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Cold seep communities in the deep eastern Mediterranean Sea: composition, symbiosis and spatial distribution on mud volcanoes

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Abstract

Two mud volcano fields were explored during the French–Dutch MEDINAUT cruise (1998) with the submersible NAUTILE, one south of Crete along the Mediteranean Ridge at about 2000 m depth (Olimpi mud field) and the other south of Turkey between 1700 and 2000 m depth (Anaximander mud field) where high methane concentrations were measured. Chemosynthetic communities were observed and sampled on six mud volcanoes and along a fault scarp. The communities were dominated by bivalves of particularly small size, belonging to families commonly found at seeps (Mytilidae, Vesicomyidae, Thyasiridae) and to Lucinidae mostly encountered in littoral sulfide-rich sediments and at the shallowest seeps. Siboglinid polychaetes including a large vestimentiferan *Lamellibrachia* sp. were also associated. At least four bivalve species and one siboglinid are associated with symbiotic chemoautotrophic bacteria, as evidenced by Transmission Electronic Microscopy and isotopic ratio measurements. Among the bivalves, a mytilid harbors both methanotrophic and sulfide-oxidizing bacteria. Video spatial analysis of the community distribution on three volcanoes shows that dense bivalve shell accumulations (mainly lucinids) spread over large areas, from 10% to 38% of the explored areas (2500–15000 m^2) on the different volcanoes. *Lamellibrachia* sp. had different spatial distribution and variable density in the two mud volcano fields, apparently related with higher methane fluxes in the Anaximander volcanoes and maybe with the instability due to brines in the Olimpi area. The abundance and richness of the observed chemosynthetic fauna and the size of some of the species contrast with the poverty of the deep eastern Mediterranean.

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The presence of a specialized fauna, with some mollusk genera and species shared with other reduced environments of the Mediterranean, but not dominated by the large bivalves usually found at seeps, is discussed.

1. Introduction

Communities of benthic animals associated with cold seeps have been discovered in recent decades at several places on active and passive continental margins of the Pacific and Atlantic oceans (see for review Sibuet and Olu, 1998; Sibuet and Olu-Le Roy, 2002). Mud volcanoes, geological structures where mud and fluid are seeping through the seafloor, are one of the most common environments favoring their development. Mud volcanism is a phenomenon typically driven by overpressured sediment in subduction zones and by salt tectonism in passive margins (Milkov, 2000; Bohrmann et al., 2002). Large quantities of methane are often released at the surface of the mud volcanoes (Ginsburg et al., 1999; Martin et al., 1997; Vogt et al., 1997; MEDINAUT/MEDINETH, 2000; Mac-Donald et al., 2000; Charlou et al., 2003). The methane is produced either through biological degradation of organic matter in the upper sediments or through migration of thermogenic gas from deeper hydrocarbon deposits. At cold seeps, archae and bacteria consortia use this methane and produce sulfides in the sediment by chemoautotrophic processes (Masuzawa et al., 1992; Boetius et al., 2000; Pancost et al., 2000) whereas symbiotic bacteria use methane or sulfide to sustain high biomass production in large size invertebrates (review in Fiala-Médioni and Felbeck, 1990; Fisher, 1990). Such ecosystems have been described on mud volcanoes along passive and active margins, for example on the Barbados accretionary prism (Jollivet et al., 1990; Olu et al., 1996, 1997), in the Gulf of Mexico (MacDonald et al., 1989, 1990a; Carney, 1994; Neurauter and Roberts, 1994; MacDonald et al., 2003), and on the Costa Rica accretionary prism (Bohrmann et al., 2002). A circular structure considered to be a mud volcano in Monterey Bay is colonized by vesicomyid bivalves (Barry et al., 1996). Finally a mud volcano (Haakon Mosby Mud Volcano) has been described in the Norwegian Sea (Vogt et al., 1997; Milkov et al., 1999). The mud volcano chemosynthetic communities include bivalves (mainly vesicomyids and mytilids) and polychaetes Siboglinidae[1] (vestimentiferans and perviate pogonophorans), that dominate both Barbados and Louisiana slope cold seep communities. Lucinid and thyiasirid infaunal clams occur at the shallowest seep sites, generally associated with subsurface lithification (Olu et al., 1996; MacDonald et al., 1990a). The Haakon Mosby Mud volcano chemosynthetic community is characterized by the absence of bivalves and dominance of small-sized Siboglinidae polychaetes (Frenulata) (Milkov et al., 1999). East Pacific cold seep sites are characterized by the absence of Mytilidae, except on the Costa Rica prism (Bohrmann et al., 2002) that has been attributed to less methane availability (Barry et al., 1996; Sibuet and Olu, 1998).

Chemosynthetic communities have been recently discovered on mud volcanoes at two distinctive settings in the eastern Mediterranean Sea south of Crete (Mediterranean Ridge) and south of Turkey (Corselli and Basso, 1996; Woodside et al., 1998; MEDINAUT/MEDINETH, 2000) (Fig. 1). Some shells have been previously sampled by dredges and cores; preliminary identification indicated lucinids and vesicomyids (Corselli and Basso, 1996), and one lucinid species has recently been described from these collections (Salas and Woodside, 2002). Nevertheless the species composition and extent of the chemosynthetic community had not been

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[1]According to McHugh (1997); Rouse and Fauchald (1997); Southward (1999), the perviate pogonophorans (Frenulata and Monilifera) and the vestimentiferans (Obturata), at one time regarded as self standing phyla, are to be included in Polychaete family Siboglinidae.
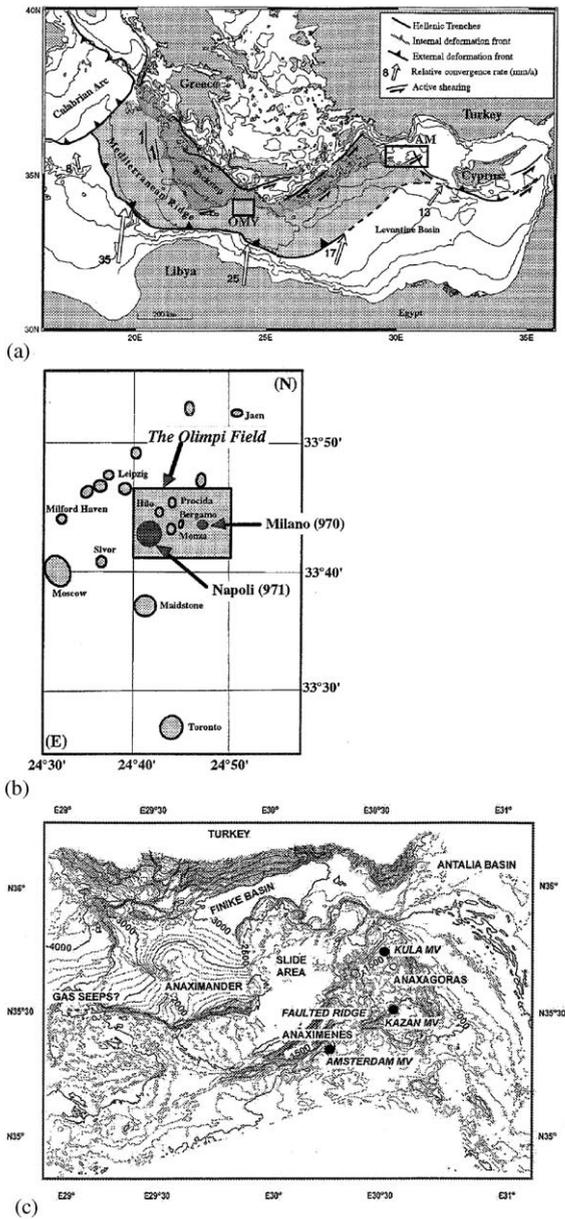
Fig. 1. (a) Location map showing the Olimpi Mud Volcano field (OMV) and Anaximander Mountains (AM) in their general Eastern Mediterranean context (from MEDINAUT/ MEDINETH, 2000). (b) Detailed position of the mud volcanoes in the Olimpi area (OMV), Napoli, Milano, Maidstone and Moscow were investigated during the diving MEDINAUT cruise. (c) Bathymetric map of Anaximander mountains (AM) with the four geological structures (Amsterdam, Kula, Kazan, Faulted Ridge) investigated by Nautile during the MEDINAUT cruise.

studied prior to the MEDINAUT submersible cruise, realized in 1998. Dives and video observations revealed exceptionally large fields of bivalve shells, and living large siboglinids (vestimentiferans), though less abundant, were also observed in association with carbonate crusts. In this paper, we first describe the composition of the seep fauna and symbiosis in four species associated with methane release on several mud volcanoes south of Crete and Turkey, and outline the main features of the chemosynthetic fauna in the deep Mediterranean, with hypothesis for colonization processes. The spatial distribution of the benthic megafauna colonizing the volcanoes is mapped based on video processing and these features are compared between several mud volcanoes in relation with environmental patterns. This paper describes for the first time a diverse chemosynthetic community in the deep Mediterranean. These new data increase our knowledge of cold seep community structure on mud volcanoes and the biogeography of cold seep species.

2. Geological setting

Intense emission of methane occurs at Mediterranean mud volcanoes and along related fault systems (MEDINAUT/MEDINETH, 2000; Charlou et al., 2003). The Olimpi Mud Volcano field (OMV area) is located on the Mediterranean Ridge (Cita et al., 1996; Mascle et al., 1999), the accretionary prism associated with subduction along the Hellenic Arc south of Greece (Fig. 1a and 1b). The other area of mud volcanoes is the Anaximander Mountains (AM area; Fig. 1a and 1c) formed by an older group of continental fragments now caught up in the plate convergence between Africa and Eurasia (Woodside et al., 1998). The OMV and AM settings have in common strong compressional tectonics with superposed faulting. The mud volcanoes are conical topographic features several km across and on the order of 100 m high, while a few have relatively flat summit areas with low relief. Fresh mud flows were observed on some volcanoes, whereas others may lie dormant for a relatively long time before reactivating. An extensive field of gas hydrate was sampled on both Amsterdam and Kula mud volcanoes. Large methane concentrations measured in the water
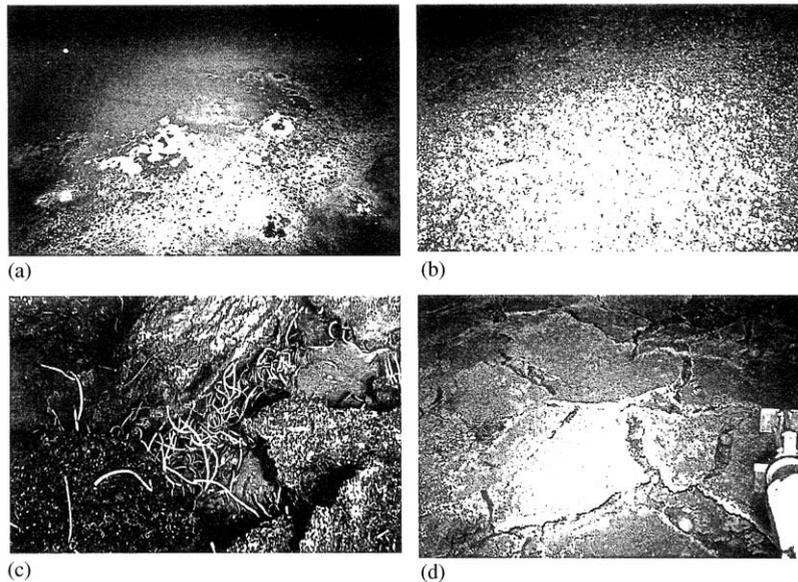
Fig. 2. (a) Brine lake on Napoli mud volcano, (b) Lucinid shells and echinids, on Kazan mud volcano, (c) *Lamellibrachia* sp. on Amsterdam mud volcano, (d) Sea urchins and small size *Lamellibrachia* sp. between carbonate crusts.

column above the mud volcanoes indicate intense degassing (MEDINAUT/MEDINETH, 2000; Charlou et al., 2003). Brine pools rich in methane occur on some mud volcanoes such as Napoli (Fig. 2a). Anaerobic methane oxidation by a consortium of Archaea and Bacteria has been evidenced in the mud volcano sediments (Pancost et al., 2000). Carbonate crusts, formed by oxidation of methane from mud flows at the seafloor (Aloisi et al., 2000) by methane-consuming Archaea (Aloisi et al., 2002), form pavements up to several tens of centimeters thick, round slabs, and circular mounds on the central most active part of the mud volcanoes. When the crusts are absent, mud flows are presumed to be freshly erupted.

3. Methods

3.1. Dives and sampling

Two "mud fields" were explored during the MEDINAUT cruise (Dutch–French collaboration) with the submersible Nautile: the Olimpi mud field south of Crete (about 34°N, 25°E, about 2000 m depth) with the Napoli, Milano, Maid-

stone and Moscow mud volcanoes, and the Anaximander mud field south of Turkey (35°30′N, 30°E, 1700–2000 m depth) with the Kula, Kazan and Amsterdam mud volcanoes and the Faulted Ridge. Two brine lake areas were surveyed, one related to the mud field in the Olimpi area (Nadir brine lakes) and the other in the Urania area south of Greece (Urania brines).

Benthic species of chemosynthetic communities were collected on 6 volcanoes among 7 explored (Napoli, Milano, Maidstone, Amsterdam, Kazan, and Kula) and along the Faulted Ridge, using different sampling devices (Table 1). Nevertheless, as only a few samples were taken by cores, no quantitative data are available to estimate densities or for statistically valuable comparison of species richness or diversity between sites. Specimens were formalin fixed on board and sorted in the laboratory (Centob, Ifremer DRO-EP) and identified by specialists for each group.

3.2. Transmission electronic microscopy

Small pieces of gills were fixed in 3% glutaraldehyde in 0.4 M Nacl, buffered with 0.1 M cacodylate pH 7.4 and post-fixed in 1% osmium

Table 1
Number of dives and fauna sampling realized on the different geological structures surveyed by Nautile during the Medinaut cruise, and subsequent video analyses

Area	Structure name	Number of dives	Bottom time	Fauna sampling	Video analysis
Olimpi	Napoli (M.v.)	4	20h06	8 BT, 1 BC, 1 TC	X
	Milano (M.v.)	3	15h39	5 BT, 2 BC	X
	Maidstone (M.v.)	1	1h00	1 BT	
	Moscow (M.v.)	1	5h25		
	Nadir (B.l.)	1	6h54		
Anaximander	Amsterdam (M.v.)	4	20h39	7 BT, 2 TC	X
	Kazan (M.v.)	2	10h40	6 BT, 1 TC	X
	Kula (M.v.)	1	5h23	1 BT	
	Faulted Ridge	1	5h12	4 BT, 1 TC	
Urania	Urania (B.l.)	1	3h05		

The volcanoes Napoli, Amsterdam and Kazan have been studied in detail. Additional data from Milano volcano (video data) and Faulty ridge (sampling data) have been included.
M.v. = Mud volcano, B.l. = Brine lake.
BT: bottom grab, BC: box core, TC: tube core.

tetroxide for 1 h in the same buffer. The specimens were dehydrated through a graded ethanol series and embedded in Araldite. Semi-thin sections were stained with toluidine blue. Ultra-thin sections were contrasted with uranyl acetate and lead citrate, and observed under a Hitachi H600 transmission electron microscope.

3.3. Isotopic ratios

Stable carbon and nitrogen isotope analyses of the tissues were made on samples frozen and stored in liquid nitrogen then freeze dried and acidified in the laboratory. The acidified samples were combusted to CO_2 and N_2 using standard methods before being passed through a mass spectrophotometer (Finnigan Delta E). Carbon results are reported relative to PDB (PeeDee Belemnite) and nitrogen relative to atmospheric molecular nitrogen.

3.4. Video analysis

A video study was made of chemosynthetic communities observed around seeps at three mud volcanoes (Table 1). Dive tracks were plotted on bathymetric maps using the Adelie software, an extension of ArcView GIS developed at Ifremer for submersible data processing. Large siboglinids (vestimentiferans) were enumerated every 30 s and an average density was computed along continuous tracks with a sampling unit of about 50 m², following the method described in Olu et al. (1997). Their densities were mapped along the dive tracks, and the mean density on the whole active surveyed area of each volcano was estimated by dividing the total number of individuals by the total active area explored. The active area is defined as the area where living siboglinids were observed. In order to test if the density is significantly different between two sites, means were compared using the Kolmogorov–Smirnov test as the data did not fit the normal distribution. The patterns of distribution (patchy or regular) are compared by the variance/mean ratios of the densities with the sampling unit of 50 m². A different method was used to map bivalve shells along the tracks, because the species are small and partially or totally buried. Three categories were delimited: bivalve clusters (dense aggregations on restricted areas from 0.1 to 1 m²), bivalve fields (dense aggregations from 10 to more than 50 m²) and scattered bivalves (on areas from 10 to more than 100 m²). Dense aggregations (clusters or fields) are mostly continuous layers on the bottom (Fig. 2b). The surface of each aggregation was

estimated using the empirical relationship between the submersible altitude and the surface covered by the camera field. Crabs, fishes, shrimps and sea urchins were enumerated, abundance of each group was mapped, and the mean densities were estimated for each volcano.

4. Results and discussion: community composition

4.1. The fauna sampled on mud volcanoes

4.1.1. Bivalves

The sampled bivalves belong to five species in four families, Mytilidae, Vesicomyidae, Lucinidae and Thyasiridae. Numbers of specimens and shells sampled for each species at the main sites are indicated in Table 2a. The Mytilidae *Idas modiolaeformis* (Sturany, 1896) (Fig. 3) appears to be relatively ubiquitous in both Olimpi and Anaximander areas as it was sampled at all the sites, except on the Kula volcano where only one grab was taken.

Shell lengths of sampled specimens are comprised between 2 and 17 mm. The Vesicomyidae *Isorropodon perplexum* Sturany (1896) (revised by von Cosel and Salas, 2002) (Fig. 3) was sampled at the five well-explored sites. Nevertheless, no living specimens were collected in the Olimpi area and only a few on Amsterdam and Kazan (Anaximander area) whereas the shells and dislocated valves are very numerous (15 live specimens and more than 1000 valves sampled on Amsterdam). This species was reported from shells taken by cores on Napoli dome by Corselli and Basso (1996) as *Vesicomya* sp. Shell lengths of our specimens ranged from 2 to 14 mm. The Lucinidae, *Myrtea amorpha* (Sturany, 1896) (Fig. 3), was collected at the five main sites and on Kula mud volcano. Living specimens were only be found in the Anaximander area on Kazan and Kula. A second Lucinidae is the recently described species *Lucinoma kazani* Salas and Woodside (2002) sampled only in the Anaximander zone, with a few living juveniles on Kazan (Fig. 3). Shells of *M. amorpha* were of small size (8–20 mm in length) whereas *Lucinoma* is larger (up to 40 mm). Finally, living and dead specimens of the Thyasiridae *Thyasira*

striata Sturany (1896) (Fig. 3) were collected on Amsterdam and Kazan mud volcanoes in the Anaximander area. Four of the five bivalve species, *I. perplexum*, *I. modiolaeformis*, *M. amorpha*, and *T. striata* were described from the same dredge sampling in the Nile delta by Sturany (1896).

4.1.2. Gastropods

Numerous gastropods, all less than 2 cm were collected at the five main sites (Table 2b). A total of 11 species in eight families has been collected. All the species were present in the Anaximander area, whereas only four species (only one living) were sampled in the Olimpi area. Empty shells of the Turridae *Taranis moerchi* (Malm, 1861) were collected at all the sites and were particularly abundant in the Kazan samples (176 shells in one grab). The Trochidae include *Putseysia wiseri* (Calcara, 1841) sampled on the five sites, with dominance of dead individuals, but a few living ones in both areas, and *Clelandella* n. sp., also common but with living specimens only in the Anaximander area. The limpet *Lepetella* sp. collected on Kazan, is likely living on polychaete tubes (maybe on *Lamellibrachia* sp.), as other species of this genus, and *Odostomia* sp. is likely commensal of the abundant polychaetes as this is an obligate way of life (Fretter and Graham, 1962). A few specimens of *Lurifax vitreus* (Warén and Bouchet, 2001), recently described from hydrothermal sites on the Mid Atlantic Ridge, were interestingly sampled on Amsterdam.

4.1.3. Polychaetes Siboglinidae and others

All the sampled siboglinids of the Obturata group (Vestimentifera) belong to the same, likely new species of *Lamellibrachia* (E. Southward, pers. com.). Specimens were of various sizes (up to 80 cm long) and were observed on various substrata. However, they were always near or in close relation to carbonate crusts (e.g. in mud with small crust fragments, or attached under crusts, or in fractures inside large size blocks) (Fig. 2c and 3). They sometimes occupied sinuous tubular hollows in carbonate crusts, which form when methane-charged mud flows are oxidized at the seafloor (Fig. 3 and Aloisi et al., 2000). This species has

Table 2
Sampled fauna on the different mud volcanoes and Faulty Ridge

	Olimpi area		Anaximander area			Relation to seeps
	Napoli	Milano	Amsterdam	Kazan	Faulted ridge	
(a) Bivalves						
Idas modiolaeformis	L	L	L	L/D	L	?Endemic
Myrtea amorpha	D	D	D	L/D	D	?Endemic
Lucinoma kazani	—	—	D	L (juv)/D	D	?Endemic
Isorropodon perplexum	D	D	L/D	L/D	D	?Endemic
Thyasira striata	—	—	L/D	L/D	—	?Endemic
Yoldiella sp.				R		Vagrant
(b) Gastropods						
Taranis moerchi	D	D	D	D	D	?Colonist
Putseysia wiseri	L/D	D	L/D	L/D	D	Colonist
Clelandella n. sp.	D	D	L/D	L/D	L/D	?Colonist
Akritogyra conspicua		R	L/D	L/D	L/D	?Colonist
Lurifax vitreus			L/D			Endemic
Odostomia n. sp.				D		?Colonist/endemic
Drilliola loprestiana				R		Vagrant
Benthomangelia macra				R		Vagrant
Xylodiscula sp.			D			?Vagrant
Benthonella tenella			D	D		Vagrant
Lepetella sp. (limpets)				L		On vestim. tubes

	Napoli	Milano	Amsterdam	Kazan
(c) Other groups				
Porifera: *Rhizaxinella pyrifera*	X			
Actiniaria	R			
Nemertina				X
Nematoda	X	X	X	X
Polychaeta	X		X	X
Polychaeta: Serpulidae		X		X
Polychaeta: Siboglinidae (vestimentifera): *Lamellibrachia* sp.	X	X	X	X
Polychaeta: Siboglinidae (Monilifera): *Siboglinum* sp.	X	X	X	X
Echiurida: *Bonellia viridis*				X
Aplacophora: *Falcidens* sp.			X	X
Amphipoda	X		X	X
Decapoda: *Munidopsis marionis*				X
Munidopsis acutispina				X
Chaceon mediterraneus	X		X	
Tanaidacea: *Paranarthrura intermedia*			X	
Echinodermata: *Echinus* sp.	D			X

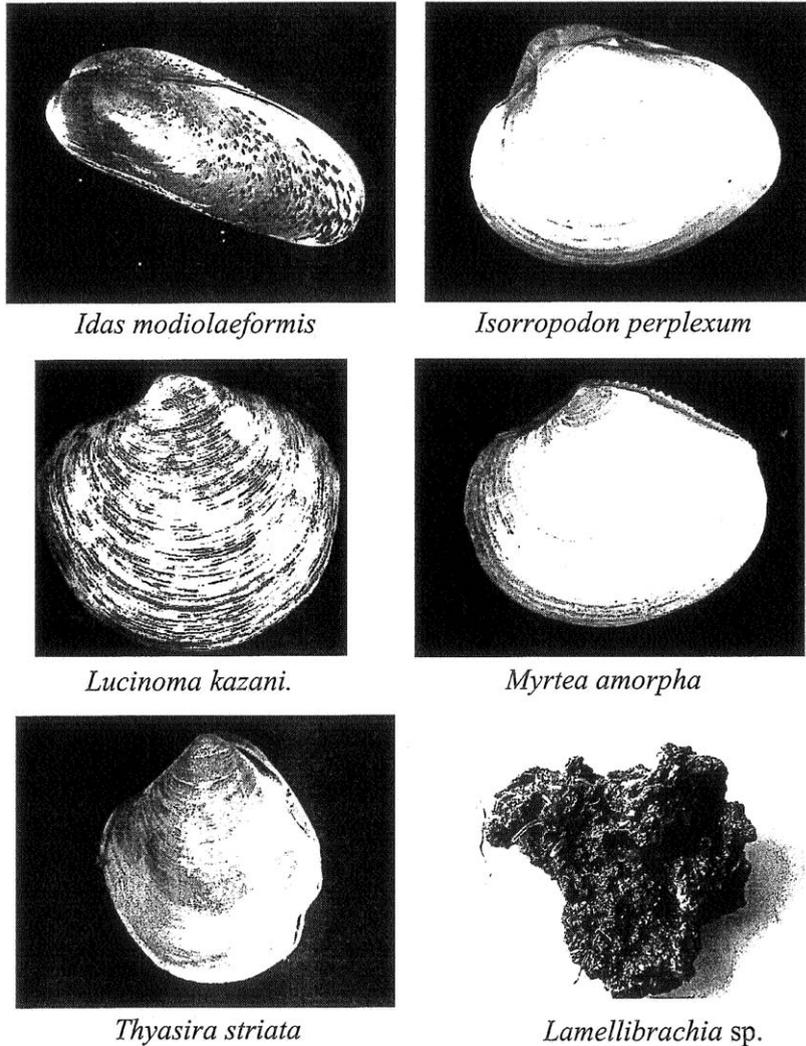L—living specimens, D—dead ones, juv.—juveniles, X—presence, R—rare.

Fig. 3. Invertebrate species sampled on Mediterranean mud volcanoes, known or assumed to be associated with chemautotrophic symbiotic bacteria. Bivalve shells and tubes of *Lamellibrachia* sp. attached in a close relationship with carbonate concretion. *I. modiolaeformis*: 15 mm long, *I. perplexum*: 14 mm, *L. kazani*: 40 mm, *M. amorpha*: 20 mm, *T. striata*: 10 mm. Photos Patrick Briand (Ifremer).

been sampled on each volcano and at Faulted Ridge. The exception was the Maidstone mud volcano, where it was not observed during the 1-h dive. Small Siboglinidae of the Monilifera goup were collected on Napoli, Milano, Amsterdam and Kazan mud volcanoes. Most of them belong to a probably new species of *Siboglinum* sp., that is different from the only species described in Mediterranean, *S. carpinei* (Ivanov, 1970) from west of Corsica (E. Southward, pers. comm.).

Tubes of another family of Polychaetes were also observed in abundance during dives, especially on Napoli and Amsterdam mud volcanoes. Unfortunately, they were not sampled living and could not be identified to a genus or species.

4.1.4. Other phyla

The other groups are listed in Table 2c. Exceptional large specimens of the Suberitidae sponge *Rhizaxinella pyrifera* (Delle Chiaje, 1828),

common in the bathyal Mediterranean, were sampled on the Napoli mud volcano (J. Vacelet, pers. com.). However, the sampled specimens have an unusually large size probably linked to their life at seeps. Among Crustaceans, the galatheids were sampled at active sites on Kazan, and the large crab, *Chaceon mediterraneus* (Manning and Holthuis, 1989) (D. Guinot, pers. com.) was sampled on the Napoli and Amsterdam mud volcanoes The Echinoidea *Echinus* sp. (M. Sibuet, pers. com.) were observed in large density at active seep sites, together with bivalves and *Lamellibrachia* sp. and between carbonate crusts (Fig. 2b and 2d), in the Anaximander area. They occur as dead individuals around Napoli volcano brine lakes.

4.2. Nutritional strategies

4.2.1. Symbiotic species

The bivalves and siboglinids colonizing the two mud volcano areas belong to families which are typical component of cold seeps, where they live in symbiosis with chemoautotrophic bacteria (Sibuet and Olu, 1998; Sibuet and Olu-Le Roy, 2002). Table 3 summarizes transmission electronic microscopy (TEM) observations and isotopic $^{13}C/^{12}C$ ratios of the presumed symbiotic species sampled in the Anaximander area. TEM analysis of transversal sections of gill filaments of the lucinid *M. amorpha* (Fig. 4a) and of the vesicomyid *I. perplexum* (Fig. 4d), as well as of the trophosome of the siboglinid *Lamellibrachia* sp. (Fig. 4b) show that they are mainly composed of bacteriocytes

hosting number of bacteria. Similar observations were made with scanning electron microscopy (SEM) on the gills of *L. kazani* by Salas and Woodside (2002). Observations of the mytilid *I. modiolaeformis* (Fig. 4c) show that the great majority of its gill cells contain two distinct morphological types of symbionts. The first type consists of small (mean diameter: 0.35 μm) coccoids or short rods, resembling to the sulfide-oxidizing symbionts already observed in *Bathymodiolus thermophilus* from the Pacific hydrothermal vents (Fiala-Médioni and Felbeck, 1990). The second type are large (mean diameter: 1.25 μm) coccoid-shaped bacteria looking type I methylotrophs with stacked intracytoplasmic membranes as observed in *B. puteoserpentis* (Cavanaugh et al., 1992; Robinson et al., 1998) or *B. azoricus* (Fiala-Medioni et al., 2002) and identified as methanotrophic symbionts.

$^{13}C/^{12}C$ and $^{15}N/^{14}N$ isotopic ratios were determined on the same tissues as observed in TEM. Carbon isotopic ratios on gills of *M. amorpha* and *I. perplexum* and *Lamellibrachia* sp. trophosome (Table 3) are in the range of those of other symbiotic chemosynthetis-based bivalves or siboglinids hosting sulfide-oxydizing symbionts, found in different vent or cold seep sites (Van Dover and Fry, 1989; Kennicutt et al., 1992; Fisher, 1995). The isotopic ratios measured on the mytilid *I. modiolaeformis* sampled on the Kazan m.v. are more depleted than those of the lucinids and vesicomyids; ranging from − 44.2‰ to −44.6‰ : they reflect more likely the presence

Table 3
Carbon isotope ratios and results from TEM observations of different species of the seep community

Species	$\delta^{13}C$	$\delta^{15}N$	TEM observations
Idas modiolaeformis	−44.2‰ to −44.6‰	0.2‰ to 0.4‰	Sulfur-oxidizing symbionts Probably type I methylotroph
Isorropodon perplexum	−29.3‰ to −30.1‰	–1.6‰ to 1.9‰	Sulfur-oxidizing symbionts
Myrtea amorpha	−27.7‰ to −30.5‰	−1.0‰ to 1.4‰	Sulfur-oxidizing symbionts
Lucinoma kazani			Intracellular symbionts[a]
Lamellibrachia sp.	−23.6‰ to −26.6‰	−0.8‰ to 0.2‰	Sulfur-oxidizing symbionts
Echinus sp.	−36.12‰ (muscle) −42.05% (stomach)		No observation
Rhizaxinella pyrifera[b]	−27.6 to −28.1‰		No symbionts

[a] Salas and Woodside (2002).
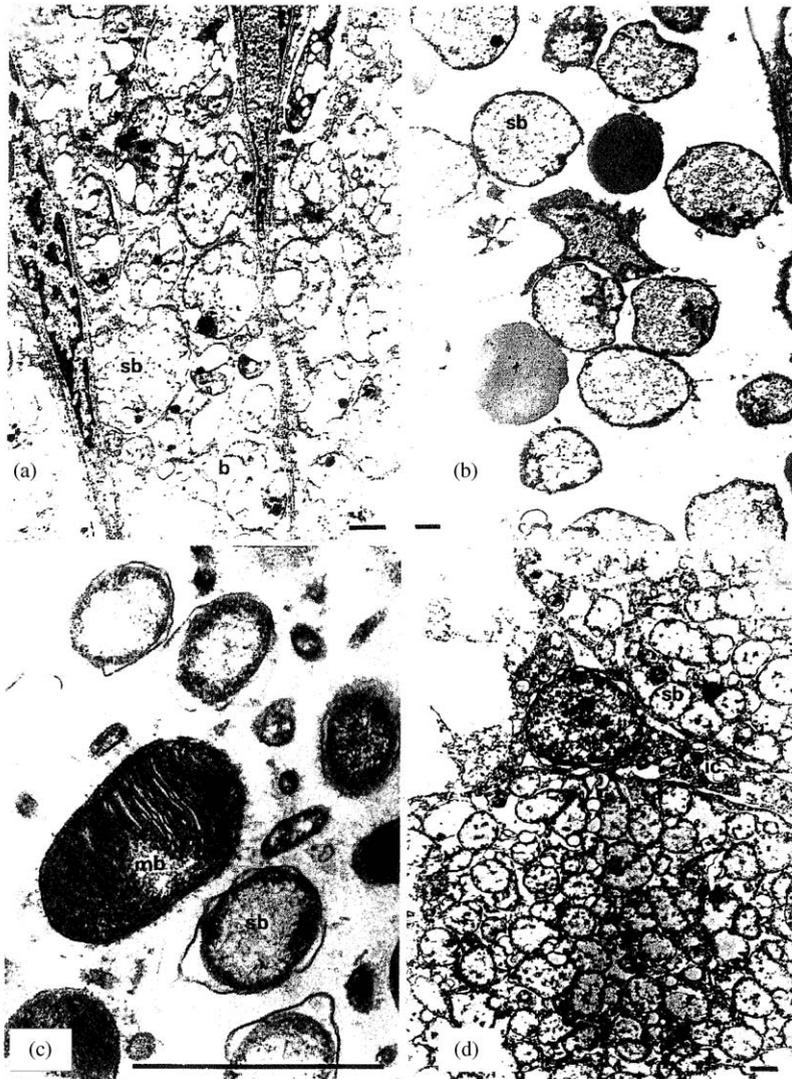[b] Fiala-Médioni and Vacelet (unpublished).

Fig. 4. Transmission electron micrographs of endosymbiotic bacteria on transversal sections: (a) TEM showing bacteriocytes in the gill of *M. amorpha*; (b) TEM of bacteria in the trophosome of *Lamellibrachia* sp.; (c) TEM of methylotrophic and sulfur-oxidizing bacteria in the gill of mytilid *I. modiolaeformis*; (d) TEM showing bacteriocytes with sulfur-oxidizing bacteria in the gill of *I. perplexum*; b: bacteriocyte; ic.: intercalary cell; sb: sulfur-oxidizing bacteria; mb: methylotrophic bacteria. Scale bars: 1 μm.

of methylotrophic symbionts which may use methane as energy source as the cold seep mussel *B. childressi,* ($\delta^{13}C = -37.5‰$ to $-67.1‰$) (Nelson and Fisher, 1995; Kennicutt et al., 1992). The $^{15}N/^{14}N$ isotopic ratios measured in *M. amorpha*, *I. modiolaeformis, I. perplexum* and *Lamellibrachia* sp. are also within the range of values reported from other symbiotic organisms (2.0‰ to –12.9‰).

Perviate siboglinids ("pogonophorans") are known to be associated with methylotrophic or sulfur-oxidizing symbionts (Southward et al., 1981, 1986; Southward, 1982). This probably the case of the sampled *Siboglinum* sp. Thyasiridae are known from coastal reduced environments or cold seeps to be associated with thioautotrophic symbionts and maybe another undetermined type of

bacteria (Fujiwara et al., 2001; Fullarton et al., 1995; Schmaljohann et al., 1990). Nevertheless, thyasirids may live at some periods, with little input of carbon from their symbionts (Dando and Spiro, 1993).

4.2.2. Accompanying species

These "accompanying "species, assumed to not contain symbionts, are tentatively classified as endemic to the seep community or to reducing environments in general, colonist, or vagrants, according to Carney (1994). This is summarized in Table 2. Four gastropods collected once at our sites, and commonly encountered in the Mediterranean Sea, are considered as accidental (vagrant), while the others, abundant in samples, can be considered as component of the seep (endemic or colonist). *T. moerchi* may be endemic of reduced environment or seep communities, considering its abundance on mud volcanoes, and its record (as *T. alexandrina* Sturany (1896), a synonym according to Bouchet and Warén, 1980) in the Nile delta together with symbiotic bivalves, where similar environmental conditions may occur. Nevertheless, this turrid could be an active predator, as others of this family, and be attracted by the concentration of biomass on the mud volcanoes rather than being a resident part of the community. *P. wiseri* (Trochidae), rare in the Mediteranean Sea, is assumed to be attracted to the seep sites where its density is clearly higher than elsewhere. Exceptionally high densities of *Putseysia* were also observed off Lanzarote, Canary Islands, possibly related to hydrothermal activity (Engl, 1995). Both trochids, *Putseysia* and *Clelandella* n.sp. are assumed to graze bacterial mats as suggested for other gastropods at seeps or vents, and seems to be colonists (opportunists), with higher densities at seeps. Finally, *L. vitreus*, originally described from the Mid Atlantic Ridge hydrothermal vents and only recently cited from the Mediterranean (Tyrrhenian Sea, Smriglio and Mariottini, 2002) is likely endemic of the seep/vent environments.

Polychaetes have been observed in abundance around the brine lakes on Napoli mud volcano and at the active sites on Amsterdam. High densities of large size tubicolous polychaetes have

been reported from the Japan Trench seeps (Juniper and Sibuet, 1987) and from Barbados prism mud volcanoes (Olu et al., 1997). They were found around the seeps or in areas of diffuse fluid emission and are assumed to benefit from local higher production. The galatheids *Munidopsis marionis* and *M. acutispina* sampled on Kazan, are probably scavengers or predators as other galatheids of these genera associated both with active or extinct cold seep communities (Chevaldonné and Olu, 1996). Brachyuran crabs are less commonly reported from cold seep sites than anomurans, except from the shallowest areas such as the Louisiana slope where two vagrant *Chaceon* spp. occur in and around mussels beds (Carney, 1994). Another *Chaceon* species is known feeding on mussel beds at the shallowest hydrothermal site of the Azores Triple Junction, characterized by a lower fluid toxicity than other vent sites (Desbruyères et al., 2001). The exceptional size of sampled sponges *R. pyrifera* suggests that they could have adapted to the seep environment by developing particular nutritional strategies. This has been tentatively attributed to their possible association with symbiotic bacteria, like those found in other cold seep sponges (Harrison et al., 1994; Vacelet and Boury-Esnault, 1995; Vacelet et al., 1996), but methylotrophic symbiotic bacteria were not observed by microscopic observations. Isotopic measurements (Table 3) neither indicate dependence on chemosynthesis-originated carbon.

The sea urchins of genus *Echinus* have a patchy distribution and are absent in the inactive areas, which suggests either a link between this species and the fluid emission, or an increase in food supply within the active area. The $\delta^{13}C$ value of the tissues of one specimen (from –35.67 to –42.33‰) may be indicative of such reliance on chemosynthesis originated carbon, as they lie between those of methanotrophic bacteria associated mytilids (– 44.2 to – 44.6‰) and those of bivalves and *Lamellibrachia* sp. associated with sulfur-oxidizing symbionts (from –23 to –30.5‰). Further studies are required however to determine if the echinids contain symbiotic bacteria or graze on chemosynthetic bacteria living on sediment. The burrowing echinoid *Echinocardium cordatum*, living in shallow water reduced environment, is

known to live in symbiosis with sulfur-oxidizing bacteria (Temara et al., 1993). Nevertheless, this species can possibly ingest sulfides in sediment, which can serve as substrate for its symbionts, while the Mediterranean seep *Echinus* sp. is a regular urchin, probably grazer, which more likely feeds from bacterial mats on the sediment surface.

4.3. Particularities of the Mediterranean cold seep fauna

The amount of organisms collected during the Medinaut cruise reaches at least 25 species. Despite a relatively limited sampling, this figure appears high by the standards of oriental deep-water Mediterranean fauna, usually extremely poor in macrofauna at similar depths (Chardy et al., 1973a; Pérès, 1985; Bogi & Galil, 2004). On all the mud volcanoes where a reasonable sampling effort was conducted, *Lamellibrachia* sp. and *Siboglinum* sp. were collected. A total of three to five species of bivalves belonging to 4 families were sampled on the different volcanoes and on Faulted Ridge. The total of at least 7 symbiont-bearing species in the Anaximander area and 4 in the Olimpi area stands high compared to other seep sites at similar depths even if the sampling effort was probably different (Sibuet and Olu-Le Roy, 2002). The remarkable richness of symbiotic species and gastropods may be favored by time duration of fluid and gas emission. The exceptional abundance of shells also suggests perennial seepage that could have persisted from several hundreds to thousands of years, and that maybe were more colonized in the past. Episodic eruptions of the mud volcanoes dating back over 1 Ma (Robertson and Kopf, 1998), and the fact that some may lie dormant while others are reactivated, may contribute to maintain a pool of specialized fauna able to colonize successively each of them when conditions are favorable.

The typical cold seep or hydrothermal vent genera (*Calyptogena*, *Bathymodiolus*…) are surprisingly absent. The same holds for gastropod families more closely associated with vent or seep environments (e.g. Provannidae; see Warén and Bouchet, 2001). This absence is likely related to the severe biogeographical isolation of the eastern

Mediterranean. The consequence of this present day isolation is that the deep-water fauna of the Mediterranean is currently much more impoverished than it was in some cold epochs of the Pleistocene (Pérès, 1985). The sole species shared with North Atlantic hydrothermal or seep areas, the gastropod *L. vitreus* and maybe the undetermined *Lamellibrachia* sp., may be therefore relicts from such an epoch, or are recent colonists from the Atlantic chemosynthetic environments. The absence of more specialized competitors would then explain that the bulk of the species living on the Eastern Mediterranean mud volcanoes is derived from more widespread environments. Although *I. washingtoni* from cold seeps of the Gulf of Mexico was provisionally placed in this genus (Gustafson et al., 1998), *Idas* is mostly represented in organic remains and sunken wood (Warén and Carrozza, 1990, for NE Atlantic species). The occurrence of *I. modiolaeformis*, as well as *I. perplexum, T. striata* and *T. alexandrina* in the Nile Cone (Sturany, 1896) suggests that reduced environment and maybe cold seepages may exist at this place. Coleman and Ballard (2001) also reported clam and mussel shells, as well as polychaete tubes at an active seep characterized by carbonates and gas bubbles south of Cyprus, east of the Nile cone, between 300 and 800 m depth. A remarkable feature at the sites described in the present paper is the dominance of lucinids, absent from vents and from deep cold seep settings although present in shallower seep areas up to 1200 m (Turner, 1985; Ohta, 1990; Sibuet and Olu, 1998). The lucinids are common in coastal waters, burrowed in organically rich mud where their long and specialized foot allow them to obtain either sea water at the sediment surface or sulfide in the deeper sediment (Turner, 1985). Two lucinids co-occur in Norwegian fjords at 33 m depth with thyasirids and *Siboglinum* sp. (Dando et al., 1985). Considering the bivalve fauna, the seep community living on the mud volcanoes is therefore closer to coastal reducing environments than to deep cold seep settings. This Mediterranean seep bivalve fauna could appear less specialized than usually observed at cold seeps and hydrothermal vents, but adapted to a wider range of reducing habitats with high sulfur concentrations, that may indicate

a relatively recent settlement. Nevertheless, instability of the seep environment due to episodic mud flow eruptions or brines, and possibly explosive emissions (MEDINAUT/MEDINETH, 2000), may prevent the establishment of seep endemic species and favor the dominance of less specialized fauna pre-adapted to organic-rich environments. In the same way, Levin et al. (2000) observed that insufficient persistence of seeps along the northern California slope and shelf does not allow chemosynthesis based specialization by most infauna while it favors normal slope and shelf species pre-adapted to organic-rich, reducing environments. At the Mediterranean seeps however, the emergence of neo-endemic species such as *Clelandella* n.sp. and *L. kazani*, and maybe *Lamellibrachia* sp. would point to a rather perennial existence of the chemosynthetic community of the mud volcanoes. Geological data indicate that the volcanoes are active over 1 Ma, which is sufficient for speciation.

The bivalves are curiously of small size (less than 2 cm, up to 4 cm for *Lucinoma* sp.) compared to species known from other cold seep sites or vents whose adult shell lengths are generally comprised between 10 and 30 cm. Assumed high growth rates and/or long-lived bivalves of large size as observed at other cold seep sites seem not to be favored at the Mediterranean seeps. The small size of the Mediterranean deep-sea macrofauna, compared to the Atlantic one, has been already pointed out by Chardy et al. (1973b). Interestingly, there is a report of a typical large size cold seep fauna from the Miocene Mediterranean, with large mussels tentatively attributed to the genus *Bathymodiolus*, lucinids (*Lucina hoernea*), vesicomyids (*Calyptogena* sp.) and buccinid gastropods (*Neptunea*) (Taviani, 1994), attributed to widespread hydrocarbon venting. At such epoch the Atlantic deep-sea cold seep/vent species was certainly able to colonize the Mediterranean seeps via deep corridors between the Miocene Mediterranean basins and oceanic fauna reservoirs, and this seep/vent fauna was likely exterminated by the Messinian salinity crisis. At the present-day seeps, only the gastropod *L. vitreus* described from the MAR vents and *Lamellibrachia* sp. are typical cold seep endemic species and are likely post-Miocene

colonizers of the deep Mediterranean, via the Strait of Gilbraltar. Comparative studies with the fauna of other reducing environments in the deep and shallow Mediterranean are required for a better understanding of the Mediterranean seep colonization and evolution.
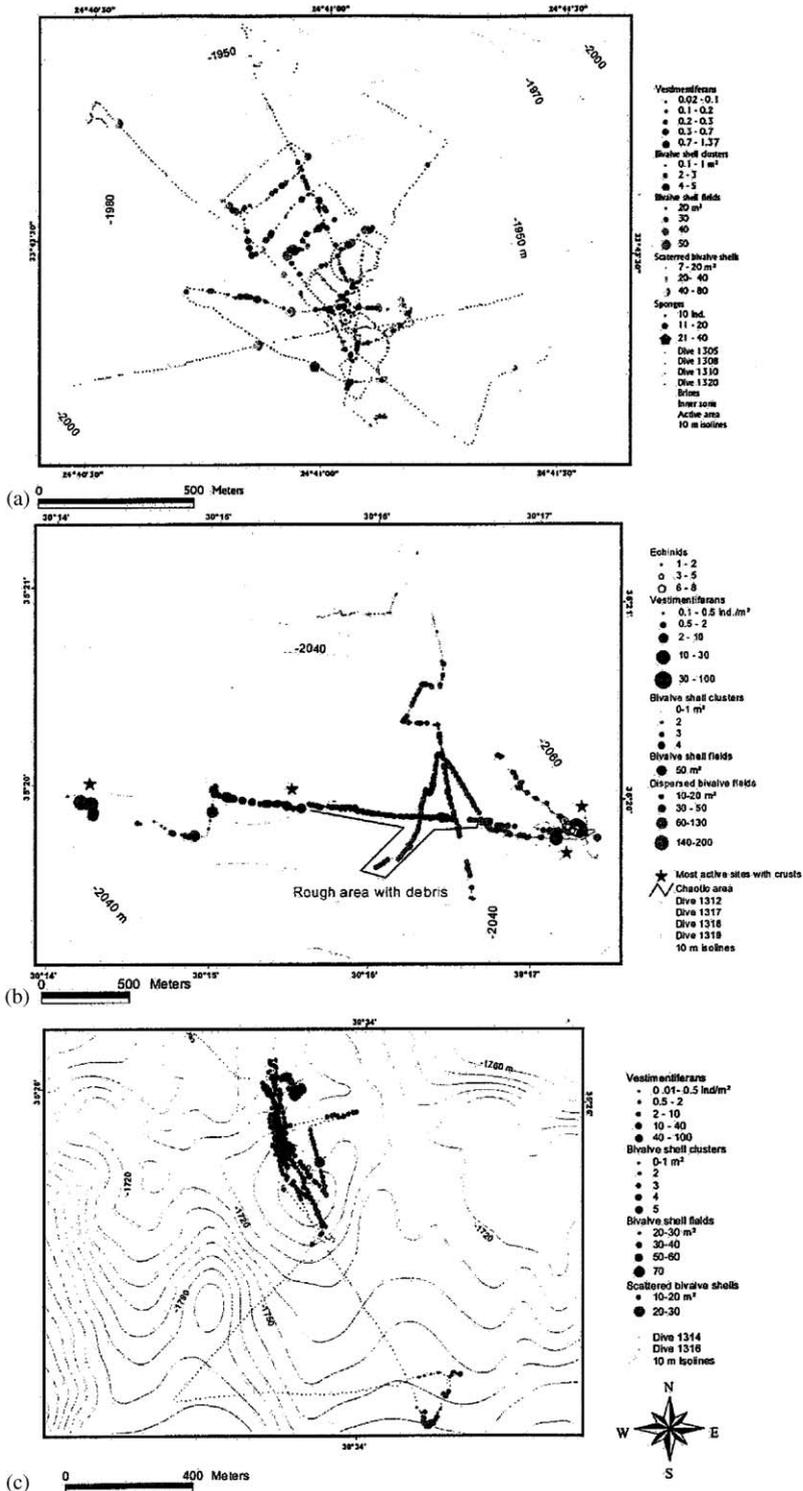
4.4. Distribution and abundance of the fauna on three mud volcanoes

Large bivalve shell fields, assumed to belong mainly to lucinids, because they are large enough to be visible on video, were observed during dives on Napoli, Amsterdam, Kazan and Milano mud volcanoes. On these volcanoes, the chemosynthetic megafauna (*Lamellibrachia* sp. and the densest shell clusters) was mostly concentrated around large-sized carbonate crusts (Fig. 2c) also linked to fluid emissions. *Lamellibrachia* sp. were often attached under the crusts.

No large-size chemosynthetic fauna was observed during dives on the Maidstone mud volcano and only a little on Moscow mud volcano (OMV area). The Nadir Brine lakes showed only sparse evidence for fossil cold seep communities at the edge of an empty brine lake and there was no life at all in the active Urania Brine lake showing very high salinity of $120 \, g \, l^{-1}$ (Charlou et al., 2003) and anoxia. In the Anaximander area, only scattered *Lamellibrachia* sp. were observed at the top of the Kula mud volcano. Such contrast in the chemosynthetic fauna associated to the different mud volcanoes can be explained by an asynchronous activity of the volcanoes of the same field. Moreover, it appears that high salinity brine lakes prevent biological colonization on some volcanoes. Along the steep scarps of the Faulted Ridge, *Lamellibrachia* sp. along fractures and mytilids were observed, as well as bivalve shell patches at the top of cliffs.

4.4.1. Napoli Mud volcano
The Napoli Mud volcano is a circular dome lying at 1950-m depth in a trough. It is about 200 m high (Fig. 5a) with a roughly circular almost flat summit of about 1100 m in diameter. A few to several hundred square meters brine lakes with up to $83 \, g \, salt \, l^{-1}$ (Charlou et al., 2003) were observed

(a)

(b)

Rough area with debris

(c)

on the summit plateau (Fig. 5a). The bivalve shells and sparse *Lamellibrachia* sp. were mostly observed in an area of 700 m × 900 m on the western part of the summit, including the brine lake zone. In the "inner zone", active seeps with crusts and live *Lamellibrachia* sp. are the most developed (Fig. 5a) while the "outer zone" appeared to be an area of previous activity with large bivalve cemeteries and mostly dead *Lamellibrachia* sp. There was obviously no fauna inside the brine lakes except a few dead fishes and shrimps. Nevertheless bivalve shell clusters associated with crusts and dense polychaete aggregations, and also dead urchins, were observed very close to the lakes or on islands in the middle of them. Several large sponges *Rhizaxinella pyrifera* were observed with bivalves in the outer active area.

Scattered bivalve shells were observed in abundance (fields from 7 to 80 m^2, 6% of the active area) as well as denser fields (3% of the active area) especially in the inner area (Fig. 5a, Table 4). High-density bivalve shell clusters of less than 1 m^2 were very numerous. The total area covered by the bivalve shells is about 2500 m^2 or 10.5% of the active area (see Fig. 5a). The mean density of *Lamellibrachia* sp., estimated from video enumeration, is 0.025 ind m^{-2} in the active area and generally less than 1 ind m^{-2} along the dive tracks (mean density on 50 m^2). *Lamellibrachia* sp. was observed isolated or in small groups of most often less than 10 individuals. The density of *Lamellibrachia* sp. along the dive tracks is generally less than 1 individual per m^2 (mean density on 50 m^2, Fig. 5a). Most are concentrated in the west part of the active area but seem to avoid the proximity of brines. However, the value of the variance–mean ratio ($\sigma^2/\mu = 0.24$) indicates a regular distribution of *Lamellibrachia* sp. along the dive tracks. The motile fauna (shrimps, fishes and crabs) was relatively abundant in the whole surveyed area (Table 4) and not significantly more abundant in the inner zone. Nevertheless, a small white brachyuran crab was frequently seen at the active sites, while *Chaceon mediterraneus* presented a more regular distribution in the active area.

4.4.2. Amsterdam mud volcano

Amsterdam is a large 3 km across mud volcano with a very rough morphology with depressions and abrupt scarps, particularly in the central top (2040 m depth), due to an erupted mud flow over 300 m thick. The substratum is characterized by the presence of a lot of carbonate crusts and by muddy areas. Bivalve shells and *Lamellibrachia* sp. were observed along all the dive tracks (3200 m from West to East and 1000 m Northward), except along the northern flank. However their distribution is not uniform and spots of higher activity were observed at the western and at the eastern ends (stars on Fig. 5b). The most active sites show very dense groups of the *Lamellibrachia* sp., dense bivalve aggregations and living sea urchins. The central part is a very rough area probably formed by freshly expelled mud, with debris and scattered shells and *Lamellibrachia* sp.

Areas of scattered bivalve shells covering very large areas, up to 200 m^2, and represents more than 20% of the surveyed area (Fig. 5b, Table 4). On the other hand high-density shell fields were not abundant (only 0.3% of the active area) and clusters are numerous but fewer compared to Napoli (108, 0.2% of active area). *Lamellibrachia* sp. was very abundant with more than 75% arranged in dense clusters of several hundreds of individuals, and less than 6% observed in low density aggregations or isolated (Fig. 5b). Variance by mean ratio ($\sigma^2/\mu = 35.4$) indicates a patchy distribution. The estimated mean density is 0.19 ind m^{-2} in the active area. Considering only scattered individuals or in small clusters of less than 100 individuals, this density is 0.04 ind m^{-2}. Motile fauna showed densities from 1.2×10^{-3} ind m^{-2} for shrimps to 2.5×10^{-3} ind m^{-2} for fishes. Crabs were mostly observed in active areas with crusts and *Lamellibrachia* sp.

Fig. 5. Distribution of symbiotic species on the mud volcanoes along the Nautile dive tracks: mean density of *Lamellibrachia* sp. on 50 m^2 sampled areas, different size of lucinid bivalve shells aggregations (bivalve shell clusters and fields and scattered bivalve shells), abundance of sponge *R. pyrifera* (Napoli m.v.) and *Echinus* sp. (Amsterdam m.v.). (a) Napoli m.v., (b) Amsterdam m.v., (c) Kazan m.v.

Table 4
Areas covered by bivalve shells and mean density of *Lamellibrachia* sp. and mobile species in the active areas of the 4 studied volcanoes

	Olimpi area		Anaximander area	
	Napoli	Milano	Amsterdam	Kazan
Surveyed area (m^2)	43,200	14,700	72,700	43,000
Active surveyed area	24,100	14,700	72,700	20,100
Bivalves				
Areas covered by shells				
total (m^2)	2529		15,156	7752
% Of active area				
Clusters (%)	0.92		0.23	0.98
Fields (%)	3.21		0.29	4.7
Scattered (%)	6.36		20.33	32.88
Total (%)	10.5		20.85	38.57
Lamellibrachia sp.				
Abundance (ind.)	657	711	14013	8904
Density (ind/m^2)	0.025	0.048	0.19	0.44
Shrimp density (ind./m^2)	1.53×10^{-3}		1.18×10^{-3}	0.78×10^{-3}
Fish density	3.17×10^{-3}		2.49×10^{-3}	1.48×10^{-3}
Crab density	1.02×10^{-3}		1.68×10^{-3}	0.49×10^{-3}
Echinid density	Dead		0.89×10^{-3}	1.55×10^{-3}

4.4.3. Kazan mud volcano

The Kazan mud volcano has a diameter of over 2 km, and a high of 40–50 m with a steep slope southward and a gentle slope to the north. It is located at 1750 m depth on the edge of a plateau in an area of rough topography. The two dives explored the central area of the mud volcano and on both the south and north flanks (Fig. 5c). Two areas of activity were observed, separated by a gap about 600 m long with bare sediment. A small center of activity (a few tens of square meters), with bivalves and *Lamellibrachia* sp., was observed along the south flank. A larger area, about 500 m long, located north of the summit, is colonized by dense *Lamellibrachia* sp. bushes and a lot of bivalves, and showed large carbonate crusts. High ridges located at the Northwest of the summit were covered by white patches suggesting bacterial activity in this area, where a few scattered bivalve shells and *Lamellibrachia* sp. were observed.

The area covered by bivalve shells is more than 25% of the area surveyed and 38% of the active area (Table 4), defined by the occurrence of *Lamellibrachia* sp. Scattered bivalve fields were very abundant (33% of the active area) and

extended a few hundreds of meters southward and eastward of the main activity zone. Dense bivalve fields and bivalve clusters were concentrated in the most active area, characterized by the abundance of siboginids (Fig. 5c, Table 4). In these fields and clusters a lot of living individuals were sampled. *Lamellibrachia* sp. were numerous and mainly concentrated in dense clusters of several hundreds of individuals, at two closely located sites that comprised 90% of the observed individuals. This is evident in the variance–mean ratio ($\sigma^2/\mu = 67.9$) that indicates a patchy distribution. The mean density in the total surveyed active area is 0.44 and 0.04 ind m^{-2} considering only scattered individuals or small-sized clusters. Motile fauna is less abundant than on the two other volcanoes (from 0.5 ind m^{-2} for crabs to 1.5 ind m^{-2} for fishes). Crabs and sea urchins were associated with *Lamellibrachia* sp. around the few large crusts.

4.5. Comparison of the different mud volcanoes

The chemosynthetic communities showed common features on the mud volcanoes studied south of Crete and South of Turkey with very large

accumulation of bivalve shells, occurrence of small bivalves more or less buried in the sediment, abundance of lucinids, *Lamellibrachia* sp. in bushes or isolated, and carbonate crusts. This activity linked to fluid expulsion was concentrated at or near the summit of the volcanoes, on areas of several hundreds of meter long and up to a few kilometers on Amsterdam. The distribution of the fauna showed spatial variability inside these active areas with spots of higher density, particularly on Amsterdam and Kazan corresponding to eruption centers of limited spatial extent, sometime on parasitic cones, and areas of more scattered or absent fauna. On Napoli, an outer area with mostly dead animals was defined, surrounding the inner zone more active. The instability due to brines (on Napoli) or to fresh mud flows (on Amsterdam) clearly influences this distribution and precludes the development of the seep community at some places on the volcanoes, as observed in the central conduits of diatrem-type volcanoes (Olu et al., 1997). These spatial patterns may probably vary during time as brines and mud flows are likely episodic phenomenon (MEDINAUT/MEDINETH, 2000). The absence or rarity of seep communities on the other surveyed volcanoes or brines in this region may be due to similar unstable environments on such structures.

Despite the difficulty to estimate the real extent of the bivalve populations, mainly composed by small partially buried individuals, the quantitative distribution of shells and *Lamellibrachia* sp. can be compared between the studied volcanoes. The coverage by bivalve shells is higher in the Anaximander area (Amsterdam and Kazan) than in the Olimpi area, with percentage in the active area about twice higher on Amsterdam (20.8%) and nearly 4 times higher on Kazan (38.6%) compared to Napoli (10.5%). The largest continuous areas of scattered shells were encountered on Amsterdam (up to 200 m^2) whereas denser bivalve shell fields and clusters are twice as abundant (in number of clusters) on Napoli and Kazan compared to Amsterdam. The distribution of shells appears more homogeneous on Amsterdam than on Napoli and Kazan, while the variance–mean ratios indicate that the distribution of *Lamellibrachia* sp. was regular on Napoli while it was

patchy on both Amsterdam and Kazan. These differences observed in the spatial distribution the present-day or past chemosynthetic activity may reflect different fluid emission patterns. According to MEDINAUT/MEDINETH (2000), general degassing probably occurs throughout fresh mud flows initially, linked to continuous or episodic slow mud motion inside the volcanoes. This can explain the largest area of continuous bivalve shells observed on Amsterdam, while the high biological activity spots are probably supplied by specific vents, that developing later where pathways have formed through the flows. These focused seeps are likely fed to deeper and less ephemeral sources. On Napoli, the numerous high density clusters are probably related to focused vents from small source holes linked to brine pools by shallow channels. Parasitic cones as centers of mud eruption on Kazan and Amsterdam also correspond to high chemosynthetic activity areas.

The mean density of *Lamellibrachia* sp. in the active area is one order of magnitude lower on Napoli (0.025 ind m^{-2}) than on Amsterdam (0.19 ind m^{-2}) and Kazan (0.44 ind m^{-2}). The mean densities were significantly different between Napoli and Amsterdam or Kazan and between Amsterdam and Kazan at the 95% confidence level (Kolmogorov–Smirnov test, approximate *P*-value = 0.0). The density of scattered *Lamellibrachia* sp. (without considering dense bushes with more than 100 individuals) is still higher on Amsterdam (0.043 ind m^{-2}) and Kazan (0.039 ind m^{-2}) than on Napoli (0.02 ind m^{-2}). Moreover, part of isolated *Lamellibrachia* sp. (in the "outer zone") appeared to be dead on Napoli. Although the seep siboglinids may be long-lived (Bergquist et al., 2000), these results suggest that current chemosynthetic activity is higher on both Amsterdam and Kazan than on Napoli. This hypothesis is supported by the sampling of young *Lamellibrachia* sp. in the Anaximander area and not on the Napoli m.v. As shown on the histograms of the number of *Lamellibrachia* sp. observed along the dive tracks (Fig. 6), aggregations of several tens or hundreds of individuals are frequent on Amsterdam and a few were observed on Kazan. They are in contrast rare on Napoli where *Lamellibrachia* sp. were observed isolated or in small groups of
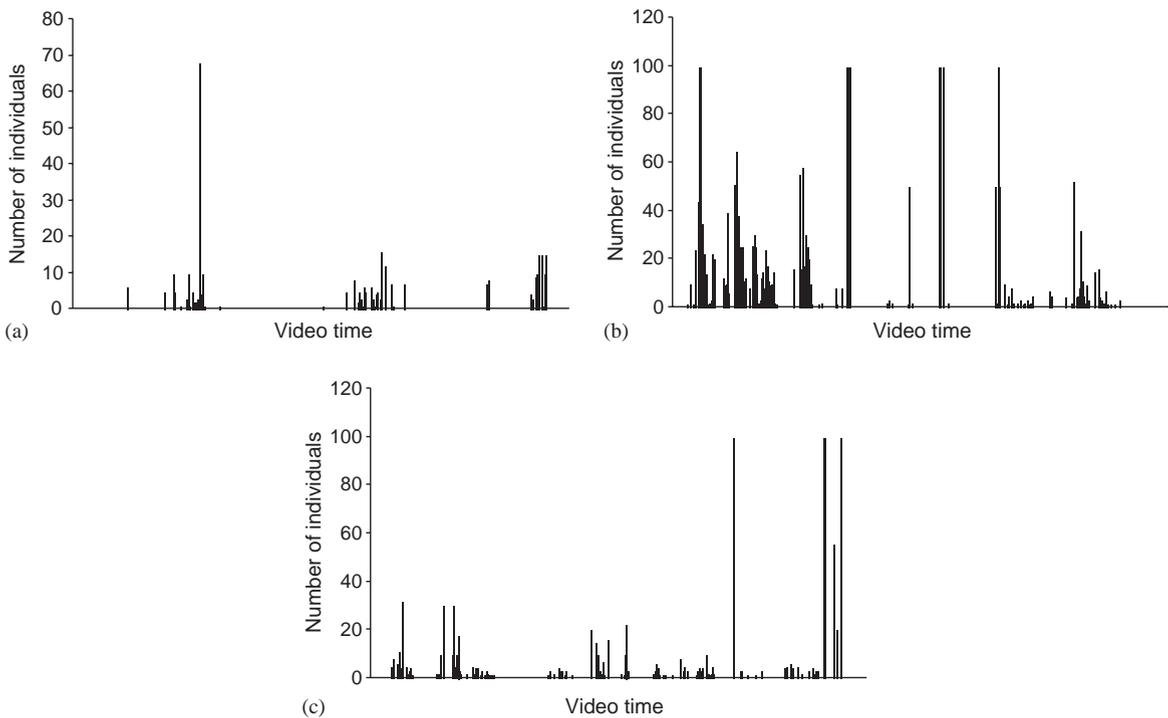
Fig. 6. Distribution of the number of *Lamellibrachia* sp. along the dive tracks issued from video processing. The time scale is divided in 30 s intervals. The abundance of more than 100 individuals include values up to 2000 individuals: (a) Napoli m.v., (b) Amsterdam m.v., (c) Kazan m.v.

less than 10 individuals. Although sampling effort seems insufficient to give valuable comparison of species richness, it can be noticed that five species of bivalves were sampled alive on Kazan; four of them (including three living) were sampled on Amsterdam, while *I. modiolaeformis* is the sole bivalve collected alive on Napoli. Nevertheless, greater sampling effort will be required to test this hypothesis, particularly for bivalves and gastropods.

The differences observed in the biological activity can be related to variability of fluid expulsion intensity between the volcanoes. The methane concentration measured a few meters above the sea floor is, except just above the brine lakes, about ten times lower on Napoli than on Amsterdam (Charlou et al., 2003), which is the same order of magnitude than the difference in *Lamellibrachia* sp. density between both volcanoes. According to the same authors, nephelometre profiles indicated

highly turbid fluid above Amsterdam. This is consistent with the occurrence of fresh mud flows on Amsterdam while large carbonate crusts were most abundant on Napoli, suggesting recent fluid expulsion on Amsterdam and older on Napoli (T. Zitter, pers. com.). The spatial variability of methane degassing at the volcano scale could also explain the distribution of the chemosynthetic fauna: on Napoli, the inner zone had methane concentration of 100–200 nmols kg^{-1} while a maximum concentration of 10 nmols kg^{-1} was measured in the outer zone. Likewise on Kazan the same difference of one order of magnitude was observed between the active area and outside. The Napoli brine lakes are characterized by very high methane degassing (up to 770 nmols kg^{-1}). As such high concentrations have been measured on Amsterdam, the high salinity of brines is likely the limiting factor for fauna settlement, instead of high methane concentrations. Nevertheless MacDonald

et al. (1990b, 2000) observed abundant chemosynthetic communities near brine lakes. Methane expulsion may be concentrated in the brines, and not sufficiently spread around them to sustain current chemosynthetic activity on Napoli. Other species, like sea-urchins, dead on Napoli and living on Amsterdam and Kazan may indicate higher activity in the past than at present on Napoli. The presence of anoxic brine lakes may not favor the maintenance of chemosynthetic macrofauna communities. The tubicolous polychaetes present in high density in the whole active area on Napoli may either profit from organic enrichment or use free living bacteria (bacterial mats are abundant around the brine lakes), as suggested at Japan seeps by Juniper and Sibuet (1990). Mobile fauna is about as abundant on the three volcanoes, although a little more dense for shrimps and fishes on Napoli.

5. Conclusion

The benthic community linked to methane-rich fluid expulsion on mud volcanoes of the eastern Mediterranean includes a lot of symbiotic macrofauna and megafauna species (at least 7 species assumed or proved to harbor symbionts) which stands high compared to similar communities at similar depths in other oceans (Sibuet and Olu-Le Roy, 2002). This seems to result from the occurrence of neo-endemic species favored by sufficient long duration of seepages, together with a less specialized fauna, although including symbiotic species, adapted to organic rich environments. The symbiotic fauna shared some families (Mytilidae, Vesicomyidae, Lamellibrachiidae,…), but only a few genera, with Atlantic and Pacific oceans cold seep communities, and contrasts by the small size of the bivalves. Comparison of chemosynthetic communities of volcanoes in two areas of the eastern Mediterranean evidences higher activity in the Anaximander area (Amsterdam and Kazan mud volcanoes) than in the Olimpi area (Napoli volcano), which can be explained by lower fluid flow at present (Charlou et al., 2003) and maybe instability due to brines on the Napoli mud volcano.

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