

Biodiversity of Cold Seep Ecosystems Along the European Margins

BY ANN VANREUSEL, ANN C. ANDERSEN, ANTJE BOETIUS,
DOUGLAS CONNELLY, MARINA R. CUNHA, CAROLE DECKER,
ANA HILARIO, KONSTANTINOS A. KORMAS,
LOÏS MAIGNIEN, KARINE OLU, MARIA PACHIADAKI, BENEDICTE RITT,
CLARA RODRIGUES, JOZÉE SARRAZIN, PAUL TYLER,
SASKIA VAN GAEVER, AND HELEEN VANNESTE

ROV *Victor 6000* taking a water sample above a bush of
Siboglinidae polychaetes in the central Nile Deep Sea Fan.
Image courtesy of Ifremer/MEDECO Cruise 2007

ABSTRACT. During the European Commission's Framework Six Programme, HERMES, we investigated three main areas along the European margin, each characterized by the presence of seep-related structures exhibiting different intensity of activity and biological diversity. These areas are: (1) the Nordic margin with the Håkon Mosby mud volcano and many pockmarks, (2) the Gulf of Cádiz, and (3) the eastern Mediterranean with its hundreds of mud volcanoes and brine pool structures. One of the main goals of the HERMES project was to unravel the biodiversity associated with these seep-associated ecosystems, and to understand their driving forces and functions, using an integrated approach. Several multidisciplinary research cruises to these three areas provided evidence of high variability in ecosystem processes and associated biodiversity at different spatial scales, illustrating the "hotspot" nature of these deep water systems.

INTRODUCTION

Soon after the discovery of the spectacular hydrothermal vent communities 30 years ago, other types of chemosynthetic assemblages—so-called "cold seeps"—were found along continental margins during submersible dives to the deep Gulf of Mexico (Paull et al., 1984), subduction zones off Oregon in the eastern Pacific (Suess et al., 1985), and trenches off Japan in the western Pacific (Juniper and Sibuet, 1987). Cold seeps are now among the most geologically diverse and widely distributed of the deep-sea reducing environments explored to date, and new sites are still being discovered every year. Since their initial discovery, active seeps have been reported from shallow to hadal (> 6000-m) depths (Sibuet and Olu-Le Roy, 2002; Levin, 2005, and references therein), along other active and passive margins, and from all parts of the global ocean, even Antarctic regions (Domack et al., 2005). It is only during the last decade that more intense

observation of the European continental margins using in situ video and photography with adapted deep submersibles provided evidence for a wide range of active cold-seep ecosystems associated with fluid, gas, and mud escape structures. These structures include pockmarks (seafloor depressions), brine lakes, and elevated or flat mud volcanoes.

As with hot vents, cold seeps are characterized by the flow of reduced chemical compounds from the subsurface to the seafloor, but they are not directly associated with high thermal anomalies. Most known cold seeps are associated with reduced environments that are linked indirectly to gas hydrates or to hydrocarbon reservoirs. Hence, in contrast to the majority of marine deep-water ecosystems, which depend on photosynthetically derived food (phytoplankton and plant material), methane and other hydrocarbon seeps are colonized by specific anaerobic subsurface microbiota; these organisms use hydrocarbons as an energy source

(Sloan, 1990) to transform seawater sulfate, thus producing high fluxes of hydrogen sulfide (Jorgensen and Boetius, 2007). Chemosynthetic microorganisms are the primary producers in seep food webs, depending on such reduced chemicals as methane and sulfide as their energy sources.

Similar to their hydrothermal vent counterparts, most cold seeps support highly productive ecosystems (high biomass) that consist of specialized metazoan communities dominated by a few adapted taxa that can cope with elevated concentrations of chemical compounds and low oxygen levels at and below the sediment-water interface. Other harsh conditions, such as high concentrations of hydrocarbons or high-salinity brines, may locally reduce faunal diversity (MacDonald et al., 2004; Bergquist et al., 2005). Among the most remarkable of the fauna exploiting the abundant chemical energy of seeps are the symbiont-bearing invertebrate species, often similar or related to the fauna

found at hydrothermal vents. These large taxa, such as vesicomysids (clams), bathymodiolid (mussels), and siboglinids (formerly known as Pogonophora or tube worms), and some motile species such as shrimps and gastropods, cluster in areas where fluids rich in reduced chemicals reach the seafloor (Sibuet and Olu, 1998; Sibuet and Olu-LeRoy, 2002; Bergquist et al., 2003; Van Dover et al., 2003; Cordes et al., 2007).

During HERMES, three main areas harboring prominent seep ecosystems were investigated, including the Nordic margin and its Håkon Mosby mud volcano, the Gulf of Cádiz, and the eastern Mediterranean. After a short introduction of the three main study areas, which are more extensively discussed elsewhere (see Foucher et al., this issue), we provide an overview of the main results from biodiversity studies performed during HERMES. An integrated approach combined detailed habitat mapping and characterization of associated fauna.

Both symbiont-bearing and associated nonsymbiotic fauna were investigated, as well as different size classes, from fish and large invertebrates (megafauna), to small endofaunal organisms (meio- and macrofauna), including the very specific seep-related microbial communities. In addition to biodiversity patterns in relation to the high habitat heterogeneity within a region, similarities in communities among regions are currently under investigation in order to gain better insight into the biology, biodiversity, and biogeography of seep-associated biota along Europe's continental margins.

HERMES COLD SEEP STUDY SITES

Along the Nordic margin, the highly active Håkon Mosby mud volcano (72°N) at 1280-m water depth on the Barents Sea slope south of Svalbard, was the target of several multidisciplinary cruises (Figures 1A, 2). The Storegga slide at 64°N and associated Nyegga

pockmarks were also visited (Figure 1A). Håkon Mosby mud volcano was first observed in 1989 during a side-scan sonar survey (Vogt et al., 1997). An expedition in 1995 measured very high temperature gradients in the sediments, recovered methane hydrate from 2-m subbottom depth and sampled siboglinid polychaetes, suggesting active chemosynthesis (Vogt et al., 1997). The concentric structure of the mud volcano can be divided into several subhabitats characterized by different biogeochemical sediment conditions (de Beer et al., 2006; Niemann et al., 2006b).

The Storegga area is well known for its giant Holocene slide, one of the largest ever mapped on continental margins (Paull et al., 2008). On the northeastern flank of the Storegga slide, complex pockmarks are located in the so-called Nyegga area at 740-m water depth. These pockmarks are circular in plan view and feature up to 190-m-long ridges of carbonate rock (Hovland et al., 2005).

Ann Vanreusel (ann.vanreusel@ugent.be) is Professor, Marine Biology Research Group, Universiteit Gent, Gent, Belgium. **Ann C. Andersen** is Professor, Université Pierre et Marie Curie (UPMC), and a researcher in Equipe Ecophysiologie: Adaptation et Evolution Moléculaires, UMR 7144 - Centre national de la recherche scientifique (CNRS) - UPMC, Station Biologique, Roscoff, France. **Antje Boetius** is Head, Microbial Habitat Group, Max Planck Institute for Marine Microbiology, and Professor, Jacobs University Bremen, Germany. **Douglas Connelly** is a researcher in the Geology and Geophysics Group, National Oceanography Centre, University of Southampton, Southampton, UK. **Marina R. Cunha** is Professor, Centro de Estudos do Ambiente e do Mar (CESAM), Departamento de Biologia, Universidade de Aveiro, Campus Universitário de Santiago, Aveiro, Portugal. **Carole Decker** is PhD Candidate, Deep-Sea Ecosystem Department, Institut français de recherche pour l'exploitation de la mer (Ifremer), Centre de Brest, Plouzané, France. **Ana Hilario** is a postdoctoral researcher at CESAM, Departamento de Biologia, Universidade de Aveiro, Campus Universitário de Santiago, Aveiro, Portugal. **Konstantinos Ar. Kormas** is Assistant Professor, Department of Ichthyology and Aquatic Environment, University of Thessaly, Nea Ionia, Greece. **Loïs Maignien** is PhD Candidate, Laboratory of Microbial Ecology and Technology (LabMET) and Renard Center for Marine Geology (RCMG), Universiteit Gent, Gent, Belgium. **Karine Olu** is a researcher in the Deep-Sea Ecosystem Department, Ifremer, Centre de Brest, Plouzané, France. **Maria Pachiadaki** is PhD Candidate, Department of Chemistry, University of Crete, Heraklion, Greece. **Benedicte Ritt** is PhD Candidate, Deep-Sea Ecosystem Department, Ifremer, Centre de Brest, Plouzané, France. **Clara Rodrigues** is PhD Candidate, CESAM, Departamento de Biologia, Universidade de Aveiro, Campus Universitário de Santiago, Aveiro, Portugal. **Jozée Sarrazin** is a researcher in the Deep-Sea Ecosystem Department, Ifremer, Centre de Brest, Plouzané, France. **Paul Tyler** is Professor, National Oceanography Centre, University of Southampton, Southampton, UK. **Saskia Van Gaever** is a postdoctoral researcher in the Marine Biology Research Group, Universiteit Gent, Gent, Belgium. **Heleen Vanneste** is PhD Candidate, School of Ocean and Earth Science, University of Southampton, UK.

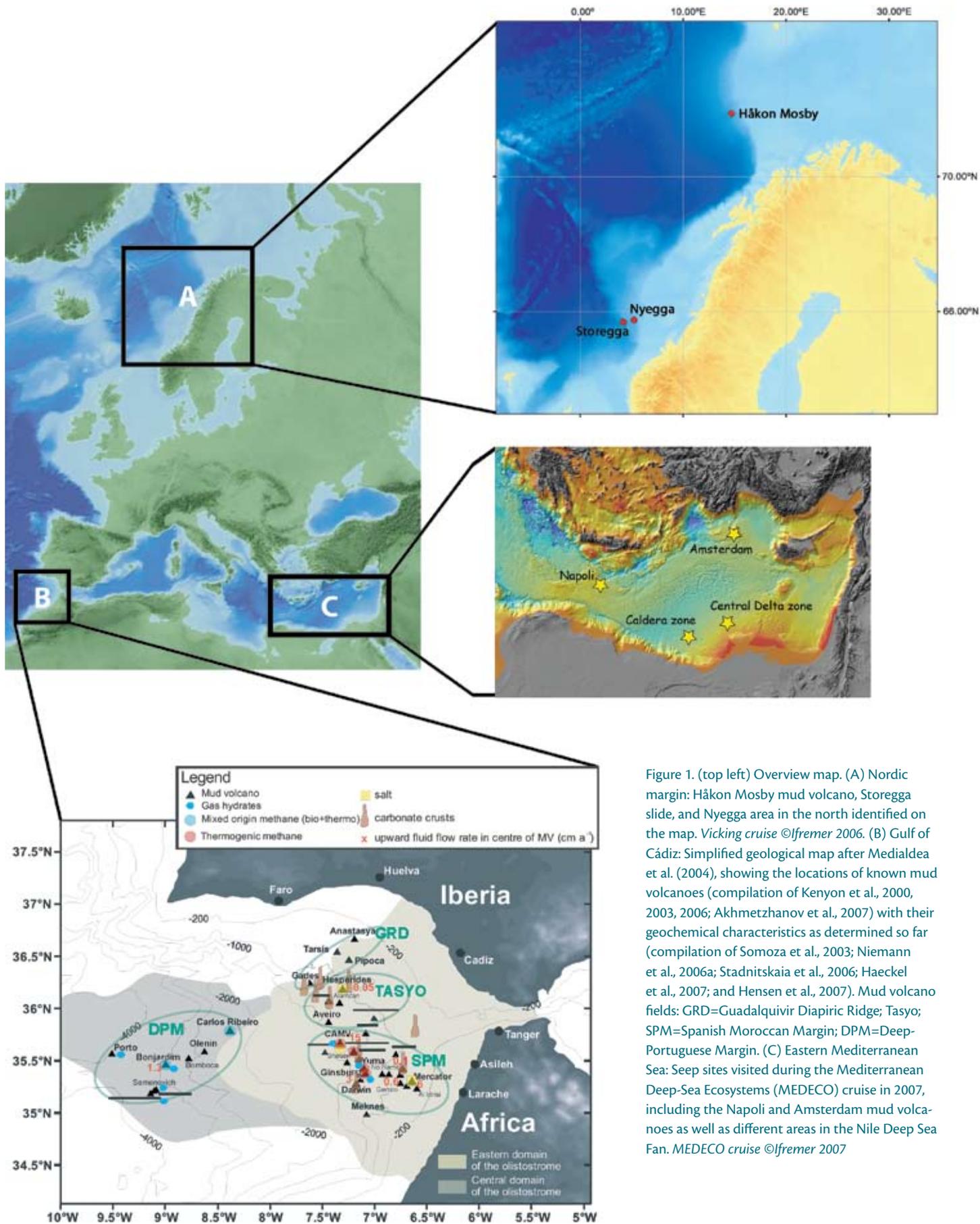


Figure 1. (top left) Overview map. (A) Nordic margin: Håkon Mosby mud volcano, Storegga slide, and Nyegga area in the north identified on the map. *Vicking cruise* ©Ifremer 2006. (B) Gulf of Cádiz: Simplified geological map after Medialdea et al. (2004), showing the locations of known mud volcanoes (compilation of Kenyon et al., 2000, 2003, 2006; Akhmetzhanov et al., 2007) with their geochemical characteristics as determined so far (compilation of Somoza et al., 2003; Niemann et al., 2006a; Stadnitskaia et al., 2006; Haeckel et al., 2007; and Hensen et al., 2007). Mud volcano fields: GRD=Guadalquivir Diapiric Ridge; Tasyo; SPM=Spanish Moroccan Margin; DPM=Deep-Portuguese Margin. (C) Eastern Mediterranean Sea: Seep sites visited during the Mediterranean Deep-Sea Ecosystems (MEDECO) cruise in 2007, including the Napoli and Amsterdam mud volcanoes as well as different areas in the Nile Deep Sea Fan. *MEDECO cruise* ©Ifremer 2007

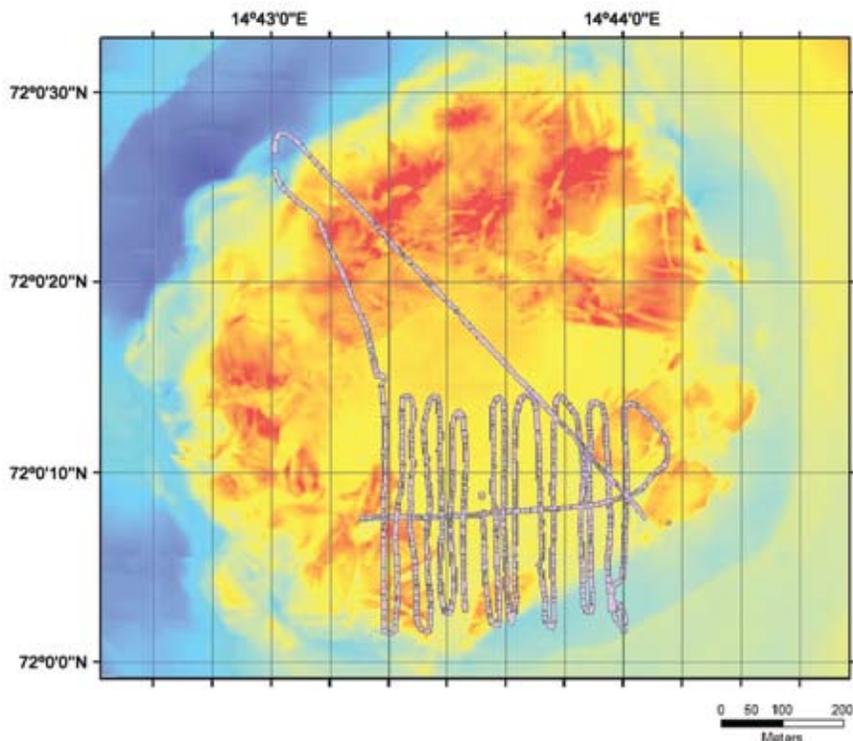


Figure 2. OTUS image of the Håkon Mosby mud volcano in the north. Area covered by optical survey (OTUS camera), representing 30% of a 400 x 400-m area. Footprints of each photo are georeferenced. *Vicking cruise ©Jfremer 2006*

The Gulf of Cádiz is located between Iberia and Africa on the Atlantic side, between 9°W and 6°45'W, and 34°N and 37°15'N. The hydrography of the study area is complex, with the influence of Mediterranean outflow water on the shallower eastern mud volcanoes, and evidence for input of high-nutrient Antarctic Intermediate Water in the deeper western regions (Van Aken, 2000). The area has a complex tectonic history and is now dominated by thick sedimentary deposits. Since their initial discovery in the area in 1999 (Kenyon et al., 2000), a large number of mud volcanoes have been identified, located in four main fields and exhibiting different but generally very localized hydrocarbon seepage (Niemann et al., 2006b; Figure 1B). The presence of carbonate chimneys indicates past activity. At most

of them, the majority of the methane is consumed within the sediments, and does not reach the hydrosphere.

Different seep sites are also present in the eastern Mediterranean Sea (Figure 1C), where intense emission of methane occurs from the center of active mud volcanoes and along related faults (MEDINAUT/MEDINETH 2000, Charlou et al., 2003; Dupré et al., 2007). During the last decade, three major areas were the focus of multidisciplinary cruises using submersibles: the Olimpi mud volcano field, located on the Mediterranean Ridge south of Crete (Masclé et al., 1999); the Anaximander Mountains, south of Turkey, caught up in the plate convergence between Africa and Eurasia (Woodside et al., 1998); and the seafloor of the Nile Deep Sea Fan (Nile delta turbidic system; Loncke et al.,

2004). The Olimpi mud volcano field and Anaximander Mountain areas, located at depths between 1700 m and 2000 m, are characterized by strong compressional tectonic processes superimposed by faulting. They harbor large conical mud volcanoes several kilometers across but only a few hundred meters high. Fresh mud flows, brines, clasts, and carbonate crusts were observed on their surface, depending on the current activity of the volcano (Zitter et al., 2005). In a passive margin context, the Nile deep turbidic system displays many fluid-releasing structures on the seafloor, including mud volcanoes, mud pies, and pockmarks (Loncke et al., 2004).

MAPPING HABITAT HETEROGENEITY AT COLD SEEPS

Cold seeps are characterized by the patchy occurrence of sulfide and/or methane-dependent biota, including microbial mats and symbiont-bearing invertebrates (Bivalvia, Polychaeta) that can form small clusters or spread over large fields in high densities. This high spatial variability at scales of tens to hundreds of meters has been attributed to the magnitude of fluid flow and the correlated chemical depth profiles (Henry et al., 1992; Barry et al., 1997; Olu et al., 1997; Sahling et al., 2002; Levin et al., 2003; de Beer et al., 2006). Low net flow rates appear to provide sufficient methane from depth to fuel the near-surface biological communities while still allowing downward transport and mixing of oxygen- and sulfate-rich seawater in the upper few centimeters of the sediment (de Beer et al., 2006). More intense fluid flow is associated with altered pore-water composition and elevated sulfide

concentrations extending to the sediment surface, thus allowing the growth of microbial mats (Tryon and Brown, 2001; Levin et al., 2003).

To understand the spatial and temporal scales at which seep ecosystem processes change, a crucial initial phase in seep research is mapping the size and distribution of different habitats and identifying the associated communities (Sibuet and Olu-Le Roy, 2002). A great step forward in the precision of habitat mapping has been achieved in the last few years with use of remotely operated vehicles (ROVs), which allows regular transects over long distances in combination with more precise positioning methods. There is also rapid progress in optical camera resolution and data processing using Geographic Information System (GIS)-supported image analysis. On the Håkon Mosby mud volcano, the concentric distribution of habitats around a central crater, apparently not colonized, was first described from towed video systems and observations by submersibles (Milkov et al., 1999; Gebruk et al., 2003).

The first predictive habitat map was based on ROV video mosaics processed by Jerosch et al. (2007) using geostatistical analysis. These authors estimated the percent coverage for each targeted habitat: mud apparently devoid of life in the center, surrounded by areas densely inhabited by microbial mats, particularly in the south; and hummocky outer parts colonized dominantly by siboglinids. During the HERMES Vicking cruise (2006), a new habitat mapping survey was conducted (Figure 2) by means of parallel transects at 8-m altitude above the seafloor, using the black and white high sensibility camera OTUS mounted

on the ROV *Victor 6000* survey module. At this altitude, each picture covers a surface of ~ 64 m² (Figure 3A, B). The new habitat map suggests changes in the colonization of mud flows by microbial mats and siboglinids between 2003 (Jerosch et al., 2007) and 2006 (recent work of authors Olu, Fabri, Deep-Sea Ecosystem Department of Ifremer, and others). A similar spatial organization of habitats (central seep area surrounded by microbial mats and siboglinid fields in the external ring) was also observed at small individual pockmarks along the Storegga slide and in the Nyegga area, but at a much smaller scale (decimeter to meter) (recent work of author Olu).

In 2007, the MEDECO cruise aboard RV *Pourquoi pas?* visited several different seep sites in the eastern Mediterranean, four of which were the focus of systematic ecological studies at different spatial scales: the Napoli mud volcano south of Crete, the Amsterdam mud volcano south of Turkey, and a pockmark field and the Cheops mud volcano located in the Nile delta. Five different habitats were recognized by the presence of visible features such as key megafaunal taxa (Bivalvia, Siboglinidae) or microbial mats on the seafloor. More extensive habitat and megafaunal distribution surveys on the Napoli mud volcano (33°43.7777'N, 24°40.9495'E; 1750–1934-m depth) were based on regularly spaced transects at 10-m altitude with each OTUS picture covering a surface of ~ 100 m². This survey showed that numerous brine pools characterized the southeast sector (Figure 3C), corresponding to depressions on the microbathymetric map. Small tubeworms were rarely observed. The northern part showed a more disturbed environment,

colonized by siboglinids. Abundant empty bivalve shells were lying on the seafloor, possibly indicating the extinction of previous bivalve-dominated communities (recent work of authors Olu and Sarrazin).

ENDOSYMBIONT-BEARING CHEMOSYNTHETIC FAUNA

Megafaunal species comprise organisms larger than 2 cm that are generally visible on seafloor optical images. Cold seep ecosystems provide niches for chemo-synthetic communities based on reduced compounds such as methane and sulfide, which are energy sources for CO₂-fixing symbiotic bacteria. These symbioses between invertebrates and sulfur-oxidizing and/or methanotrophic bacteria are only found in highly reduced environments, and are an obvious example of how cold seep ecosystems add biodiversity to marine deep-water life. During our HERMES research, we were able to identify the dominant chemosynthetic symbioses on Europe's continental margins, but most likely much more remain to be discovered.

At the Håkon Mosby mud volcano, megafauna are dominated by siboglinids (tubeworms) that lack both mouth and gut and live in symbiosis with sulfur-oxidizing bacteria stored inside their bodies (Lösekann et al., 2008). Wide areas in the periphery of this mud volcano are covered with the curled brownish tubes of the species *Sclerolinum contortum* (Figure 3D), buried up to 70-cm deep in soft sediment. In some areas, clusters of the straight black tubes of *Oligobranchia haakonmosbiensis webbi* (Smirnov, 2000) stand erect about 5 cm above the seafloor. Both species are also found further south on pockmarks

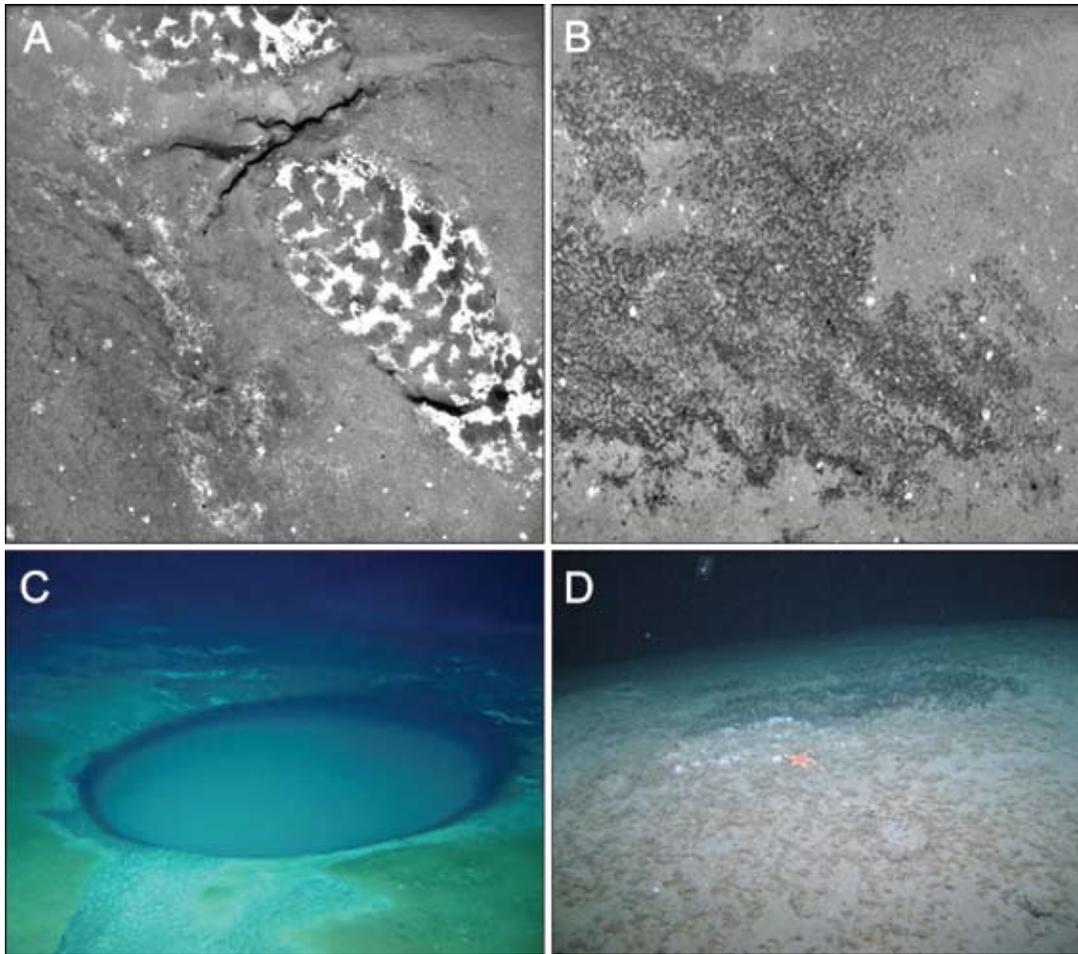


Figure 3. Example of OTUS photos showing (A) microbial mats and zoarcid fishes and (B) a patch of siboglinid polychaetes. Photo size is 64 m². *Viking cruise* ©Ifremer 2006. (C) A brine pool on the summit of the Napoli mud volcano. MEDECO 2007 ©Ifremer. (D) View of a wide field of siboglinid polychaetes at Håkon Mosby mud volcano. The curled brownish *Sclerolinum* surrounds a large spot of black, straight *Oligobrachia* tubes. An orange sea star lies in front of a *Sclerolinum* patch covered by whitish bacterial filaments. *Viking cruise* ©Ifremer 2006

at the Storegga slide and at Nyegga, where they surround every dark spot of methane seepage (recent work of author Andersen). Many small symbiont-bearing bivalves belonging to the family Thyasiridae have been sampled on these sites, especially in siboglinid fields at the Håkon Mosby mud volcano, whereas numerous larger Vesicomidae shells have also been observed at the Storegga and Nyegga pockmarks (recent work of authors Decker and Olu).

At Nyegga, 1-m-high pillow structures

covered with a carpet of siboglinids are known as “submarine pingoes;” they are described by Hovland and Svensen (2006) as local hydrate (ice) accumulations. However, during the HERMES *Viking cruise* in 2006, observation and sampling by ROV *Victor 6000* showed the pingoes to be composed of mud accumulations with entangled *Sclerolinum*, soft enough to be sampled by blade core. In all the explored areas, *Sclerolinum* seem to dominate, whereas *Oligobrachia* has a discrete, highly patchy

distribution (recent work of authors Andersen and Olu). The factors that control the spatial distribution of these two species remain unclear. The local sediment chemistry, the penetration of the worms into the seabed, and some of the worms’ physiological adaptations concerning their hemoglobins seem to differ between the two species, and may affect their habitat selection (recent work of author Andersen). However, other factors such as their reproduction and dispersal may also play a role. Filamentous

bacteria often cover their tubes and the spaces between tubes provide shelter to a highly diversified macrofauna, particularly between the twisted creeping tubes of *Sclerolinum*. *Sclerolinum* can therefore be compared to other habitat-providing species such as deep-water corals, as it harbors a great epifaunal biodiversity on the otherwise barren soft sediments of the Norwegian deep margin.

In contrast to the Håkon Mosby mud volcano, where permanently high fluxes of reduced compounds are readily indicated by the presence of large aggregations of siboglinids and bacterial mats, the mud volcanoes in the Gulf of Cádiz do not show evidence of dense aggregations of living chemosynthetic megafauna. An initial ROV transect at 1100-m depth on the Darwin mud volcano during a sampling campaign with RRS *James Cook* in 2007 revealed a mass of mytilids identified as *Bathymodiolus mauritanicus* (Figure 4A) on the top of this mud volcano. However, most of this accumulation comprised empty shells. Further along the transect, rock exposures (Figure 4B) and vast carbonate outcrops (Figure 4C) were observed with both live and dead mussels within cracks in the carbonates. These observations suggest that the Darwin mud volcano had once been very active and that the release of methane was sufficient to support a considerable population of mytilids. Cessation of seep activity probably leads to the death of the population. This event took place relatively recently as many of the shells remained intact and articulated. A small area (about 100 cm²) of soft, blue-grayish-colored sediment (Figure 4D) was observed in the northwest corner of the mud volcano that, when disturbed, released

considerable quantities of methane, but contained no obvious chemosynthetic fauna. Other megafauna, not directly chemosynthesis-dependent, consisted of stylasterine corals attached to the carbonate cap (Figure 4E), scavenging crabs (Figure 4F), and corals (Figure 4G).

Aside from the dead mytilid fields, the chemosynthetic species of the Gulf of Cádiz live mostly buried inside the sediments, a distribution that is probably related to the shallow (< 30 cm) depth of the sulfide/methane gradient. The most common species include

siboglinid polychaetes (*Siboglinum* spp.) and solemyid bivalves (*Acharax* sp., *Petrasma* sp.), but also other frenulate (*Polybrachia*, *Spirobrachia*, *Bobmarleya*, *Lamellisabella*) (Figure 5) and bivalve taxa (*Lucinoma*, *Thyasira*, *Bathymodiolus*, *Vesicomysidae*) (Génio et al., 2008; Hilário and Cunha, 2008; Rodrigues et al., 2008). The first results of stable isotope analyses ($\delta^{13}\text{C}$, $\delta^{15}\text{N}$, $\delta^{34}\text{S}$) support the occurrence of chemosynthetic production in these species, with thiotrophy (H_2S) being the main metabolic pathway in the benthic food

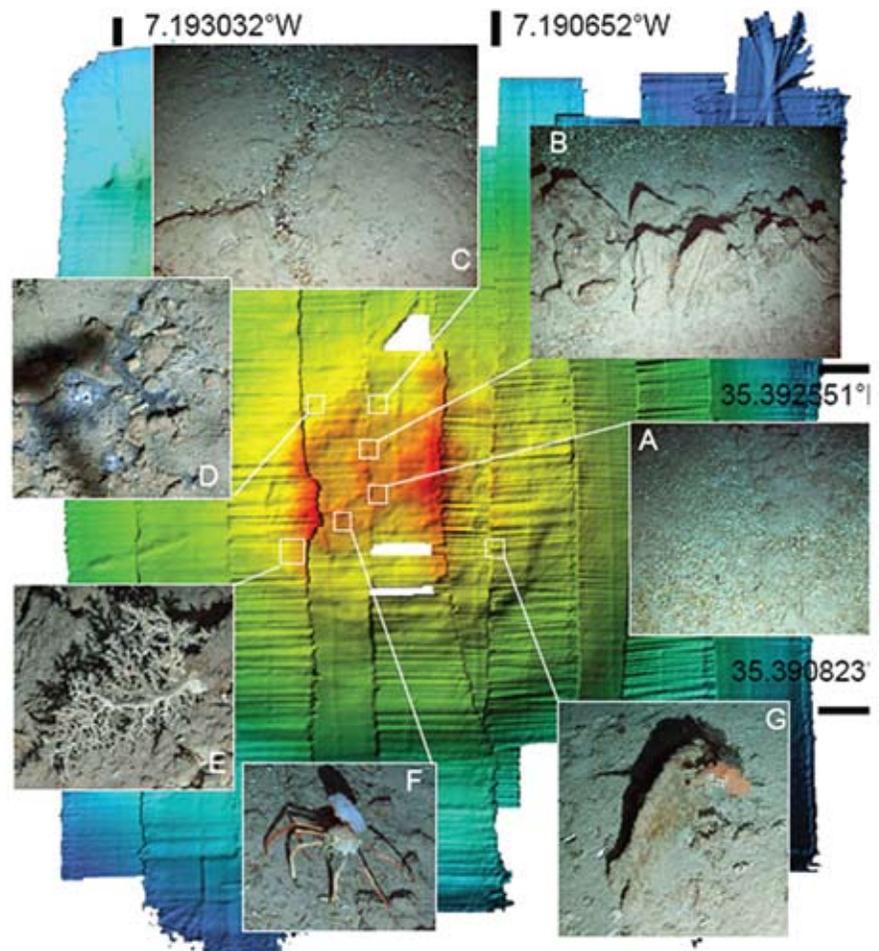


Figure 4. Bathymetry and habitats associated with the Darwin mud volcano: (A) *Bathymodiolus mauritanicus*, (B) rock exposure, (C) carbonate outcrops, (D) soft, blue-grayish-colored sediment, (E) stylasterine corals, (F) scavenging crabs, and (G) corals attached to an upturned piece of carbonate crust.

web (recent work of author Rodrigues and colleagues).

The first data on Mediterranean cold seep communities were acquired during the French-Dutch MEDINAUT cruise (1998). Chemosynthetic communities from the Olimpi and Anaximander mud fields were mostly concentrated near the summits of the volcanoes, where fluid expulsion is concentrated (Olu-Le Roy et al., 2004). The communities were dominated by small-sized bivalves from four families common to cold seep environments (Mytilidae, Vesicomidae, Thyasiridae), or to shallower sulfidic-rich habitats (Lucinidae). However, large-size bivalve genera typical of cold seeps (*Bathymodiolus* and *Calyptogena*) were absent. Siboglinids of the Obturata group (genus *Lamellibrachia*) were found nearby or in close relation to carbonate crusts (Figure 6A). Differences in the biological activity can be related to the variability and intensity of fluid expulsions between the volcanoes (Olu-Le Roy et al., 2004), as supported by variable methane concentrations measured above the seafloor (Charlou et al., 2003). Small Siboglinidae of the Frenulata group (*Siboglinum* sp.) were also collected.

During the HERMES BIONIL cruise to the Nile Deep Sea Fan in 2006, new samples from the three dominant symbiont-bearing macrofaunal species were collected. The clam *Lucinoma* aff. *kazani* was found within the sediments, the mytilid *Idas* sp. was attached to different hard substrata (crusts, tubes, urchins), and siboglinid tubeworms occurred either on reduced sediments (Amon mud volcano) or on carbonate crusts (central pockmark area). Symbiotic associations have been described for *Lucinoma* aff. *kazani* and for the small mytilid *Idas* sp., the latter harboring an exceptional number of symbionts in its gills (Duperron et al., 2006, 2007).

“NONSYMBIOTIC” MEGAFUNA

The megafauna at seeps also include many nonsymbiont-bearing species, which profit in many different ways from the large biomass and productivity of chemosynthetic megafauna. They are attracted by the heterogeneity of the habitats, the abundance of prey, or possibly to the elevated topographic position provided by mud volcanoes. At the Håkon Mosby mud volcano, the most abundant species in the megafaunal size

class is the fish of the Zoarcidae family, *Lycodes squamiventer* (Gebruk et al., 2003) (Figure 6B). Image analysis from the Vicking cruise (2006) confirmed previous observations of Gebruk et al. (2003) on the distribution of this zoarcid fish: they show the highest abundances in the most active area of the volcano, and are particularly associated with microbial mats (recent work of authors Olu and Decker). Zoarcidae is the typical fish family encountered at hydrothermal vents and cold seeps, with some endemic species likely having adapted to the toxic environment.

On the Storegga slide and in Nyegga pockmarks, nonsymbiotic megafauna are much more abundant and diverse, probably for two reasons. First, the cold seeps are much smaller compared to the Håkon Mosby mud volcano, they are sparsely distributed, and methane concentrations in seawater are quite low (J.L. Charlou, Marine Geosciences Department, Ifremer, *pers. com*, 2008). Second, the water depth is shallower (600–1000 m) and the Storegga slide is likely to be a site of high detritus input that favors the presence of suspension and deposit feeders. The very large ophiurid *Gorgonocephalus* sp. (Figure 6C), reaching up to 0.5-m diameter, is the most striking species of this background megafauna, but abundant comatules, crinoids, and pedonculate sponges were also observed.

In the Gulf of Cádiz, several nonchemosynthetic species were observed associated with different mud volcanoes at various water depths. In contrast to the shallower mud volcanoes, the Carlos Ribeiro mud volcano at 2200-m water depth has a more diverse nonchemosynthetic-dependent

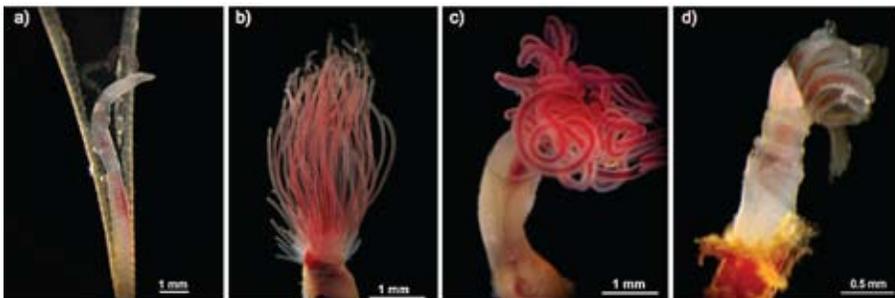


Figure 5. Frenulata collected during TTR17 cruise in the Gulf of Cádiz: (a) *Siboglinum* sp. (from Darwin mud volcano), (b) *Polybrachia* sp. 1 (from Porto mud volcano), (c) *Polybrachia* sp. 2 (from Sagres mud volcano), and (d) *Polybrachia* sp. 3 (from Soloviev mud volcano). Photos by Ana Hilário

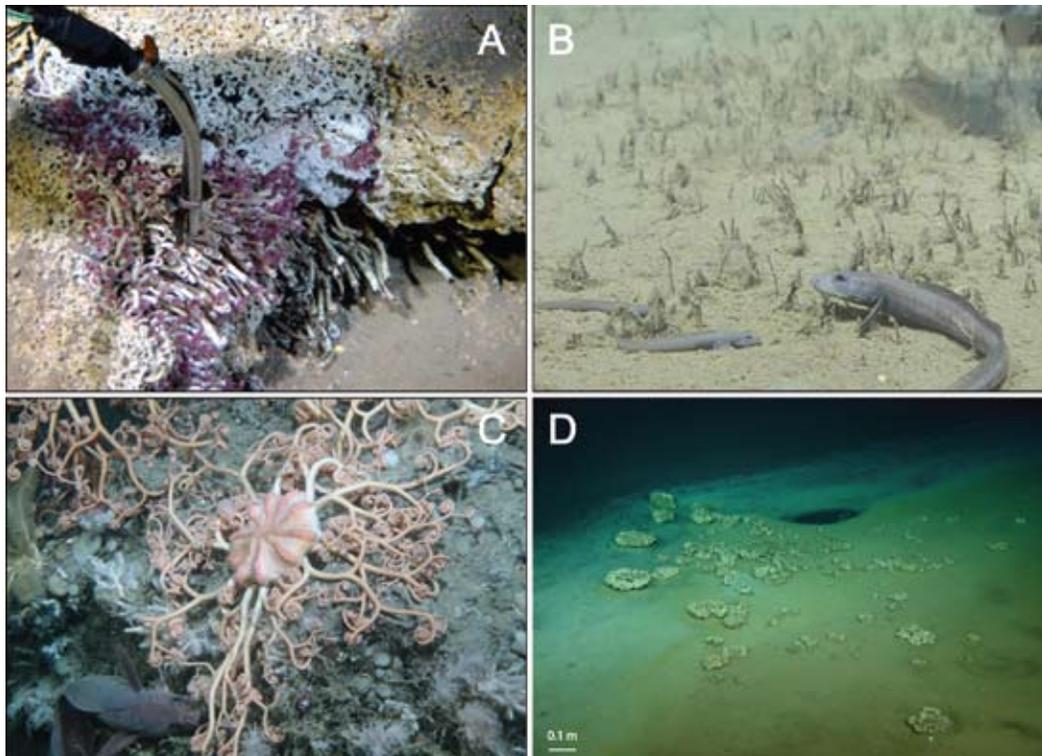


Figure 6. (A) *Lamellibrachia* from the pockmark area in the Nile Deep Sea Fan. MEDECO cruise ©Ifremer 2007. (B) *Lycodes squamiventer* (Zoarcidae) in a patch with siboglinid tubeworms at the Håkon Mosby mud volcano. ARKXXII cruise ©MARUM, University of Bremen. (C) Richness of the background megafauna on Storrega slide, with the giant ophiurid *Gorgonocephalus* sp. Vicking cruise ©Ifremer 2006. (D) Unusual large specimens of the sponge *Rhizaxinella pyrifer* on Napoli mud volcano. MEDECO cruise ©Ifremer 2007

megafauna. The mud volcano center consists of series of concentric ridges that support very few megafauna except siboglinid tubeworms (Figure 7A) and a mobile echinothurid sea urchin found close to the “eye” of the volcano (Figure 7I). Most of the more extensive megafauna comprise suspension-feeding cnidarians situated at the periphery of the mud volcano, including poriferans (Figure 6B), the sea pen *Umbellula* (Figure 7C), and dense gorgonian bushes (Figure 7F, G). Further off the mud volcano the enigmatic athecate hydroid *Monocaulus* (Figure 7D) was observed. At some time in the past, mud overflowed the volcano’s crest and slid down its southeast side, where huge numbers

of deposit-feeding holothurians were observed (Figure 7H).

In the eastern Mediterranean, polychaetes are abundant around the Napoli brine lakes and on the active sites on Amsterdam mud volcano. Other associated species include unusually large specimens of the Suberitidae poriferan *Rhizaxinella pyrifer* (Figure 6D) that was sampled on Napoli mud volcano. Crustaceans such as galatheids, shrimps, and *Chaceon mediterraneus* crabs were equally abundant at all sites. Large densities of *Echinus* sp. were observed at active sites, suggesting some sort of dependence on fluid emission (Olu-Le Roy et al. 2004; recent work of authors Ritt, Olu, and Sarrazin).

SMALL-SIZED ENDOFAUNA

Since their discovery, much seep research has focused on the chemosynthetic megafauna as well as the associated microbiota. Infaunal organisms, usually of smaller size (macro- and meiofauna), such as nematodes, harpacticoid copepods, polychaetes, amphipods, tanaids, gastropods, ostracods, and kinorhynchans, have been studied to a much lesser extent. The HERMES project aimed at painting a full picture of seep biodiversity by investigating all size classes of the benthos. The macrofaunal and meiofaunal communities at active cold seeps on the Nordic margin and in the Gulf of Cádiz and the eastern Mediterranean were studied for the first time, and analysis

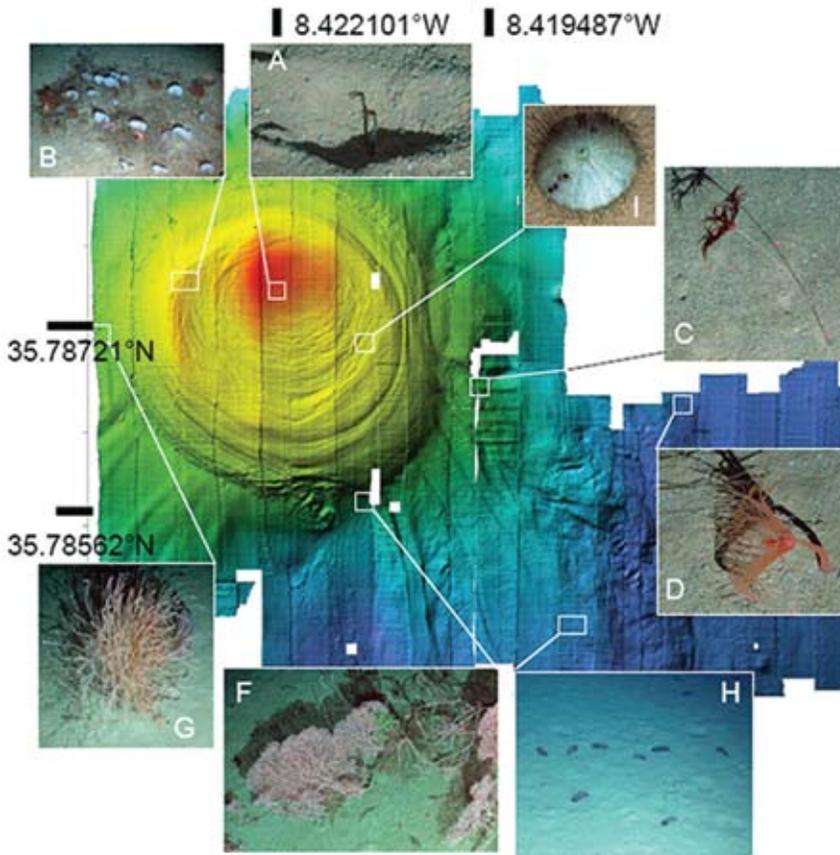


Figure 7. Bathymetry and fauna associated with Carlos Ribeira mud volcano: (A) Siboglinid polychaetes, (B) Porifera, (C) the seapen *Umbellula*, (D) athecate hydroid *Monocaulus*, (F, G) gorgonian bushes, (H) holothurians in high numbers, and (I) echinothurid sea urchin.

of the samples is currently ongoing. Initial results from the Nordic margin, including different habitats found on the Håkon Mosby mud volcano (in its center, microbial mats and siboglinid polychaete fields) as well as the Storegga and Nyegga cold seeps, show that the macrofauna (those between 500 μm and 1–2 cm) are generally dominated by polychaetes. A quantitative analysis revealed highly contrasting densities among the habitats (recent work of authors Decker, Van Gaever, and Olu); the highest abundances were associated with siboglinid fields (from 6700 to 56,000 ind. m^{-2}). Macrofauna were much less abundant in the microbial

mats (1000 to 1600 ind. m^{-2}) and even less abundant in the central area (55–170 ind. m^{-2}). Great discrepancies among habitats were also observed in the taxonomic diversity, because only a few species are able to colonize the more sulfidic and oxygen-depleted sediments at the microbial mat sites. There, the macrofauna were dominated by a polychaete belonging to the genus *Capitella* (Figure 8A), whose shallow-water species from the *Capitella capitata* complex is adapted to organic and sulfide-rich environments and is used as an indicator of pollution. In contrast, Siboglinidae fields were colonized by a higher taxonomic diversity

with at least seven polychaete families (M. Morineaux, Deep-Sea Ecosystem Department, Ifremer, *pers. com.*, 2008) and other groups, including poriferans, molluscs (*Bivalvia*, *Gastropoda*), and crustaceans (*Amphipoda*, *Tanaidacea*, *Isopoda*). Similar differences in community structure and dominant taxa among habitats were observed in the Storegga and Nyegga pockmarks (recent work of authors Decker, Van Gaever, and Olu). Ongoing work on dominant taxa will compare Storegga/Nyegga and Håkon Mosby mud volcano communities at higher taxonomic levels to test the influence of geographic patterns compared to habitat influence on the structure of communities.

Significant differences in diversity and abundance of the meiofaunal communities (organisms passing through a 1-mm sieve and retained on a 32- μm sieve) associated with different habitats were also found. The bare, muddy sediments from the active center yielded the lowest nematode densities, but unusually high benthic copepod abundance (271 ± 37 ind. 10 cm^{-2} ; Van Gaever et al., 2006). In contrast, one single nematode species, *Halomonhystera disjuncta* Bastian 1865 (Figure 8B), previously described from shallow-water habitats, was found in extremely high abundances ($> 11,000$ ind. 10 cm^{-2} ; Van Gaever et al., 2006) in the bacterial sediment-covering *Beggiatoa* mats. Biomarker fatty acid and stable carbon isotope analyses of *H. disjuncta* revealed that this species was thriving on chemosynthetically derived food sources, in particular, on the *Beggiatoa* bacteria (recent work of author Van Gaever and colleagues). The uncommon ovoviparous reproduction of *H. disjuncta*

at Håkon Mosby mud volcano has been identified as an important adaptation of parents to secure the survival and development of their brood in this anoxic environment. This nematode species was not found on the adjacent Storegga slide or in the Nyegga area, nor in any of the other cold seeps studied in the Gulf of Cádiz or the eastern Mediterranean. Here, the reduced sediments host a very impoverished nematode assemblage, in terms of both diversity and density, that is dominated by one or two species belonging to the genera *Terschellingia*, *Thalassomonhystera*, *Sabatieria* or *Desmodora* (Van Gaever et al., in press). At least three of these dominant species (i.e., *Halomonhystera disjuncta*, *Terschellingia longicaudata* De Man 1907, and *Sabatieria mortenseni* Ditlevsen 1921) were already described as common inhabitants of intertidal, organically enriched mudflats. Seep sediments that are strongly affected by reduced fluids and characterized by harsh environmental conditions (such as oxygen depletion, toxic sulfide levels) generate a habitat that is difficult for most of the typical deep-sea nematode

species to exploit. Only some oxygen-stress-resistant, shallow-water nematode species with an extensive geographical range are able to thrive in these deep-sea reduced environments. In contrast, the seep sediments colonized by siboglinid polychaetes display very diverse nematode communities, highly similar in terms of generic diversity compared to the surrounding background sediments. Siboglinidae are known to strongly affect the geochemical conditions in the sediment surrounding the tube through their intense ventilation activity (Julian et al., 1999; Bergquist et al., 2002). Consequently, well-oxygenated sediment down to 5-cm depth is created (de Beer et al., 2006), providing a suitable habitat for a wide range of nematode species. Siboglinidae fields and “control” samples of deep-sea sediments yield comparable highly diverse nematode assemblages, but a shift in dominant families and genera was detected (Van Gaever et al., in press).

The endobenthic community of the mud volcanoes in the Gulf of Cádiz displays wide variability in species composition and structure. Densities commonly vary from a few hundred to thousands

per square meter, but local patches of greater than 20,000 ind. m⁻² often occur. The shallower mud volcanoes of the Moroccan field (200–1000-m depth) show higher densities and number of species but a low degree of endemism, while the few samples taken from the Portuguese field (2000–3000-m depth) show lower densities and species number but suggest that endemism is clearly higher at these deeper mud volcanoes as many of the species collected (including the chemosynthetic ones) do not match the available descriptions of similar taxa.

In the eastern Mediterranean, different habitats were selected for systematic sampling (recent work of authors Ritt, Sarrazin, and colleagues). Preliminary results on the Napoli mud volcano show that the “*Lamellibrachia* habitat” has higher macrofaunal densities (5133 ± 3993 ind. m⁻²) than the “bivalve habitat,” where the highest density reaches only ~ 2117 ± 226 ind. m⁻² (recent work of authors Ritt and Sarrazin). Despite lower faunal density, the latter habitat exhibits a higher taxonomic richness. Characterization of the physico-chemical conditions is not yet

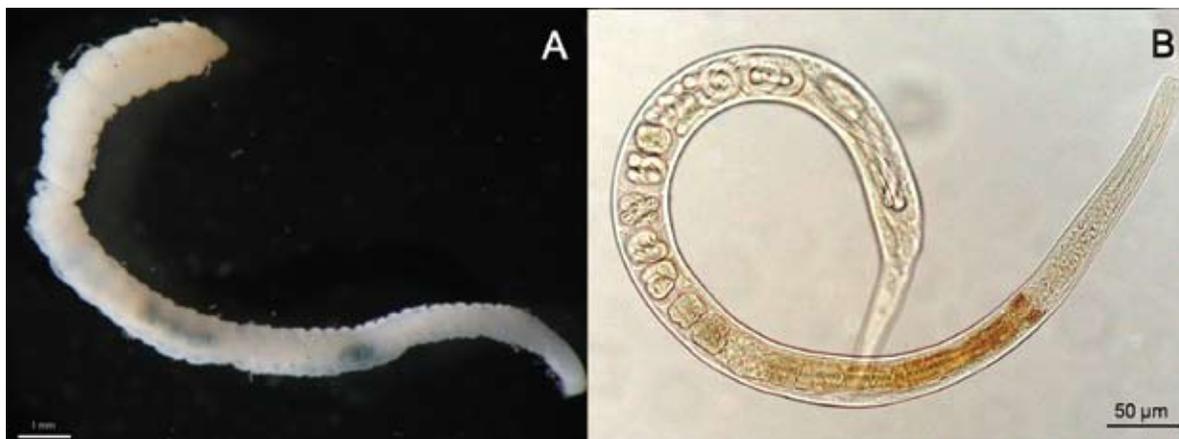


Figure 8. Small-sized endofauna with (A) the polychaete *Capitella* sp. and (B) the nematode *Halomonhystera disjuncta*.

finalized but a significant difference in oxygen penetration in the sediments was measured—limited to a few millimeters into the sediment in the “*Lamellibrachia* habitat” but reaching several tens of millimeters in the “bivalve habitat” (recent work of authors Ritt and Sarrazin). At the rim of the Amon mud volcano, a muddy brine flow characterized by blackish sulfidic sediments was sampled. Preliminary results from analyses of the macrofauna from the BIONIL cruise (RV *Meteor/Quest*, 2006) show that this particular habitat (only fauna > 1 mm have been sorted so far) exhibits a high abundance of polychaetes in addition to the presence of three families of bivalves (Lucinidae, Thyasiridae, Vesicomidae) typical of chemosynthetic or reduced habitats. Species richness on this probably short-lived habitat appears to be lower than on other seep habitats studied in this region. On the pockmark area (1700 m, Nile Delta), the “reduced sediment” sample contained a high abundance of dorvilleid polychaetes, characteristic of reduced habitats.

Overall, the preliminary results obtained during the BIONIL (2006) and MEDECO cruise (2007) show a vast heterogeneity of habitats and faunal assemblages, even within tens to hundreds of meters within different geological structures. More data need to be analyzed to highlight faunal and environmental similarities among similar habitats (bivalve, siboglinid) sampled from different mud volcanoes.

MICROBIAL COMMUNITIES AT COLD SEEPS

Similar to hot vents, cold seeps support an enormous biomass of free-living and symbiotic microbial life that is

nourished by the oxidation of methane, higher hydrocarbons, and sulfide. In fact, methane-fueled microbial communities in anoxic sediments above gas hydrates and gas vents have the highest biomass known to occur in marine ecosystems, with up to 10^{12} cells per cm^3 (Boetius et al., 2000). Because of their distinct metabolic abilities, which are adapted to the exploitation of reduced chemical compounds, methanotrophs, hydrocarbon degraders, and sulfate-reducing and sulfide-oxidizing bacteria are the key functional groups at cold seep ecosystems (Jorgensen and Boetius, 2007). Unfortunately, environmentally relevant representatives of these functionally relevant bacterial and archaeal clades have not yet been isolated, but a variety of nucleic acid and membrane lipid-based molecular identification methods have been instrumental in HERMES investigations of the microbial diversity of European cold seeps. The main groups at cold seeps can be summarized as follows. Hydrocarbon degradation is usually dominated by sulfate-reducing bacteria of the Deltaproteobacteria (Knittel et al., 2003). In contrast to most other seafloor habitats, cold seep sediments host a high proportion of archaea, mainly methanotrophic and methanogenic *Euryarchaeota* and uncultured *Crenarchaeota* (Knittel et al., 2005). The microorganism-mediated anaerobic oxidation of methane (AOM) with sulfate is the dominant process at cold seep ecosystems and the cause of the observed high sulfide fluxes. The organisms mediating AOM are anaerobic methanotrophic (ANME) archaea that form consortia with sulfate-reducing *Deltaproteobacteria* of the *Desulfosarcina* (Boetius et al., 2000) or *Desulfobulbus*

groups (Lösekan et al., 2007).

A key indicator community of active cold seep ecosystems is microbial mats, some of which cover hundreds of meters of seafloor, for example, at the Håkon Mosby mud volcano (Niemann et al., 2006b). These mats typically consist of giant, vacuolated sulfur-oxidizing bacteria, such as *Beggiatoa*, *Thioploca* and *Thiomargarita* spp., which exploit the high AOM-derived sulfide fluxes at the seafloor (Figure 9). Such bacteria can use internally stored nitrate to oxidize sulfur and fix carbon dioxide for growth, thus coupling the carbon, nitrogen, and sulfur cycles in seep sediments. Their diversity is much higher than anticipated, and each population has distinct adaptations to enable the use of the steep gradients of sulfide, nitrate, and oxygen that develop in the methane-rich sediments (Preisler et al., 2007). Microbial mats at seeps are often very patchy and mainly white (caused by the reflection of the intracellular sulfur granules), but they are also found in shades of yellow, orange, or grey. Thiotrophic mats usually comprise a diverse mixture of mostly bacterial taxa, but are dominated by large filamentous sulfide-oxidizing bacteria or by small thiotrophic Epsilonproteobacteria such as *Arcobacter* (Omeregíe et al., 2007). Some of these “giant” sulfide-oxidizing microbes, such as the filamentous *Beggiatoa* bacteria, have a gliding movement with which they position themselves within a steep gradient of oxygen and sulfide (Preisler et al., 2007); others depend on a flux of sulfide to the bottom-water interface.

Lösekan et al. (2007) describe in detail the relationship among the bacterial mats and the methanotrophic and sulfate-reducing bacteria found at the

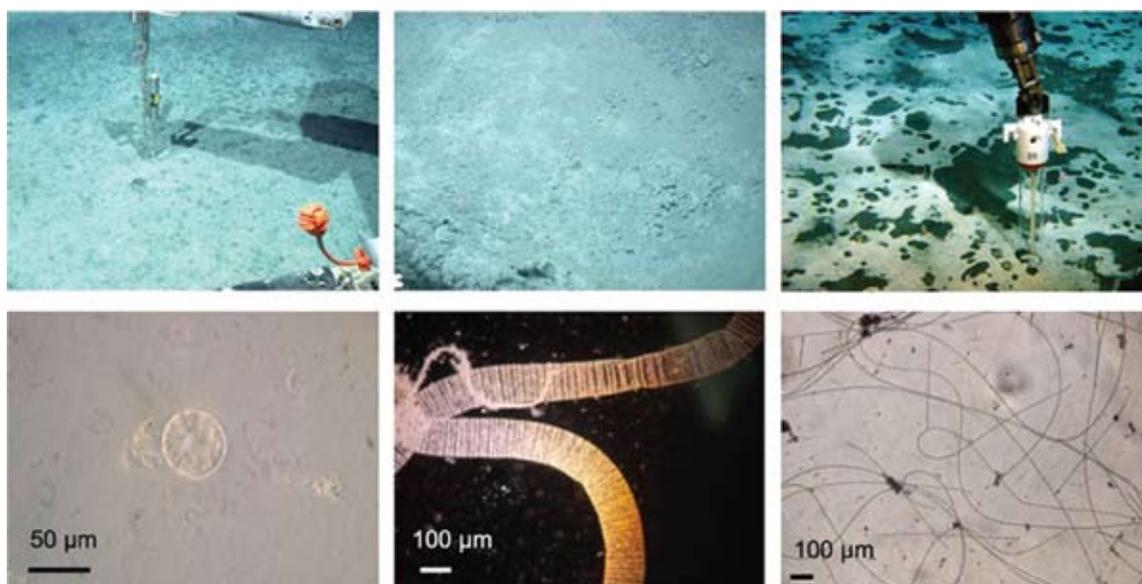


Figure 9. Macroscopic and microscopic images of microbial mats at cold seep ecosystems. (Upper panel, left) *Thiomargarita* mat in a fault surrounding the Amon mud volcano. ©MARUM/MPI. (Middle) Thin filamentous mats at the center of the Amon mud volcano. ©MARUM/MPI (Right) Thick filamentous mats surrounding the center of the Håkon Mosby mud volcano. ©Ifremer/AWI. The micrographs in the lower panel show the respective mat-forming thiotrophic bacteria. (Left) Sphere-like vacuolate *Thiomargarita* cell. (Middle) Giant filamentous vacuolate gammaproteobacteria. (Right) Thin filamentous vacuolate *Beggiatoa* cells. ©Stefanie Grünke, MPI/AWI

Håkon Mosby mud volcano. Briefly, in the active volcano center, the main methane-consuming process was bacterial aerobic oxidation. In this zone, aerobic methanotrophs belonging to three bacterial clades closely affiliated with *Methylobacter* and *Methylophaga* species accounted for $56 \pm 8\%$ of total cells. In sediments below the *Beggiatoa* mats encircling the center of the Håkon Mosby mud volcano, methanotrophic archaea of the ANME-3 clade dominated the AOM. They form cell aggregates with sulfate-reducing bacteria of the *Desulfobulbus* (DBB) branch, comprising $94\% \pm 2\%$ of the total microbial biomass at 2–3 cm below the surface. At the outer rim of the mud volcano, the seafloor is colonized by siboglinids. Here, both aerobic and anaerobic methane oxidizers were found in lower abundances, but distributed over a much

larger vertical and horizontal zone. Microbial diversity was higher at this site compared to the central and *Beggiatoa*-covered part of the Håkon Mosby mud volcano. Obviously, microbial diversity and community structure are closely related to different fluid-flow regimes at the Håkon Mosby mud volcano, providing distinct niches for aerobic and anaerobic methanotrophs.

Mud volcanism in the Gulf of Cádiz is characterized by a wide diversity of processes and environmental settings, such as different types of fluid migration pathways, tectonic activity and/or salt diapirism, migration velocity, fluid composition and alteration processes, depth, sea bottom temperature (from 4°C to 13°C if under the influence of the Mediterranean outflow water), and the presence of gas hydrate. Because these parameters create a wide array of unique

ecological niches for the seep microbial communities, the Gulf of Cádiz is an ideal natural laboratory for exploring the diversity and activity of seep microbes in relation to their environment. In the Gulf of Cádiz, overall AOM activity is typical for low to moderately active seeps. For instance, maximum methane turnover is typically around $20 \text{ nmol cm}^3 \text{ day}$ at both Captain Aryutinov and Carlos Ribeiro mud volcanoes (Niemann et al., 2006a; recent work of author Maignien and colleagues). However, some mud volcanoes deviate from this trend: at the Darwin mud volcano, thick carbonate crusts and plates seal methane escape routes. Discrete AOM hotspots have been observed at the rim of the crater, suggesting a relocalization of seep activity. In these hotspots, AOM activity is one order higher than at Carlos Ribeiro mud volcano and was found to sustain

the development of white bacterial mats. In contrast, salt diapir-driven mud volcanoes such as Mercator have hypersaline pore water that probably inhibits microbial activity, which was found to be one order of magnitude lower than at Carlos Ribeiro mud volcano, although methane and sulfate are present in large amounts. Interestingly, these environmental setting variations and AOM activity are reflected by diverse microbial community compositions. Inventories of archaeal and bacterial phylotypes in methane-rich sediments reveal that the three microbial consortia known to perform this reaction (ANME-1, -2, and -3) are active with different distribution patterns (Figure 10). Another major feature of mud volcanoes from the Gulf of Cádiz is the very deep origin of migrating fluid and mud reaching the surface (Hensen et al., 2007).

HERMES work also shed some light on the bacterial and archaeal diversity of the mud volcanoes at the Anaximander Mountains, eastern Mediterranean Sea. The Anaximander Mountains comprise a group of three main mountains between the Cyprus and Hellenic arcs (Zitter et al., 2005). The first gas hydrate sampling in the Anaximander Mountains

took place in 1996 at the Kula mud volcano (Woodside et al., 1997, 1998). Today, hydrates have also been sampled from four other mud volcanoes in the area. High seafloor methane fluxes are associated with the mud volcanoes as well as with the accompanying cold vents and seeps (Charlou et al., 2003), and the available gas provides energy for rich benthic communities, including chemosynthetic symbiotic fauna (Olu-Le Roy et al., 2004). Carbonate crusts derived from anaerobic oxidation of methane are formed in these environments (Aloisi et al., 2002). Of the five known mud volcanoes in the province, microbial diversity data exist only for prokaryotes (Bacteria and Archaea) from the Amsterdam (ca. 2030 m) and Kazan (ca. 1700 m) mud volcanoes. Based on 16S rRNA gene diversity, the Amsterdam mud volcano harbors a rather diverse bacterial community. Shannon diversity index H' (a tool for comparing two distinct habitats by combining the quantifiable terms of species richness and species equitability; high H' values indicate more diverse communities—an H' value of 0 indicates a community with one species) varies between 3.33 (carbonate crusts)

and 5.93 (sediments) (Heijs et al., 2006, 2008; recent work of author Pachiadaki and colleagues). The most abundant phylotypes in carbonate crusts are related to *Actinobacteria*, *Clostridia*, and *Alpha*-, *Gamma*- and *Deltaproteobacteria* as previously described (Heijs et al., 2006). Regarding sediments, the majority of the phylotypes are closely related to gas hydrate bearing sediments (Knittel et al., 2005). High numbers of *Deltaproteobacteria* phylotypes are present, as well as *Actinobacteria*, *Acidobacteria*, *Alpha*-, *Gamma*-, *Epsilon*- and *Deltaproteobacteria*, *Firmicutes*, *Cytophaga-Flexibacter* group, and candidate division WS3. Several phylotypes have also been found from the *Chloroflexi* and candidate division JS1. More rare phylotypes are related to *Planctomycetes*, *Firmicutes (Bacilli)*, *Bacteroidetes*, and candidate divisions OD1, OP8, OP11, and GN06. (Heijs et al., 2008; recent work of Pachiadaki and colleagues).

Archaeal communities show lower diversity with H' (base e) values ranging from 2.14 to 2.68 for sediments while in carbonate crusts H' is 2.93 (Heijs et al., 2006, 2008; recent work of author Pachiadaki and colleagues). Most of the archaeal sequences found in Amsterdam mud volcano carbonate crusts belong to the *Crenarchaeota*, Marine Group I (MGI). The remainder of the crenarchaeal sequences fall into a hitherto unclassified novel group of *Crenarchaeota* whose related sequences have previously been obtained from deep-sea sediments (Vetriani et al., 1999). The euryarchaeal sequences are related to novel *Thermoplasmata* or *Methanosarcinales*. The latter are affiliated to ANME-2 sequences (Heijs et al., 2008; recent work of author Pachiadaki

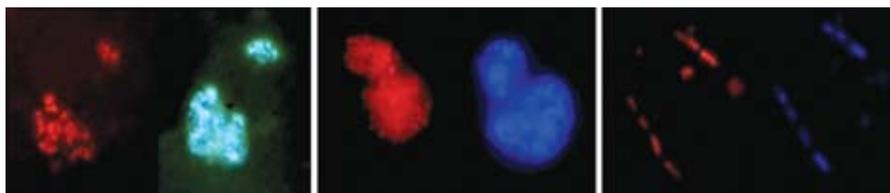


Figure 10. Diversity of anaerobic oxidation of methane (AOM) communities from the Gulf of Cádiz mud volcanoes shown with fluorescent in situ hybridization imaging. (Left) ANME-2 type aggregates with multiple archaeal cores surrounded by bacteria as observed in the Carlos Ribeiro mud volcano AOM zone. (Middle) ANME-2 type clusters with a single archeal core in a shallow microbial community of the Darwin mud volcano. (Right) ANME-1 type of archaeal filaments dominate the Mercator mud volcano microbial community. Microbial cells are stained with the archaeal specific probe Arch915 (red) and DAPI staining (blue).

and colleagues), while all three groups—ANME-1, ANME-2, and ANME-3—have been found in Amsterdam mud volcano sediments. Other sequences are related to *Methanomicrobiales*, marine benthic group D (MBG-D), and the *Thermoplasmata*. In comparison, the sediments of the Kazan mud volcano harbored less diverse bacterial assemblages. The H' index varied between 2.52 and 3.63. The Kazan phylotypes are related to the *Acidobacteria*, *Actinobacteria*, *Bacilli*, *Clostridia*, *Chloroflexi*, *Spirochaetes*, *Nitrospira*, *Planctomycetes*, *Alpha-*, *Gamma-* and *Deltaproteobacteria*, OP11, WS3, and a few unclassified *Bacteria* (Heijs et al., 2007; Kormas et al., 2008; recent work of author Pachiadaki and colleagues). Archaeal diversity was also lower, varying between 1.63 and 2.57. The occurring phylotypes are related to MGI, novel *Crenarchaeota*, *Halobacteriales*, *Methanosarcinales*, *Thermoplasmata*, and unclassified *Archaea* (Heijs et al., 2007; Kormas et al., 2008; recent work of author Pachiadaki and colleagues). Further work on the microbial biodiversity of eastern Mediterranean mud volcanoes and pockmarks of the Nile Deep Sea Fan is underway, focusing on the variety of microbial mats and associated communities (Omorgie et al., 2007).

OUTLOOK

Seeps, vents, and other reduced ecosystems contain a variety of organisms with unique functions related to chemoautotrophy, respiration, detoxification, mineral precipitation and dissolution, attachment, and sensing (chemotaxis). Among the most remarkable observations regarding different size groups and taxa, from bacteria to fish, are the

high heterogeneities from small to large scales. Every seep region along the European margin is different in terms of community composition and biodiversity, and high patchiness and other differences are observed within regions. However, many questions remain in the quest to unravel the diversity, functioning, and genomic capacity of chemosynthetic organisms, including free-living microbes and symbiotic associations with invertebrates. Chemosynthetic habitats are isolated and highly fractured ecosystems in which the organisms require distinct environmental features and cues to maintain their populations (temperature, presence of sulfide and CH_4 , hard ground, particle flux). The life history of animals and microbes restricted to chemosynthetic ecosystems and their dispersal remains a key limitation in understanding the interconnectivity and resilience of these dynamic ecosystems. Interconnectivity can be studied at different geographical scales as well as among vents, seeps and other habitats, which requires a combination of biological, oceanographic, and biogeographic studies, including population biology using genomic markers to assess gene flow. Furthermore, in a changing ocean it becomes critical to assess variations in biodiversity across all habitats in order to distinguish between natural and anthropogenic effects. The first long-term observatories at cold seeps are planned, and will provide data on the link between environmental fluctuations and the fate of the benthic ecosystem.

ACKNOWLEDGEMENTS

First, we would like to thank Myriam Sibuet who was one of the initiators of the HERMES project and the first

leader of the Cold Seep Workpackage. We also want to thank Phil Weaver and Vikki Gunn, respectively, the coordinator and manager of the HERMES project, which was funded under the European Commission's Framework Six Programme (EC contract no. GOCE-CT-2005-511234). We acknowledge the captains, the crews, ROV teams, and chief scientists of the cruises:

- ARKTIS XIX/3b (2003): *Polarstern* (Michael Klages);
- BIONIL (2006): *Meteor* (Antje Boetius);
- Vicking (2006): *Pourquoi pas?*, *Victor 6000* (Hervé Nouzé);
- MEDECO (2007): *Pourquoi pas?*, *Victor 6000* (Jozée Sarrazin and Catherine Pierre). 

REFERENCES

- Akhmetzhanov, A.M., M. Ivanov, N.H. Kenyon, and A. Mazzini. 2007. Deep-water cold seeps, sedimentary environments and ecosystems of the Black and Tyrrhenian Sea and the Gulf of Cádiz. Preliminary results of investigations during the TTR-15 cruise, RV *Professor Logachev*, June–August 2005, Intergovernmental Oceanographic Commission, Paris, 99 pp. plus appendices.
- Aloisi, G., I. Bouloubassi, S.K. Heijs, R.D. Pancost, C. Pierre, J.S. Sinninghe Damste, J.C. Gottschal, L.J. Forney, and J.-M. Rouchy. 2002. CH_4 -consuming microorganisms and the formation of carbonate crusts at cold seeps. *Earth and Planetary Science Letters* 203:195–203.
- Barry, J.P., R.E. Kochevar, and C.H. Baxter. 1997. The influence of pore-water chemistry and physiology on the distribution of vesicomyid clams at cold seeps in Monterey Bay: Implications for patterns of chemosynthetic community organization. *Limnology and Oceanography* 42:318–328.
- Bergquist, D.C., C. Fleckenstein, J. Knisel, B. Begley, I.R. MacDonald, and C.R. Fisher. 2005. Variations in seep mussel bed communities along physical and chemical environmental gradients. *Marine Ecology Progress Series* 293:99–108.
- Bergquist, D.C., I.A. Urcuyo, and C.R. Fisher. 2002. Establishment and persistence of seep vestimentiferan aggregations from the upper Louisiana slope of the Gulf of Mexico. *Marine Ecology Progress Series* 241:89–98.
- Bergquist, D.C., T. Ward, E.E. Cordes, T. McNelis, S. Howlett, R. Koisoff, S. Hourdez, R. Carney, and

- C.R. Fisher. 2003. Community structure of vestimentiferan-generated habitat islands from Gulf of Mexico cold seeps. *Journal of Experimental Marine Biology and Ecology* 289:197–222.
- Boetius, A., K. Ravensschlag, C. Schubert, D. Rickert, F. Widdel, A. Gieseke, R. Amann, B.B. Jørgensen, U. Witte, and O. Pfannkuche. 2000. A marine microbial consortium apparently mediating anaerobic oxidation of methane. *Nature* 407:623–626.
- Charlou, J.L., J.P. Donval, T. Zitter, N. Roy, P. Jean-Baptiste, J.-P. Foucher, J. Woodside, and The MEDINAUT Scientific Party. 2003. Evidence of methane venting and geochemistry of brines on mud volcanoes of the eastern Mediterranean Sea. *Deep-Sea Research Part I* 50:941–958.
- Cordes, E.E., S.L. Carney, S. Hourdez, R.S. Carney, J.M. Brooks, and C.R. Fisher. 2007. Cold seeps of the deep Gulf of Mexico: Community structure and biogeographic comparisons to Atlantic equatorial belt seep communities. *Deep-Sea Research Part I* 54:637–653.
- de Beer, D., E. Sauter, H. Niemann, U. Witte, and A. Boetius. 2006. In situ fluxes and zonation of microbial activity in surface sediments of the Håkon Mosby Mud Volcano. *Limnology and Oceanography* 51:1,315–1,331.
- Duperron, S., C. Bergin, F. Zielinski, A. Blazejak, A. Pernthaler, Z.P. McKiness, E. DeChaine, C.M. Cavanaugh, and N. Dubilier. 2006. A dual symbiosis shared by two mussel species, *Bathymodiolus azoricus* and *Bathymodiolus puteoserpentis* (Bivalvia: Mytilidae), from hydrothermal vents along the northern Mid-Atlantic Ridge. *Environmental Microbiology* 8:1,441–1,447.
- Duperron, S., A. Fiala-Medioni, J.C. Caprais, K. Olu, and M. Sibuet. 2007. Evidence for chemoautotrophic symbiosis in a Mediterranean cold seep clam (Bivalvia: Lucinidae): comparative sequence analysis of bacterial 16S rRNA, APS reductase and RubisCO genes. *FEMS Microbiology Ecology* 59:64–70.
- Dupré, S., J. Woodside, J.-P. Foucher, G. de Lange, J. Masclé, A. Boetius, V. Mastalerz, A. Stadnitskaia, H. Ondreas, C. Huguen, and others. 2007. Seafloor geological studies above active gas chimneys off Egypt (Central Nile Deep Sea Fan). *Deep-Sea Research Part I* 54:1,146–1,172.
- Domack, E., S. Ishman, A. Leventer, S. Sylva, V. Willmott, and B. Huber. 2005. A chemotrophic ecosystem found beneath Antarctic ice shelf. *Eos, Transactions, American Geophysical Union* 86:269–276.
- Gebbruk, A.V., E.M. Krylova, A.Y. Lein, G.M. Vinogradov, E. Anderson, N.V. Pimenov, G.A. Cherkashev, and K. Crane. 2003. Methane seep community of the Håkon Mosby mud volcano (the Norwegian Sea): Composition and trophic aspects. *Sarsia* 88:394–403.
- Génio, L., S.B. Johnson, R.C. Vrijenhoek, M.R. Cunha, P.A. Tyler, S. Kiel, and C.T.S. Little. 2008. New record of “*Bathymodiolus mauritanicus* Cosel 2002 from the Gulf of Cádiz (NE Atlantic) mud volcanoes. *Journal of Shellfish Research* 27:53–61.
- Haeckel, M., C. Berndt, V. Liebetrau, P. Linke, A. Reitz, J. Schonfeld, and H. Vanneste. 2007. Genesis and rates of fluid flow at the Mercator mud volcano, Gulf of Cádiz. *Geochimica et Cosmochimica Acta* 71:A367.
- Heijs, S.K., G. Aloisi, I. Bouloubassi, R.D. Pancost, C. Pierre, J.S. Sinninghe-Damsté, J.C. Gottschal, J.D. van Elsas, and L.J. Forney. 2006. Microbial community structure in three deep-sea carbonate crusts. *Microbial Ecology* 52:451–462.
- Heijs, S.K., R.R. Haese, P.W. van der Wielen, L.J. Forney, and J.D. van Elsas. 2007. Use of 16S rRNA gene based clone libraries to assess microbial communities potentially involved in anaerobic methane oxidation in a Mediterranean cold seep. *Microbial Ecology* 53:384–398.
- Heijs, S.K., A.M. Laverman, L.J. Forney, P.R. Hardoim, and J.D. van Elsas. 2008. Comparison of deep-sea sediment microbial communities in the eastern Mediterranean. *FEMS Microbiology Ecology* 63:362–377.
- Henry, P., J.P. Foucher, X. Le Pichon, M. Sibuet, K. Kobayashi, P. Tarits, N. Chamot-Rooke, T. Furuta, and P. Shultheiss. 1992. Interpretation of temperature measurements from the Kaiko-Nankai Cruise: Modeling of fluid flow in clam colonies. *Earth and Planetary Science Letters* 109:355–371.
- Hensen, C., M. Nuzzo, E. Hornibrook, L.M. Pinheiro, B. Bock, V.H. Magalhaes, and W. Bruckmann. 2007. Sources of mud volcano fluids in the Gulf of Cádiz: Indications for hydrothermal imprint. *Geochimica et Cosmochimica Acta* 71:1,232–1,248.
- Hilário, A., and M.R. Cunha. 2008. On some frenulate species (Annelida: Polychaeta: Siboglinidae) from mud volcanoes in the Gulf of Cádiz (Northeast Atlantic). *Scientia Marina* 72:361–371.
- Hovland, M., and H. Svensen. 2006. Submarine pingo: Indicators of shallow gas hydrates in a pockmark at Nyegga, Norwegian Sea. *Marine Geology* 228:15–23.
- Hovland, M., H. Svensen, C.F. Forsberg, H. Johansen, C. Fichler, J.H. Fosså, E. Jonsson, and H. Rueslåtten. 2005. Complex pockmarks with carbonate-ridges off mid-Norway: Products of sediment degassing. *Marine Geology* 218:191–206.
- Jerosch, K., M. Schlüter, J.-P. Foucher, A.G. Allais, M. Klages, and C. Edy. 2007. Spatial distribution of mud flows, chemoautotrophic communities, and biogeochemical habitats at Håkon Mosby mud volcano. *Marine Geology* 243:1–17.
- Jørgensen, B.B., and A. Boetius. 2007. Feast and famine—Microbial life in the deep-sea bed. *Nature Microbiology Reviews* 5:770–781.
- Julian, D., F. Gaill, E. Wood, A.J. Arp, and C.R. Fisher. 1999. Roots as a site of hydrogen sulphide uptake in the hydrocarbon seep vestimentiferan *Lamellibrachia* sp. *Journal of Experimental Biology* 202:2,245–2,257.
- Juniper, S.K., and M. Sibuet. 1987. Cold seep benthic communities in Japan subduction zones: Spatial organization, trophic strategies and evidence for temporal evolution. *Marine Ecology Progress Series* 40:115–126.
- Kenyon, N.H., M.K. Ivanov, A.M. Akhmetzhanov, and G.G. Akhmanov. 2000. Multidisciplinary study of geological processes on the North East Atlantic and western Mediterranean margins. Preliminary results of geological and geophysical investigations during the TTR-9 cruise of R/V *Professor Logachev*, June–July 1999, Intergovernmental Oceanographic Commission, Paris, 102 pp. plus appendices.
- Kenyon, N.H., M.K. Ivanov, A.M. Akhmetzhanov, and G.G. Akhmanov. 2003. Interdisciplinary geoscience research on the North East Atlantic margin, Mediterranean Sea and Mid-Atlantic Ridge. Preliminary results of investigations during the TTR-12 cruise of RV *Professor Logachev*, June–August 2002, Intergovernmental Oceanographic Commission, Paris, 112 pp. plus appendices.
- Kenyon, N.H., M. Ivanov, A.M., Akhmetzhanov, and E.V. Kozlova. 2006. Interdisciplinary geoscience studies of the Gulf of Cádiz and western Mediterranean basins. Preliminary results of investigations during the TTR-14 cruise, RV *Professor Logachev*, July–September 2004, Intergovernmental Oceanographic Commission, Paris, 115 pp. plus appendices.
- Knittel, K., A. Boetius, A. Lemke, H. Eilers, K. Lochte, O. Pfannkuche, P. Linke, and R. Amann. 2003. Activity, distribution, and diversity of sulfate reducers and other bacteria above gas hydrate (Cascadia Margin, OR). *Geomicrobiology Journal* 20:269–294.
- Knittel, K., T. Lösekann, A. Boetius, R. Kort, and R. Amann. 2005. Diversity and distribution of methanotrophic archaea at cold seeps. *Applied and Environmental Microbiology* 71:467–479.
- Kormas, K.A., A. Meziti, A. Dählmann, G.J. de Lange, and V. Lykousis. 2008. Characterization of methanogenic and prokaryotic assemblages based on *mcrA* and 16S rRNA gene diversity in sediments of the Kazan Mud Volcano (Mediterranean Sea). *Geobiology* 6:450–460.
- Levin, L.A. 2005. Ecology of cold seep sediments: Interactions of fauna with flow, chemistry and microbes. *Oceanography and Marine Biology—An Annual Review* 43:1–46.
- Levin, L.A., W. Ziebis, G.F. Mendoza, V.A. Growney, M.D. Tryon, K.M. Brown, C. Mahn, J.M. Gieskes, and A.E. Rathburn. 2003. Spatial heterogeneity of macrofauna at northern California methane seeps: Influence of sulfide concentration and fluid flow. *Marine Ecology Progress Series* 265:123–139.
- Loncke, L., and J. Masclé. 2004. Mud volcanoes, gas chimneys, pockmarks and mounds in the Nile deep-sea fan (Eastern Mediterranean): Geophysical evidences. *Marine and Petroleum Geology* 21:669–689.
- Lösekann, T., K. Knittel, T. Nadalig, B. Fuchs, H. Niemann, A. Boetius, and R. Amann. 2007. Diversity and abundance of aerobic and anaerobic methane oxidizers at the Haakon Mosby mud volcano, Barents Sea. *Applied and Environmental*

- Microbiology* 73:3,348–3,362.
- Lösekan, T., A. Robador, H. Niemann, K. Knittel, A. Boetius, and N. Dubilier. 2008. Endosymbioses between bacteria and deep-sea siboglinid tubeworms from an Arctic cold seep (Haakon Mosby mud volcano, Barents Sea). *Environmental Microbiology* 10(12):3,237–3,254, doi:10.1111/j.1462-2920.2008.01712.x.
- MacDonald, I.R., G. Bohrmann, E. Escobar, F. Abegg, P. Blanchon, V. Blinova, W. Brückmann, M. Drews, A. Eisenhauer, X. Han, and others. 2004. Asphalt volcanism and chemosynthetic life in the Campeche Knolls, Gulf of Mexico. *Science* 304:999–1,002.
- Masclé, J., C. Huguen, J. Benkhelil, N. Chamot-Rooke, E. Chaumillon, J.-P. Foucher, R. Griboulaud, A. Kopf, G. Lamarche, A. Volkonskaia, and others. 1999. Images may show start of European-African plate collision. *Eos, Transactions, American Geophysical Union* 80:421.
- Medialdea, T., R. Vegas, L. Somoza, J.T. Vázquez, A. Maldonado, V. Díaz-del-Río, A. Maestro, D. Córdoba, and M.C. Fernández-Puga. 2004. Structure and evolution of the “Olistostrome” complex of the Gibraltar Arc in the Gulf of Cádiz (eastern Central Atlantic): Evidence from two long seismic cross-sections. *Marine Geology* 209:173–198.
- Milkov, A., P. Vogt, G. Cherkashev, G. Ginsburg, N. Chernova, and A. Andriashev. 1999. Sea-floor terraces of Haakon Mosby Mud Volcano as surveyed by deep-tow video and still photography. *Geo-Marine Letters* 19:38–47.
- Niemann, H., J. Duarte, C. Hensen, E. Omeregíe, V.H. Magalhães, M. Elvert, L. Pinheiro, A. Kopf, and A. Boetius. 2006a. Microbial methane turnover at mud volcanoes of the Gulf of Cádiz. *Geochimica Cosmochimica Acta* 70:5,336–5,335.
- Niemann, H., T. Lösekann, D. de Beer, M. Elvert, T. Nadalig, K. Knittel, R. Amann, E.J. Sauter, M. Schlüter, M. Klages, and others. 2006b. Novel microbial communities of the Haakon Mosby mud volcano and their role as methane sink. *Nature* 443:854–858.
- Olu, K., F. Lance, M. Sibuet, P. Henry, A. Fiala-Médioni, and A. Dinat. 1997. Cold seep communities as indicators of fluid expulsion patterns through mud volcanoes seaward of the Barbados accretionary prism. *Deep-Sea Research Part II* 44:811–841.
- Olu-Le Roy, K., M. Sibuet, S. Gofas, A. Fiala-Médioni, J.-P. Foucher, and J. Woodside. 2004. Cold seep communities in the deep eastern Mediterranean Sea: Composition and spatial distribution on mud volcanoes. *Deep-Sea Research Part I* 51:1,915–1,936.
- Omeregíe, E.O., V. Mastalerz, G. de Lange, K.L. Straub, A. Kappler, H. Roy, A. Stadnitskaia, J.-P. Foucher, and A. Boetius. 2007. Biogeochemistry and community composition of iron- and sulfur-precipitating microbial mats at the Chefren mud volcano (Nile Deep Sea Fan, eastern Mediterranean). *Applied Environmental Microbiology* 74:3,198–3,215.
- Paull, C.K., B. Hecker, R. Commeau, R.P. Freeman-lynde, C. Neumann, W.P. Corso, S. Golubic, J.E. Hook, E. Sikes, and J. Curray. 1984. Biological communities at the Florida Escarpment resemble hydrothermal vent taxa. *Science* 226:965–967.
- Paull, C.K., W. Ussler III, W.S. Holbrook, T.M. Hill, R. Keaten, J. Mienert, H. Haflidason, J.E. Johnson, W.J. Winters, and T.D. Lorenson. 2008. Origin of pockmarks and chimney structure on flanks of the Storegga slide, offshore Norway. *Geo-Marine Letters* 28:43–51.
- Preisler, A., D. de Beer, A. Lichtschlag, G. Larvik, A. Boetius, and B. Joergensen. 2007. Biological and chemical sulfide oxidation in a *Beggiatoa* inhabited sediment. *ISME Journal* 1:341–353.
- Rodrigues, C.F., P.G. Oliver, and M.R. Cunha. 2008. Thyasiroidea (Mollusca: Bivalvia) from the mud volcanoes of the Gulf of Cádiz (North-east Atlantic). *Zootaxa* 1572:41–56.
- Sahling, H., D. Rickert, R.W. Lee, P. Linke, and E. Suess. 2002. Macrofaunal community structure and sulfide flux at gas hydrate deposits from the Cascadia convergent margin, NE Pacific. *Marine Ecology Progress Series* 231:121–138.
- Sibuet, M., and K. Olu. 1998. Biogeography, biodiversity and fluid dependence of deep-sea cold-seep communities at active and passive margins. *Deep-Sea Research Part II* 45:517–567.
- Sibuet, M., and K. Olu-Le Roy. 2002. Cold seep communities on continental margins: Structure and quantitative distribution relative to geological and fluid venting patterns. Pp. 235–251 in *Ocean Margin Systems*. G. Wefter, D. Billett, D. Hebbeln, B.B. Jørgensen, M. Schlüter, and T. Van Weering, eds, Springer-Verlag, Berlin Heidelberg.
- Sloan, E.D. 1990. Natural-gas hydrate phase-equilibria and kinetics: Understanding the state-of-the-art. *Revue de l'Institut Français du Pétrole* 45:245–266.
- Suess, E., B. Carson, S.D. Ritger, J.C. Moore, L.D. Kulm, and G.R. Cochrane. 1985. Biological communities at vent sites along the subduction zone off Oregon. Pp. 475–484 in *The Hydrothermal Vents of the Eastern Pacific: An Overview*. M.L. Jones, ed., Bulletin of the Biological Society of Washington, volume 6.
- Smirnov, R.V. 2000. Two new species of Pogonophora from the arctic mud volcano off northwestern Norway. *Sarsia* 85:141–150.
- Somoza, L., V. Díaz-del-Río, R. León, M. Ivanov, M.C. Fernández-Puga, J.M. Gardner, F.J. Hernández-Molina, L.M. Pinheiro, J. Rodero, A. Lobato, and others. 2003. Seabed morphology and hydrocarbon seepage in the Gulf of Cádiz mud volcano area: Acoustic imagery, multibeam and ultra-high resolution seismic data. *Marine Geology* 195:153–176.
- Stadnitskaia, A., M.K. Ivanov, V. Blinova, R. Kreulen, and T.C.E. Weering. 2006. Molecular and carbon isotopic variability of hydrocarbon gases from mud volcanoes in the Gulf of Cádiz, NE Atlantic. *Marine and Petroleum Geology* 23:281–296.
- Tryon, M.D., and K.M. Brown. 2001. Complex flow patterns through Hydrate Ridge and their impact on seep biota. *Geophysical Research Letters* 28:2,863–2,866.
- Van Aken, H.M. 2000. The hydrography of the middle-latitude Northeast Atlantic Ocean II: The intermediate water masses. *Deep-Sea Research Part I* 47:789–824.
- Van Dover, C., P. Aharon, J.M. Bernhard, E. Caylor, M. Doerries, W. Flickinger, W. Gilhooly, S.K. Goffredi, K. Knick, S.A. Macko, and others. 2003. Blake Ridge methane seep: Characterization of a soft-sediment, chemosynthetically based ecosystem. *Deep-Sea Research Part I* 50:281–300.
- Van Gaever, S., L. Moodley, D. de Beer, and A. Vanreusel. 2006. Meiobenthos at the Arctic Håkon Mosby mud volcano with a parental caring nematode thriving in sulphide-rich sediments. *Marine Ecology Progress Series* 321:143–155.
- Van Gaever, S., K. Olu, S. Derycke, and A. Vanreusel. In press. Metazoan meiofaunal communities at cold seeps along the Norwegian margin: Influence of habitat heterogeneity and evidence for connection with shallow-water habitats. *Deep-Sea Research Part I*.
- Vetriani, C., H.W. Jannasch, B.J. MacGregor, D.A. Stahl, and A.-L. Reysenbach. 1999. Population structure and phylogenetic characterization of marine benthic Archaea in deep-sea sediments. *Applied and Environmental Microbiology* 65:4,375–4,394.
- Vogt, P.R., A. Cherkashev, G.D. Ginsburg, G.I. Ivanov, K. Crane, A.Y. Lein, E. Sundvor, N.V. Pimenov, and A.V. Egorov. 1997. Haakon Mosby mud volcano: A warm methane seep with seafloor hydrates and chemosynthesis-based ecosystem in late Quaternary Slide Valley, Bear Island Fan, Barents Sea passive margin. *Eos, Transactions, American Geophysical Union* 78:187–189.
- Woodside, J.M., M.K. Ivanov, and A.F. Limonov. 1997. Neotectonics and fluid flow through the seafloor sediments in the Eastern Mediterranean and Black Seas. Part I: Eastern Mediterranean Sea. *IOC Technical Series* 48:1–128.
- Woodside, J.M., M.K. Ivanov, A.F. Limonov, and Shipboard Scientists of the Anaxiprobe expeditions. 1998. Shallow gas and gas hydrates in the Anaximander Mountains region Eastern Mediterranean Sea. Pp. 177–193 in *Gas hydrates: Relevance to World Margin Stability and Climate Change*. J.-P. Henriot and J. Mienert, eds, Special Publication 137, Geological Society, London.
- Zitter, T.A.C., C. Huguen, and J.M. Woodside. 2005. Geology of mud volcanoes in the eastern Mediterranean from combined sidescan sonar and submersible surveys. *Deep-Sea Research Part I* 52:457–475.