzek)		4	1	1	2			1		3		
Sigmoilopsis celata (Costa)	5	1				10	3	6	6			1
Sigmoilopsis schlumbergeri (Silvestri)		10	2	11	11	1	1		1	5	16	7
Siphotextularia affinis (Fornasini)	5	2	3	2	2	4	4	5	2	1	1	
Spirillina vivipara Ehrenberg	2											
Spiroloculina canaliculata d'Orbigny		4	1	3	1	1	4	1		1	3	
Spiroplectammina wrighti (Silvestri)	12	10	5	8	12	15	16	9	14	13	19	12
Spiropthalmidium acutimargo concava (Wiesner)		1	2		1		1		1	1	1	1
Textularia aciculata d'Orbigny	2	4	3	4	7		2	1	3	7	5	4
Textularia sagittula Defrance	8	4		1		1				1	1	
Triloculina tricarinata d'Orbigny	1						1					
Uvigerina mediterranea Hofker	8	6		1	1	1		4	1		1	1
Others	2	1	3	4	7	1	3	1		3		
Total specimens counted	308	325	304	313	335	308	329	313	315	361	307	309
(S)	43	48	47	44	49	42	43	44	41	47	42	41
H(S)	3,26	3,42	3,25	3,23	3,24	3,13	3,19	3,24	3,26	3,15	3,13	3,12
Microfaunal density	6844	4514	4903	3130	3384	4338	4700	4173	4200	2242	1129	868