I - EXECUTIVE SUMMARY

This summary, initiated during the workshop discussions, was consolidated over several months thereafter as participants and CIESM colleagues each provided inputs in their particular area of expertise: Joan Batista and Joan Moranta (ichthyofauna), Bella Galil (biogeographic trends, megafauna, macrofauna), Cristina Gambi (meiofauna), Rolf Koppelmann (zooplankton) and Tassos Tselepides (macrofauna) all contributed ideas and words to the sections concerning the deep-sea fauna; Laura Giuliano – in collaboration with Christian Tamburini and Mikhail Yakimov – to microbiology, brine pools and pollution, Gerhard Herndl to prokaryotes diversity, David Billett (abyssal plains), Daniel Desbruyères (cold seeps), Paul Snelgrove (continental slopes; emerging technologies) to patterns and processes, Anna Metaxas to technologies and, along with Aline Fiala Medioni, to cold seeps. Frédéric Briand took care of what was left and of the overall synthesis, thankfully assisted in the final editing and in the writing up of the introduction by Paul Snelgrove.

1. INTRODUCTION

The Workshop was held in Heraklion, Greece, from 2 to 5 July 2003, at the Institute of Marine Biology of Crete. In welcoming the participants, Frédéric Briand, Director General of CIESM, first expressed his gratitude to Tassos Tselepides, Acting Director of the Center and one of the pioneers of deep sea ecology in the Cretan Sea, for facilitating the venue and the logistics, and to Angelo Tursi, Chair of the CIESM Committee on the Marine Ecosystem, for his early, enthusiastic support in selecting this theme. He then presented the general background and objectives for the workshop.

Background and objectives

Today much more is known about planet Mars than about life in the deep sea regions of our own planet. Despite our powerful fascination – since ancient times – about undersea worlds, deep-ocean exploration lags very far behind the exploration of outer space, and the global number of scientific deep-sea submersibles looks pale in comparison to that of satellites and spaceships. In the Mediterranean, deep-sea exploration is truly in its infancy, and our knowledge of the bathyal biota, dismal. To draw attention to this perplexing state of affairs, CIESM brought together some of the best experts in the discipline in order to summarize the scant, but developing knowledge on Mediterranean deep-sea biology and to identify the most crying gaps.

It is in the Mediterranean that deep-sea organisms were first discovered and studied : Antoine Risso, an apothecary from Nice published a series of papers between 1810 and 1827 on fish and crustaceans collected by fishermen at depths of 600 to 1,000 m in the Gulf of Genoa. Risso’s papers were thoroughly ignored at the time for they contradicted the “paradigm” formulated by Edward Forbes that below 550 m the deep ocean was a lifeless zone. It was only in 1861, when Alphonse Milne-Edwards identified molluscs and corals attached to a piece of telegraph cable brought up from 1,800 m deep between Algeria and Sardinia, that Risso's findings were vindicated.

Today we know several species that live below 4,500 m, deep in Mediterranean trenches. Yet our current level of knowledge of Mediterranean deep-water communities remains extraordinarily limited as very few biologists have sampled deep-sea regions. Few deep-sea habitats in the world ocean are thoroughly known, but in those areas that have been studied scientists did find a plethora of new species (Grassle and Maciolek, 1992), new habitats, and even new types of ecosystems (e.g. Grassle, 1986). New data on deep-sea seamounts and deep-water reefs elsewhere in the world (e.g. Koslow *et al.*, 2001; Hall-Spencer *et al.*, 2002) suggest that these environments often support taxonomically unique communities that are highly vulnerable to human-induced disturbance; there is every reason to expect that seamounts in the Mediterranean will be as distinct and vulnerable.

More generally, in view of the small sampling effort invested so far in deep areas of the Mediterranean Sea, there is little doubt that future investigations will generate exciting new discoveries. At the very least, many species that are new to science will be discovered. It is also possible that completely novel processes and environments will be found and that we have only scratched the surface with some of our recent discoveries. The discovery of hydrothermal vents in the Eastern Pacific, hardly 30 years ago, has revolutionized the way that ecologists view the Earth. Given the very limited state of our knowledge of the Mediterranean, it is quite possible that discoveries of equal magnitude await.

There is also a real sense of urgency : surrounded by dense coastal human populations and cities, subjected to intense fishing, fast-growing maritime traffic, the Mediterranean deep sea may be among the most heavily impacted deep-sea environments in the world. Yet it is also among the least known areas in terms of biodiversity resources. It may well be that a significant loss of biodiversity is currently taking place before scientists have had a chance to document its existence. In the following pages we will share our concerns over the initial commercial exploitation of deep water species; over the mounting threats posed by oil drilling, gas and mineral exploration. *In fine* a significant, complex issue looms large: given the unknown level of sustainability of deep-sea ecosystems, what would be the wisest policy for their conservation ?

The Mediterranean Deep-Sea : a major research opportunity

The morphology of the Mediterranean Sea, its geological history, and proximity of research laboratories make it an ideal location for innovative deep-sea research. The Mediterranean deep-sea is unique among deep-sea environments in being uniformly warm (>13°C), which in itself offers opportunities for testing fundamental concepts about deep-sea communities (e.g. reduced rates of metabolism, links between physiological adaptations and diversity patterns, colonization, etc.). The contrasting material flux in western and eastern basins (described below) offers an ideal mensurative experimental opportunity to evaluate how material flux influences patterns of diversity, whereas the unusual complexity of bottom habitats (see below) offers an excellent framework in which to examine how habitat heterogeneity influences rates of species turnover. The topographic and hydrological isolation, the frequent extinction and reinvasion cycles, also provide an intriguing background for biogeographic studies, particularly in tandem with novel techniques (e.g. molecular clocks, phylogenic analyses, etc.).

Perhaps the most compelling aspect of the Mediterranean as a research locale for deep-sea investigations and explorations is the proximity of many well-equipped laboratories. Process-oriented studies in other areas of the world are seriously constrained by the cost and logistics of mounting major seagoing expeditions to deploy and retrieve experiments. In the Mediterranean it is possible to access deep-water environments in day trips, thereby greatly increasing the range of possible experiments. In short, the Mediterranean Sea has the potential to become a major focal point for deep-sea diversity studies.

2. Mapping deep-sea habitats

In the past few years, the access of the oceanographic community to multibeam echo-sounding technologies has allowed the mapping of vast areas of the deep Mediterranean Basin, revealing a sea bottom of far greater complexity than that known from standard bathymetric maps, and providing a tremendous resource – rarely available over such a large scale – for biodiversity studies.

In 2001 a joint campaign of French and Dutch researchers on board the Ifremer R/V *L'Atalante* collected data that led to the co-production by CIESM and Ifremer of two important compilation maps – bathymetry and acoustic imagery – of the Mediterranean Ridge and adjacent areas in the Eastern Mediterranean Basin. Both maps offered, with a degree of resolution never achieved before, a brand-new vision and understanding of the vast bulge created at the subduction zone between the African and European plates. This effort was followed in 2003 by the co-production by CIESM and Geosciences Azur (Observatory of Villefranche s/mer, France) of maps of bathymetry and acoustic imagery of the Nile deep-sea Fan (see http://www.ciesm.org/publications/misce.html).

In recent months, swath mapping data have been collected and analysed for most areas of the Western Mediterranean Basin – from the Alboran Sea, the Algerian margin, the Balearic Sea, the Gulf of Lions, to the Tyrrhenian and Ionian Seas. Spanish, French and Italian research teams (from the University of Barcelona, IEO, Ifremer, University of Brest, Geosciences Azur, IGM Bologna) are currently cooperating under the aegis of CIESM and Ifremer, to produce a new compilation map which will synthesise these new findings at a scale of 1:200,000 with a grid resolution of 500 m. This map should be available by the end of 2004.

Before long, by integrating additional data collected by Eastern Mediterranean Institutes and French teams in the Aegean Sea, the Gulf of Lybia, the Nile system and Levantine waters, CIESM will be able to produce a comprehensive map of the entire Deep-Sea Mediterranean. A formidable challenge will be then to couple this knowledge with our emerging, fragmented picture of deep-sea biodiversity.

3. Biodiversity patterns

Investigations of the Mediterranean deep-sea fauna are at a very early stage: below 1,000 m, where systematic *quantitative* sampling has been extremely limited, most faunal groups remain largely unknown and there are basins in the Eastern Mediterranean and in southern waters where effectively nothing is known about deep-sea biology. At depths shallower than 800 m the sampling effort is considerably better, except in the southern Mediterranean which remains largely unsampled for any deep-sea fauna. While the basis of our knowledge of deep-sea zooplankton and microbes is somewhat stronger – at least in the Western Basin – the pelagic ichthyofauna is hardly known. Indeeed meso- and bathypelagic fishes represent a wide-open area for research.

3.1. Megafauna

Our knowledge of deep megafaunal communities (fishes, crustaceans and cephalopods) in the Mediterranean is mainly limited to the bathymetric range over which commercial fishing operates, usually down to 800 m. Below this range we have only fragmented data on the bathyal biota of the Western and Levantine basins.

Although there are past collections of megafauna in the Mediterranean, most of these have either been non-quantitative or have collected scattered samples without any systematic coverage. For example, the scientific exploration of the Levantine bathyal biota commenced with the extensive voyages of the *Pola* (1890-1893). In 1910 the Danish Oceanographic Expedition to the Mediterranean, aboard the *Thor*, sampled nine sites along the western limits of the Levantine Sea, from Cyrenaica to Rhodes. However, as that expedition focused mainly on plankton, and "dredgings were only occasionally made … the result will thus necessarily be but poor" (Stephensen, 1915). The Lamont Geological Observatory research vessel *Vema* collected few deep benthic samples in 1958 (Barnard, 1964). The German research vessel *Meteor* in 1987 collected benthic samples at sites between Crete and Israel at depths ranging between 95 and 4396 m during a ten-day sojourn (Janssen, 1989). The handful of deep-sea fishes gathered persuaded Klausewitz (1989) that "this collecting trip confirmed the paucity of the deep-sea fish fauna in the eastern basin of the Mediterranean". The total of 167 trawl hauls conducted since 1988 by the Israel Oceanographic and Limnological Research, down to 1,550 m, have not significantly altered this perception (Galil, in press).

Further down, photographic investigations have collected valuable *qualitative* information on the Levantine bathyal ichthyofauna. Two surveys conducted at a 15-year interval utilized bait, thus attracting facultative or obligate scavengers. Gilat and Gelman (1984) employed a free-fall camera positioned on top of a bait holder, taking still photographs at 5 minutes intervals for 18 hours, at six sites between Israel and Cyprus. Off Cyprus, at a depth of 1490 m, they found that the sharks *Centrophorus granulosus* and *Etmopterus spinax* were the most abundant species, constituting over 83% of the records. More recently Jones used an autonomous unmanned lander platform equipped with cameras and sonars for tracking movements of fish attracted to bait in the Cretan sea, Rhodos basin, and Ierapetra Basin (see Jones *et al.*, 2003). In the Cretan Sea (1,500-3,850 m) and Rhodos Basin (2,300-3,850 m), again sharks (*Hexanchus griseus*, *Galeus melastomus*, *Centrophorus* spp., *Centroscymnus coelolepis*, and *Etmopterus spinax*) predominated, together with the teleost fishes *Chalinura mediterranea* and *Lepidion lepidion* (Jones, *in* Priede and Bagley, 2000).

In a recent survey transacted without resorting to bait, Galil (in press) documented the site-typical faunal complement. She concluded that "there is no doubt that the abundance of the Levantine deep water megafauna is remarkably low: in 80 hours of video documentation only three fishes, *B. mediterraneus, Cataetyx laticeps,* and *Chauliodes sloani*, were recorded. Although the methods utilized were not sufficient for quantitative comparison with studies made in the Western Mediterranean, the small number of species and specimens confirms the ichthyofaunal scarcity of the Levantine Sea, not only compared with the adjacent Atlantic Ocean (Haedrich and Merrett, 1988), but also with the Western Mediterranean".

In the Western basin valuable quantitative information is available on the bathymetric distribution and community structure of deep megafaunal groups in the north-western area, essentially down to 2,200 m. This has been complemented recently by a few samples (only 10 bottom trawls were conducted) taken as far down as 4000 m in western and central deep-sea areas (Sardà, 2001).

3.2. Zooplankton

Although temporal variability is insufficiently covered, the sampling effort for zooplankton is better than in most other groups. Since the Straits of Gibraltar act as an ecological barrier to the immigration of many deep Atlantic species (see Scotto di Carlo *et al.*, 1984), the Mediterranean Sea is characterized by an impoverished bathypelagic fauna. Thus, in contrast to the rather diverse zooplankton found down to ca. 200 m (e.g. Mazzochi *et al.*, 1991; Siokou-Frangou *et al.*, 1997; Deudero and Morales-Nin, 2001; Fernández de Puelles *et al.*, 2003) in the Mediterranean epipelagic zone, only few typical bathypelagic species have been identified so far and the deep waters appear to be populated by several midwater species adapted to living at anomalously great depths (Vinogradov, 1968; Lapernat and Razouls, 2001).

Table 1 lists some of the main references published on deep-sea zooplankton of the Mediterranean Sea in the last fourty years. As there are more publications and reports (several in non-English language) available, the list is only suggestive and does not claim for completeness. The reader is refered to Scotto di Carlo *et al.* (1991) for a summary of investigations on copepod communities until the end of the 1980s, to Gasser *et al.* (1998) for updated information from the Western Mediterranean, and to Andersen *et al.* (2001a,b), Laval *et al.* (1992) and Mills *et al.* (1996) for data on gelatinous zooplankton down to mesopelagic depths. Information on deep mesozooplankton from the Eastern Mediterranean will be found in Pancucci-Papadopoulou *et al.*

Table 1. Major deep-sea zooplankton investigations in the Mediterranean Sea

Region	**max. Depth**	**Group**	**Source**
Balearic Sea	2800 m	Zooplankton	Furnestin and Arnaud, 1962
Balearic Sea	2800 m	Copepoda	Mazza, 1962
Ionian Sea	3000 m	Microzooplankton	Greze, 1963
Eastern Mediterranean Sea	3000 m	Microzooplankton	Delalo, 1966
Eastern Mediterranean Sea	2000 m	Zooplankton	Kimor and Berdugo ,1967
Mediterranean Sea	3000 m	Zooplankton	Vinogradov, 1968
Tyrrhenian, Adriatic Seas	1000 m	Copepoda	Hure and Scotto di Carlo, 1974
Tyrrhenian Sea	2000 m	Copepoda	Scotto di Carlo *et al.*, 1975
Levantine Sea	4400 m	Zooplankton	Kimor and Wood, 1975
Alboran Sea	1000 m	Zooplankton	Vives *et al.*, 1975
Western Mediterranean Sea	1000 m	Copepoda	Vives, 1978
Tyrrhenian, Ionian Seas	2000 m	Copepoda	Vaissière and Seguin, 1980
Tyrrhenian Sea	3000 m	Copepoda	Ianora and Scotto di Carlo, 1981
Tyrrhenian Sea	3000 m	Mesozooplankton	Scotto di Carlo *et al.*, 1984
Eastern Mediterranean Sea	4000 m	Near-bottom Plankton	Christiansen 1989
Ligurian Sea	700 m	Gelatinous Zooplankton	Laval *et al.* 1989
Levantine Sea	4000 m	Micronekton	Weikert, 1990
Levantine Sea	4000 m	Mesozooplankton	Weikert and Trinkaus,1990
Mediterranean Sea	2500 m	Mesozooplankton	Scotto di Carlo *et al.*, 1991
Western Mediterranean Sea	1000 m	Micronekton	Andersen and Sardou, 1992
Western Mediterranean Sea	1000 m	Gelatinous Zooplankton	Andersen *et al.*, 1992
Ligurian Sea	400 m	Gelatinous Zooplankton	Laval *et al.*, 1992
Levantine, Aegean Seas	3000 m	Mesozooplankton	Pancucci-Papadopulou *et al.*, 1992
Levantine Sea	4000 m	Mesozooplankton	Weikert and Koppelmann, 1993
Levantine Sea	1850 m	Microcopepoda	Böttger-Schnack, 1994
Alboran Sea	850 m	Gelatinous Zooplankton	Mills *et al.*, 1996
Western Mediterranean Sea	1000 m	Macropl. Micronekton	Sardou *et al.*, 1996
Levantine Sea	1850 m	Microzooplankton	Böttger-Schnack, 1997
Ligurian Sea	1000 m	Mesozooplankton	Gasser *et al.*, 1998
Ligurian, Tyyrhenian Seas	700 m	Macropl., Micronekton	Andersen *et al.*, 1998
Western Mediterranean Sea	1355 m	Near-bottom Plankton	Cartes, 1998
Ionian Sea	3700 m	Copepoda	Lapernat and Razouls ,2001
Levantine Sea	4250 m	Mesozooplankton	Weikert *et al.* 2001
Western Mediterranean Sea	1000 m	Mesozooplankton	Andersen *et al.*, 2001a,b
Levantine Sea	4250 m	Mesozooplankton	Koppelmann *et al.*, 2003

(1992), Weikert and Koppelmann (1993) and Weikert *et al.* (2001) plus in studies cited therein. Microzooplankton investigations down to 1,850 m were conducted by Böttger-Schnack (1994, 1997) in Levantine waters.

Overall, deep-sea zooplankton abundance and diversity decrease from west to east. Two taxa are dominant in the deep water of the Levantine Sea: *Eucalanus monachus* at mesopelagic depths and *Lucicutia longiserrata* in the bathypelagic zone. Scotto di Carlo *et al.* (1991) stated that the deep community structure showed only little or no diel and seasonal variability. However, significant changes occur occasionally or on time-scales not assessed so far. Weikert *et al.* (2001) found high amounts of zooplankton in the deep water body of the Levantine Sea in 1993. This change in zooplankton composition and abundance is likely associated with a change in hydrography, the Eastern Mediterranean Transient (see CIESM Workshop n°10, 2000; Klein *et al.*, 1999), which started in 1988/1989. Since the available data are sparse and fragmentary, all studies suffer from the lack of time-series investigations.

3.3. Macrofauna

Despite the thorough review of Fredj and Laubier (1985) on qualitative aspects of the benthic macrofauna composition of the deep Mediterranean Sea, quantitative data remain generally scarce, when not completely lacking. It is not an exaggeration to state that the vast majority of the deep Mediterranean basin is virtually unknown. Indeed, the Eastern Mediterranean which is the deepest of the two basins, long remained one of the most poorly studied areas of the world. During the second half of the 20th century some general ecological and faunal surveys provided valuable but scattered information on various areas (e.g. Pérès and Picard, 1958; Tchukhtchin, 1964; Ledoyer, 1969; Guille, 1970; Desbruyeres *et al.*, 1972; Vamvakas, 1970 and 1973; Di Geronimo, 1974). In the deepest area – the Cretan Sea – the earliest records of macrobenthic organisms were those given by Forbes (1844), Raulin (1870) and Jeffreys (1881,1883). With the establishment of the Institute of Marine Biology of Crete a number of benthic studies appeared in the last twelve years (e.g., Tselepides and Eleftheriou, 1992; Koutsoubas *et al.*, 1992; Karakassis and Eleftheriou, 1997; Koutsoubas *et al.*, 2000; Tselepides *et al.*, 2000), contributing new information on the macrobenthic fauna distributed from the outer continental shelf to the upper and mid-slope of Crete.

Until recently, however, knowledge of the deep (bathyal to abyssal) macrobenthic fauna remained sparse, derived primarily from material collected from the early *Pola* Expedition (Sturany, 1896) to the Eastern Mediterranean. In recent years (1987-2000) a more coherent, quantitative sampling effort was undertaken, largely through EU-funded projects and the logistic support of national deep sea initiatives such as the German *Meteor* Expeditions (see Janssen, 1989; Fiege *et al.*, 1994; Ben Eliahu and Fiege, 1996; Fiege *et al.*, 2000). Even so the number of quantitative macrobenthic samples acquired is very low (probably less that 100), as most of these studies were conducted as part of large multidisciplinary biogeochemical projects and therefore little effort and time was devoted to sampling the benthic community. The western basin remains also grossly understudied as very few quantitative studies did focus on the bathyal and abyssal macrofauna. A recent comprehensive quantitative investigation, undertaken by Stora *et al.* (1999) analysed samples from within and along the flanks of the Toulon Canyon. Valuable quantitative information on the community structure of the bathyal suprabenthos in the Catalan Sea, showing relatively high diversity at mid-bathyal depths, has been added by Cartes *et al.* (2001).

“The floro-faunistic impoverishment of the Eastern Mediterranean compared with the Western Mediterranean richness in species” (Sarà, 1985) has been generally accepted, as well as the perception of a gradational decrease from west to east that is more conspicuous for the deep benthos than for the whole fauna (Fredj and Laubier, 1985). Thus the Levantine deep water fauna has long been presumed the poorest in species and abundance in the whole Mediterranean (Fredj, 1974), although Bellan-Santini (1990) recognized that particularly “limited amount of sampling [was] carried out in the eastern basins”, even suggesting that “the relative species richness of (…) faunas of the different sectors of Mediterranean is better correlated with the level of research effort than the true species richness”. It seems realistic, however, to assume that the low number of species and specimens recorded does not stem solely from limited effort or inefficient gear.

Information on biogeography can be extracted from non-quantitative historical studies, but considerable caution is neeed. Although Marenzeller (1893) reported that species occur deeper in the Levant than elsewhere in the Mediterranean, his records were later considered suspect, an artifact resulting from a “systematic mistake on the depth measurements” that “needs to be cleared up in the future” (Fredj and Laubier, 1985: 128). Recent studies published greater depth records in the Levantine Sea than in the Western Mediterranean for 14 serpulid species, with a third of the depth extensions > 400 m (Ben Eliahu and Fiege, 1996). Several molluscs have been collected from greater depths in the Levantine Sea than in the southern Tyrrhenian Sea (Di Geronimo *et al.*, 2001). Extension of the depth records were also reported for five of the bathyal amphipods, in one case for as much as 1,100 m (Sorbe and Galil, 2002). The Levantine Sea bathyfaunal scarcity – theoretical ecologists might evoke a “niche vacuum” – may cause such extensions of the bathymetric distributions.

The present-day Mediterranean deep water fauna is less closely related to the Atlantic bathyal than was the case in the Pleistocene (Barrier *et al.*, 1996). This disparity is attributed in part to the shallow Gibraltar sill that bars the deep water of the Atlantic Ocean from entering the Mediterranean, and to the Mediterranean outflow that bars the entry of the deep water Atlantic fauna into the Mediterranean (see Salas, 1996). The onset of warmer climates led to the demise of many cold stenothermic and stenohaline species and to the eventual impoverishment of the bathybenthos. In addition, the extreme oligotrophy of the Levantine Sea prevented settlement by members of the Atlantic bathyal that would have been able to cross the shallow Gibraltar Straits and the Siculo-Tunisian sill (<400 m) (Pérès, 1985). The recurring stagnant (dysoxic and anoxic) Quaternary episodes resulted in a reduction, or even extinction of deep bottom-living fauna unable to avoid annihilation by adapting to shallower depth: Van Harten (1987) reported that “several species of deep-water ostracodes that are still common in the Western Mediterranean became extinct in the Eastern Mediterranean basin at the onset of early Holocene S1 sapropel deposition”. Bacescu (1985) believed that the bathyal bottoms of the Levant are still “unfavourable”, or even “azoic”, after the last sapropelic event, dated between 9,000 and 6,000 years B.P., following the suggestion (George and Menzies, 1968) “that sufficient time has not elapsed to allow colonization of the deep-sea floor”.

Bouchet and Taviani (1992) suggested that much of the Mediterranean deep-sea fauna is made-up of non-reproducing pseudopopulations that have entered the Mediterranean as meroplankton with the Atlantic inflow at Gibraltar. However, the populations of the most common benthic molluscs in depths greater than 1,000 m in the Levantine Sea are composed of both adult and juvenile specimens. Moreover gravid benthic decapod crustaceans and fish have been collected repeatedly from the depths of the Levantine Sea (Galil and Goren, 1994; Goren and Galil, 1997; Fishelson and Galil, 2001). Though much reduced in diversity and richness compared with the deep sea fauna of the western and central basins of the Mediterranean, the Levantine bathybenthos appears to be composed of autochthnous, self-sustaining populations of opportunistic, eurybathic species that have settled there following the last sapropelic event.

Often considered a “biological desert”, the deep Mediterranean Sea certainly holds many surprises: it was recently found that certain areas display such high activity as to be characterised as “benthic hotspots” (see Fiege *et al.*, 2000; Danovaro *et al.*, 2001; Tselepides and Lampadariou, in press). It appears likely that other areas, relatively rich in species – including some new ones to science – await discovery.

3.4. Meiofauna

Literature on meiofauna assemblages and nematode diversity in deep-sea Mediterranean is very scant. The only available information on meiofauna concerns the continental shelf and upper slope of the Western Mediterranean Basin and the Cretan Sea (Vivier, 1978; Guidi, 1987; Soetaert *et al.*, 1991; Bovée de *et al.*, 1990; Danovaro *et al.*, 1999; Grémare *et al.*, 2002; Dinet, 1976; Danovaro *et al.*, 1995, 1999). It appears that the Western Basin is characterised by significantly higher meiofaunal densities. In spite of this, meiofaunal assemblages display exactly the same community structure and indeed are strongly dominated by nematodes (80-90% of total meiofaunal density) followed by harpacticoid copepods and polychaetes (Danovaro *et al.*, 1999).

Nematodes are the most abundant metazoans on Earth, accounting for 80% of all multicellular animals on the planet; they are ubiquitous in marine habitats and sensitive to environmental changes. Moreover, nematodes become increasingly important with increasing water depth (Carmen *et al.*, 1987; Cook *et al.*, 2000). This phylum is characterised by a very high species number : more than 20,000 have been described on morphological and anatomical features, and potential species richness is in excess of 1 million (Lambshead and Boucher, 2003). As nematode genera and species are often cosmopolitan (the same genera can be found at tropical and polar latitudes), nematodes provide interesting “models” for investigating benthic biodiversity patterns.

However, despite their ubiquity and dominance, the knowledge of nematode diversity in the deep-sea sediments is limited to few areas such as the upper abyssal zone of the North and Central Atlantic, the eastern Central and Southern Pacific, the Arctic and the Weddell Sea, Antarctica

(Tietjen, 1976, 1984, 1989; Dinet and Vivier, 1979; Thistle and Sherman, 1985; Jensen, 1988; Renaud-Mornant and Gourbault, 1990; Soetaert and Heip, 1995; Thistle *et al.*, 1995; Vanreusel *et al.*, 1997, 2000; Vanhove *et al.*, 1999; Lambshead *et al.*, 1983, 1994, 2000; Gambi *et al.*, 2003).

Information on nematode biodiversity in the Mediterranean is scant and limited to the Western Basin (Cassidaigne Canyon, Vitiello, 1976; transect off Calvi, Corsica by Soetaert *et al.*, 1991). Moreover, long-term times series on deep-sea meiofaunal diversity are completely lacking and almost no information is available for extreme environments such as the Mediterranean abyssal plain, cold seeps, brine pools and sea-mounts or along the Mediterranean latitudinal and longitudinal gradients. No information is available yet on meiofaunal or nematode diversity in deep-sea sediments of the Southern Mediterranean sector, and a single study has been conducted across the Mediterranean to investigate the role of the longitudinal gradient at depths of ca 3,000 m (work in progress).

3.5. Prokaryotes

Since prokaryotic organisms largely lack distinct morphological features, assessment of prokaryotic species diversity relies on the use of molecular tools such as fingerprinting techniques, cloning and sequencing of the ribosomal RNA gene (see Stahl *et al.*, 1984; Giovannoni *et al.*, 1990; Muyzer *et al.*, 1993, CIESM Workshop n°11, 2000). Over the past decade it became clear that marine environments harbour an enormous phylogenetic diversity of prokaryotes. It is estimated that only less than 1% of all the marine prokaryotic species have been isolated thus far.

Diverse members of the two domains of life, *Bacteria* and *Archaea*, make up the prokaryotic component of marine plankton. *Bacteria* are phylogenetically more diverse than *Archaea* and about 80% of the *Bacteria* clones fall among nine phylogenetic groups (Giovannoni and Rappé, 2000). As indicated by fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE) and terminal restriction fragment length polymorphism (T-RFLP), the composition of the bacterioplankton community changes significantly between adjacent strata of the water column, even in the Mediterranean Sea despite the comparatively high temperature of Mediterranean deep waters. Thus depth is one of the major factors affecting bacterial community structure (Massana *et al.*, 1997; Murray *et al.*, 1998; Acinas *et al.*, 1999; Massana *et al.*, 2000).

The generally slightly higher richness of bacterial species in the euphotic layer is probably a reflection of the higher dissolved organic matter concentration and diversity as compared to deeper layers (Moeseneder *et al.*, 2001). In the euphotic layer, phytoplankton are releasing parts of the photosynthetically fixed organic matter directly into the water and grazing on phytoplankton by the proto- and metazoan food web also contributes to the production of fresh dissolved organic matter which can be utilized efficiently by the bacterioplankton consortia. Remarkably, the richness of bacterial species declines only by about 25% from the surface waters to the lower mesopelagic and bathypelagic realm (Moeseneder *et al.*, 2001) while overall bacterial abundance declines concurrently by about one order of magnitude. This paradox calls for more extensive investigations on the substrate requirements of deep water bacteria under i*n situ* pressure conditions.

High-pressure conditions typical of the deep-sea have long been known to affect the metabolism of marine bacteria (see for instance Zobell and Oppenheimer, 1950; Jaenicke, 1987; Straube *et al.*, 1990; Bianchi and Garcin, 1994; Poremba, 1994; Yayanos, 1995; Tholosan *et al.*, 1999; Tamburini *et al.*, 2003). Even if these conditions are not necessarily lethal for bacteria (Sharma *et al.*, 2002), they will likely affect the bacterial community composition. Due to the homeothermic features of its deep-sea layers (around 13°C), the Mediterranean Sea offers an unique opportunity to study the effects of the high pressure on the deep sea microbial communities without the compounding effects of low temperature which characterize all other deep oceanic environments.

The metabolic activities of deep-sea bacteria are strictly depending on the particle flux through the water column (Nagata *et al.*, 2000; Tamburini *et al.*, 2003). A large fraction of the bacterial consortia (>32 x 10^9 cells m^{-2} d^{-1}; Turley and Mackie, 1995) are transported into the deep-sea by settling particles (Turley and Mackie, 1994; Turley and Mackie, 1995). Attached bacteria play an important role in the degradation of aggregates. They convert the particulate organic carbon (POC) to dissolved organic carbon (DOC), bacterial carbon and carbon dioxide (CO_2) through ectoenzymatic hydrolysis (Cho and Azam, 1988; Smith *et al.*, 1992; Turley, 1993). In order to better understand how attached bacteria contribute to POC mineralization through the water column, the biological mechanisms controlling dissolution of ballast minerals should be studied (Armstrong *et al.*, 2001). Preliminary essays simulating the descent of diatoms through the water column have demonstrated that enzymatic hydrolysis rates of attached bacteria are strongly affected by the increase in pressure during particles sinking. There is evidence now that particle-associated bacterial consortia are phylogenetically remarkably different from free-living bacterial communities (Moeseneder *et al.*, 2001) at any given depth stratum of the Mediterranean Sea water column. Thus, the concept that free-living bacteria are becoming entrapped in particles and exhibit high metabolic activity there and releasing their progeny into the ambient water might not always reflect the actual situation. Thus studies on the phylogenetic composition of bacterial communities have to be combined more intensively with metabolic rate measurements in the future to shed light onto the complex interactions between free-living and particle-associated bacteria.

The second prokaryotic domain of life, the *Archaea*, are ubiquituously distributed throughout the global ocean. Planktonic *Archaea* fall almost invariably in two phylogenetic groups, the *Crenarchaeota* and the *Euryarchaeota*. Recently it has been shown that the relative abundance of *Crenarchaeota* increases with depth, equaling roughly bacterial abundance in the layers below 1,000 m (Karner *et al.*, 2001). *Euryarchaeota* generally make up no more than 15% of the total prokaryotic cells and do not vary in abundance with depth. At present the energy sources of the *Archaea* are still unknown, due to the lack of culturing these organisms. There is indication, however, that they may take amino acids (Ouverney and Fuhrman, 2000) and utilize inorganic carbon as well (Wuchter *et al.*, 2003). The relatively larger contribution of *Archaea* to total prokaryotic abundance in the deep ocean than in surface layers suggests that they are better competitors for available substrate than *Bacteria* in deep waters.

In summary, information available on microbial diversity in the deep oceans is still scarce, as is information on the regulating mechanisms (CIESM Workshop n°21, 2003). Deep-sea environments are likely to contain numerous novel and widespread major prokaryotic lineages. Given the enormous volume represented by this habitat and typical bacterial abundances, the previously unknown Archaeal and Bacterial groups may well be the most abundant organisms on Earth (Fuhrman and Davis, 1997).

3.6. Extreme environments

3.6.1. Cold seeps

Seepage of cold fluids, enriched in sulfide, methane, hydrocarbons, as well as nutrients are common both in active and passive margins from 400 to 7,326 m. They are known to sustain exuberant deep-sea, chemosynthesis-based communities, which are usually dominated by bacterial mats, bivalves (mussels and clams) and tube worms, both metazoans associated with endosymbiotic chemo-autotrophic bacteria (see review in Sibuet and Olu, 1998; Kojima, 2002; Tunnicliffe *et al.*, 2003). At all sites, chemosynthesis-based communities are restricted to areas where methane seep out along tectonic features leading to accumulation of hydrogen sulfide in the sediment through microbial sulfate reduction processes (Fiala-Médioni *et al.*, 1993; Henry et *al.*, 1996; Guezennec and Fiala-Médioni, 1996). Mud volcanoes have been identified as one of the geological contexts favoring the exploitation by chemosynthetis-based symbioses of cold seeps rich in methane (Henry *et al.*, 1996).

Although exploration is still in its infancy, several cold-seep communities have been located and described in the Eastern Mediterranean Sea, south of Crete and Turkey, on mud volcanoes or along faults associated with high flux of methane (Camerlenghi *et al.*, 1992; Charlou *et al.*, 2003) and observed from 1,700 to 2,000 m (Medinaut/Medineth Shipboard Scientific parties, 2000; Olu *et al.*, 2001). These observations followed the discoveries on the Napoli mud volcano (1,900 m) of abundant thanathocoenoses of bivalves attributed to *Myrtea* sp. (Lucinidae) and *Vesicomya* sp. (Vesicomyidae) (Corselli and Basso, 1996). More recently similar communities were also observed north of Egypt in the Nile delta (*Nautinil* cruise, 2003).

The communities are dominated by small bivalves belonging to four families often encountered at seeps (Lucinidae, Vesicomyidae, Mytilidae and Thyasiridae) and by pogonophorans including a large vestimentiferan *Lamellibrachia* sp. Associated fauna included a Suberitidae sponge *Rhizaxinella pyrifera*, Polychaeta (other than pogonophorans), galatheids *Munidopsis marionis* and *M. acutispina*, one large crab, *Chaceon mediterraneus* and a sea urchins belonging to the genus *Echinus* (Olu-Le Roy *et al.*, 2001).

3.6.2 - Brine pools microbiology

The deep hypersaline anoxic basins (DHABs) of the Eastern Mediterranean Sea are unique environments created by the flooding of ancient evaporites from the Miocene period (5.5 Million years B.P.). Compared to smaller hypersaline ponds, the composition of these basins, ranging in area from 5 to 20 km^2, is fairly constant perhaps due to their deep location (deeper than 3,300 m). The DHABs are characterised by a total salinity above 30% (close to the saturation point of NaCl which would mark the upper limit of resistance of all biological forms) (De Lange *et al.*, 1990a), absence of light, elevated pressure (>30 MPa), variable pH values and ionic composition. The transition from normal seawater to brine occurs at about 3,300 m depth. At this transition, salinity increases sharply with depth, whereas the concentration of dissolved oxygen drops rapidly to zero. This change is likely to occur within a depth of 1 m. The sharp density difference between brines and the upper normal deep seawater acts as a barrier to the oxygen exchange between water and brines. Several reports indicate that the seawater-brine interface is a very stable physical configuration (Wiesenburg *et al.*, 1985; De Lange *et al.*, 1990b).

The DHAB environments represent one of the last frontiers that challenge the life sciences today. A great diversity of microorganisms have been detected by cultivation-independent methods along the seawater-brine gradient, and hundreds of individual isolates were obtained in pure cultures. Compared to other anoxic marine hypersaline lakes that have been more largely studied for their bacterial diversity, namely the Kebrit Deep and the Shaban Deep basins (Red Sea) (Antunes *et al.*, 2003; Eder *et al.*, 1999, 2002), the DHABs showed a highly complex structure and a very high abundance of newly described prokaryotic taxonomic groups, particularly in the interface. According to these findings, both the *Bacteria* and *Archaea* domains are well represented in the different layers of the DHABs, particularly at the interface where the hydrolysis rates of biopolymers appear clearly higher than in the immediately overlying oxygenated water (Biodeep data).

4. New research directions - Patterns

The Mediterranean Sea offers a fascinating location for studying issues in deep-sea biodiversity because of its unique geological history, its physiography and easy access of its deeper areas (see Fig. 1). Unlike the open ocean, the Mediterranean Sea has been subjected to drying out and then inundation from the Atlantic, a process that may have occurred as many as 17 times (Maldonado, 1986). The most recent event occurred about 5 million years ago, making the Mediterranean abyssal zones very “young” (biologically speaking) in comparison to the oceans. In addition, since the shallow sill (300 m) at the Strait of Gibraltar has prevented migration of abyssal species into the Mediterranean Sea (see section 3.3), unique opportunities exist of studying the influence of migration *vs* speciation in influencing species richness. The presence of a number of abyssal basins at varying distances from the connection to the Atlantic, and the separation of the deep Eastern and Western Mediterranean by the Strait of Sicily sill (400 m) provides further opportunities of assessing the effects of topographic separation on biodiversity.

Apart from unique geological features, the Mediterranean Sea has a number of hydrographic features that will also have a different effect on biodiversity in comparison to "normal" abyssal settings. Most notable of these is the temperature of the water, which does not fall below 13°C making the Mediterranean the warmest abyss in the World's oceans. Normal rules that apply to cold abyssal fauna elsewhere are not found in the Mediterranean, offering novel comparisons on the combined physiological effects of temperature and pressure. The deep Mediterranean is also affected by temporal variation in the formation of cold water during winter months at a number of locations, but most notably in the Aegean Sea, off Rhodes and the Adriatic (see CIESM Workshop n° 10, 2000; Lascaratos *et al.*, 1999).

4.1. Bathyal patterns on continental slopes

Continental slope environments include some of the most heterogeneous sedimentary environments in the deep sea. Bathymetry is an overriding variable that must be considered in any comparison between slope communities and likely contributes significantly to broad-scale patterns. Process-related studies will be considered separately (see section 6 below), but in terms of pattern there are several questions that are particularly compelling in the Mediterranean.

One key question is how patterns of diversity change as a function of depth along the continental slope and rise, and how the quality, quantity, and frequency of organic input contribute to this pattern. Different areas of the Mediterranean are known to vary in terms of surface production, sedimentation rates, and morphology (e.g. Danovaro *et al.*, 1999). The degree to which the faunas of these areas differ remains poorly known, beyond the recent comparative study of Galil (in press) on the biodiversity patterns of bathyal mega- and macro-benthos in the Western and Levantine basins.

Among other questions :

1) Does diversity peak at intermediate depths (as e.g. in the north Atlantic, Rex, 1983) in all of these areas or do areas with different material flux show different depth-diversity relationships?

2) What is the extent of bathymetric zonation (e.g. Cartes and Sarda, 1993) in different taxonomic groups in all areas of the Mediterranean? Comparative studies between regions with nearby geographic regions with contrasting flux regimes (e.g. Levin and Gage, 1998) are likely to be particularly informative.

3) Do topographic features such as canyons support unique faunas within and adjacent to canyon areas (see 4.4. below)? This should be linked to zooplankton studies, given the potential zooplankton may play in influencing material flux.

4) Are there latitudinal or longitudinal gradients within the Mediterranean?

Another area of interest is whether specific areas of the Mediterranean slope represent "hotspots" of diversity, and therefore should be prioritized for conservation efforts. Because this region is poorly sampled for most groups, except in a few key areas, representative, quantitative sampling for different faunal groups (e.g. megafauna, macrofauna, meiofauna, microbes) is urgently called for in areas where these data are completely lacking. Topographic features (e.g. canyons, seamounts) should also be targeted given that these areas are likely to support relatively distinct faunas. Ideally, studies of different groups should be undertaken collaboratively, so that it may be possible to test whether groups such as macrofauna and meiofauna are similar in diversity pattern; evidence from the ocean basins suggests possible conflicting patterns among groups (Snelgrove and Smith, 2002), but comparisons are difficult because collaborative studies that examine different size groups from the same location are extremely rare.

4.2. Abyssal plains patterns

Although the abyssal basins occur at more or less the same latitude, there are significant differences in surface productivity between the eastern and western basins. In the Western

Mediterranean seasonal changes occur with maximum (mesotrophic) production of about 400 mgC m^{-2} d^{-1} (Morel and André, 1991) in May. In contrast, the Eastern Mediterranean is oligotrophic with a maximum surface production in offshore areas of about 60 mgC m^{-2} d^{-1} (Danovaro *et al.*, 1999) leading to a much lower flux of organic matter to the seabed in the two regions (Kerhervé *et al.*, 1999). There are distinct differences therefore in the quantity and quality of organic matter deposited on the seabed which will have important biological consequences. In addition, recent observations reported elsewhere in this volume highlight that, as in the major oceans (Smith and Druffel, 1998; Billett *et al.*, 2001), there are long-term radical changes in the deep-sea fauna, which may be related to variation in hydrography and organic matter flux.

The Mediterranean deep-sea clearly offers a variety of different and unique environmental settings in a continuum that stretches longitudinally from 4° to 36°W. Yet, as emphasized in section 3, little scientific use has been made of these different settings, and we face a striking lack of information on species richness and community structure. The apparent low diversity of the deep Mediterranean Sea has not encouraged sampling. A concerted effort, therefore, is needed in relation to the major gradients (environmental, geological and historical) and would yield rich rewards. Such a programme should focus on all size classes from bacteria to fish, with the greatest effort on the smaller size fractions. It should also use molecular and morphological techniques. The former would be especially useful in the study of small size fractions and for studying speciation in relation to the environmental and physical gradients.

With reference to the location of the abyssal basins in the Mediterranean (see Fig. 1) the following questions should be addressed:

1) What changes occur in species richness, species dominance and community structure between the Algero-Provencal, Tyrrhenian, Ionian and Levantine Basins?
2) How do these patterns differ between taxa, size classes (micro-, meio-, macro- and mega-fauna) and functional groups?
3) How do these patterns differ in relation to reproductive strategies of the fauna?
4) What is the level of endemism within each basin?

In terms of pattern it is particularly important to characterise regional biodiversity, within each basin, in the Western and Eastern Mediterranean, and in the whole Mediterranean in comparison with the Atlantic Ocean, particularly abyssal plain regions in close proximity to the Strait of Gibraltar.

4.3. Deep-sea zooplankton

If we have some knowledge about the vertical distribution of the mesozooplankton main taxa in various basins, there is a dearth of information on temporal changes. The information on microzooplankton including protozoans is very sparse in the Western and Eastern Mediterranean (see Tanaka and Rassoulzadegan, 2002) and the same holds true for gelatinous organisms. Quantitative information available for the latter group is from mesopelagic depths in the western part of the Sea, only. To gain better insights into the distribution and dynamics of the deep-sea zooplankton, the temporal and spatial distribution of different size classes of zooplankton from protozoa over micro-, meso- and macrozooplankton to micronekton and gelatinous organisms must be investigated. The sampling design should cover diurnal to decadal time scales to detect daily and ontogenetic migrations as well as changes associated with global change. The spatial resolution should cover the full-depth vertical distribution in different basins. These investigations will help to define key species for process studies.

Zooplankton sampling should be combined with investigations of the benthos. Information on the abundance, composition and ecological role of the near bottom fauna as a linking element between the plankton and the benthos (pelago-benthic and bentho-pelagic coupling) should be gained. Moreover, fine-mesh samples in the near-bottom environment are needed to investigate the dispersal of benthic larvae.

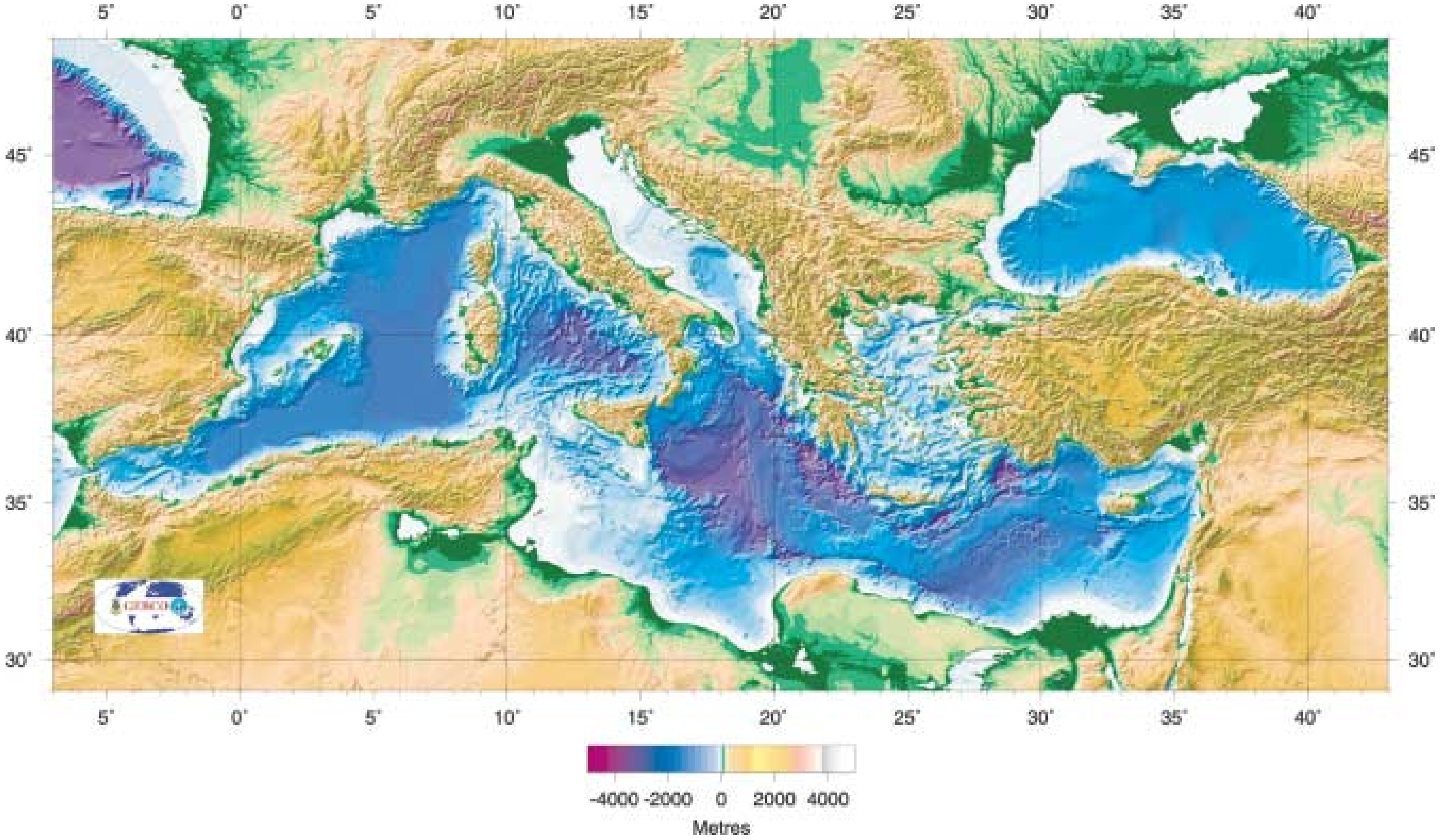

Fig. 1. General bathymetry of the Mediterranean Sea (adapted from GEBCO data).

4.4. Canyons

Several submarine canyons cross the continental slope of the Western and Central Mediterranean (Reyss, 1971). These submarine features are viewed as hotspots of species diversity and endemism (Gili *et al.*, 1998, 1999), likely to play an important role on structuring the populations and life cycles of the planktonic fauna (Macquart-Moulin and Patriti, 1993, 1996; Gili *et al.*, 2000) and benthic megafauna fishery resources dwelling on their influence (Cartes *et al.*, 1994; Sardà *et al.*, 1994a,b; Stefanescu *et al.*, 1994). Faunal density and biomass are usually higher inside the canyons when compared to similar depths in the surrounding habitat, but individual size is significantly smaller than on the adjacent open slope. Although information relating submarine canyons and biological aspects of the fauna is still very scarce, morphologic and oceanographic features of the canyons are understood to be the main factors of this linkage (Gardner, 1989; Vetter and Dayton, 1998).

4.5. Extreme environments

4.5.1. Cold seeps

Extensive exploration and characterization of these communities along geological structures which can be located by multibeam echosounding and other geophysical tools have yet to be initiated. Due to their spatial patterns, the distribution and characteristics of these highly fragmented habitats could be only assessed using large facilities, including oceanographic vessels and manned or remotely operated deep-sea vehicles in a framework of an interdisciplinary approach (geophysics, geochemistry, biology and microbiology). A large section of the bottom mapping has been published and provides a very important base for upcoming benthic exploration (see section 2 above).

The isolation of the Mediterranean seeps and vent habitats from the Atlantic Ocean after the Messinian crisis led to the development of unique communities, which are likely different in composition and structure from those in the Atlantic Ocean. For example, the bivalve mollusc populations found in Mediterranean cold seeps are very specific, with species much smaller in size than those from other seep sites. The use of modern molecular markers, as well as more classical taxonomy, would lead to a better understanding of the origin and evolution of these communities (e.g. their relationship to shallow water sulfuretum organisms and/or to cold seep communities in the Atlantic Ocean).

Once the major spatial patterns in community structure are established, the food web structure should be described using enzymatic and isotopic methods, as well as "*in situ* simulated" techniques, aboard research vessels. Bacterial symbioses and consortia which form the base of chemo-autotrophic functioning can be described using both ultrastructural methods, as well as molecular markers. The relationship between the microdistribution of biota and fluid patterns can be examined using *in situ* analysis, microsensors, flow meters, and water sampling associated to optical imagery. The Mediterranean Sea provides a unique opportunity to understand the relative roles of temperature and pressure in adaptive ecophysiology, particularly for organisms inhabiting high sulfide environments.

4.5.2. Brine pools

A number of DHABs isolates and individual DNA clones from the metagenomic expression libraries have been selected as candidates for potential biotechnological applications. All the organisms from those samples were derived from enrichment cultures set under the conditions simulating the DHAB environments (temperature, chemical composition etc.) except for the pressure. Therefore apparent baro/piezophilic organisms cannot be uncovered with that cultivation strategy. The lack of isolates belonging to the new taxonomic groups that were described by means of culture independent methods could support the hypothesis of their pressure dependence. Almost no attempts have been made to detect the possible presence of fungi and viruses and to study their influence on the microbial population dynamics in the DHABs.

5. DEEP-SEA SAMPLING METHODS AND EMERGING TECHNOLOGIES

Our capacity to quantify biodiversity in deep-sea environments is most severely constrained by the sheer size and remoteness of the habitat, the cost of sample collection and processing, and a shortage of qualified taxonomists. If, for the most part, the technologies necessary to sample deep-sea organisms for diversity studies have been developed, there are recognized deficiencies with any single approach. Moreover, a given sampling approach usually targets specific size groupings of organisms, integrates over specific spatial and temporal scales, and typically underestimates certain taxa because of behavioural response or physical disturbance of individuals. Deep-sea ecologists are aware of these shortcomings and try to minimise their effect, recognizing the limitations of their samples.

For megafaunal organisms, various types of trawls and nets can provide quantitative, though not absolute, estimates of species abundance. Submersibles (e.g. Uzmann *et al.*, 1977) and towed still and video camera systems (e.g. Russel *et al.*, 1986) have been used for ground truthing. These approaches are particularly useful in rugged or fragile habitats where trawling is not possible (e.g. Kreiger *et al.*, 2000). Imaging quality is constantly improving and facilitating identification, and the advent of digital imagery has allowed quantification at mm scales. However, still and video camera images are limited in that they do not provide voucher specimens. For this reason, combining trawl efforts with photography, video, or sampling by submersible provides the most accurate measurements of diversity and abundance.

A variety of approaches have been developed for sampling organisms associated with bottom sediments. The first semi-quantitative sampling of deep-sea macrofaunal benthos was the epibenthic sled (Hessler and Sanders, 1967) which is dragged through the surficial sediment on the seafloor. Although epibenthic sleds are known to significantly undersample sedimentary fauna, they are still used to sample small hyperbenthos – the organisms that live just above the seafloor (e.g. Mees and Jones, 1997). Alternative quantitative approaches for sampling hyperbenthos include novel applications of moored plankton pumps (e.g. Garland *et al.*, 2002) and video plankton recorders (e.g. Davis *et al.*, 1992) that can provide quantitative time series data *in situ.* Interestingly, in deep-sea systems there is still a constraint that sampling volume may be a limiting factor for some organisms because concentrations (e.g. meroplankton of some taxa) may be too low to be effectively sampled by current versions of these instruments.

For sedimentary macrofauna, box corers have been a standard quantitative sampler since their development in the early 1970s (Hessler and Jumars, 1974). Nonetheless box corers create a bow wave that can disperse meiofauna and light surperficial sediments, resulting in underestimates of meiofauna and lost “fluff” layer from the sediment-water interface. A multiple corer that is engineered to enter the sediment slowly and produce no bow wave was therefore developed to resolve this problem (Barnett *et al.*, 1984). Very recent comparisons of macrofaunal samples collected with box corers and multiple corers reveal the alarming finding that box corers may underestimate macrofauna in several instances (Horton *et al.*, 2003). A large multiple corer (megacorer) may represent the next generation of sediment sampler. A distinct disadvantage with all such samplers is that samples are collected blindly, and the immediate neighbouring spatial environment is not known. In mixed environments or areas with rough topography, sampling with bulky and not highly targeted equipment may be particularly difficult. One solution is to combine a corer or grab with a video system (e.g. Mortensen *et al.*, 2000). Another approach is to use submersibles or remotely operated vehicles (ROVs) equipped with manipulators to push small box corers or tube corers into the sediment (e.g. Grassle and Morse-Porteous, 1987). The limitation of this latter approach is the relatively small number of samples that can be collected, and the high cost of submersibles and ROVs.

For hard substrate environments ranging from bedrock to mixed gravel, towed video and photographs from submersibles (e.g. Grassle, 1985) or remotely operated vehicles (e.g. Sarrazin *et al.*, 1999) are effective for megafauna, whereas grab and suction samplers offer a mechanism for obtaining voucher specimens and sampling smaller organisms (e.g. Tsurumi and Tunnicliffe, 2001). Whereas video and still imagery can provide quantitative descriptors of biodiversity, grab and suction samples have been strictly qualitative. As with sedimentary habitats, plankton pumps and video plankton recorders can be utilized to quantify the hyperbenthos on hard substrates.

Process studies that aim to address regulation of biodiversity often require different types of approaches that are not adequately represented in the brief “snapshots” provided by trawl or video transects from discrete cruises. Bottom time-lapse camera (e.g. Lampitt and Burnham, 1983) and lander vehicles (e.g. Smith *et al.*, 2001) that remain *in situ* for extended periods can provide key data on variables such as particle flux and respiration. However, except for megafauna that can be enumerated by *in situ* cameras, a physical sample for faunal analysis is still required. At present, a ship or submersible/ROV is required to accomplish that task. Continuous access to shallow-water ecosystems has been achieved with the development of ocean observatories, fixed stations that are linked to shore by a fiber optic cable which provides power to sensors and transmits data to land (e.g. Van Alt *et al.*, 1997). Based on the same concept, project NEPTUNE, currently underway in the Northeast Pacific (www.neptune.washington.edu), will run a network of 3,000 kilometers of fiber optic cables throughout the Juan de Fuca plate at depths up to ~3,000 m, and instrument multiple areas with suites of sensors. This approach will provide two-way interactive sampling and has significant potential to advance our understanding of deep-sea diversity regulation and the role of biodiversity in ecosystem function.

One other area where new technologies have the capacity to accelerate efforts to map abundance patterns of fishes is the use of acoustics. Acoustics is being used increasingly in fisheries stock assessments and has the advantage of covering large swaths of relatively deep sea-bottom while enumerating individual fishes (e.g. Boyer *et al.*, 2001). The main drawbacks of this approach are very poor taxonomic resolution and significant underestimates of abundances of fishes living above rugged topography (e.g. Starr *et al.*, 1996).

The issue of taxonomy remains one of the great challenges in assessment of deep-sea diversity. The development of molecular techniques has greatly improved our capacity to resolve taxonomic patterns in difficult groups such as microbes (e.g. Lopez-Garcia *et al.*, 2003), and population structure in metazoans (e.g. France, 1994), as well as biogeographic relationships (e.g. Baco *et al.*, 1999). The pairing of morphological taxonomists with molecular biologists is the most effective means of moving forward in taxonomy of deep-sea organisms and ultimately improved understanding of patterns and regulation of biodiversity in deep-sea ecosystems.

6. PROCESSES – PRIORITY STUDIES

6.1. Slope environments – Process studies

Deep-sea continental slope studies in the Mediterranean should focus on several key areas. Description of bathyal patterns of diversity (see section 4.1 above) should be complemented by process-oriented studies that address why patterns of diversity exist and what factors are responsible for these patterns.

Patterns of diversity and why they exist

The Mediterranean offers an ideal environment in which to test the role of seabed heterogeneity, both in terms of morphological features such as canyons and slump areas, as well as biological features such as feeding disturbances and patches of organic matter. This question requires combined observational efforts and experimental approaches, where effects of disturbance and organic input are manipulated at small scales in order to infer how broad-scale variation in disturbance and organic input regimes influence maintenance of biodiversity pattern. Particularly for experimental work, the Mediterranean has a tremendous advantage over most deep-sea settings; the proximity of research laboratories and scientific expertise to slope environments facilitates repeat visits to recover experiments and resample areas to a degree that has never been achieved elsewhere.

The relatively small scale of the Mediterranean in comparison with ocean basins also facilitates comparison of local *versus* regional diversity and rates at which species turn over spatially. This question is wide open for all deep-sea systems, but is more tractable in the Mediterranean than elsewhere because of the proximity of environments. Specific questions include: How do slope species compare between the Eastern and Western Mediterranean, and how does this relate to organic input? Do rates of species turnover change with depth in contrasting these regions? Can experimental manipulation of organic input alter species composition to make local compositions similar among regions? Does the comparatively warm Mediterranean deep sea influence rates of species change with depth, since the temperature variable is significantly reduced in importance? Are there physiological differences in the temperature tolerances of species inside and outside the Mediterranean, and can this explain diversity patterns? Does this relationship affect affinities of different depth slope communities with Atlantic fauna?

Biogeographic links to the Atlantic

An obvious question regarding geographic patterns in Mediterranean slope communities is the degree to which they are “self-seeding” or dependent upon colonizers supplied from the Atlantic, and whether the degree to which this happens is depth-dependent. This work feeds in directly to abyssal communities (see below). A two-pronged approach is warranted. First, molecular studies could help address the distinctness of Mediterranean organisms. A more complex, but intriguing approach, would be to look at flux of reproductive propagules through the Strait of Gibraltar. How does self-seeding vary from one taxonomic group to the next, particularly as a function of reproductive mode?

Time series

The deep sea is perhaps the most deficient marine environment in terms of time series data to evaluate role of processes that change on seasonal or decadal basis. The use of cabled observatories in shallow water areas (e.g. LEO-15; www.marine.rutgers.edu/mrs/) is now being extended into the deep sea through international collaboration (www.Neptunecanada.ca, www.neptune.washington.edu), and a similar approach in the Mediterranean could help to address how seasonal flux of organic and inorganic material to slope environments influences patterns of diversity. Long-term changes, such as those caused by climate change or changes in land use practices (e.g. hydrology changes) would also benefit from this type of approach. The importance of long-term time series data is becoming increasingly apparent in all ecosystems as human footprints expand, and the deep sea is no exception (see CIESM Workshop n°22, 2003). Such data could also address the sensitivity of slope systems to human perturbations, either land-based or related to seagoing activities. Although a cabled observatory would provide the most temporally comprehensive data set to address these questions, the proximity of slope environments to multiple marine laboratories does suggest the alternative approach of establishing long-term monitoring stations that could be visited on at least a yearly basis.

Biodiversity and ecosystem function

Slope environments receive higher levels of organic input than abyssal regions, and may therefore play a more critical role in remineralization or organic matter. Natural patterns in diversity within the Mediterranean could be utilized to conduct experimental studies that examine the effects of diversity on rates of carbone metabolism and nutrient efflux from sediments.

Historical issues

The unusual geological history of the Mediterranean also offers opportunities to utilize paleo-oceanographic records to enhance our understanding of modern distribution patterns. Taxa particularly well preserved in geological records (e.g. Ostracoda, Foraminifera, Mollusca) could be particularly enlightening.

6.2. Abyssal plains – process studies

Once the basic patterns of species richness, dominance, endemism and community structure are known in relation to environmental gradients, then more specific sampling and experimental programmes can be formulated to study critical processes, such as downslope migration and speciation processes; whether the reduced diversity of the Mediterranean Sea has an effect on ecosystem functioning; or whether the Mediterranean Sea is more vulnerable to anthropogenic and catastrophic (e.g. turbidite) impacts because of its reduced biodiversity?

A way forward is to develop Mediterranean deep sea biodiversity research within the framework of the Census of Marine Life (CoML) international research programme. This project has a specific field programme to compare species richness, species distributions, dominance and community structure in major abyssal basins in all the World's oceans. A CoML workshop was held recently to coordinate research in all deep-sea sediment environments. Its report (Horton, Wigham and Billett, in prep.) will be made available on : www.coml.org. Mediterranean researchers could contribute unique data to this programme, thus allowing to compare the diversity of the Mediterranean region with the major Atlantic abyssal plains.

6.3. Zooplankton

The role of deep-sea zooplankton within the ecosystem should be assessed by determining the synecological significance of functional groups and key species for remineralisation processes. Energetic and somatic material fluxes have to be determined to estimate the role of these faunal elements in biogeochemical cycles. Remineralisation processes are difficult to observe and quantify, and they remain poorly characterized throughout the entire water column. The depth dependence of nutrient remineralisation and of the controlling factors are important features of biogeochemical cycles. The character of organic and inorganic material transported to the deep-sea reflects the ecological structure in the upper ocean and influences the remineralisation rate. Furthermore, the sensitivity of the deep pelagic community to climate change has to be investigated to predict changes in remineralisation at depth and feedback mechanisms to the surface. To obtain such data, needed for individual-based models and in oceanic and global material flux budgets, *in situ* measurements and observations and experiments under *in situ* conditions are necessary.

6.4. Canyons

The deep-sea grounds of the western and central basins of the Mediterranean exhibit several submarine canyons of varying size that may form an important link between the coastal zone and the deep sea zone. These canyons may act as conduits of coastal detritus (organic and inorganic) to the deep-sea grounds (Gardner, 1989). However, geological and biological processes related to canyons remain largely unstudied and consequently poorly understood. Enhanced productivity (Macquart-Moulin and Patriti, 1996; Vetter and Dayton, 1998) and the local currents related to canyons may play a role in larval drift and juvenile recruitment because of the relationship between canyons and the formation of intermediate nepheloid layers (Company and Sardà, 1997; Puig *et al.*, 2001).

6.5. Extreme environments

6.5.1. Cold seeps

In these extreme environments, some of the most interesting processes occur at the organismal level. For example, molecular, enzymatic, and physiological adaptations can be expected in relation to the low oxygen concentration and the possibly toxic levels of compounds such as H_2S, CH_4 and trace metals in the seeping fluid, and to the extremely high salinity of the fluids in brine pools. Since the communities in these habitats are most likely chemosynthetically fueled, the understanding of chemosynthetic metabolic pathways in the microbes, and of microbe-metazoan interactions, is imperative. In cases of symbiosis, modifications in the metabolic pathways of the metazoans are most likely present. The co-evolution of the host-symbiont complex has probably resulted in efficient mechanisms of nutrient uptake and delivery by the host to the symbiont, and of carbohydrate reduction and distribution from the symbiont to the host. Trophic strategies other than symbiosis, such as grazing, detritivory and suspension feeding, will also be present, perhaps adapted to the source, density and availability of particulates on the substratum and in the water column.

Interestingly the structure and function of populations in these chemosynthetic environments are strongly influenced by fluid-fauna interactions. Spatial and temporal patterns in the distribution of metazoan species are directly related to the spatial pattern and temporal fluctuations in fluid flux (e.g. Juniper and Sibuet, 1997). However, faunal individual growth and population proliferation can alter (or even inhibit) the direction and rates of fluid flux, thus deteriorating access to their required source of nutrition.

Trophic structure, carbon and material flows are particularly interesting in these extreme environments. While most of the carbon that flows through the ecosystem originates chemosynthetically, photosynthetically-derived carbon also enters in the form of sinking particulates, such as phytodetritus, marine snow, fecal pellets, as well as dead plankton and fish. The relative importance of the two sources of carbon in these systems is not understood and should be investigated.

Chemosynthetic environments, such as cold seeps and brine pools, are patchily distributed on the ocean floor and can be separated by distances of hundreds to thousands of kilometers (Sibuet and Olu, 1998). Additionally, most organisms that inhabit these environments are highly endemic and sessile (Sibuet and Olu, 1998). Thus the processes of larval dispersal, colonization and recruitment on which little is known (Young, 2003), are of particular interest in these habitats. Most likely the larval life history stage lasts for periods of weeks to months, and dispersal occurs along the direction of the dominant flow, since most metazoan larvae are weak swimmers. It is possible that other chemosynthetic environments, such as wood and whale falls, are used as stepping-stone habitats to extend the dispersal range of these larvae (Smith *et al.*, 1989, Sibuet and Olu, 1998; Smith and Baco, 1998; Distel *et al.*, 2000). Molecular markers are extremely useful tools in determining range of dispersal through patterns in relatedness (e.g. Vrijenhoek, 1997). The processes of invertebrate colonization and recruitment, and of vertebrate migration have not been studied in these systems to date. Understanding their importance will allow a better assessment of connectivity among these chemosynthetic, extreme environments.

6.5.2. Brine pools

Very little is known concerning the biological changes that characterise the DHABs. Different hypotheses have been made about the origin of the bacteria inhabiting the Basins that could (i) sink with the particulate matter through the upperlying water column, accumulate first at the interface, and then continue a slow flowing through the brine layers; (ii) precipitate during landslide of the DHABs surrounding oxygenated sediments; (iii) descend from populations that became trapped in salt when the evaporites formed (Hübner, 2002; McGenity *et al.*, 2000).

The DHABs are undersampled with respect to bacterial functional diversity so that, in spite of the evidence for diagenetic production of several elements (e.g. sulphur, iodine, phosphate) (Henneke *et al.*, 1997; Hübner, 2002; Luther *et al.*, 1990; Ullman *et al.*, 1990), nothing is known about the bacterial intervention in the DHABs biogeochemical cycles. The bacterial phylogenetic diversity of four DHABs was investigated by means of 16S rDNA based analyses and the results suggested the presence of novel *Bacteria* and *Archaea* thriving under extreme environmental conditions. Interestingly, some correspondence with similar environments (Orca and Cariaco basins in the Gulf of Mexico; Shaban and Kebrit basins in the Red Sea) has been found concerning several eubacterial taxa, mainly members of the g, d, and e subdivisions of the *Proteobacteria*, and archaeal sequences felling into the marine groups of the *Crenarchaeota* and *Euryarchaeota*. Compared to the other hypersaline anoxic basins, the DHABs and therefore the Urania Basin harbour a much higher diversity of unknown sequences, most of which belonging to new candidate divisions.

Up to date, neither representatives of the novel detected bacterial taxa nor the majority of deeply branching e-*Proteobacteria*, and the marine groups of the two above-mentioned archaeal kingdoms have ever been obtained in culture. Therefore their metabolic features and their role within their natural source ecosystems are still unknown. Evidence of the active role of some novel Bacterial and Archaeal taxa (e.g. KB1-related clones, similar to those obtained from the Kebrit Deep basin) natural microbial populations has been obtained by preliminary RT-PCR based analyses of the whole community 16S rRNA at the interface of the Urania Basin. The presence of prokaryotic activities in all the sampled layers (interface and brines) of the four DHABs has been detected by measurement of functional hydrolytic ectoenzymes activities, bacterial biomass production and ^{14}C-glutamic acid assimilation and respiration rates.

7. ARE DEEP-SEA RESOURCES SUSTAINABLE ?

At a time when the Mediterranean Sea is confronted to unprecedented, cumulative “assaults” ranging from global climatic change to oil drilling, from overfishing to massive pollution, it is no longer possible to simply brush off the question.

7.1. Hydrological change

Changes in surface water hydrology will also affect intermediate and deep-water masses on decadal to centennial scales. Because it is a sensitive ecosystem (Turley, 1999), hosting a deep-sea fauna well adapted to live under a combination of high pressure, high temperature and high salinity (see Scotto di Carlo *et al.*, 1984), the Mediterranean Sea is particularly worth monitoring to detect early warning signals of change. Hydrological data collected in past decades from the surface down to the greatest depths in many areas, plus several one-year time series collected since 1999 (as deep as 3,400 m) in the Tyrrhenian and the Ionian sub-basins, point with few exceptions to a continuous increase in both temperature and salinity. Whether this is a long-term trend, whether it reflects climatic changes at a Mediterranean scale (see Bethoux *et al.*, 1999), at a global scale via the influx of Atlantic water at Gibraltar (C. Millot, pers. com.), or is driven more directly by man through changes in the water budget following river diversions and daming in Mediterranean riparian countries (Bryden *et al.*, 1994), are open questions. The new Hydrological Programme launched by CIESM will hopefully cast light on this issue, through a grid of autonomous CTD stations deployed in key areas of the Mediterranean Sea – straits, channels, zones of dense water formation, deeper areas (see CIESM Workshop n°12, 2002 and http://www.ciesm.org/ people/task2.html).

7.2. Pollution

Massive urban development, industrialization and touristic development on its shores, fast-growing maritime transport, plus oil, mineral and gas exploration in its deeper areas, all seriously threaten the quality of Mediterranean waters. Many international Agreements – notably the 1978 Barcelona Convention – Protocols and Action programmes aim to regulate the management of Mediterranean pollution. Yet deep-sea environments are not included into the available legislation, despite evidence (see Fig 2; and Galil *et al.*, 1995) that litter can reach the Mediterranean seabed at great depths. In general, the impact of new types of contaminants – primarily persistent, hydrophobic compounds that are nonvolatile, relatively insoluble, and resistant to biodegradation – on the deep sea ecosystem remains unknown.

Developing oil and gas exploration in the deep waters off the southeastern Mediterranean coast is a cause of further concern. Unfortunately no information is actually available on the natural oil biodegradation mechanisms active at the deep-sea layers as was highlighted during the recent, dramatic heavy-fuel oil spill caused in 2001 by the *Prestige* accident off Galicia.

Exploration and production activities place stresses upon the offshore ecosystems: gas production platforms are point sources of chronic contamination and disturbance. Drilling discharges are composed of cuttings and drilling fluids. The large cuttings descend directly to the bottom,

Fig. 2. Solid waste collected from a trawl conducted at 2,200 m deep off the Peloponese, Greece, during the DESEAS campaign (2001).

where in areas of relatively low current speed, piles of up to 30 m in diameter and 1 m in height have been observed (Zingula and Larsen, 1977). The effects of drilling discharges on the benthic biota can be profound. The biota in the immediate vicinity of the operation is smothered by the rapid accumulation of the cuttings. Those mounds of cuttings alter the local physical environment. The cuttings may be later colonized by a variety of organisms, many of which are capable of living only on hard substrates and therefore are not naturally present in the area.

Deep-sea tailings placement of mines located close to the coast is now one of several options available for disposing of mine wastes. While there is yet no generally accepted definition for deep-sea tailings placement, several proposals have been made for placing such tailings at depths below the upper mixed layer or upwelling zone. There is an urgent need to develop improved or additional evaluation and monitoring techniques to assess and demonstrate conclusively where and when this form of tailings placement is environmentally safe, where it is the preferred and lowest risk option, and where and when it is not acceptable.

Among the minerals of economic interest, the occurrence of metaliferous oxides and metaliferous sulfides in the deep ocean has long been known. Thus manganese nodules were collected by the HMS *Challenger* in 1872 and their untapped potential has generated grandiose plans ever since. Yet deep ocean mining of these minerals is still in its infancy. Polymetallic sulfide deposits are produced by seawater circulation through the hot volcanic rocks which well up along spreading ridges of the oceans and back-arc basins. Upon coming in contact with cooler water, the minerals precipitate, producing mineral deposits of zinc, copper, lead, barium, silver, and gold in widely varying proportions in sulfides and oxides. Some deposits in heavily sedimented environments appear to contain several millions of tons of ore, which compare well with some of the largest massive sulfide deposits that are being mined on land. Except for the Atlantis II Deep in the Red Sea, none of the deposits have been surveyed and sampled sufficiently to determine their grade and tonnage. Deposits are known from about one hundred locations in the Pacific, two in the Atlantic, one in the Mediterranean, one in the Indian Ocean, and in several "deeps" of the Red Sea. Today there are many specialists who believe that polymetallic sulphides will be extracted from the sea before mining of ferromanganese crusts and nodules takes place. While the resource is plenty, unfavorable metal prices plus problems linked to recovery, processing and environmental concerns make it unlikely that exploitation will begin soon.

7.3. Fisheries

Most living marine resources are exploited on the continental margins at depths less than 400 m, but recently deep-sea fisheries have been developed all over the world. Traditional trawl and long-line fisheries capable of reaching depths down to nearly 1,000 m have been operating in the Mediterranean Sea for many years, mainly in the western basin. These fisheries target shrimps as well as hake and sharks. In this context, the development of a deep-water fishery in terms of sustainability is a legitimate, major concern. The key questions are how deep-sea fisheries exploitation may affect the deep-sea grounds and whether Mediterranean deep-sea resources can sustain a commercial exploitation. The answers to these questions well extend beyond the biological realm.

Some Mediterranean deep-sea species may attain, at present, high biomass levels between 1,000 and 1,400 m depth, making attractive as resources to fishermen. Indeed construction of trawlers capable of working depths below 1,000 m is now getting under way. Based on limited available knowledge, it appears that the deep-sea bed is inhabited by generally slow-growing species that are less dependent on the production pulses that occur closer to the surface. Such species tend to be more specialised feeders, partly because of their morphological and functional adaptations to great depth, low light levels, and low food availability. Their populations will typically have lower densities. As these environments are subjected to more constant conditions than the coastal environment, they are presumably more vulnerable to (i.e., less able to recover from) exploitation and other anthropogenic disturbances.

8. SELECTED KEY RESEARCH QUESTIONS

By way of conclusion the following, clearly non exhaustive, list of key research questions or issues is suggested:

- How do faunal dominance and diversity vary as a function of depth across different regions of the Mediterranean ?
- What is the rate of spatial turnover of species, and how does this vary with habitat heterogeneity? Does reproductive strategy within groups influence biogeographic turnover rate?
- Is nanism – the size differential of conspecific Atlantic and Mediterranean deep sea biota – a general rule ?
- Do some areas of the Mediterranean represent "hotspots' of diversity that should merit some degree of protection from human-related disturbance? How do natural forms of disturbance, such as seasonal pulsing of food or predator feeding, influence these patterns? Can this input explain between-basin differences in pattern, as well as depth-related differences?
- Is high endemism a general pattern in the Mediterranean deep sea ? To what extent is recruitment local ? How do Atlantic, Mediterranean abyssal and slope communities influence one another in this respect? Do some regions serve as sources and others as sinks? What are the sources of reproductive propagules ?
- Can the geological record of past events in the Mediterranean tell us how patterns have changed in the past, and thus how they are currently regulated, and how global climate change and other perturbations may affect these environments in the future?

- What sampling protocols/ strategies are required to ensure quantitative collection of biological data (spatial and temporal scales)?
- How is deep-sea ecology impacted overall by surface conditions ?
- What are material and process links between chemosynthetic ecosystems and the surrounding deep-sea?
- What is the connectivity among individual chemosynthetic habitats?
- Does pressure affect contaminant toxicity to deep-sea organisms (Are deep-sea organisms any more/less sensitive to contaminants than surface water organisms) ?
- What is the bioavailability/toxicity of dissolved and particulate materials to deep-sea organisms?

Gaps in Mediterranean deep-sea megafaunal biology and fisheries

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As biomasses of predatory fish suffer serious depletion worldwide (see Myers and Worm, 2003) since the 1980s, the fishing industry has directed increasing attention to the potentially exploitable deep-sea living resources. Waters deeper than 1000 m cover around 60% of our planet and many new deep-sea habitats have been discovered in the last three decades (e.g. hydrothermal vents, cold seeps, gas hydrates and cold-water coral reefs). Some of these habitats, such as seamounts (Rogers, 1994; Koslow, 1997) and canyons (Sardà *et al.*, 1994a), have been identified as hot-spots of biological production. Even so, these ocean depths remain virtually unknown to us, and this lack of knowledge is one of the main reasons making these ecosystems highly vulnerable to exploitation (Roberts, 2002).

Although it seems obvious that "*before any attempt is made to exploit living resources anywhere in the world, prior monitoring of the state of such resources must be undertaken*", this cautious approach is failing in relation to the exploitation of deep-sea living resources, repeating the failure which decimated coastal and shelf fisheries (Myers and Worm, 2003) in the past. Thus, 40% of the world's trawling grounds are now in waters deeper than the continental shelves and many seamount fisheries have been exhausted in as short a period of time as three to four years (Roberts, 2002).

Ecological processes are one of the challenges to be addressed by marine scientists. However, with regard to megafaunal species, we have many baseline gaps in our knowledge that need to be addressed before we can even consider studying these processes. For example, we still have not described baseline aspects such as megafaunal species composition and geographical distribution in many Mediterranean regions below 1000 m depth and no data are presently available regarding the reproductive biology of many highly abundant deep-sea species. What we think we know about the physiological adaptations of these species is still at the level of hypotheses. The objective of this paper is to report on some of the gaps in knowledge that we have investigated for megabenthonic species dwelling in the Mediterranean Sea below 1000 m depths, from species community level to individual physiology going through species life histories. We consider that all these aspects need to be studied prior to undertaking any potential exploitation of these species.

GAPS IN KNOWLEDGE REGARDING THE GEOGRAPHICAL DISTRIBUTION OF SPECIES AND SPECIES ASSEMBLAGES

Data on the geographical distribution of deep-sea species and species assemblages and bathymetric boundaries are now available for many regional seas worldwide. However, this baseline level of knowledge, which is one of the first steps needed prior to undertaking any further investigations, has still not yet been carried out for most of the Mediterranean basin below 1000 m depth. Until recently, descriptive and quantitative research on these aspects had only been conducted in the north-Western Mediterranean waters. Thus, while both Spanish fishery managers and industry have started to consider developing the fishing of deep-sea species such as *Mora moro* and *Alephocephalus rostratus* (Sardà *et al.*, 2002a), we did not know until recently if these species were even present in other Mediterranean regions. A trans-Mediterranean survey (Sardà, 2001) has been conducted in three regional seas of the western and central Mediterranean down to 4000 m depth : these data, obtained using otter trawl gear, are all that are currently available on megafaunal species distribution and biomass in the Ionian Sea below 1000 m depth. As far as the authors are aware, nothing is known at sites further east in terms of deep-sea species distribution and assemblages. Figure 1 shows how the overall biomass of fish at depths from 1000 to 1300 m in the Western Mediterranean is higher than at depths above and below this bathymetric range. This relatively high abundance of fish was not found at the other two sites sampled during the same oceanographic survey (Sardà, 2001). This west-east gradient of megafaunal abundance has been correlated with the increasing oligotrophy of the central and Eastern Mediterranean waters in comparison with the western area (Company *et al.*, in press; D'Onghia *et al.*, in press).

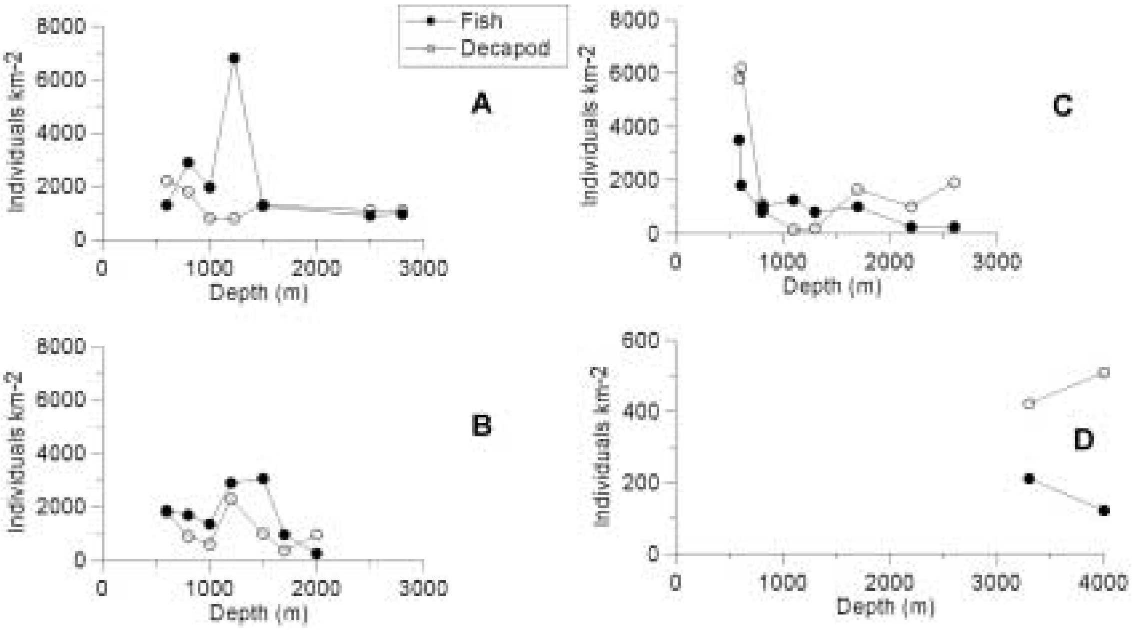
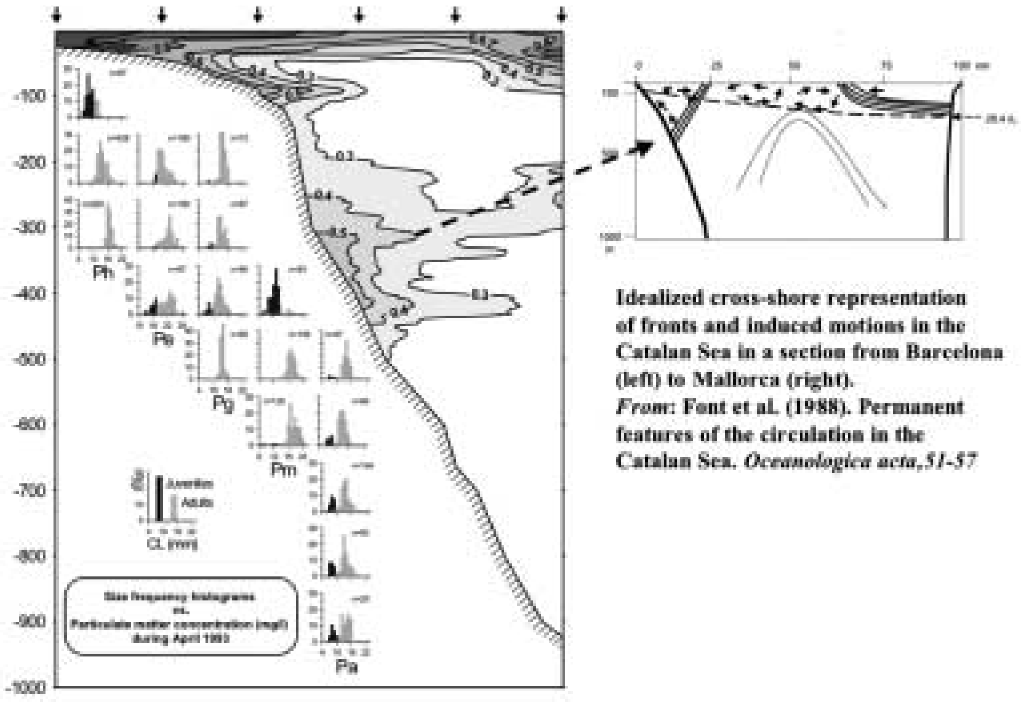


Fig. 1. Total decapod abundance *versus* total fish abundance along the bathymetric range sampled during the DESEAS oceanographic survey (as number of individuals per km^2). **A**: Western Mediterranean (Balearic sea south Majorca Island); **B:** central Mediterranean (western Ionian sea; south Taranto Gulf); **C**: central Mediterranean (eastern Ionian sea; south Peloponnesian Peninsula); **D:** abyssal site (Abyssal plain of the Ionian sea).

GAPS IN KNOWLEDGE REGARDING LIFE HISTORIES OF SPECIES AND POPULATION STRUCTURES

Information regarding the life histories of species and their population structures is of great value and must be obtained before conducting any commercial exploitation. This kind of data is another baseline research needed in order to understand some of the processes linked to the overall life histories of the species, such as larval processes (distribution and dispersion) and recruitment processes (which might help to identify nursery grounds requiring protection from fishery activity). At present, no information on any of these aspects is available for deep-sea Mediterranean species and it is even scarce for shallower dwelling species. Puig *et al.* (2001) show how the larval and recruitment processes of five deep-water pandalid shrimps seem to be related to nepheloid detachments at the continental margins along a narrow bathymetric range at around 400 m depth (Fig. 2). This nepheloid layer is related with the overall water mass circula-



Idealized cross-shore representation of fronts and induced motions in the Catalan Sea in a section from Barcelona (left) to Mallorca (right).
***From*: Font et al. (1988). Permanent features of the circulation in the Catalan Sea. *Oceanologica acta*,51-57**

Fig. 2. Distribution of nepheloid layers and population size structure of five *Plesionika* species along the continental margin in the northWestern Mediterranean Sea in April 1993, the month with the highest presence of juvenile individuals. Comparison of the histograms by 100 m depth intervals for each species revealed statistically significant differences between all depth strata in *Plesionika heterocarpus* (Ph), *P. edwardsi* (Pe), *P. gigliolii* (Pg), and *P. martia* (Pm) ($p < 0.01$, Kolmogorov-Smirnov test), but not in *P. acanthonotus* ($p > 0.05$, Kolmogorov-Smirnov test). Note that for the five species, the size of the smallest female with active gonads or ovigerous (which was considered juvenile), is located approximately between two size-class modal frequencies. Note the association between the juveniles (black bars) and the shelf-break or slope INL in all four species (Ph, Pe, Pg, and Pm) whose depth ranges spanned the nepheloid layers and the absence of any spatial trend for juveniles of the species (Pa) whose depth range did not cross either of the nepheloid layers. Arrows at the top of the cross-margin section mark the locations of the hydrographic profiles compiled from the CTD casts (from the shelf to ~ 1000 m) [n = number of individuals] (Partially modified from Puig *et al.*, 2001).

tion in the area (north-western frontal structure). These frontal structures located at around 400 m depth are very widespread in oceans worldwide (Puig *et al.*, 2001).

Other studies directed at describing the population structure of several deep-sea species indicate that nursery and recruitment grounds for a highly important fishery resource such as the rose shrimp *Aristeus antennatu*s (Risso, 1816) might be located below 1000 m depth (Sardà *et al.*, 2002b). This species is exploited along the entire Mediterranean basin at deep-water depths of between 200 and 1000 m, depending on the location. This species supports high fishing rates and no evidence of overexploitation has been reported to date. However, if fishermen start to exploit grounds below 1000 m, this species may rapidly present overexploitation symptoms due to the juveniles being captured.

The reproductive biology of Mediterranean deep-sea species is another area where scarce information is available. However, a recent study on the duration of the reproductive periods of 17 deep-water decapod species (some of them dwelling below 1000 m depth) shows that their reproductive periods are more much seasonal than those of species dwelling along the shelf and upper-slope continental margins (Fig. 3) (Company *et al.*, 2003). The shorter reproductive periods shown by these deep-sea dwelling species is an aspect of their life histories that might effect the exploitation of their populations. The reproductive output of deep-sea species could be affected if any exploitation is undertaken during their short reproductive periods and furthermore a short reproductive period might also be correlated to a lower annual reproductive output in comparison with shallower dwelling species.

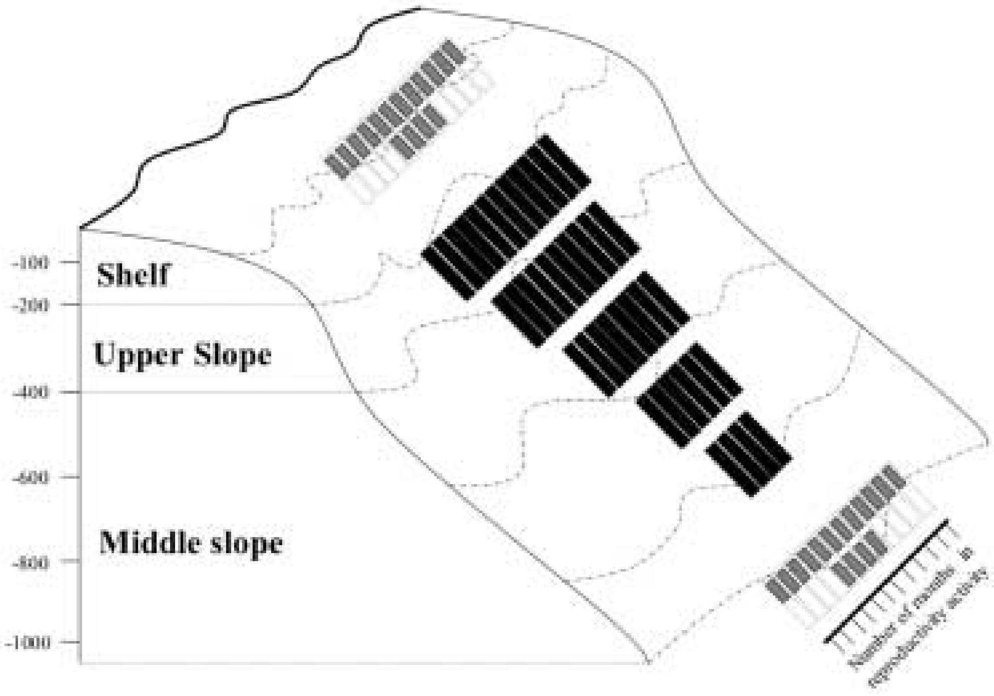


Fig. 3. Diagram of the relationship between the duration of the reproductive periods and depth of distribution of the megafauna dwelling across the continental margins of the Northwestern Mediterranean Sea. Black bars: hypothesised number of months in which species may undertake their reproductive activity supported by the data presented in this study. Grey bars: hypothesised co-occurrence of seasonal and continuous reproductive species at shelf and lower-slope depths (from Company *et al.*, 2003).

GAPS IN KNOWLEDGE REGARDING INDIVIDUAL PHYSIOLOGY

Although no information about the physiological adaptation of Mediterranean deep-sea species is available (Company and Sardà, 1998), the data available from other oceans indicate that deep-sea megafaunal species are completely unsuitable for fishery exploitation due to their peculiar biochemical and physiological adaptations (Childress, 1995; Koslow, 1997). Besides certain specific biological and physiological adaptations on endemic species dwelling at unique deep-sea habitats (seamounts and hydrothermal vents), the most widespread physiological and biochemical adaptations of deep-sea species are: 1) low metabolic rates, 2) low protein and lipid content, and 3) high water contents. Low metabolic rates allow deep-sea megafaunal species to attain high growth efficiencies (higher than many shallow species), to attain extraordinary ages (over 100 years old; see Roberts, 2002) and to attain large sizes. Low protein and lipid content and high water content are adaptations to the absence of light in these ecosystems (not to the reduced food availability, as was hypothesised until recently). Powerful muscles are not needed in an environment where predator-prey interaction is rather passive. Thus, strong swimming capabilities are not needed in these dark environments. Due to the poor musculature and the extraordinary ages attained by deep-sea species (which usually means that maturity is only attained at an advanced age), any fishery activity will obviously fail because the flesh of these fish is not pleasant to eat and because stocks will be depleted rapidly.

CONCLUSION

Ecological processes of the Mediterranean deep-sea environments are unstudied, but before even trying to understand any of these processes, we first need to study: 1) species distribution; 2) species assemblages; 3) community boundaries; 4) biomass distribution; 5) population structure; and 6) reproductive biology, among others. Aboce all, a cautious approach must be taken before proceedding to the exploitation of Mediterranean deep-sea living resources.

Differences in biomass composition and size-related structure between Mediterranean and Atlantic deep-sea fish assemblages

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There are studies available from Mediterranean and Atlantic deep-sea ecosystems on the fish community structure (e.g. Haedrich and Merrett, 1988; Koslow, 1993; Stefanescu *et al.,* 1992a, 1993; Moranta *et al.,* 1998), population ecology (e.g. Stefanescu *et al.*, 1992b) and the distribution and biology of the main species, (e.g. Mauchline and Gordon, 1983; Gordon and Duncan, 1985; Morales-Nin *et al.*, 1996; Carrasón *et al.*, 1997; Carrason and Matallanas, 1998; Massutí *et al.*, 1995).

Differences between Mediterranean and Atlantic deep-sea fish assemblages concern not only species composition (e.g. Haedrich and Merrett, 1988; Stefanescu *et al.*, 1992a), but also the maximum size of individual species (Tortonese, 1960; Stefanescu *et al.*, 1992b). However, since misleading conclusions in comparative studies can arise from the use of different methodologies, the importance of sampling gear when comparing fish assemblages must be highlighted (Merrett *et al.*, 1991; Gordon and Bergstard, 1992). Our aim is to make a standardised comparison between biomass composition and size-related structure for deep-sea fish assemblages from different areas of the Western Mediterranean (Algerian and Balearic basins) and eastern North Atlantic (Rockall Trough and Porcupine Seabight). These areas are characterized by distinctly different and well-documented oceanographic conditions, surface productivity and seasonality, biogeographical affinities, biodiversity and fishing exploitation patterns.

MATERIAL AND METHODS

Data were derived from a series of bottom trawl surveys carried out between 1978 and 1998 by different groups of researchers, studying the distribution and ecology of deep-sea benthic and benthopelagic communities in four different areas: two in the Western Mediterranean and two in the eastern North Atlantic (Fig. 1). The Western Mediterranean is a subtropical semi-enclosed area, separated from the Atlantic by a sill in the Strait of Gibraltar, with a high degree of environmental stability for both temperature and salinity below a depth of 200 m. The two sampled basins of the Western Mediterranean are characterized by different oceanographic and geomor-

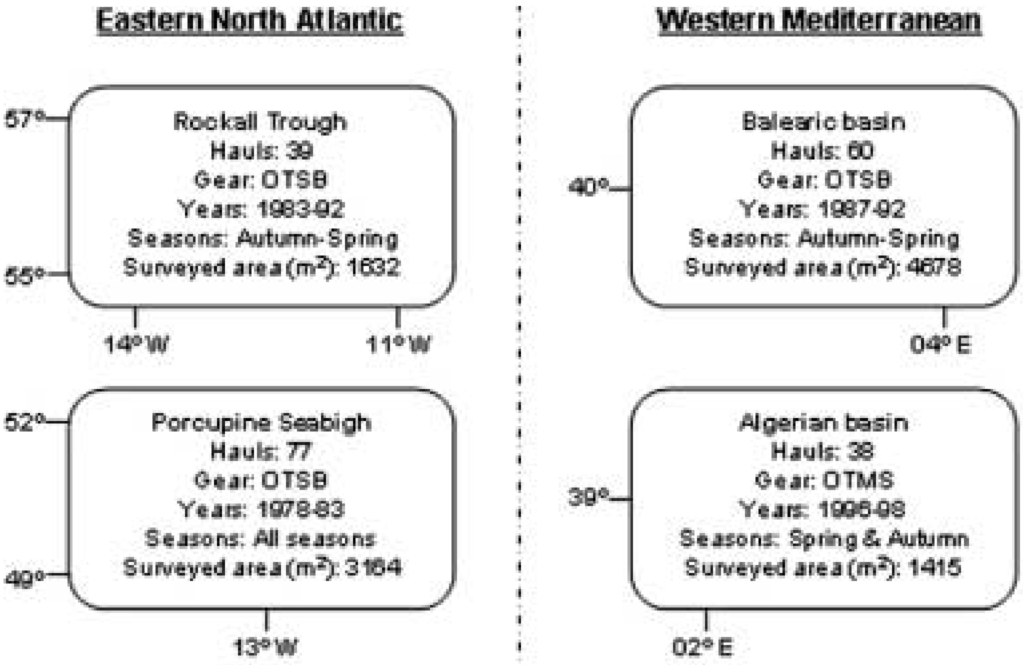


Fig. 1. Diagram showing the geographic coordinates and sampling data (number of hauls, trawl gear used, period, area surveyed) of the four study areas: Balearic and Algerian basins, in the Western Mediterranean; and Rockall Trough and Porcupine Seabight, in the eastern North Atlantic.

phological conditions (e.g. EUROMODEL Group, 1995; Beckers *et al.*, 1997) and are connected by a series of sills at depths ranging from 100 to 800 m, which play an important role in the general circulation and transport of water masses between these areas. Available estimations give the annual primary production in the northWestern Mediterranean as between 77 and 100 gCm^{-2} year^{1}, although only about 1-5 gCm^{-2} may reach the benthic ecosystems at 800 to 1000 m depth (Miquel *et al.*, 1994).

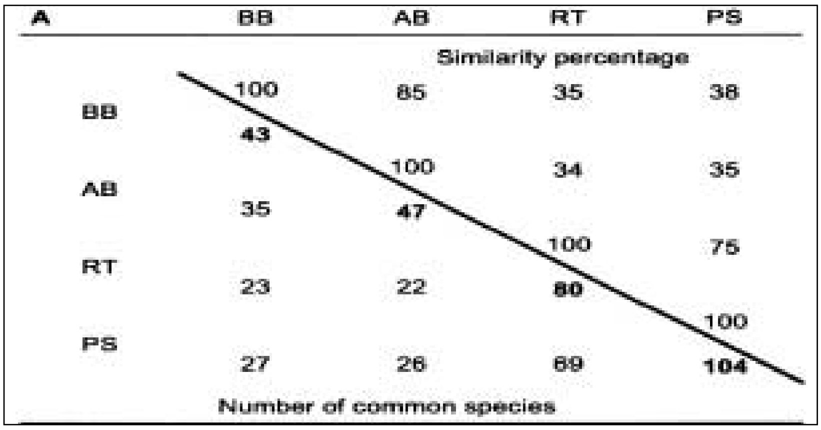
The two sampled areas in the Atlantic present a seasonal thermocline but, below this, the environmental conditions are virtually constant throughout the year, with a gradual decrease in temperature with increasing depth, from about 10°C at 600 m depth to about 3.5ºC at 2000 m depth (Ellett *et al.*, 1986; Rice *et al.*, 1991). In these areas the upper layers, down to about 1200 to 1500 m, are comprised of North Atlantic Central Water of the western Atlantic and below this lies Labrador Sea Water (Ellett *et al.*, 1986). Other water masses are detectable, notably the Norwegian Sea overflow water in the Rockall Trough and a layer of Mediterranean (or Gulf of Gibraltar) water at intermediate depths in the Porcupine Seabight. There is some evidence that the Rockall Trough and Porcupine Seabight could be a marginal oceanic region in the eastern Atlantic, where greater than average production takes place compared with adjacent regions. Although direct measurements of annual primary production are not available, the general rate for these regions appears to be within the range 60-100 gCm^{-2} year^{1}, with a vertical flux to the deeper layers of about 12-20 gCm^{-2}.

Catches from different years and seasons have been pooled, representing a total of 214 hauls taken between 400 and 1993 meters depth (Fig. 1). Samples were obtained by a single warp trawl: a OTSB-14 was used In the Rockall Trough, Porcupine Seabight and the Balearic basin, whereas in the Algerian basin the OTMS-27.5 was used. For the analysis the data were grouped into pre-established depth intervals approximately corresponding to the upper slope (400 to 800 meters depth), middle (800 to 1400 meters depth) and lower slope (from 1400 to 2000 meters depth). Standardised catch data at 1000 m^{2} were pooled for these depth intervals, and the ecological parameters of species richness, biomass and abundance were determined. Analysis of variance was used to test differences between the mean values of these ecological parameters. Biomass by size class for each assemblage was estimated following standard methodologies.

RESULTS

The number of species captured was 43 and 47 in the Balearic and Algerian basin and 80 and 104 in the Rockall Trough and the Porcupine Seabight, respectively (Table 1).

Table 1.Total number of species (in bold), common species and similarity percentage (from Bray-Curtis similarity index) between the studied areas: Balearic basin **(BB)**, Algerian basin **(AB)**, Rockall Trough **(RT)**, Porcupine Seabight **(PS)**.



Similarity percentage

A	BB	AB	RT	PS
BB	100 **43**	85	35	38
AB	35	100 **47**	34	35
RT	23	22	100 **80**	75
PS	27	26	69	100 **104**

Number of common species

The similarity percentage and common species within Mediterranean and Atlantic areas, separately, were much higher than between the Mediterranean and Atlantic assemblages. The highest density values were from the Rockall Trough, the lowest from the Mediterranean areas (Fig. 2). Biomass followed the same trends in all areas, presenting highest values in the Rockall Trough and in the middle slope in each zone. Non significance differences in biomass and abundance were noted between the Mediterranean areas. By depth groups the middle slope marked

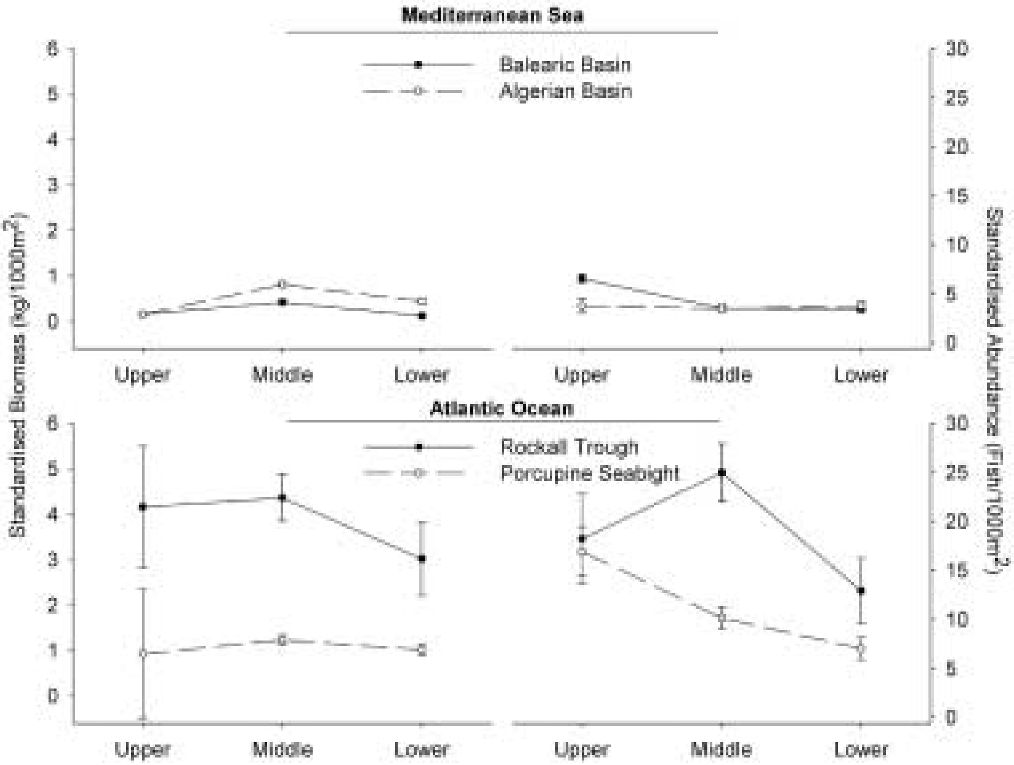


Fig. 2. Mean standardised biomass and abundance (standard error in vertical lines) obtained in the Mediterranean and Atlantic assemblage (Upper: 400-800 m depth, middle: 800-1400 m depth, lower: 1400-2000 m depth).

the difference in the case of biomass but only differences between upper and lower slope were detested in the case of abundance.

In the Mediterranean, there was no obviously predominant family over the whole depth range (Fig. 3). The dominant families were Gadidae and Scyliorhinidae in the upper slope assemblages, Moridae and Alepocephalidae on the middle slope and Alepocephalidae on the lower slope. In both Atlantic areas, the family Macrouridae was the most important throughout the whole depth range surveyed along with Gadidae and Chimaeridae on the upper slope, Moridae and Alepocephalidae on the middle slope and Synaphobranchidae on the lower slope.

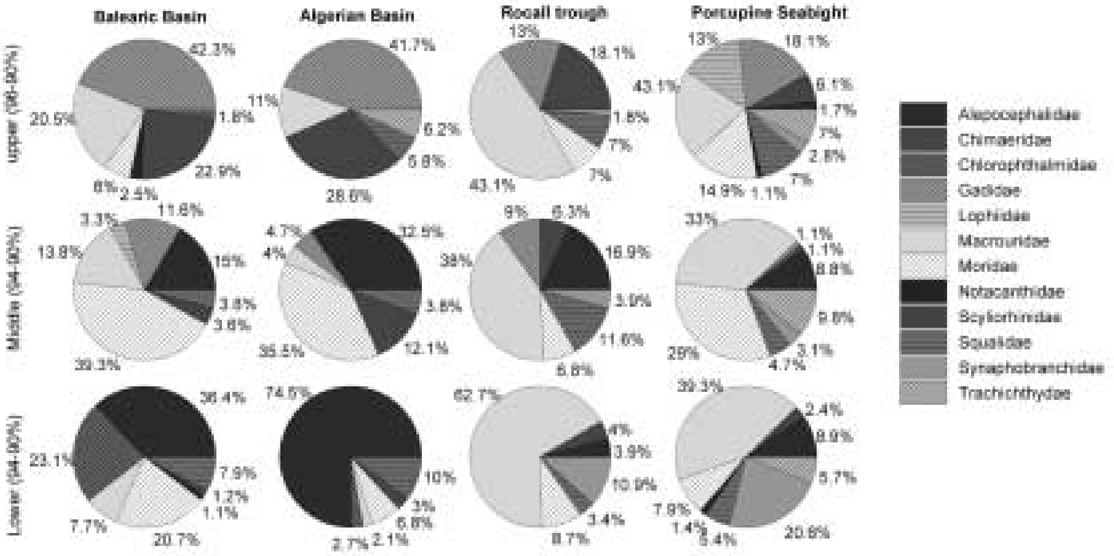


Fig. 3. Biomass composition (in percentage) for the most important families, obtained by depth-strata in each area. These families accounted for more than 90% of the total biomass (in brackets) in each assemblage.

As shown in Fig. 4, Atlantic assemblages display the largest size classes. The biomass spectra showed clear differences with the Mediterranean, except on the lower slope, where the biomass spectrum from the Algerian basin was similar to those found in the Atlantic assemblages. The largest differences were on the upper slope.

The bathymetric distribution, based on the minimum and maximum depth of occurrence, of twelve species common in the four areas studied were very similar between Mediterranean and Atlantic, except in the case of *Galeus melastomus*, *Alepocephalus rostratus*, *Coelorhynchus labiatus* and *Coryphaenoides guentheri* (Fig. 5). Clear size differences were also observed, at a species level, by comparing the length of nine species captured in significant numbers within the four areas (Fig. 6). In all cases, both maximum and mean lengths were higher in the Atlantic than in the Mediterranean. By contrast, minimum lengths were more similar, excep for *Centroscymnus coelolepis* where the minimum size in the Atlantic was at least double that found in the Mediterranean.

DISCUSSION

The standardised comparisons of this study confirm the clear differences between the deep-sea demersal fish fauna of the Mediterranean and the Atlantic, which were already suggested four decades ago by Tortonese (1960) and more recently shown, at a species level, by Stefanescu *et al.* (1992b) and D'Onghia *et al.* (1998).

The density indices were lower for the Mediterranean assemblages than for the Atlantic. Although the surface productivity on demersal megafauna is poorly understood, pelagic production and its arrival on the sea floor has been reported as one of the most important sources of organic matter for deep-sea ecosystems. Similar ranges of surface productivity have been esti-

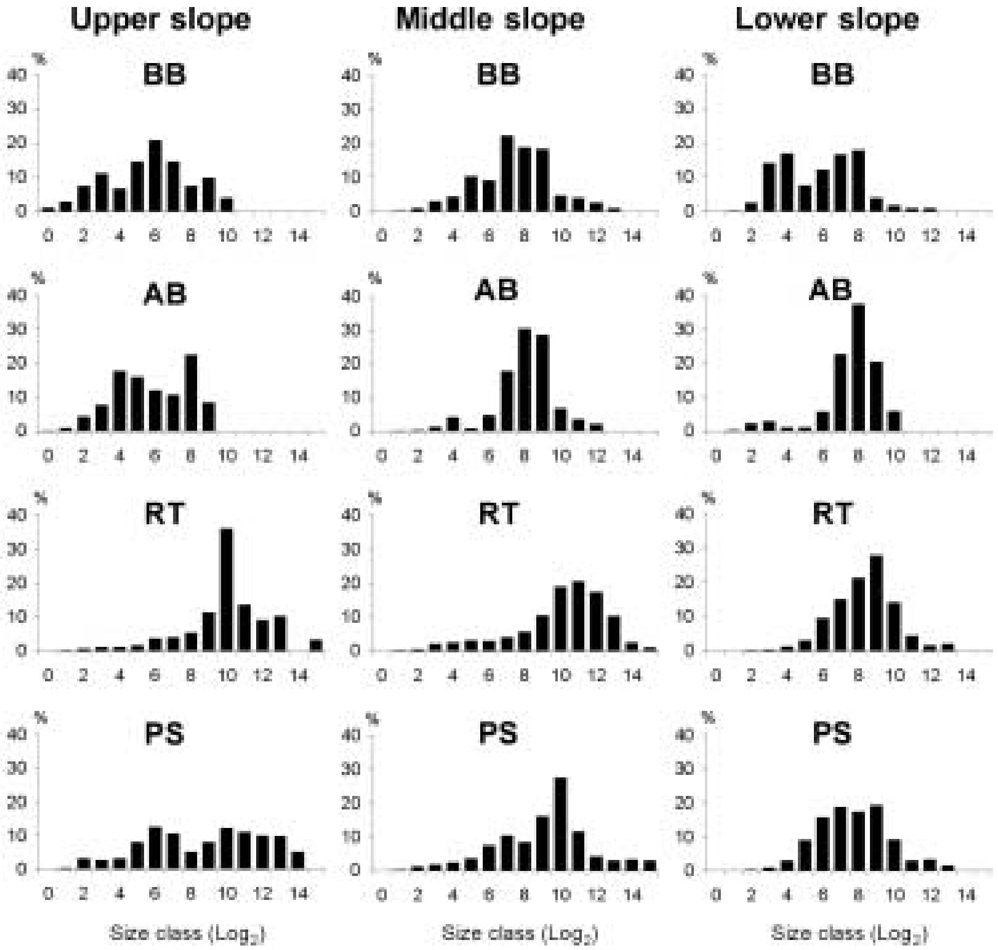


Fig. 4. Percentage of total biomass by size-class and depth-strata in the four studied areas: Balearic **(BB)** and Algerian **(AB)** basins, in the Western Mediterranean; and Rockall Trough **(RT)** and Porcupine Seabight **(PS)**, in the eastern North Atlantic (Upper slope: 400-800 m depth, middle slope: 800-1400 m depth, lower slope: 1400-2000 m depth).

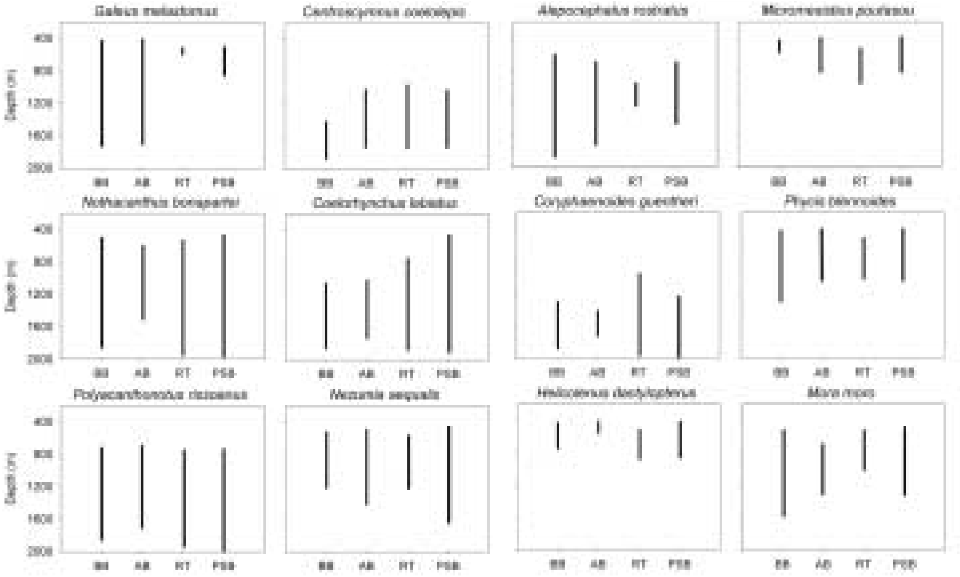


Fig. 5. Bathymetric distribution of twelve common species captured in the four studies areas: Balearic **(BB)** and Algerian **(AB)** basins, in the Western Mediterranean; and Rockall Trough **(RT)** and Porcupine Seabight **(PS)**, in the eastern North Atlantic.

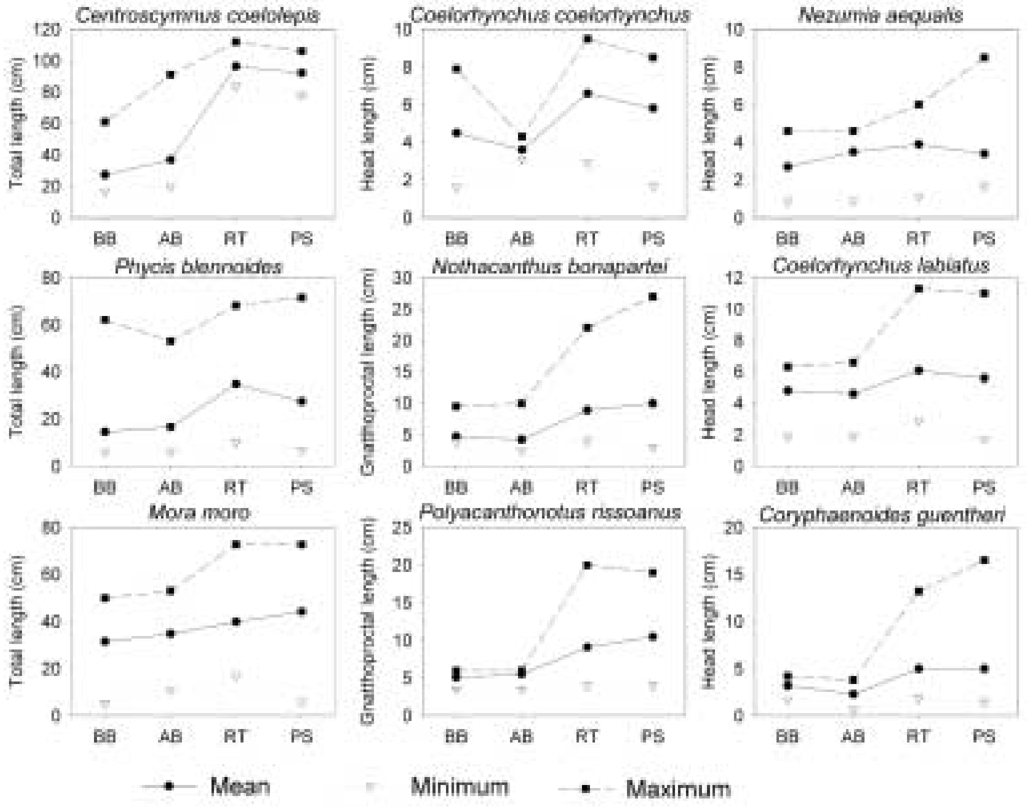


Fig. 6. Average (arithmetic Mean), minimum and maximum length of the species appearing in the four assemblages (**BB:** Balearic basin; **AB:** Algerian basin; **RT:** Rockall Trough; **PS:** Porcupine Seabight), in which more than ten specimens could be measured

mated in the studied areas but the availability of organic matter on the Atlantic seabed which is an order of magnitude higher than in the Mediterranean, could contribute to the differences in density of fish fauna between both areas. In our opinion, three main factors could contribute to the differing amounts of energy reaching the seabed in both areas: (i) efficient transfer of surface production via overlapping food chains and vertical migration of preys. In the eastern North Atlantic this energy transfer mechanism has been estimated to reach about 1500 m depth but only about 1000 m in the Western Mediterranean; (ii) water temperature: the higher temperature below 200 m in the Mediterranean could contribute to a faster decomposition of the organic matter before its arrival on the sea floor as food source; and (iii) different food-web structures in both areas. In the Rockall Trough and Porcupine Seabight the abundance of epipelagic species (e.g. *Scomber scombrus* and *Trachurus trachurus*) tends to be confined to the continental shelf. By contrast, the great abundance of large and middle-sized pelagic fishes in the oceanic waters of the Mediterranean (e.g. dolphinfish, swordfish and tunas), which are likely to exploit production in the surface layers, could result in a lower flux of energy to the seabed in this area. Moreover, in the Western Mediterranean, decapod crustaceans represent an important or even dominant group at the upper slope – to a greater extent than in the North Atlantic – and they could also exploit trophic resources in Mediterranean deep-sea ecosystems.

Atlantic assemblages are composed of a larger number of species than those of the Mediterranean. The more constant physical environment and the recent origin of the deep-sea fauna of the Mediterranean (Pérès, 1985) probably accounts for the fewer number of species. In the Atlantic, the Porcupine Seabight has a greater number of species than the Rockall Trough. In addition to biogeographic factors, such as some affinities with the northwest African slope, where there is a high diversity in some families, and the presence of some species attributed to the influence of Mediterranean water clearly identified in this area, the proximity of the slope of the Porcupine Seabight to abyssal depths also accounts for a high species number in this area, as demonstrated by the capture of juveniles of abyssal-living species (e.g. *Coryphaenoides leptolepis* and *Coryphaenoides carapinus*).

There are some very clear differences in the families that dominate the biomass between the Mediterranean and the Atlantic. In the Mediterranean, a different family dominates each bathymetric zone: Gadidae on the upper slope, Moridae on the middle slope and Alepocephalidae on the lower slope. By contrast, in the Atlantic the family Macrouridae is dominant across bathymetric gradient. The different food availability and the partitioning of the main trophic resources within these ecosystems may explain some of these differences. The dominant Macrouridae in the Atlantic feed on small macroplankton, probably at some distance above the seabed. However, on the upper and middle slope of the Mediterranean, this trophic resource may also be used by benthopelagic decapods, which in turn could provide food for the dominant fish or/and for their prey.

The biomass spectra show clear differences between Mediterranean and Atlantic assemblages where, respectively, small-middle and middle-large size classes predominate. Where the same species occurin both areas, those in the Mediterranean tend to attain a smaller adult size. These differences were greatest on the upper slope, most likely related to the fishing exploitation of this bathymetric range in both Mediterranean areas. Fishing exploitation s known to be an important factor affecting distribution of biomass within benthic fish assemblages (Macpherson and Gordoa, 1996).

The small size of Mediterranean deep-sea fishes has already been discussed by Tortonese (1960) and more recently by Stefanescu *et al.* (1992b). According to these authors, it is evident that this phenomenon is very complicated and results from a combination of factors of disparate origin (e.g. latitudinal gradients on fish size, ecological factors affecting body size). More limited resources and an elevated temperature in the Mediterranean (~13ºC compared with 10 to 4ºC in the eastern Atlantic areas) are also likely to play an important part.

The differences found in demersal deep-sea fish assemblages between the Mediterranean and the Atlantic reinforces not only the general view of the Mediterranean deep-sea as a poor ecosystem (e.g. Gage and Tyler, 1991; Bouchet and Taviani, 1992), but also that important differences exist in the size-related structure of the fish populations. The primary cause of the differences in size structure is probably a result of adaptations at both the species and higher level to different conditions. The historical evolution of the Mediterranean basin, its geomorphology and high environmental specificity are additional factors.

Deep-sea biodiversity in the Mediterranean Sea: a comparison at different spatial scales

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Earlier indications that depth-related marine biodiversity patterns are unimodal, with a peak at intermediate depths and lower diversity values at upper bathyal and abyssal depths (Rex, 1981; Etter and Grassle, 1992), are contradicted by an increasing number of studies which indicate that such patterns are not universal (Rex *et al.*, 1997; Stuart *et al.*, 2001).

If the distribution of biodiversity in the deep sea is far from being clarified, even more uncertain is our comprehension of the mechanisms driving biodiversity attributes and distribution. Among the factors more frequently invoked for explaining deep-sea biodiversity patterns, the following variables/processes have found experimental and field evidence: a) sediment grain size and substrate heterogeneity; b) productivity, organic content and/or microbial features; c) food resources; d) oxygen availability; e) current regimes and f) catastrophic disturbance (see Lambshead *et al.*, 1994; Danovaro *et al.*, 1995; Paterson and Lambshead, 1995; Cosson-Sarradin *et al.*, 1998; Levin *et al.*, 2001; Lambshead *et al.*, 2002). However, these factors are in most cases site specific and constrained by local (or regional) conditions. Moreover, when attempts are made to develop general rules, all these factors are subjected to a strong debate and "deep" criticisms.

Deep-sea biodiversity obviously lacks a general theory explaining observed patterns (Ellingsen and Gray, 2002). Such lack is also the result of the limited data set available. In particular, there are very few studies investigating biodiversity patterns at different spatial scales (Clarke and Lidgard, 2000; Izsak and Price, 2001; Ellingsen and Gray, 2002), especially in the deep sea (Lambshead and Boucher, 2003). In contrast, much information is available on terrestrial ecosystems, where it has been concluded that local species richness is linearly related with regional richness (Griffiths, 1997; Ricklefs, 2000; Cornell and Karlson, 1996; Rex *et al.*, 1997). As we still do not know whether biodiversity patterns respect the same rules in the deep sea, it becomes essential to investigate benthic biodiversity at different spatial scale in this ecosystem, the largest on Earth.

The Mediterranean Sea can be considered the optimal environment for such studies since it behaves as a miniature ocean, able to rapidly respond to all environmental clues. Here we used nematodes (the most abundant organisms on the deep sea, representing ca 80-90% of total meio-macrofaunal abundance; Heip *et al.* 1985), which account for 60% of metazoans on Earth, as a model for investigating biodiversity patterns in the deep Mediterranean Sea.

In order to investigate nematode biodiversity at different spatial scales we collected sediment samples according to a hierarchical strategy at local, meso- and regional scales. This approach was conducted in both the deep Eastern and Western Mediterranean basins at ca 3000 m depth in two sampling periods (spring and autumn). Sediment samples were collected with Multi-Corer and/or USNEL-type box-corer.

We define: i) biodiversity “at local scale” as the biodiversity measured on samples collected at a distance <7 km; ii) biodiversity “at mesoscale” as the biodiversity measured on samples collected at a distance of ca 30 km; and iii) biodiversity “at regional scale” as the biodiversity measured on samples collected at a distance between sampling areas of >1000 km.

The analysis of these samples revealed that meiofauna was characterised by low densities in the deep Mediterranean Sea, at ca 3000 m depth, with values five times higher in the Western than in the Eastern Basin (102.3±6.0 ind 10 cm^{-2} and 22.0±8.0 ind 10 cm^{-2} respectively). Nematodes, as expected, dominated meiofauna assemblages, accounting for 73-96% in the Western basin and 83-91% in the Eastern basin.

Overall 226 species (belonging to 34 families) were identified: 124 genera and 168 species were found in the Western Mediterranean *versus* 39 genera and 46 species in the Eastern Mediterranean. The most abundant families were Desmoscolecidae, Chromadoridae, Xyalidae, Oxystominidae, Leptolaimidae, Cyatholaimidae, Comesomatidae. Interestingly, the families Leptolaimidae, Draconematidae, Ethmolaimidae, Haliplectidae, Pandolaimidae, Paramicrolaimidae, Siphonolaimidae, Trefusidae were encountered only in the deep Western Mediterranean. In constrast, the genera *Adoncholaimus, Araelaimus, Axonolaimus, Epacanthion, Lauratonema, Micoletzkyia, Rhynchonema* were exclusively encountered in the Eastern Mediterranean.

Nematode biodiversity was lower than in other equally deep sediments world-wide (Jensen, 1988; Tietjen, 1984, 1989), and lower than in values reported for canyons (down to 1500 m depth) of the Mediterranean (Soetaert *et al.*, 1991).

In the Western Mediterranean, independently from the diversity indices utilised, biodiversity distribution at mesoscale reflected biodiversity at local scale. The different values and distribution in nematode diversity in the Western and Eastern Mediterranean may be related to the different amounts and spatial distribution of the food sources.

Conversely, independently from the spatial scale considered, the evenness (J) did not change significantly between Eastern and Western basins, suggesting a rather high homogeneity of individual nematode distribution among species in the deep Mediterranean Sea.

β-diversity, here defined as the turnover diversity (i.e., the percentage of species similarity among different sampling areas) was very high. The SIMPER analysis (Primer 5.0) revealed that the similarity coefficient among stations at local and meso-scale ranged from ca 30 to 50%, while at regional scale (i.e., between Eastern and Western basins) it ranged from 3 to 40%. Moreover, the ANOSIM analysis of our data indicated that species composition in the Western and Eastern basin was significantly different.

Functional diversity was investigated by means of the Maturity Index (MI) and of the trophic diversity. The Maturity Index was slightly higher in the Western than in the Eastern basin, suggesting a different ecological strategy: in the Western Mediterranean, the larger food availability allowed the coexistence of a higher number of species belonging to the ecological group of “persisters” (comparable to K-strategists). Conversely, in the Eastern Mediterranean, the highly fluctuating availability of food resources resulted in a higher number of “colonisers” (nematodes comparable to r-strategists; Bongers and Ferris, 1999).

Also nematode trophic structure and diversity changed significantly between the Western and Eastern Mediterranean. In the Eastern Mediterranean, trophic structure changed significantly with values depending on food availability, and predator nematodes (Wieser, 1953) usually of large size, were far more abundant than in the Western Mediterranean, where trophic composition did not change between seasons.

Organic loads (and, more generally, food availability) are an important factor for sustaining a high species number (Levin *et al.*, 2001) and could influence the ecological strategy of the species investigated (Bongers *et al.* 1991; Bongers and Ferris, 1999; Levin *et al.*, 2001; Gambi *et al.*, 2003). Although both areas were characterised by oligotrophic conditions (as suggested by the low quantity of organic matter available in the sediments; Danovaro *et al.*, 1993, 1998, 1999, 2000) significant differences can be observed comparing the two basins. The Western Mediterranean displayed higher concentrations and an “homogenous” distribution of labile organic compounds (i.e., available food for consumers), whereas the Eastern Mediterranean displayed lower concentrations and a more heterogeneous distribution. In both areas all the diversity indices changed significantly with season according to changes in the food supply, suggesting that the variation of food availability between sampling periods can influence species number (Fig. 1).

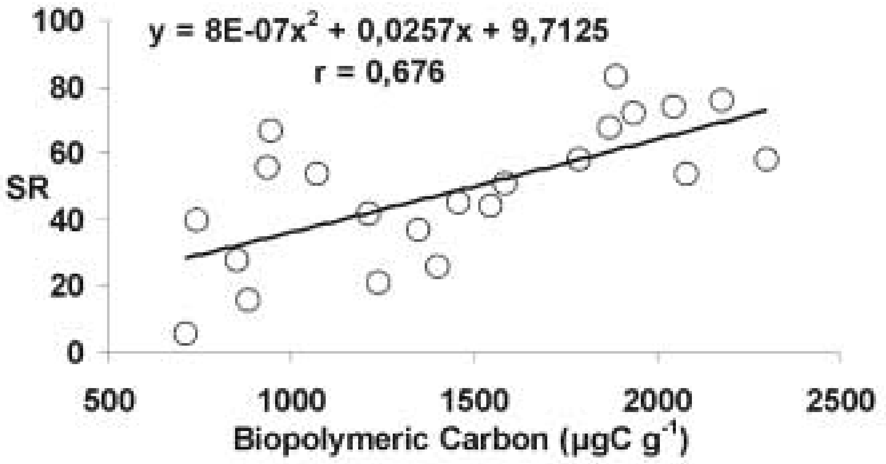

Fig. 1. Relationship between species richness and amounts of available food in the sediment.

For instance, in the Eastern Mediterranean the increased organic matter content observed from spring to autumn determined not only the increase of species number but also a clear species substitution. These results suggest that food availability might by the key factor regulating spatial distribution and temporal changes in nematode biodiversity in the deep Mediterranean.

Deep-sea zooplankton ecology of the Eastern Mediterranean. State-of-the-art and perspectives

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The Mediterranean Sea is a semi-enclosed basin in an arid region. Due to its anti-estuarine circulation and low external nutrient supply from the atmosphere or rivers, the production decreases from west to east. In the western part of the Basin, phytoplankton and bacterial production is up to three times higher than in the Eastern Mediterranean Sea (Turley *et al.*, 2000), which is one of the most oligotrophic marine regions in the world (Dugdale and Wilkerson, 1988). The deep water body in this basin deviates from the open ocean (2-4°C, 35 PSU) by higher temperatures (>13.3°C) and higher salinities (around 38.9 PSU). The stability of the deep water body was interrupted in 1988/89. Due to cold winters and a reduced precipitation in summer, high-density water from the Aegean Sea filled the Levantine Basin of the Eastern Mediterranean (Roether *et al.*, 1996; Lascaratos *et al.*, 1999) and probably shifted the nutricline partly into the euphotic zone (Klein *et al.*, 1999). This change, named the Eastern Mediterranean Transient (EMT), is well documented in a previous report of this collection (CIESM Workshop n°10, 2000). Associated with this change in hydrography, Weikert (1995) observed a faunistic change in mesozooplankton composition and abundance in June 1993 and Danovaro *et al.* (2001) documented an influence on the benthos community.

Zooplankton investigations in the Eastern Mediterranean are largely restricted to the epi- and upper mesopelagic zones (Scotto di Carlo *et al.*, 1991; Mazzocchi *et al.*, 1997; Siokou-Frangou *et al.*, 1997; Kovalev *et al.*, 2001; and literature cited in these studies). Quantitative investigations of the deep water column were done by Delalo (1966b), Kimor and Wood (1975), Pancucci-Papadopoulou *et al.* (1992) and by our group which focused on mesozooplankton (Weikert and Trinkaus, 1990; Weikert *et al.*, 2001; Koppelmann *et al.*, 2003; and studies cited therein) and microzooplankton (Böttger-Schnack, 1997). Information on the ecology of protozooplankton mainly refers to the upper layers (see Pitta and Giannakourou, 2000; Pitta *et al.*, 2001). Only one study describes qualitative and quantitative aspects of the pelagic and benthic occurrence of heterotrophic nanoflagellates (Arndt *et al.*, 2003). Microbial respiration in the deep Eastern Mediterranean was measured in 1995 by La Ferla and Azzaro (2001).

SPATIAL AND TEMPORAL MESOZOOPLANKTON DISTRIBUTION

With the use of 1m^2 Double- and Single-MOCNESS's (Multiple Opening and Closing Net and Environmental Sensing System; Wiebe *et al.*, 1985), we sampled mesozooplankton by oblique and horizontal hauls during different years and at several stations over the full depth of the Eastern Mediterranean (Fig. 1). Mesozooplankton biomass standing stocks in the bathypelagic zone are very low compared to the NE Atlantic (Koppelmann and Weikert, 1999) and the Arabian

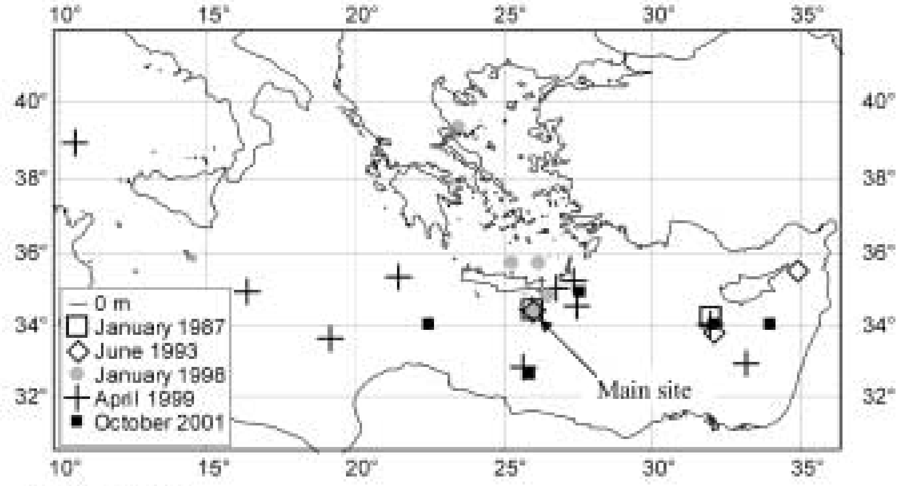

Fig. 1. Mesozooplankton sampling sites in the Eastern Mediterranean. The main site was covered by all cruises. (Source : GEBCO).

Sea (Koppelmann *et al.*, 2000), where the standing stocks are higher by factors of 7-16 and 3-5, respectively. Coupled with the surface production in the Mediterranean Sea, a decrease in zooplankton biomass exists from west to east in the upper 1050 m of the water column as well as in the bathy- and abyssopelagic zones, below 1050 m. The vertical distribution of the mesozooplankton biomass (wet weight, wwt) in January 1987, June 1993 and April 1999 shows only small differences in the upper 800 m of the vertical profiles (Fig. 2). In the bathypelagic zone, however, differences were distinct: the sharpest decline of abundance with depth (from 900 mg/1000 m^3 to 30 mg/1000 m^3) and the lowest concentrations were observed in January 1987. In June 1993, after the onset of the EMT, the values were highest and the respective concentrations (around 7000 mg/1000 m^3) were more or less stable in the whole bathypelagic zone, i.e., over a vertical extension of 3 km. The differences were associated with the predominance of two calanoid copepods, *Eucalanus monachus* and *Calanus helgolandicus* (Weikert *et al.*, 2001). The profile obtained in April 1999 occupies an intermediate position between the vertical distributions observed in January 1987 and June 1993. Between 1050 to 2050 m, the profile parallels the distribution in January 1987, but below, in the abyssopelagic zone, the biomass was higher, varying around 150 mg/1000 m^3.

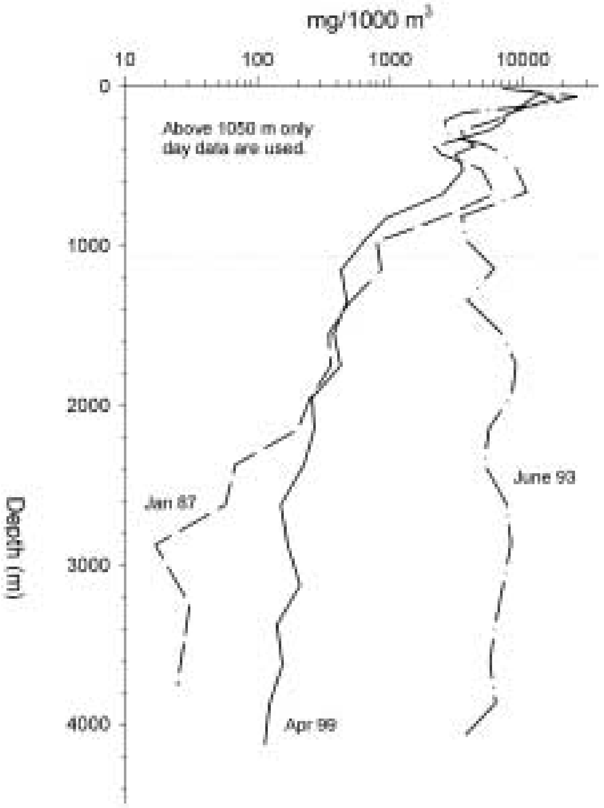

Fig. 2. Vertical distribution of mesozooplankton biomass at the Ierapetra site, SE of Crete.

The results from April 1999 indicate that the vertical succession of numerically predominant calanoid copepod taxa (Fig. 3 left) tended back to the "old" structure found in 1987 (Weikert and Trinkaus, 1990; Weikert and Koppelmann, 1993) before the EMT: *Haloptilus* spp. was abundant in the upper 250 m, comprising 16.6% of the total zooplankton, *Eucalanus* spp. was most important between 250 m and 1050 m (27.3%), and *Lucicutia longiserrata,* one of the rare true deep-sea species in the Levan-

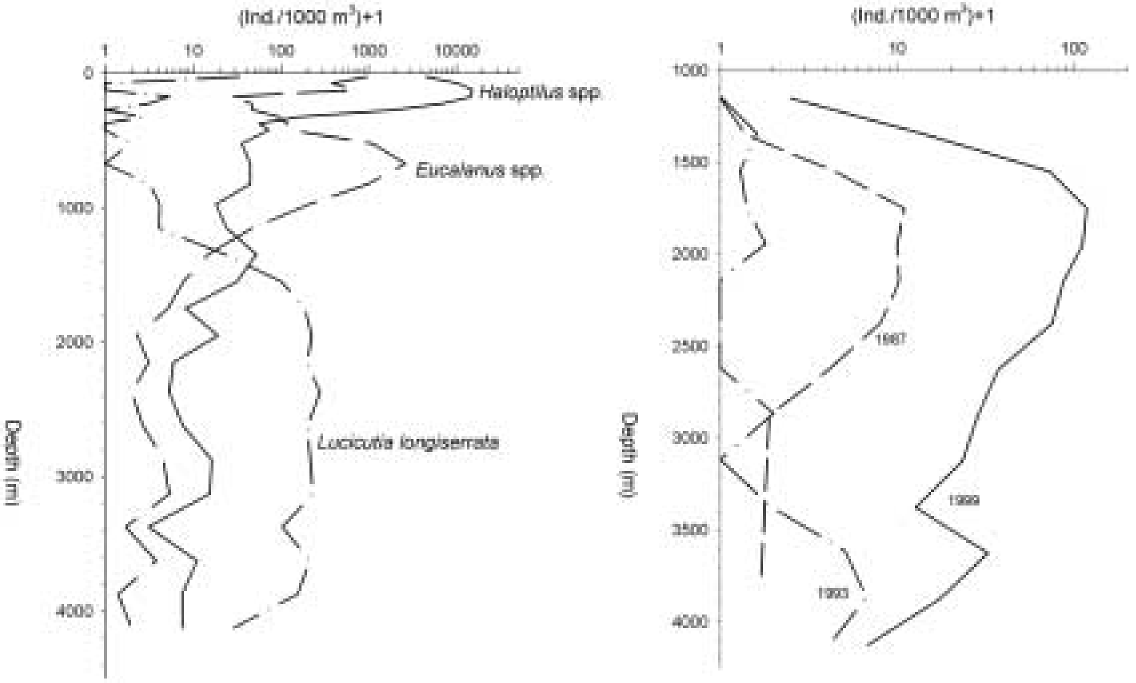

Fig. 3. Vertical distribution of zooplankton main taxa at the Ierapetra site (SE of Crete) in April 1999 and temporal distribution of *Lucicutia longiserrata* (adults only) in January 1987, June 1993 and April 1999.

tine Basin, resided at depths below 1050 m (37.8%). *Lucicutia longiserrata*, however, showed a vertically extended distribution in 1999 compared to the other years (see Fig. 3 right).

CARBON FLUXES IN THE BATHYPELAGIC ZONE

ETS activity as a measure of potential respiration was determined for mixed zooplankton and single taxa at different depths in the water column SE of Crete in April 1999 according to the method of Packard (1971). Enzyme activity was converted into respiration and carbon consumption rates using conversion factors from the literature for epipelagic zooplankton. Detailed information on the method can be found in Koppelmann *et al.* (2000) and literature cited therein. The results on carbon remineralisation rates in the deep Levantine Sea were somewhat higher than data reported from other oceanic deep-sea regions, but similar, if the open ocean data are adjusted to the *in-situ* temperature of the Levantine Basin (see Halsband-Lenk *et al.*, in press). The peak of ETS activity recorded at 2750 m depth (Fig. 4) might be attributed to the true deep-sea copepod *Lucicutia longiserrata* which consumed 1.43 µg C mgwwt^{-1}d^{-1}. The sampled material contained mainly late developmental stages of this species (copepodites IV-VI) and the high carbon demand may indicate increased consumption during gametogenesis (see also Mayzaud *et al.*, 2002; Rey-Rassat *et al.*, 2002). In order to calculate the mesozooplankton carbon demands

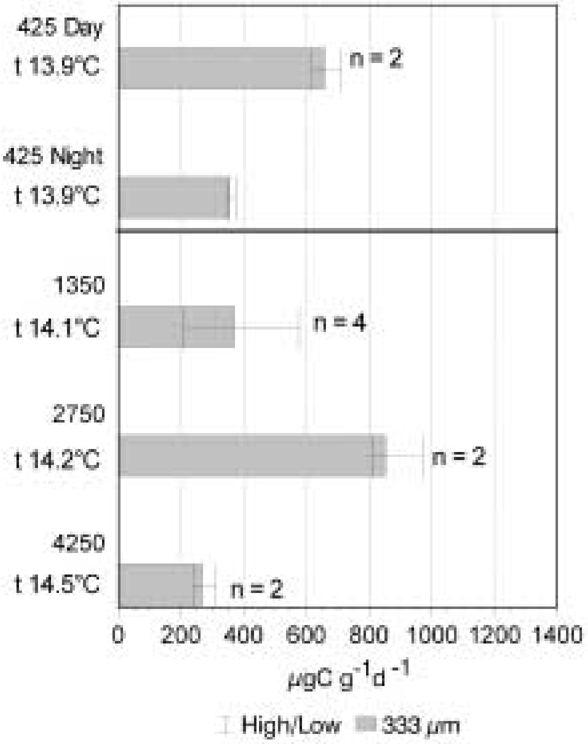

Fig. 4. ETS activity converted into carbon remineralisation per gram zooplankton (wet weight) at the Iearapetra site, SE of Crete (modified after Halsband-Lenk *et al.*, in press).

throughout the water column in 1999, the biomass of each depth interval [g m^{-2}] of the profile was multiplied by the carbon consumption rates [µg C g wet weight^{-1} d^{-1}], which were graphically determined at the midpoints of each depth interval.

The flux of organic material in 1999 was measured using sediment traps in the deep Levantine Sea, SE of Crete. The low sinking flux at 2700 m reflects the oligotrophic character of the basin. Based on the trap data, the C_{org}-flux was extrapolated to the depth (z) at the beginning and end of each zooplankton sampling interval by applying the Martin *et al.* (1987) equation [$J_z = J_T/(z/T)^{0.858}$; J_z = flux at depth z, J_T = measured flux at the depth (T) of the sediment trap]. The difference between the upper and the lower values gives a measure of the "losses" of organic carbon from the sinking POC-flux in each depth interval. This equatio often used in biogeochemistry models, is based on organic carbon flux measurements during short-term sediment trap deployments in the upper 2000 m of the NE Pacific, and its global and depth-range applicability needs to be confirmed (Francois *et al.*, 2002). We do not know whether or not the equation is applicable in the deep Levantine Sea since the higher temperature of the deep-water body in the Levantine Sea compared to the open ocean leads to the suggestion that remineralisation of organic material is enhanced due to higher metabolic activities at elevated temperatures. Nevertheless, the equation is applicable for low latitude, oligotrophic regions (Francois *et al.*, 2002), and we will apply this function to estimate the carbon losses in the water column.

Integrated over the bathypelagic zone, the mesozooplankton contributed 23 % to the carbon losses from the sinking flux, with highest values up to 55 % between 3000 and 3250 m (Fig. 5).

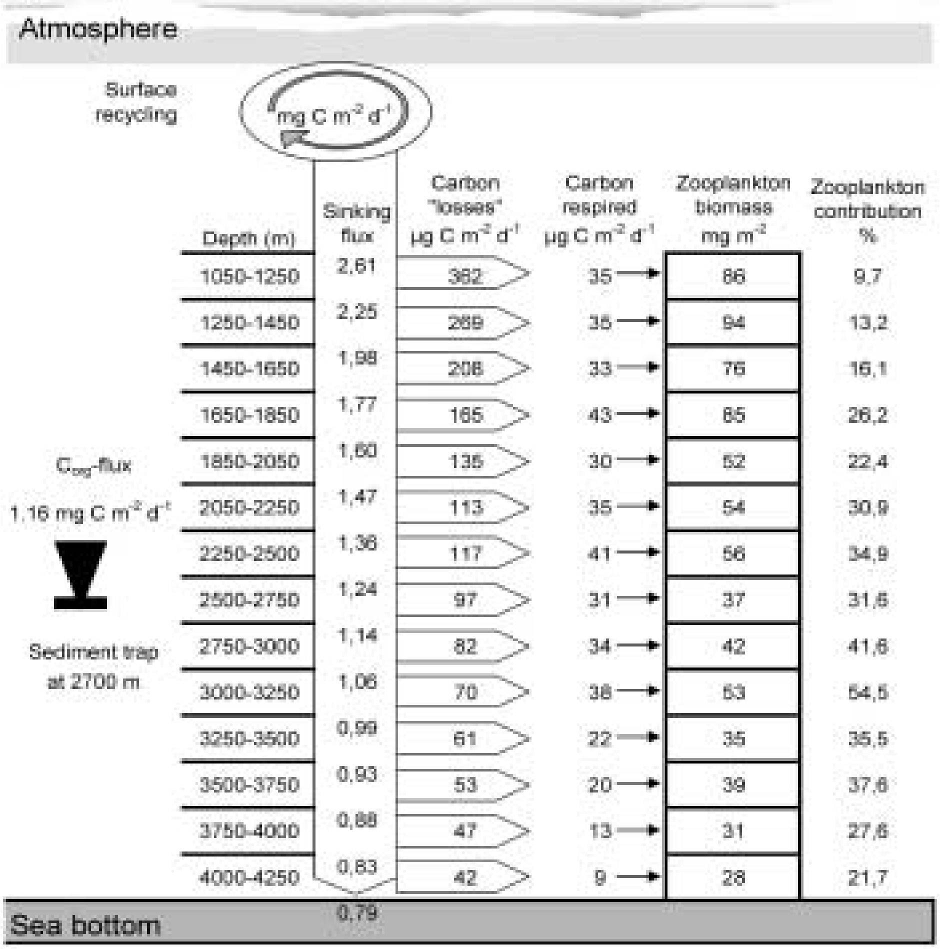

Fig. 5. Sinking organic carbon flux measured by a sediment trap at the Ierapetra site in April 1999. The flux rate is extrapolated to the depth of the beginning/end of each zooplankton sample interval and the carbon losses from the sinking flux are calculated. The amount of carbon used by the mesozooplankton for energetic reasons and its contribution to the carbon losses is indicated in the right hand panels.

Carbon remineralisation rates measured in the bathypelagic zone of the NE Atlantic (Lampitt, 1992; Christiansen *et al.*, 2001) and the Arabian Sea (Koppelmann *et al.*, 2000) resulted in lower rates (4-14%). Different rates were found at the BIOTRANS site (Koppelmann and Weikert, 1999) in the NE Atlantic, when high remineralisation rates (52-86%) were obtained from the upper bathypelagic zone (1000-2250 m) in summer 1992, which coincided with an elevated standing stock of mesozooplankton after the sinking of spring phytodetritus. In the deep zone (> 2250 m) of this site, the contribution of mesozooplankton to carbon losses from the sinking flux were lower and ranged between 10 and 19%.

If the Martin *et al.* (1987) equation is applicable for the deep Levantine Sea, the mesozooplankton, especially *Lucicutia longiserrata*, efficiently takes advantage of the sinking flux at the expense of other organisms. If not, the formula has to be adjusted to predict higher carbon losses from the sinking flux. Francois *et al.* (2002) considered that the Martin *et al.* (1987) equation, using an exponent of 0.858, underestimated the remineralisation in high productive regions and has to be adjusted for an exponent > 1. If we apply an exponent of 1.50 to our data, this will result in a mesozooplankton contribution of 10% to the carbon losses in the bathypelagic water column, which is closer to the above-mentioned low values. We suggest that the deep Levantine Sea is similar to high latitude productive regions in view of the relative deep water remineralisation rates, due to the high temperatures in this environment, albeit at a much lower level. In this case, the deep pelagic biota would efficiently take advantage of the sinking flux in the oligotrophic Levantine Sea and thus act as a filter for the nutrition of the benthos, which is extremely impoverished in the Eastern Mediterranean (Thiel, 1983; Tselepides and Eleftheriou, 1992).

TROPHIC INTERACTIONS

To determine the transfer of organic matter within the mesozooplankton food web, δ^{15}N and δ^{13}C values of different size classes (< 0.5, 0.5-1, 1-2, 2-5 mm) and single taxa were measured SE of Crete in April 1999 and October 2001. Stable nitrogen isotopes fractionate by 3.4 to 3.8‰ between trophic levels and can be used to determine the trophic level of organisms, while stable carbon isotopes can be used to track the diet of the organisms since there is little fractionation from prey to predator (see also Nyssen *et al.*, 2002). In the surface layers of the Levantine Sea, very low δ^{15}N values (2-3‰) were found in the mesozooplankton compared to other oceanic regions (Koppelmann *et al.*, 2003). This can generally be explained with the fixation of atmospheric nitrogen by diazotrophic cyanophycea (δ^{15}N 0-1‰) which lowers the isotopic baseline within the food web (see Carpenter *et al.*, 1997). Koppelmann *et al.* (2003) suggested that *Synechococcus* spp. are the main actors of nitrogen fixation in the Levantine Sea. They are common in this basin (Li *et al.* 1993, Detmer 1995) under oligotrophic conditions (see Kress, 2000; Struck *et al.*, 2001). Also, there is some evidence that isotopically light, excreted NH_4^+ (Checkley and Miller, 1989) can be used as a nitrogen source by phytoplankton under low nitrate conditions (Alcaraz *et al.*, 1994). This will additionally lower the baseline of δ^{15}N within the food web.

The low δ^{15}N values can be tracked in the mesozooplankton throughout the entire water column of the Levantine Sea (Fig. 6). Between 500 and 1000 m, the δ^{15}N measurements of all zooplankton size classes ranged between 4.0 to 6.6‰. Highest δ^{15}N values were found for the size fraction 0.51 mm. In this size class, *Eucalanus* spp. accounted for 37.7% of the standing stock of total zooplankton in the mesopelagic zone during April 1999, thus, this size class comprised 84.5% of the total standing stock of the genus in the mesopelagic zone. The δ^{15}N value of this taxon matches the value of the 0.5-1 mm size class.

A few δ^{13}C measurements obtained in October 2001 indicate that the 0.5-1 mm zooplankton (-15.7‰) as well as the dominant genus *Eucalanus* (-14.0‰) have much higher values than the zooplankton of the other size classes (-18.2 to -17.8‰) in the mesopelagic zone, and in the zooplankton of the epipelagic zone as well (19.4 to 17.2‰, including the values of *Haloptilus* spp. and *Chaetognatha*). This might indicate that *Eucalanus* spp. uses a food source different from that of the other taxa. Irrespective of possible trophic changes by upwelling events, we propose that the food of this interzonal migrator is mainly linked to the diazotrophic production in the surface layer (see also Koppelmann *et al.*, in press). Laboratory investigations on *Synechococcus* sp. showed high rates of carbon isotope fraction when the CO_2 supply was high (Erez *et al.*, 1998),

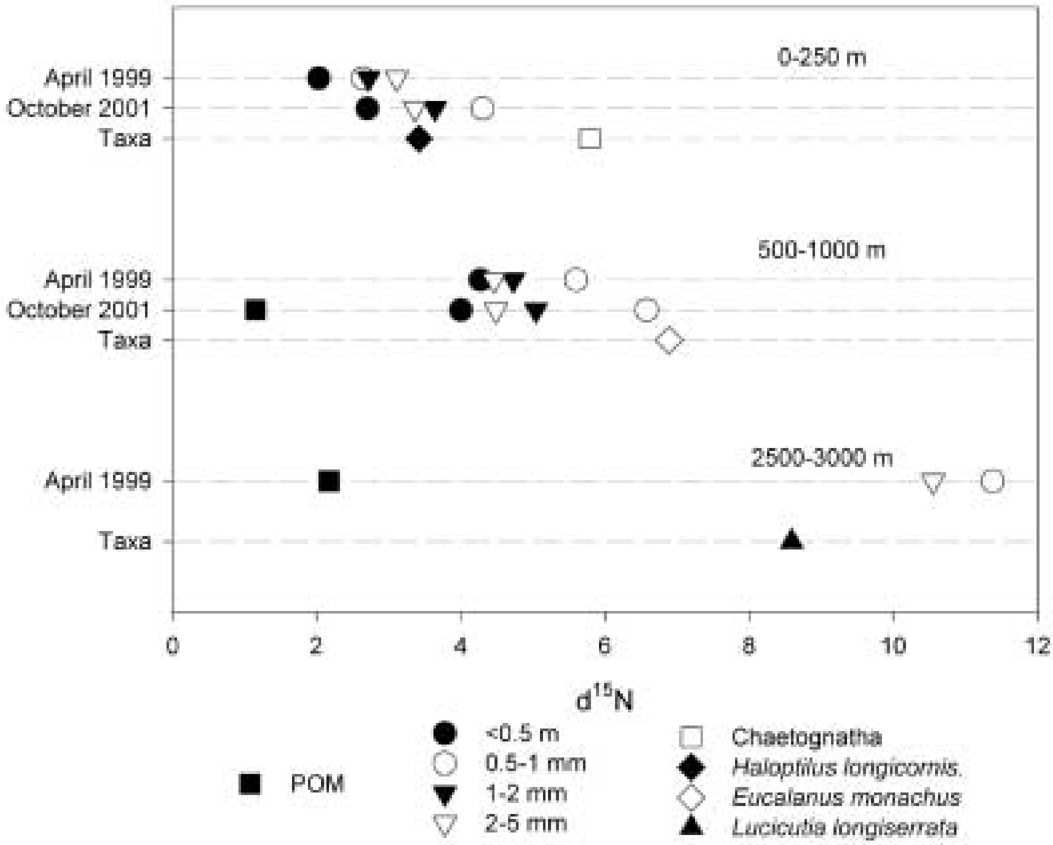

Fig. 6. δ^{15}N values of particulate organic matter (POM), different zooplankton size classes and single taxa (from Koppelmann *et al.*, in press).

and field investigations on the diazotroph *Trichodesmium* spp. reported δ^{13}C values around -12.9‰ (Carpenter *et al.*, 1997). On the other hand, assuming that sinking particulate organic matter (POM) is the primary diet in the deep-sea (see Angel, 1990), the difference of ~3.6‰ between POM (1.2‰) and the zooplankton of the size classes <0.5, 1-2, and 2-5 mm would indicate that these fractions feed directly on POM.

In the bathypelagic zone, only the δ^{15}N values of the 0.5-1 mm and 2-5 mm size classes could be measured since the material for the analysis of the smaller fractions was too scarce. The values of the larger fractions were higher (11.4 and 10.6‰, respectively) than those obtained for the zooplankton from mesopelagic depths. The differences between POM and zooplankton were 9.1‰ for the 0.5-1 mm size class and 8.4‰ for the 2-5 mm fraction, indicating that there is at least one more trophic level between POM and mesozooplankton in contrast to the mesopelagic layer. There is, however, no information available about the interaction between POM, bacteria, protozoa, micro- and mesozooplankton in the deep-sea. The δ^{15}N value of the dominant copepod *Lucicutia longiserrata* in the bathypelagic zone (37.8% of the total zooplankton) was slightly lower (8.6‰) than values of mixed zooplankton, indicating that *Lucicutia longiserrata* plays a mediating role between lower and higher trophic levels. *Lucicutia longiserrata* seems to be a key species in the deep Levantine Sea and its role in energetic and somatic carbon fluxes has to be investigated further.

PERSPECTIVES

The deep Eastern Mediterranean zooplankton fauna consists mainly of mesopelagic species and a small number of true deep-sea species like *Lucicutia longiserrata*. In general, the faunal elements are impoverished in terms of species richness and abundance. The latter feature is probably caused by the particular hydrography and the oligotrophic character of the Sea. Due to these local peculiarities, global change effects should be easily monitored in this basin, and the Eastern Mediterranean can be used as a model ocean for climate change (Bethoux *et al.*, 1999).

Many open questions remain concerning the contribution of zooplankton to material flux cycles in the Eastern Mediterranean. So far, our information is restricted to mesozooplankton at a single station. The contributions of protozooplankton, small-size zooplankton, nekton and gelatinous organisms to these fluxes in time and space are unknown. Future research in the Eastern Mediterranean should focus on the interaction between zooplankton and other trophic elements (from bacteria to nekton) and their role in mediating material fluxes.

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Community structure of meiofauna and macrofauna in Mediterranean Deep-Hyper-saline Anoxic Basins

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Deep-Hyper-saline Anoxic Basins (DHABs) are extreme habitats, associated with tectonically active zones in the deep sea. They are characterized by oxygen depletion, extremely high salinity and elevated methane and hydrogen sulphide concentrations. This reduced environment has the potential to sustain chemosynthetically based communities and thus presents a unique opportunity to study the community structure, diversity and adaptations of its meiofaunal and macrofaunal benthic components, as well as to compare protozoan and metazoan abundance and diversity. To date, several cold-seep areas that harbor also various benthic communities have been found mainly in the Atlantic and in the Eastern and Western Pacific oceans. Investigations in the neighboring mud volcanoes of the Mediterranean Ridge complex have likewise revealed the existence of cold seep communities characterized by the presence of species dependent on methane rich emissions (Corselli and Basso, 1996; Sibuet and Olu, 1998). The mega- and macrofauna and the bacterial communities of these biotopes have been the object of intensive investigations (Levin *et al.*, 2000). In contrast, the intermediate sized meiofauna have received less attention.

DHABS IN THE MEDITERRANEAN

Recently, five DHABs were discovered in the Mediterranean Sea, all below a depth of 3000 m: the Bannock, Urania, Discovery, L'Atalante and Tyro basins (Fig. 1). They represent unique deep-sea environments which originate from the dissolution of buried salt deposits (evaporates), that are

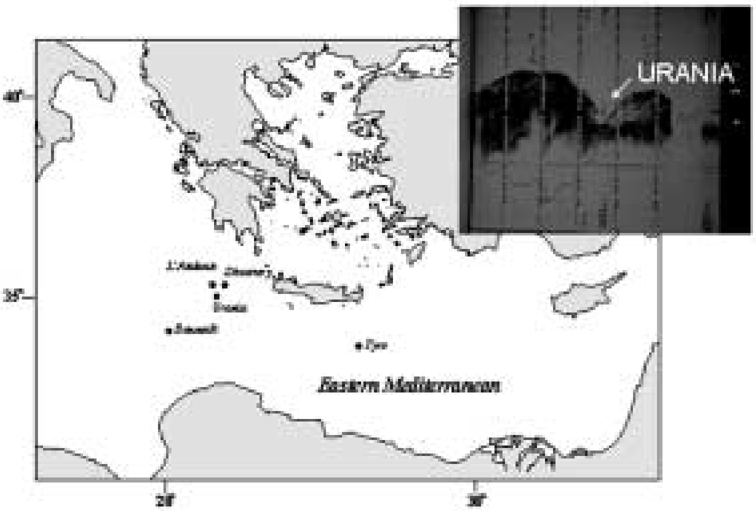

Fig. 1. Map of the Eastern Mediterranean showing the locations of the anoxic basins and the bathymetric profile along the Discovery basin.

the remains of hypersaline waters of the Miocene period (5.5. M.y. before present). DHABs are characterized by a total salinity above 30%, absence of light, elevated pressure, variable pH values and ionic compositions and are sharply separated by the overlying normal sea-water. Some basins exhibit other environmental peculiarities (e.g. Urania basin has the highest concentration of sulfide among the Earth aquatic environments; Discovery basin has hydrocarbon seeps; Urania west basin has a mean temperature of 45ºC at the brine/sediment interface). The sharp normal sea water/brines transition interface (Fig. 2), operates as a barrier to particles, which contain organic matter and pollutants, settling from the sea surface through the water column.

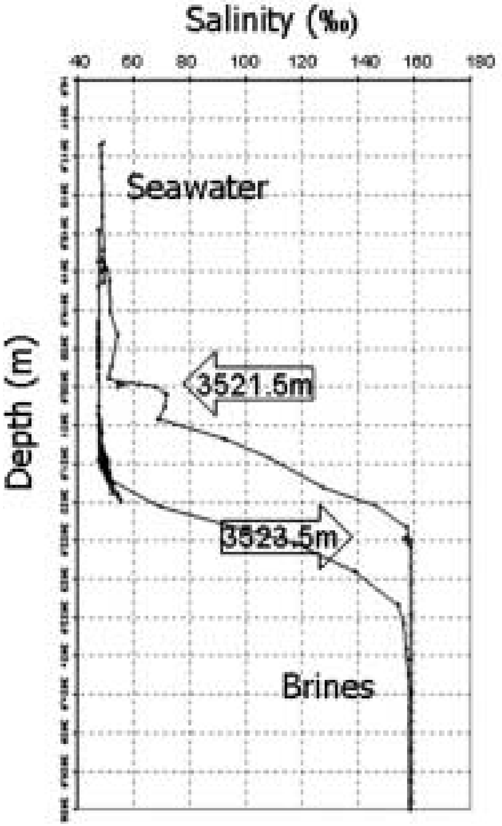

Fig. 2. The vertical profile of salinity showing the sharp normal sea water/ brine transition interface.

During this first leg (Au -Sep. 2001) of the BIODEEP project (BIOtechnologies from the DEEP) an intensive sampling effort was focused on the meiofaunal and foraminiferal assemblages of DHABs from sediments taken both within and outside the influence of brine pools. Benthic meiofauna and foraminifera were chosen as a target group because they not only consist a major element of deep-sea communities but are also considered to have a spatial distribution influenced by pore-water oxygen and methane (Rathburn *et al.*, 2000). Furthermore these organisms are known to be abundant and diverse in habitats, subjected to considerable natural, physical and chemical stress where very few if any macrofauna species remain. These extreme habitats are toxic to mega- and macrofauna; if they support any life it consists mainly of protistan and metazoan meiofaunal communities, often associated with prokaryotic symbionts (Bernhard *et al.*, 2000). Previous work has also shown that some foraminiferal species thrive in organically enriched – oxygen depleted environments and have often been found to outnumber metazoans (both meiofaunal and macrofaunal) at bathyal depths (Gooday *et al.*, 2000).

SEDIMENT CHARACTERISTICS

The physicochemical environmental parameters analysed revealed pronounced differences between the DHAB and the adjacent stations outside the influence of brines. Negative Eh values were recorded at all stations in the brine areas of all four DHABs indicative of heavy organic loading and anoxia. In contrast, Eh profiles out of the brines were characterized by positive values down to 20 cm depth and were representative of typical deep-sea conditions. The concentrations of phytoplankton pigments on the sea floor of the four DHABs were measured to obtain a general idea of sedimentation patterns of plankton derived organic material. In all non-brine sediments outside the DHAB's, the concentrations of chlorophyll *a* were very low, ranging from 0,079 (Bannock basin) to 0,175 µg g^{-1} (L'Atalante basin). As opposed to the normal sediment, the use of Mass Spectrometry showed that within the DHABs no phytoplankton pigments were present. In contrast, a high concentration of the Cholest-5-en-3β-ol sterol, which is both an algal and zooplankton marker, was found within the DHABs (Fig. 3). In a similar way, all brine sediments showed significantly higher organic carbon values compared to those found in the adjacent areas (Fig. 4).

MEIOFAUNAL ABUNDANCES

Meiofaunal density from the non-brine sites of all four basins was very low, ranging from 12 (Urania basin) to 43 ind./10cm^2 (Discovery basin). The brine samples of the L'Atalante and Discovery basins displayed higher densities compared to those from the non-brine sites (41-45 and 191 ind./10cm^2 respectively). However, at Urania and Bannock basins the opposite trend was observed (Fig. 5).

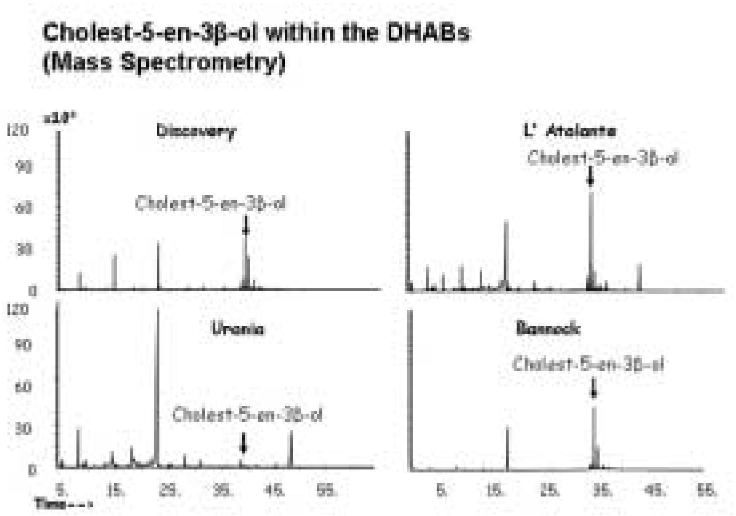

Fig. 3. Mass chromatogram showing the distribution of Cholest-5-en-3β-ol, a common phytoplankton and zooplankton biomarker, in the sediments of the four anoxic basins.

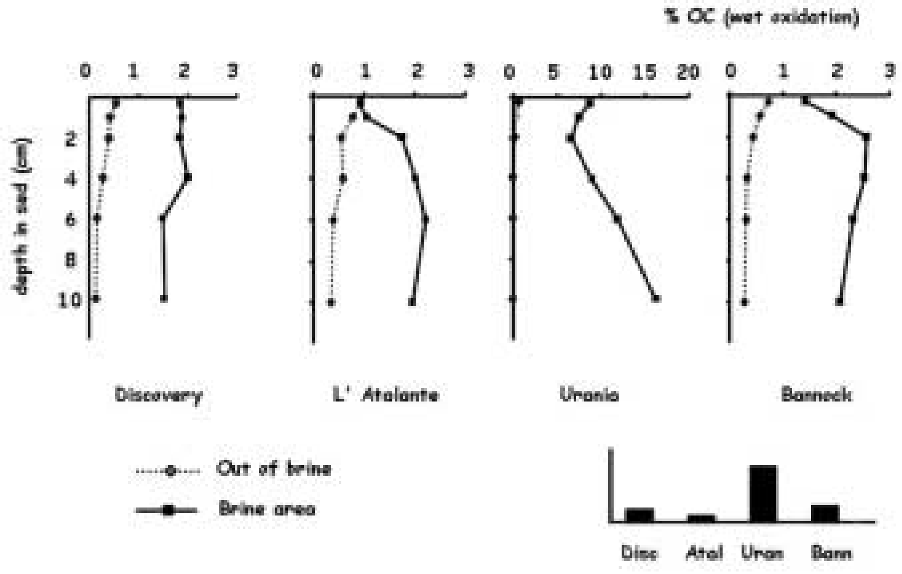

Fig. 4. Distribution of mean organic carbon values within and outside the brine areas.

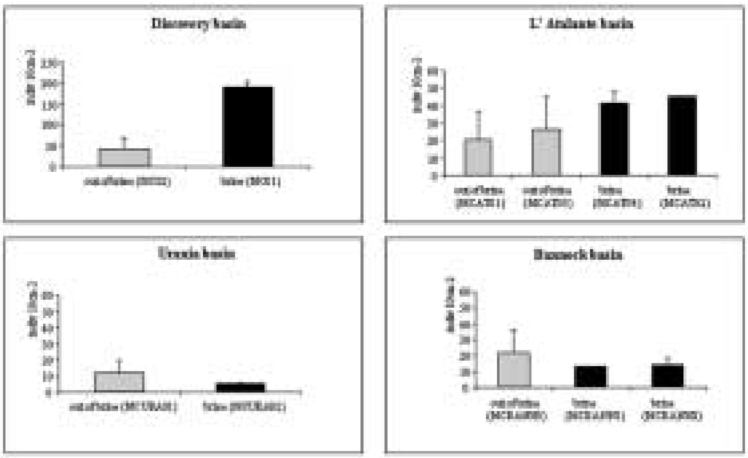

Fig. 5. Mean meiofaunal abundance (ind. 10cm^{-2}) at the four anoxic basins.

In the normal non-brine sediment and at all basins, the meiobenthic community structure was typical, with nematodes being the most abundant taxon comprising up to 64.4% of the total. In contrast, community structure in the brine sediments differed considerably among the four basins (Fig. 6). At Discovery basin meiobenthos was dominated by copepods and foraminifera (mostly planktonic species) comprising together 75% of the total, followed by juvenile bivalves, nematodes, allogromiid foraminifera and other crustacean.

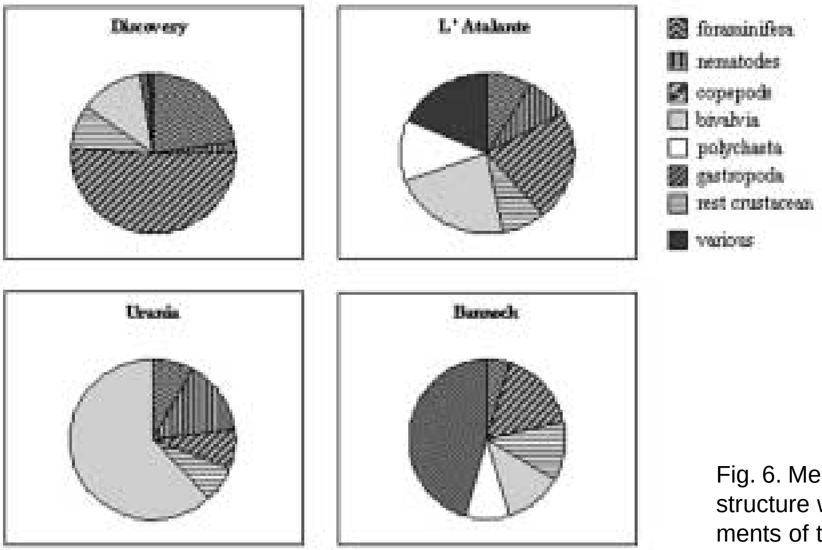

Fig. 6. Meiofaunal community structure within the anoxic sediments of the four anoxic basins.

L'Atalante basin was characterised by various meiofaunal taxa: nematodes, foraminifera, copepods, juvenile molluscs (unidentified bivalves and gastropods).

At Urania basin juvenile bivalves (unidentified species) dominated the community (61.5%) followed by copepods, foraminifera and other crustacean taxa.

At Bannock basin juvenile gastropods (unidentified species) were the most abundant taxon (45%) at station MCBANN01, followed by the above-mentioned bivalves, nematodes, planktonic copepods and foraminifera. At station MCBANN02, the meiobenthic community was comprised of various taxa such as nematodes, foraminifera, crustacean and juvenile molluscs. Figure 7 gives an overview of the different forms encountered at the brine sediments of the four basins.

MACROFAUNAL ABUNDANCE AND BIOMASS

Macrofaunal abundance values obtained from the non-brine sites of the Discovery and Urania basins ranged from 60 to 88 ind./m^2 and were normal for deep-sea environments. In contrast to this, abundance at the only brine site (Urania basin) available was much lower (24 ind./m^2). The same trend was observed for biomass, with values ranging from 0.003 to 0.005 g/m^2 outside the brines, dropping to 0.001 g/m^2 inside (Urania basin).

Macrofaunal community structure was drastically different between the non-brine and brine sites. Polychaeta abundance and biomass was dominant outside the brine sediments with values ranging from 62 to 73% and from 59 to 90% respectively, while crustacean was the second most dominant group with abundance and biomass values ranging from 20 to 38% and from 6 to 39% respectively. In contrast, the brine site (Urania basin) was dominated by crustacean (67% and 95% of the total abundance and biomass respectively).

SYNTHESIS

Up to now, sampling of meiofauna at brine areas remains rare (Jensen, 1986; Sibuet and Olu, 1998; Thiermann *et al.,* 1994) and the results are somehow contradicting. In some cases, no differences in meiofaunal abundance between brine and control sites are found (Shirayama and Ohta, 1990), whereas in others the abundance was found to be up to two orders of magnitude

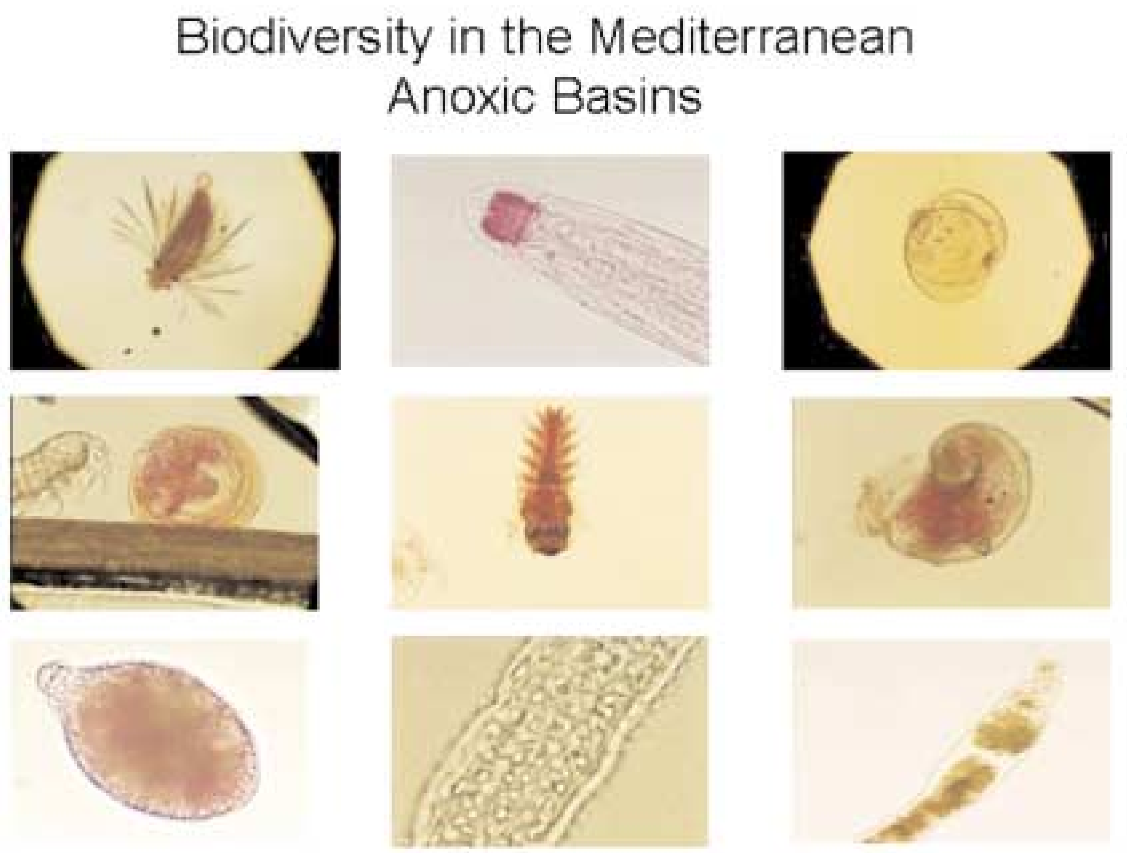

Fig. 7. Different forms found in anoxic brine sediments, below 3000 m depth in the Eastern Mediterranean.

higher than those recorded from the surrounding area (Olu *et al.*, 1997). Two hypotheses may explain these enhanced faunal abundances: the local enrichment in the sediment by chemosynthetic production which may be due (1) to free living chemoautotrophic bacteria or (2) detrital organic matter from clusters of symbiotic fauna (Sibuet and Olu, 1998).

However, the presence of a fauna living in such environments, and particularly the metazoan component, is rather unexpected due to the absence of oxygen and to the presence of hydrogen sulphide, either of which, alone, has been considered a lethal condition for all free-living metazoans (Fenchel and Riedl, 1970). The presence of the fauna implies that adaptations, which provide a long-term tolerance, exist and must be of two types. Either the animals in question can exclude the sulphide at the body wall and thereby avoid the toxicity problem or, if sulphide penetrates the body wall, they must detoxify it, something that is usually achieved with “bacterial metabolic help” (Giere, 1993).

Our results so far indicate that all four DHABs support, to a different extent, meiofaunal communities which could possibly harbour prokaryotic symbionts. Many of these taxa, including the prokaryotic symbionts are expected to be new for *science*.

The macrofaunal community was sampled only marginally and therefore much remains to be done during the next BIODEEP cruises before sound conclusions are drawn.

Deep-sea bacteria: the Mediterranean sea as a model environment

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In spite of being the largest world ecosystem, the deep-sea which accounts for almost 90% of the total global ocean volume has been classically underestimated regarding its contribution to the biosphere and represents without doubt the less known and more poorly understood environment in our planet. Due to its very unusual features (lack of light, high pressure, oligotrophy, etc.), the deep marine environment represents a very important "new frontier" for the knowledge of some unexpected behaviour of life in "extreme" conditions and, as a consequence, a great opportunity for biotechnological applications. For this reason, several industries are orienting their research fields on the analysis of deep-sea samples (e.g. Deepstar Jamstec, Japan).

The former view that the deep-sea was a rather monotonous and featureless extension of the surface water column has been often challenged in the last decades since the demonstration that a significant part of the organic matter produced at the surface layers (up to 200 m) reaches the deepest water layers linking the two world domains in a very tight way. Moreover, the Earth crust is not inert: it provides an endless range of submarine features in which the living communities, usually well diversified, often depend on the heterotrophic organic matter recycling rather than on the photosynthetic energy. Bacteria are well known to colonize this large domain to its deepest parts. During the past decade, several mechanisms of bacterial adaptations to the main conditions of the deep sea environment have been better elucidated (Kato and Qureshi, 1999; Kato *et al.*, 2000; Bartlett, 2002) and new molecular features as well as new methabolic pathways have been described that have important links with the scientific and the industrial fields (Abe *et al.*, 1995; Colquhoun *et al.*, 1998). Nevertheless, the role of the deep-sea bacteria and their relative contribution to the global biogeochemical cycles are far to be known. The main reason of the actual lack of knowledge concerning the deep sea world is related to the difficulties in sampling and sample processing that are often very expensive and time consuming activities.

The Mediterranean is a large marine area (LMA) characterized by a short continental shelf and relatively deep waters (average depth 1,500 m; maximum depth 5,150 m off the southern coast of Greece) that are easily accessible (often few hours of navigation). As the Mediterranean water column is relatively homeothermic (13°C from the subsurface to the bottom), this LMA offers a very great opportunity for studying the effect of the increase in hydrostatic pressure on the biological compartment uncoupled from the effect of the decrease in temperature (typical of the other deep sea oceanic waters). Moreover, due to its very complex bathymetry and to its peculiar history the Mediterranean deep sea harbors a high number of diversified ecological niches, several of which characterized by very unusual features and are very interesting from the biogeochemical and evolutionary perspectives (e.g. cold seeps and mud volcanoes, deep basins of evaporitic origin).

DEEP-SEA MICROBIAL ACTIVITIES: THE PRESSURE EFFECTS

In view of the increasing concentrations of carbon dioxide into the atmosphere, the role of the Ocean in the regulation of CO_2 is under close scrutiny. However, the data gathered are mainly derived from studies that have been performed in surface waters (euphotic zone) where about 50% of the organic carbon produced daily by photosynthetic organisms is processed by bacteria and used to produce new bacterial biomass or for bacterial respiration (Ducklow and Carlson, 1992). Bacterial activity rates measured in the deep-water layers of the ocean appear low relative to those described in the surface layer. However, when the measured rates are integrated through the depth of each water layer, the mineralization fluxes mediated by prokaryotic populations at depths deeper than 200 m appear far from negligible (Lefèvre *et al.*, 1996; Del Giorgio *et al.*, 1997; Bianchi *et al.*, 1998; Tamburini *et al.*, 2002). Deeper in the water column, bacteria consume between 40 and 100% of the sinking fluxes of particulate organic carbon (POC). Several models have been created to describe the relationships between the nutritive sources available for deep-sea bacteria, the role of these microorganisms in the mineralization of the peptide compounds, and their consequent implication in the global carbon cycle (Amon and Benner, 1996; Kiørboe and Jackson, 2001). A primary role has been addressed to the DOM streaming behind sinking particles of marine snow (Fig. 1). This indicates that, when the particle fluxes are sufficient, there is a tight coupling between sinking particles and free-living bacteria in the mesopelagic (Cho and Azam, 1988; Smith *et al.*, 1992) and bathypelagic layers (Nagata *et al.*, 2000). Tamburini *et al.* (2003) confirmed in the NW Mediterranean Sea (DYFAMED site) that relative high fluxes of fresh POC enhanced the efficiency of bacterial growth. However, when the flow of particles is extremely low, bacteria must extract carbon and energy from the semi-refractory part of the dissolved organic bulk.

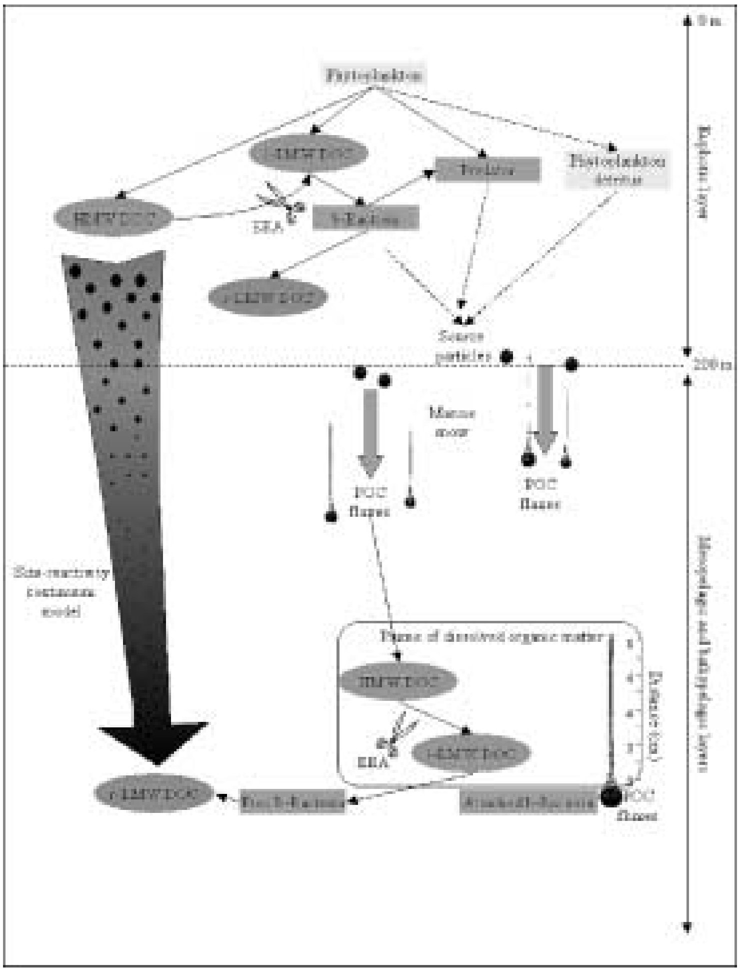

Fig. 1. Participation of microorganisms in the mineralization processes. HMW DOC: high molecular weight dissolved organic carbon; l-LMW DOC: labile low molecular weight DOC; r-LMW DOC: refractory-LMW DOC; B-Bacteria: bacteria adapted to high hydrostatic pressure; b-Bacteria: bacteria un-adapted to high hydrostatic pressure condition (Tamburini *et al.*, 2003).

Most of the available literature concerning the quantification of bacterial metabolic rates in deep sea waters (99 of 128 published articles) has been published by the French team at the Laboratory of

Marine Microbiology of Marseilles (CNRS) which has worked in different areas of the Mediterranean Sea by means of a special High Pressure retaining sampler (HPSS) that allows sampling and sample incubation at the *in situ* pressure conditions (Bianchi *et al.*, 1999). The obtained data show that changes in hydrostatic pressure when handling deep-sea samples affect all the bacterial mediated mineralization steps (from the ectoenzymatic activities to the respiration) so that reliable measurements must be carried out by maintaining the *in situ* pressure conditions (Bianchi and Garcin, 1993; Tholosan *et al.*, 1999; Tamburini *et al.*, 2002). Interestingly, this team has detected a seasonal change in the pressure effects on the bacterial metabolic activities of deep-sea samples that is mainly corresponding to the seasonal shift from the stratified (spring/fall) to the not stratified (winter) feature of the water column. The authors have concluded that the stratified conditions allow the differentiation of pressure-adapted bacterial communities in the deep layers of the Mediterranean sea (Bianchi and Garcin, 1994). Based on these findings, the bacterial response to the decompression appears as a suitable parameter for evaluating the presence of adapted bacterial populations in deep sea areas. More recently, in order to follow the silica dissolution across the water column, the same team has simulated the sinking of phytoplankton detritus aggregates through the water column, demonstrating that hydrostatic pressure affects the aminopeptidase hydrolysis rates of bacteria attached to the sinking particles.

DEEP-SEA “HOT SPOTS” OF BACTERIAL PRODUCTION

In 1977, scientists made a stunning discovery on the bottom of the Pacific Ocean (Galápagos Islands) that forever changed our understanding of our planet and life on it. They discovered the deep-sea hydrothermal vents, and – to their complete surprise – a lush community of exotic life thriving around them based on chemosynthetic primary production (Corliss, 1979). The matter of life arising at “extreme conditions” (high temperature, high pressure, etc.) together with high biotechnological interest have incited the scientific community to purchase efforts on these deep-sea peculiar sites.

The Mediterranean deep-sea harbors several peculiar environments that depend on geochemical rather than on photosynthetic energy, and where the trophic webs are mainly supported by the prokaryotic activity:

- **Mud volcanoes,** located in the Mediterranean Ridge (South Eastern Mediterranean) (e.g. Napoli Dome, Olimpi mud volcano etc.), and their related cold seep features form over great sediment accumulations in which prokariotes digest buried organic matter, producing methane as a waste product. **Cold seeps** usually involve the upward seepage of methane dissolved in water or as small bubbles. Specially evolved bacteria, mainly belonging to the g subclass of Proteobacteria, oxidize the methane, forming the foundation of a food chain. Different g Proteobacteria have evolved to oxidize the hydrogen sulfide (sulfur oxidizing Bacteria, e.g. *Beggiatoa*, Fig. 2), itself the waste product of other bacteria living below the ocean floor, which reduce sulfate ions of seawater origin. Sulfur-oxidizing bacteria dominate the free-living bacteria of many deep-sea seeps forming thin, snow like mats on the seafloor where seepage takes place. The retrieval of very peculiar giant forms of mussels in those areas has been documented (Corselli and Basso, 1996), leading to the hypothesis of symbiotic relationships between these organisms with methane and/or sulphur oxidizing bacteria. Several cold seeps have been recently discovered in the Eastern Mediterranean Sea during the French-Dutch MEDINAUT expedition in 1998 (cf. Fiala-Médioni, this volume) but no evidence of active seepages has been documented in the Western Mediterranean Sea, up to date. Nevertheless, the proven occurrence of healthy and well developed *Lophelia-Madrepora* **deep-sea coral mounds** (between 300 and 1,000 m of depth) in the Ionian Sea (North of Calabrian Arc) offshore the Apulian platform, has brought into consideration the main factors preventing the total eradication of these resilient deep-sea corals in local peculiar oceanographic conditions. Within this frame, the presence of bacterially driven food webs sustaining the coral stocks is under investigation within a National Italian Programme (FIRB, “Deep coral banks in the Apulian Plateau”, 2003). In particular, the preliminary data indicate the presence in those areas of some putative methane oxidizing bacteria (inferred physiological features to the bacterial phylogenetic lineages) acting as primary producers. Several Chrenarchaeota (Marine Group I) have also been retrieved from the analysed samples, mostly belonging to new taxonomical groups (Giuliano *et al.*, unpublished data), as described for some samples collected at the Black sea cold seeps (Pimenov, Gene Bank, unpublished data). Moreover, bacterial RNA was retrieved from the tissues of the solitary *Desmophillum* specimens (not from the *Lophelia-Madrepora* ones) and the preliminary analysis of its 16S rRNA amplified fractions lead to hypotheses on the presence of possible species-specific bacterial associations with the corals. Particularly, by extrapolating the physiological traits inferred on the basis of their phylogenetic positioning, several putative bacterial functions of which corals could take advantage have been identified within the obtained riboclones, namely nutrient acquisition, stabilization of skeleton, processing of metabolic wastes and secondary metabolite production (work in progress).

- **The deep hypersaline anoxic basins** (DHABs) of the Mediterranean Sea, namely L'Atalante, Bannock, Discovery and Urania, represent the deepest basins of evaporitic origin that have been discovered up to date. The DHABs are characterized by hypersalinity (between 160 and 300‰), high hydrostatic pressure (~35 MPa) and anoxic conditions. DHABs were most likely developed by the dissolution of 5- to 8-million year-old Messinian evaporites which became exposed to seawater during the collision of the African and Eurasian tectonic plates (Camerlenghi, 1990). Alternatively, brines of DHABs could be relics of fossil evaporated seawater in Late-Miocene sediments that had accumulated in these deep basins (Vengosh, 1998). The content of DHABs (brine) is so dense that it does not mix with the overlying seawater: indeed DHABs represent unique environments that have been isolated from the global ocean for millions of years (De Lange, 1983). Within the BIODEEP European project, the microbial activities as well as the microbial diversity have been analysed at different layers of those basins and compared to those of the overlying water column. The results show a very high bacterial diversity within the basins, with several newly described *Bacteria* and *Archaea* groups (new candidate divisions), some of which branching very far within the Bacteria domain (early evolutionary stage?) (BIODEEP consortium, data not published). Moreover, at the seawater – brine interface, the hydrolysis rates of biopolymers were clearly higher than in the immediately overlaying oxic water. Within the brines, all microbial activities, although generally lower than in the interface layer, were unambiguously detected in all samples. The bacterial metabolic rates measured in brine samples kept under ambient pressure conditions were 12.5 ± 23.6 (mean ± SD) times higher than those obtained on their decompressed counterparts. This signaled the presence of deep-sea bacterial species well adapted to the DHABs extreme environmental conditions. Unluckily, the decompression of brine samples, provoking dramatic change in the geochemical compositions, did not permit the maintenance and cultivation, under high-pressure conditions, of the piezophilic prokaryotes thriving within the DHABs. Therefore, a new line of instruments and techniques should be developed in order to enhance the knowledge of the bacterial compartment in such interesting environments. Nevertheless, several strains of potential industrial interest have been cultivated, some of which exhibiting very unusual morpho-physiological features (Fig. 2). Some newly described bacterial catabolic genes with potential application in bioremediation have also been retrieved and they are under investigation.

PERSPECTIVES

The Mediterranean deep-sea is relatively easy-to-access and offers very interesting niches for implementing our (poor) knowledge about the role of deep-sea bacteria in the global fluxes of carbon and energy. Most of these niches could harbor bacteria with novel metabolic pathways and molecular adaptation of potential industrial interest. Several European research groups have developed highly innovative techniques for advancing in this field but the work is still far from being completed. The preliminary data obtained, several of which available in the published records, show the fundamental role that deep-sea bacteria play in the global biogeochemical fluxes, and the possible links of studying deep-sea bacteria with the industrial and biotechnological fields. Particularly, the BIODEEP program has provided an enormous amount of novel materials (bacterial strains, clone libraries, etc.) whose characterization cannot possibly be completed during the remaining of the project. To realize the major benefits of this success, it will be essential to fill the gaps by specific new sets of instruments and techniques.

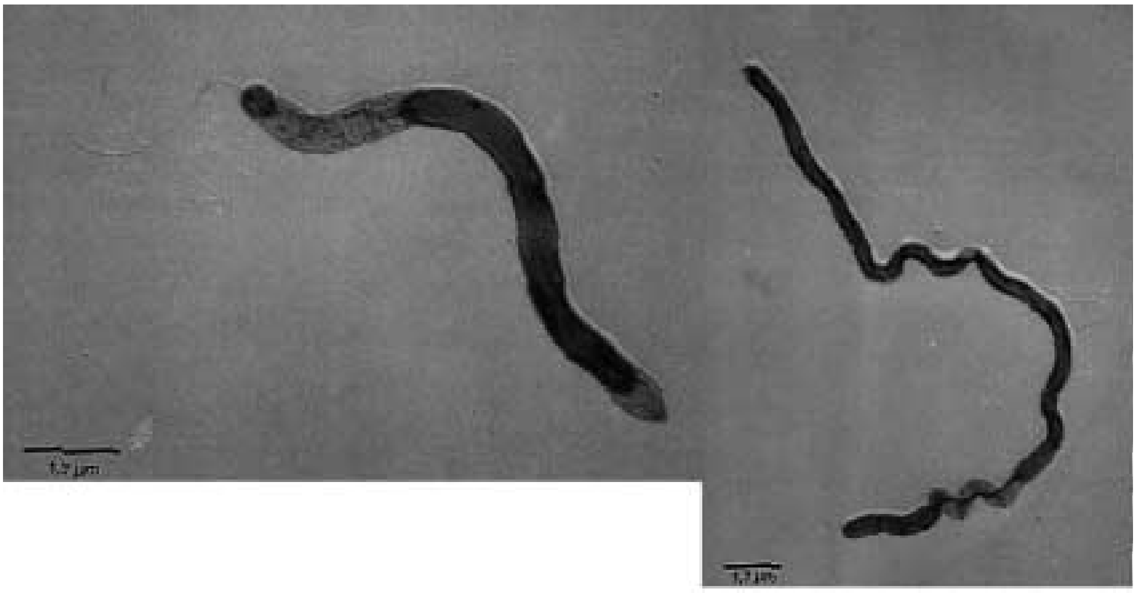

Fig. 2. Enriched pleomorphic bacterium from the Urania basin interface. This bacterium exhibits facultative anaerobic behaviour and it is capable of growing in simple mineral media amended with crude oil as single carbon and energy source, both in aerobic and anaerobic conditions (BIODEEP unpublished results).

Current challenges in the study of biological communities at deep-sea hydrothermal vents

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The discovery of hydrothermal vents in 1977 impacted profoundly our preconceptions of structure and function of marine biological communities. These extreme habitats harbour communities of exceedingly high biomass but relatively low diversity, consisting mostly of endemic, newly discovered species. The luxuriant communities at vents are sustained by chemoautotrophic production by microbes, using CO_2 as substrate and energy derived from the oxidation of hydrogen sulfide (H_2S). Over the last 25 years, research on the geological, physical, chemical and biological properties of hydrothermal vents has expanded significantly our understanding of these systems (Van Dover, 2000). Presently we are faced with our next challenge: to move forward in our understanding, we must adopt a combination of traditional approaches, which have been used in other systems for several decades, and innovative technologies, which currently are under development.

WHERE ARE VENTS?

Targeted exploration of mid ocean ridges has located hydrothermal vents in all ocean basins, and in back- and fore-arc basins and seamounts mostly in the western Pacific (Fig. 1). However, an estimate of the true extent of vents in the world's oceans remains elusive, particularly since "exploration" has lost favour as a scientific endeavour. Interestingly, the few exploratory initiatives in the last two to three years have been extremely successful. For example, Lost City is a recently (and accidentally) discovered hydrothermal vent field that, unlike all other known fields, occurs 15 km from the major spreading axis of the Mid-Atlantic Ridge, on crust that is 1.5 x 10^6 years old (Kellƒey *et al.*, 2001). Other expeditions in 2000 and 2001 discovered hydrothermal vent fields on the Central Indian

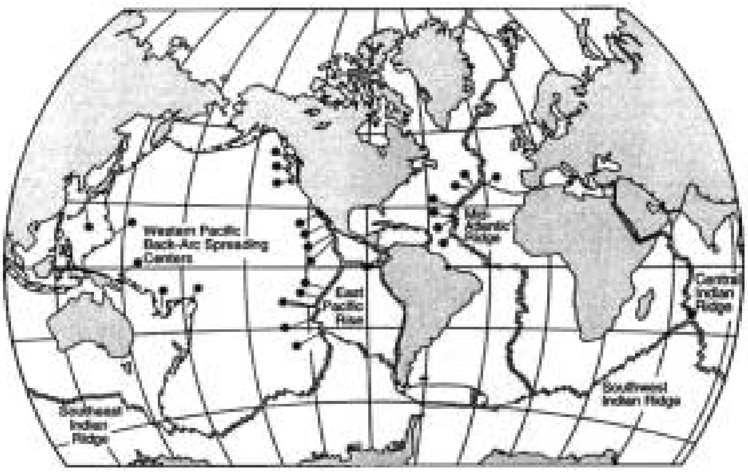

Fig. 1. Global distribution of hydrothermal vents known until 2001.Hydrothermal vents located at Gakkel Ridge not shown (from Van Dover *et al.*, 2001).

Ridge (Van Dover *et al.*, 2001) and numerous vents on the ultra-slow spreading Gakkel Ridge in the Arctic Ocean (Edmonds *et al.*, 2003). In 2002, a US/Canada multidisciplinary expedition to Explorer Ridge, which had been visited only once before (Tunnicliffe *et al.*, 1986), sampled and mapped vents in detail for the first time (Embley *et al.*, 2002, Metaxas, unpublished data). Clearly we must turn again to exploration if we are to understand the extent of these unique geological features on the ocean floor.

THE CHEMICAL ENVIRONMENT

The chemical environment at hydrothermal vents renders them a hostile habitat for most marine organisms. Because of increased volcanic activity, the ocean crust at mid ocean ridges consists of highly porous basalt (10-20% porosity), which may or may not be covered by a layer of sediment (Van Dover, 2000). Ambient seawater permeates through fissures or cracks in the seafloor and reaches the reaction zone overlying the magma chamber (which is impermeable), where it encounters temperatures of 350-400ºC. These high temperatures induce chemical reactions between the seawater and the surrounding basalt, which determine the composition of the end-member hydrothermal fluids exiting at the vents. For example, as seawater moves from the low to the high temperature reaction zones, it becomes depleted in Mg^{2+}, O_2 and $CaSO_4$ and enriched in Ca^{2+} and H^+. At temperatures >200 ºC, the fluid becomes greatly enriched (by several orders of magnitude) in H_2S, H_2, CH_4, and metals such as Mn, Fe, Zn and Cu (Van Dover, 2000). The increased temperature and pressure at the reaction zone forces the buoyant end-member fluid to rise through the crust and exit onto the ocean floor.

Hydrothermal fluids exit on the ocean floor through relatively narrow chimneys (black and white smokers) at high temperatures (300-400ºC) and flow rates (0.7-3.4 m s^{-1}) or through fissures of diffuse flow at lower temperatures (2-60ºC) and flow rates (0.5-5 cm s^{-1}) (Van Dover, 2000). Temporally the chemical composition of hydrothermal fluids may remain stable on decadal scales at equilibrium sites, or may vary on hourly to weekly scales in recently developed vents. Spatially, sharp discontinuities in chemical composition between ambient seawater and hydrothermal fluids can exist on scales of centimetres, such as along the boundary of a patch of mussels (Fig. 2). Additionally, both the rate and direction of fluid flow can be modified locally by the biota during community evolution.

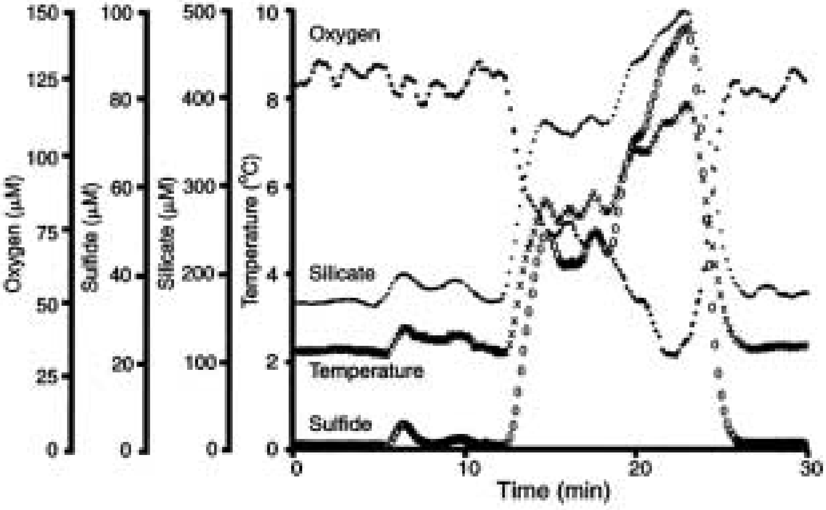

Fig. 2. Chemical composition of seawater measured in situ in Rose Garden on the Galapagos Ridge, as the sensor moved from ambient water through a clump of mussels. The sensor crosses the boundaries of the patch at 12 and 26 min. (from Van Dover, 2000).

The buoyant hydrothermal fluid rises in the water column forming a hydrothermal plume, as it draws in cold water and expands until it reaches the point of neutral density, when it begins spreading laterally by ocean currents (Van Dover, 2000). Hydrothermal plumes typically reach a height of 150-200 m above the sea floor, and they usually have characteristic and identifiable sig*nature*s used to locate hydrothermal activity. Measurable temperature, chemical and optical anomalies are derived from the increased temperature, modified chemical composition and increased particle loading of the hydrothermal fluid relative to ambient seawater. Frequently, it is the detection of these anomalies in the water column that locates (intentionally or accidentally) new sources of hydrothermal venting. For example, Miniature Autonomous Plume Recorders (MARPS) of temperature and light scatter (and thus suspended particulates) were used to explore venting on the Gakkel Ridge (Edmonds *et al.*, 2003). Such advanced technology can provide extremely efficient tools for exploration.

TROPHIC STRUCTURE OF THE BIOLOGICAL ASSEMBLAGES

The discovery of hydrothermal vents has revealed hundreds of species that are new to *science* and endemic to vents (Tunnicliffe *et al.*, 1998). These species are adapted to a suite of conditions that do not exist anywhere else in the marine realm. The most unique characteristic of vent ecosystems is the local primary production of carbon by endo- and episymbiotic, and free-living chemoautotrophic microbes that can sustain extraordinary levels of metazoan biomass.

The range in thermal and chemical conditions generates diverse microhabitats for free-living microbes. For example, hyperthermophilic *Archaea* occupy habitats ranging in temperature between 80 and 115ºC, such as the mineral matrix of sulfide chimneys, implying major adaptations in membrane and protein function (Baross and Deming, 1995). A subsurface biosphere may hold levels of biomass of thermophilic microbes (50-80ºC) similar to those on the ocean crust surface (Van Dover, 2000). Free-living microbes provide a substantial food source for grazers such as limpets and other snails.

A number of successful symbiotic relationships exist between microbes and metazoans, such as tubeworms, mussels, clams and shrimps. Typically the symbiont provides a source of carbohydrates, whereas the host provides simultaneous access to nutrients that are spatially separated (e.g. H_2S in hydrothermal fluid and O_2 in ambient seawater). Some symbionts are transmitted vertically from the female parent to the embryo (e.g. in the clams *Calyptogena magnifica* and *C. pacifica*), while others are thought to be acquired horizontally from the surrounding medium (e.g. in the tubeworms *Riftia pachyptila* and *Ridgeia piscesae*).

While most metazoan biomass at hydrothermal vents occupies by intermediate trophic levels, such as grazers and symbiont-bearing invertebrates, predators such as brachyuran crabs and zoarcid fish also are abundant. Interestingly, the contribution of carbon by symbiont-bearing species to predators, as measured with stable isotopes, may not be as great as anticipated (Van Dover and Fry, 1994).

ORGANIZATION OF BIOLOGICAL ASSEMBLAGES

One of the main goals of community ecology is to determine the relative importance of the physico-chemical environment and biotic processes in organizing biological assemblages. In the marine realm, rigorous ecological studies have pursued this goal in the intertidal or shallow subtidal habitats. One model proposed by Menge and Sutherland (1987) includes environmental stress, competition, predation and recruitment as the regulatory factors of community composition (Fig. 3): in habitats of low environmental stress, the relative importance of predation and

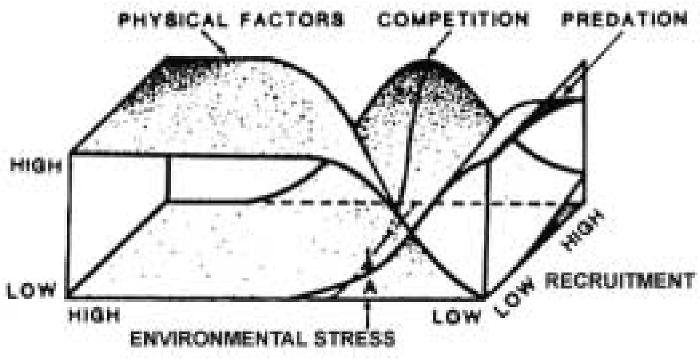

Fig. 3. Model of community regulation showing patterns in relative importance (low to high) of abiotic and biotic factors (from Menge and Sutherland, 1987).

competition is great, but it diminishes as environmental stress increases and physical factors, such as physiological tolerance, become relevant. In turn, the role of each of these factors is modulated by the magnitude of recruitment.

The presence of hydrothermal fluids defines the biological assemblages at deep-sea vents. In the absence of the appropriate chemical conditions, microbial chemoautotrophy would cease and the vent metazoan biomass would not be supported. Thus, the role of the physicochemical environment in vent community structure and organization is unequivocal. Several studies have shown spatial and temporal patterns of faunal abundance that are related to specific physical and chemical conditions (e.g. Shank *et al.*, 1998; Sarrazin *et al.*, 1999; Tsurumi and Tunnicliffe, 2001). Although the hydrothermal fluid contains concentrations of H_2S and metals that can be toxic to non-vent fauna, the species that occupy these habitats are well adapted to the extreme conditions. For example, it has been suggested that alvinellid polychaetes that inhabit sulfide chimneys may be the most thermally tolerant metazoans (Cary *et al.*, 1998). The evolution of detoxifying mechanisms, such as limited membrane diffusion, oxidation, binding and hemoglobin insensitivity has allowed vent inhabitants to exist in normally toxic concentrations of H_2S (Van Dover, 2000). Thus, because it is the hydrothermal fluid flow that provides the necessary nutrients to sustain vent fauna, it can be argued that environmental stress (as shown in Fig. 3) increases with distance from the vent opening.

The roles of biological processes in regulating communities at hydrothermal vents remain largely unknown. To my knowledge, the only study to examine the regulatory role of predation was done on East Pacific Rise at 9º50'N, and it indicated that the influence of this process was greatest nearest the venting source and decreased in more peripheral zones (Micheli *et al.*, 2002). This result corresponds well to the prediction shown in Fig. 3, if it is assumed that environmental stress increases with distance from the venting source. Although not examined to date, the role of competition will be most significant in areas of great faunal abundance, and thus possibly limiting resources, such as the physical or chemical substrate. For vent species for which access to the hydrothermal fluid is essential for survival, the limiting resource will be physical substrate near vent openings. Thus, the effect of competition on community regulation most likely will decrease with increasing distance from the venting source. This prediction also corresponds well to the model by Menge and Sutherland (1987). Knowledge of the relative importance of predation and competition can allow for the relative placement of the two corresponding surfaces in Fig. 3.

The role of recruitment in modulating the effects of abiotic and other biotic factors on the organization of vent communities is also not known. Spatial and temporal variation in recruitment has been described in several species and at different vents (e.g. Comtet and Desbruyères, 1998; Thiébaut *et al.*, 2002). These studies have indicated that the level of continuity in recruitment is species specific. Potential cues for settlement are not well understood and not consistent among taxa. Some species (e.g. the mussel *Bathymodiolus thermophilus*) settle in areas of high temperature (and thus in proximity to the source of venting fluid) (Mullineaux *et al.*, 1998). However, positive and negative interspecific interactions in settlers and recruits also have been identified and used in models of community succession at East Pacific Rise (Mullineaux *et al.*, 2000, in press). For example, during the early stages of community evolution, settlement of the tubeworm *R. pachyptila* may be facilitated by the presence of another tubeworm, *Tevnia jerichonana* (Mullineaux *et al.*, 2000). This does not seem to be true for established assemblages. Negative interactions appear more prominent near the source of venting fluid while the opposite pattern is observed for positive interactions (Mullineaux *et al.*, in press). This evidence also is in agreement with my interpretation of the model by Menge and Sutherland (1987).

Larval supply, which influences the magnitude of settlement and eventual recruitment, is in turn tightly linked to larval dispersal and retention. The ephemeral and distinct nature of hydrothermal vents implies evolutionary pressure for long-distance dispersal by larvae of vent organisms. To date, the proposed mechanisms of larval dispersal have been generated using models utilizing estimates of hydrodynamics and probable larval duration. Chevaldonné *et al.* (1997) estimated that a single migrant per generation exchanged between populations of alvinellid polychaetes was sufficient to maintain genetic mixing. To be successful this migrant must travel along-axis for 8 days or 8-30 km. For *R. pachyptila*, it was estimated that, after 20 days since their release, ~40% of larvae survive and the maximum distance travelled along-axis is ~90 km (Marsh *et al.* 2001). To my knowledge, no published studies exist that have addressed larval retention. Given the low probability of significant larval dispersal between vent active zones, most larvae within a vent zone must be produced locally to sustain populations on ecological time scales. I have found that larval availability can be in the order of thousands of individuals m^{-2} d^{-1} and does not vary spatially on scales of several hundreds of meters. These results suggest significant larval retention. Presently, our understanding of the relative importance of larval dispersal, supply, settlement and recruitment in community organization at vents is at an embryonic stage.

It is apparent that despite their long history in the study of shallow marine systems, the validity of ecological models has not been rigorously examined for communities at hydrothermal vents. Instead, most studies have been descriptive of the biota or have focussed on the physiological tolerances of different metazoans. A handful of recent studies stand as exceptions. In part, a descriptive focus is expected during the initial exploratory research in any newly discovered system. This is particularly pertinent at hydrothermal vents because of the novelty and endemicity of both the metazoan species found there and their symbiotic relationships. Additionally the harsh environmental conditions, and consequent physiological tolerances were considered of overriding importance not only in understanding the organization of these communities, but also the limitations and potential adaptations of metazoan physiology in general. Research on the community organization of rocky intertidal habitats, which are also environmentally harsh, followed a similar trajectory.

An additional constraint involves the remoteness and limited accessibility of hydrothermal vents. Currently, successful hypothesis testing and the attainment of a mechanistic understanding in ecological research involve rigorous experimental manipulations with adequate replication for powerful statistical analyses. Both these tasks are extremely time-consuming (and consequently expensive) when done from a submersible and multi-investigator teams are reluctant to complete them. A related limitation is sampling over short time intervals (cruise length) or at low frequencies (semi-annually at best; annually most likely) during fair-weather only.

These constraints will be addressed partially with cabled ocean observatories, such as the US/Canada partnership NEPTUNE. is a network of 3,000 km of underwater fibre optic cable that will span the entire Juan de Fuca plate and will provide continuous, high resolution, real-time measures and samples. Instruments positioned at selected locations along the cables will be accessible to scientists at all times of day and year and will allow for responsive sampling to physical or biological events of interest. It is believed that cabled observatories will revolutionize marine science.

THE MEDITERRANEAN SETTING

Most hydrothermal vents in the Mediterranean with described biological assemblages occur in shallow depths of <100 m, and most frequently within reach by snorkelling or SCUBA (Dando *et al.*, 1999). Consequently, the profound difference between these and the described oceanic deep-sea vents is the occurrence of photosynthetic primary production. Also, the species that inhabit shallow water Mediterranean hydrothermal vents are not endemic to these habitats, but represent a subgroup of the most tolerant species in the ambient fauna.

The only published evidence for deep-sea hydrothermalism in the Mediterranean is that of indicators of extinct activity observed on the peak of Marsili Seamount in the Tyrrhenian Basin at ~450-500 m depth (Uchupi and Ballard, 1989). Also, there is some evidence of chemosynthetically based communities associated with cold hydrocarbon seeps. Shells, belonging to families that typically harbour chemoautotrophic symbionts and are found in cold seeps elsewhere, were collected from the top of the Napoli Dome on the Mediterranean Ridge at depths of ~1900 m (Corselli and Basso, 1996). Live and dead communities of polychaetes and bivalves were found associated with cold seeps in the southEastern Mediterranean near Egypt and the Gaza Strip at depths of 500-800 m (Coleman and Ballard, 2001).

Biological communities at cold seeps have been receiving increased attention since the discovery of hydrothermal vents (Van Dover, 2000). Communities at vents and seeps share some fundamental properties such as chemoautotrophically-fueled exceptional metazoan biomass, highly specialized symbiotic relationships, physiological tolerance to harsh conditions, a distinct and pronounced resource gradient and great species endemicity. Several species, genera and families are common between the two habitats (Sibuet and Olu, 1998). Because of the high affinities between different chemosynthetic ecosystems, including hydrothermal vents and cold seeps, all such systems must be studied in combination to understand patterns of biogeography and biological diversity.

The tantalizing paucity of information on chemosynthetic ecosystems in the Mediterranean Sea lends strong support to an initiative of extensive exploration. Most likely conditions along the Mediterranean Ridge, an accretionary prism formed by the subduction of the African plate, favour the occurrence of such ecosystems. The presence of mud volcanoes and brine lakes along the Ridge are reminiscent of other areas that support deep-sea chemosynthetic ecosystems, such as the Barbados Ridge complex and the Gulf of Mexico continental slope. Technological advances, such as MARPs and deep-sea remotely operated vehicles can provide the necessary tools for effective exploration. Most of the deep-sea environment, including chemosynthetically driven ecosystems, remains unknown, despite humankind's great technological advances. Given their possible extent and uniqueness in the world's oceans, we must strive to attain a level of understanding for these ecosystems similar to that for other aquatic or terrestrial ones.

Chemosynthesis-based ecosystems in the deep Atlantic – what we do know and we don’t

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The discovery of hydrothermal vents, followed by that of cold seeps, has been one of the great interdisciplinary successes of oceanography in at least the last 25 years (Tyler *et al.*, 2002). One can hardly imagine a greater contrast to the typical soft-sediment deep sea than the exuberant oases of life at hydrothermal vents and cold seeps areas. With the steep gradient of chemistry and the high biological primary production, the ecology of vent invertebrates is probably more closely similar to that of intertidal communities than to the “regular” deep sea ones (for review see Van Dover, 2000). These oases of life are small-sized and discontinuous in space and cannot be observed and studied without the use of deep-sea submersibles. The direct linkage between fluid emission and bacterial chemo-autotrophic production makes them also discontinuous in time at different scales.

Discovered in 1977 in the Eastern Pacific (see Metaxas, this volume), the biological communities associated with active deep-sea hydrothermal vents were first observed on the Mid-Atlantic Ridge system in the mid’ 80s (Rona *et al.*, 1984; Rona, 1985). Until 1993, the deep sites (3500-3600 m) TAG and Snake Pit fields, where the fauna is dominated by swarms of the blind shrimp *Rimicaris exoculata*, remained the only known vent sites in the Atlantic (Segonzac, 1992). During the years 1993-1997, three vent fileds were discovered and described in the vicinity of the Azores Plateau (Van Dover *et al.*, 1996; Desbruyères *et al.*, 2000; Desbruyères *et al.*, 2001).

Close to the Azores plateau, the ridge axis depth decreases from 2400 m in the Rainbow vent field (c.a. 36°13’N) to 850 m in the Menez Gwen vent field (c.a. 37°35’N). The deepest site (Rainbow) is dominated as in TAG and Snake Pit by swarms of shrimps *Rimicaris exocultata*, when the shallowest sites are dominated by mussel beds of the mytilid bivalve *Bathymodiolus azoricus*. The variations observed in the structure and composition of the communities along the depth gradient are most likely due to changes in the vent fluid toxicity (metallic and sulfide content) and suspended mineral particles, which render the fluids harsher for species living there. In the shallowest sites, the main faunal difference is due to impoverishment in the hydrothermal endemic species and to the penetration in the vent field of bathyal species. This shift in biodiversity from specialized to non-specialized hydrothermal fauna is due to the phase separation and boiling of fluids and is a rather important boundary between deep-sea vents and shallow water vents which are with a rare exception (i.e. Kagoshima Bay), never colonized by specialized fauna.

A more recent description of a deep hydrothermal site was the Logatchev vent sector made of three different fields ranging from 2700 to 3050 m depth, c.a. 14°45’N. Hydrothermal communities from the three fields differ considerably: one is dominated by the Vesicoymid bivalve

Fig. 1. Typical mussel bed (*Bathymodiolus azoricus*) in the Rainbow vent site (MAR) by 2300 m depth.

Ectenagena aff. kaikoi, the second by the mussel *Bathymodiolus puteoserpentis* and large swarms of *Rimicaris exoculata*, the third one is characterized by a very impoverished hydrothermal fauna (Gebruk *et al.*, 2000). Finally, a new active site named Lost City was recently discovered at 30°N, 15 km off-axis and distinctly different from all known vent fields with relatively cold (40°-75°C) and alkaline fluids (pH- 9 to 9.8), supporting dense microbial communities but no so-called “hydrothermal vent fauna”. Fauna is limited instead to few crabs, sea urchins and abundant sponges and corals (Kelley *et al.*, 2001). No other deep hydrothermal site is known in the Atlantic north of the Azores or south of 14°N/MAR although a number of hydrothermal plumes were detected in the equatorial Atlantic, Scottia-Arc and in the Arctic Ocean (Gakkel Ridge) (Edmonds *et al.*, 2003).

In Mediterranean waters, hydrothermal vents are not associated with spreading centers but to Volcanic Arcs as the Hellenic Volcanic Arc, an area with strong volcanic activity. In Milos Island, shallow water vents (44 m) are colonized by white flocculent bacterial mats but no large-scale effect of venting on the macro-epibenthic communities (Cocito *et al.*, 2000) was observed as on most shallow water vents in the Atlantic (Fricke *et al.*, 1989).

The discovery of large ecosystems based on bacterial chemosynthetic production at continental margins off Western Florida (Paull *et al.*, 1984) and along the subduction zone off Oregon (Suess *et al.*, 1985) gave us new insights on the biogeography of the chemoautotrophic associations (see Sibuet and Olu, 1998) for review). In the Atlantic the known cold seeps are associated with passive margins in the north Carolina continental slope (Blake Ridge) (Van Dover *et al.*, 2003) and northern Gulf of Mexico from 400 to 3500 m associated with gaz and oil seepages (Kennicutt II *et al.*, 1985; Carney, 1994). In the Gulf of Mexico, the communities are dominated by mytilids *Bathymodiolus childressi* and *B.* spp. and the vestimentiferans worms *Lamellibrachia luymesi* and *Seepiophila jonesi* (Bergquist *et al.*, 2003). In the Barbados prism, at depth ranging from 1000 to 5000 m, mud volcanoes and diapirs are colonized by vesicomyid clams and cladorhizid sponges associated with methanotrophic bacteria (Olu *et al.*, 1997). In the Gulf of

Fig. 2. Swarms of *Rimicaris exoculata* in the same vent field sulfide edifices.

Guinea, recent data show that the mussel beds are dominated by a species very close to *Bathymodiolus heckerae* described from Florida and the Barbados prism. Vestimentiferan bushes are dominated by a new species closely related to *Escarpia laminata* from the Florida escarpment. The most conspicuous fauna of cold seep ecosystems are large vesicomyid clams, mytilid mussels, vestimentiferan tubeworms, cladorhizid and hymedesmiid sponges. At a supraspecific taxonomic level, the faunal composition of cold seep communities remindis hydrothermal vent associations (Van Dover *et al.*, 2002). Some vent and seep invertebrate species are immigrant from the surrounding deep-sea, when others may be derived from shallow water species. Other species have a long history of endemism, having diversified within vent and seep habitats. Massive food falls (sunken woods, carcasses, plant fibers, even “organic rich” wreck) could be also sources of reduced components and could harbour chemosynthesis-based communities (Dando *et al.*, 1992; Smith *et al.*, 1989, 1996; Allison *et al.*, 1991; Bennett *et al.*, 1994).

In the Mediterranean Sea, two mud volcanoes were explored south of Crete (see also Fiala-Medioni, this volume) along the Mediterranean Ridge (2000 m depth). Chemosynthesis-based communities are dominated by small sized bivalves (vesicomyids, thyasirids, mytilids, lucinids) and pogonophorans including large sized *Lamellibrachia* (Olu-Le Roy *et al.*, 2001). The faunal difference between Atlantic and Mediterranean seeps could be attributed to the biogeographic isolation of the basins by the Gibraltar sill since the upper Miocene and the Messinian faunal sterilization (Taviani, 2002). Multibeam mapping and side scan sonar tracts (B. Loubrieu, C. Satra, R. Cagna - Cartography by multibeam echosounder IFREMER/CIESM) reveal the existence of numerous mud-volcanoes and anoxic basins in the Eastern Mediterranean, which can harbour cold-seep type communities. A new cruise planned in the upcoming months at the Nile deep-sea fan and the Mediterranean Ridge, aims to investigate the fluid seepages and their consequences on the ecosystem in the frame of a broad European Project MEDINAUT. Several other transient habitats (e.g. plant remains or whale carcasses) can be also colonized by chemosynthesis-based communities and can act as stepping stones at an evolution time scale for deep vent (if any) and seep communities.

More than elsewhere, our knowledge of the Mediterranean communities based on bacterial primary production in sulfidic fragmented deep-sea habitats is in its infancy and deserves an international coordinated effort. The long isolation of these biological associations, as well as the unique hydrographic and physiographic features of the Mediterranean basins provide a unique opportunity to test hypotheses on the role of isolation *vs* habitat constraints on sulfophilic fauna.

Sources of energy for deep-sea benthos in Mediterranean mud volcanoes

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Following the discovery of bivalve thanatocoenoses in samples cored on the Napoli mud-volcano at 1,900m depth, with dead shells attributed to *Myrtea* sp. (Lucinidae) and *Vesicomya* sp. (Vesicomyidae) (Corselli and Basso, 1996), chemosynthesis processes were demonstrated to support dense living chemosynthesis-based communities observed by 1,700/2,000 m depth in the Eastern Mediterranean Sea, south Crete or Turkey, on mud volcanoes or along fault zones associated to methane seeps observed by 1,900/2,000 m.

These chemosynthetic populations were found in the Olympi field and the Anaximander area (southwestern Turkey) by the submersible Nautile during the French-Dutch MEDINAUT expedition in 1998 (MEDINAUT/MEDINETH Shipboard Scientific Parties, 2000).

In both areas, geochemical studies show evidence of upward flow of methane (concentrations as high as 13 ml/l and higher hydrocarbon gases associated with these mud volcanoes (Camerlenghi *et al.*, 1992; Woodside *et al.*, 1998).

The food webs of hydrothermal vent and cold seep communities have been demonstrated to rely entirely on the production of organic matter via bacterial chemoautolithotrophy (Jannasch and Mottl, 1985). At both hydrothermal vents and cold seeps , bacterial endosymbionts in gills (bivalve molluscs Vesicomyidae, Mytilidae, Solemyidae, Lucinidae or Thyasiridae) (Fiala-Médioni and Felbeck, 1990; Fisher, 1995; Fiala-Médioni *et al.*, 1993) or trophosomes (Vestimentiferan worms) (Nelson and Fisher, 1995) derive energy from oxidation of the methane and/or reduced sulfur compounds.

Seepages and associate communities have been observed on continental margins from depths ranging between 400 and 7,326 m in different geological settings (see biological overviews in Sibuet and Olu, 1998; Kojima, 2002). At all sites, chemosynthesis-based communities are restricted to areas where methane seeps out along tectonic features leading to accumulations of hydrogen sulfide in the sediment through microbial sulfate reduction processes (Henry *et al.*, 1996; Sibuet et Olu, 1998; Masuzawa *et al.*, 1992).

KAZAN MV BIOLOGICAL COMMUNITY

During Nautile explorations dense populations of small bivalves and vestimentiferan tube worms were discovered on different mud volcanoes, but living samples were however mainly obtained from the top of Amsterdam at 2,032 m depth and Kazan MV at 1,707 m depth in the Anaximender field, south of Turkey.

The large fluid realising area on the Kazan MV shows a mixture of dark grey sediment and pieces of calcareous crusts fragments where Vestimentiferan are incrusted (Fig. 1). The community appears as mainly composed of bivalves belonging to four families (Lucinidae, Vesicomyidae, Mytilidae and Thyasiridae) and pogonophorans (Annelida Siboglinidae) including a large size vestimentiferan worm.

Fig. 1. Picture of the top of the Kazan mud volcano with a cold seep community at 1,707 m. Only *Myrtea sp*. shells and scattered living tube worms are visible but small living individuals identified as *Myrtea sp., Isoropodon perplexum*, as well *Idas modiolaeformis* were found in the sediment and carbonate crusts sampled. (IFREMER picture, MEDINAUT cruise, 1999).

A remarkable fact is the abundance of Lucinidae previously only observed in shallowest reduced sediments; one identified as *Myrtea sp*. (Olu-Le Roy *et al.*, 2001 and in press) appears as the most abundant species in this community; abundant dead shells of this species were observed but a few living specimens were found in the two cores made on the site. Few samples of another lucinid was found and described as *Lucinoma kasani* (Salas and Woodside, 2002). Other living bivalves included a small vesicomyid, identified as *Isorropodon perplexum* (Olu-Le Roy *et al.*, 2001 and in press; von Cosel and C. Salas, 2001) and a small mytilid attached to carbonate crusts and identified as *Idas modiolaeformis* (Olu-Le Roy *et al.*, 2001 and in press). Some tubes of living worms belonging to *Lamellibrachia sp.* (Olu-Le Roy *et al.*, 2001 and in press) were also collected at the site. On top of a strongly reduced sediment white-grey bacterial mats were observed as well as many sea-urchins and a number of unidentified crabs (Olu-Le Roy *et al.*, 2001 and in press)

The Kazan MV community appears quite different from all other known cold seep communities which are generally dominated by large size bivalves (mytilids or vesicomyids) and to less extent by tube worms. Lucinids can be found in minor abundance at shallow sulfide-rich sites (the shallowest site being 0.25 m at St Joseph's Bay, Florida, Fisher and Hand, 1984); only dead shells have previously been found in deep sea (1,950 m) on Napoli MV in the olympi field (Corselli and Basso, 1996). The only known living Mediterranean species belonging to the genus *Myrtea* is *M. spinifera* Montagu, 1803, widely distributed on the continental shelf of the Eastern Atlantic, from the Norwegian Sea to the Azores islands, and into the Mediterranean from a few meters to about 100 m depth. At the Kazan MV site, the infaunal lucinid is typically of larger size (up to 20 mm) than the shallower mediterranean species and is associated with subsurface carbonate lithification as also found at the Napoli MV site (Corselli and Basso, 1996).

Symbiotic mytilids were found from 472 m (Kaikata Seamount, Ogasawara Islands area (Ohno *et al.*, 1996) to 3,660 m in the Marianna Back Arc Basin (Hessler and Lonsdale, 1991). Few mytilid *I. modiolaeformis* were collected at the Kazan MV site, and it has been impossible to estimate their abundance since they were all found fixed on carbonate crust samples. All the individuals are quite small when compared to mytilids from all other known vent or seep sites but they represent also an additional example of a species housing two types of symbionts. Such a dual symbiosis has only been described in two species, respectively from vent sites in the Mid Atlantic Ridge: *B. puteoserpentis* from the Snake-pit site (Cavanaugh *et al.*, 1992) and *B. azoricus* from Azores sites (Fiala-Médioni *et al.*, 2003), in two species from Atlantic cold seeps: *B. boomerang* from the Barbados accretionary prism (Fiala-Médioni *et al.*, 1994; Olu *et al.*, 1997) and in an undescribed *B. sp.* from the Gulf of Mexico (Fisher *et al.*, 1993).

Vesicomyids are widely distributed in cold seeps, colonizing sites from 400 m water depth on the Lousiana slope (Brooks *et al.*, 1987) to 7,326 m in the Japan trenches (Kojima, 2002) where they often constitute the dominant organisms. Related to its small size, the Kazan MV vesicomyid *I. perplexum* was not observed *in situ* but found in the sediment samples. Despite its unusually small size for a seep vesicomyid, this species also hosts in its gills bacterial symbionts.

Shells of a small thyasirid were also found in the samples (Olu-Le Roy *et al.*, 2001 and in press) but no living organisms were collected as for the species *L. kazani* found in very low numbers.

SOURCES OF ENERGY

Transmission electron microscopy observations show that the tube worms and the bivalve molluscs (Lucinidae,Vesycomyidae and Mytilidae) contain, respectively in their trophosome or gills, bacterial endosymbionts: the lucinid *Myrtea sp*. gill filaments cells house, number of sulfo-oxidizing like symbionts as also the vesicomyid *I. perplexum* and the trophosome of the Vestimentiferan *Lamellibrachia sp. (*Fiala-Médioni *et al.*, 2001*)*

In the gill of the mytilid two distinct morphological types of symbionts were observed: the first type consists of small (mean diameter: 0.35 µm) coccoids or short rods, resembling the sulphide-oxidizing symbionts already observed in *Bathymodiolus thermophilus* from the Pacific hydrothermal vents (Fiala-Médioni and Felbeck, 1990). The second type are large coccoid-shaped bacteria (mean diameter: 1.25 µm) looking like type I methylotrophs with stacked intracytoplasmic membranes as observed in *Bathymodiolus puteoserpentis* (Cavanaugh *et al.*, 1992) or *B. azoricus* (Fiala-Médioni *et al.*, 2003) and identified as metanotrophic symbionts *(*Fiala-Médioni *et al.,* 2001*).*

Stable carbone and nitrogen isotope analyses of the tissues confirmed the chemosynthetic fractionation of of the organic material from these species.

13C/12C ratios are ranging from -27.7‰ to -30.5‰ (n= 3) for *Myrtea sp*, from -29.3‰ to -30.1‰ (n=4) for *I. perplexum* and from -23.6‰ to -26.6‰ (n=4) for Vestimentiferan *Lamellibrachia sp*. *(*Fiala-Médioni *et al.,* 2001*)*. These values are similar to the ones of other symbiotic chemosynthetis-based bivalves or tube worms hosting sulfide-oxydizing symbionts, found in different vent or cold seep sites (Kennicutt *et al.*, 1992; Fisher, 1995); they are more depleted that the values of non symbiotic deep sea species ranging from -17.1‰ to -21.3‰ (Van Dover and Fry, 1989). The values measured on the Kazan MV mytilid *I. modiolaeformis* are more depleted than those of the lucinid ; ranging from -44.2‰ to -44.6‰ (n=3) they reflect more likely the presence of methylotrophic symbionts which may use methane as energy source.

The 15N/14N isotopic ratios are also within the range of values reported from other symbiotic organisms: +2,0‰ to -12.9‰ (Kennicutt *et al.*, 1992), values which are quite depleted in 15N when compared to other non symbiotic species organisms characterized by values between

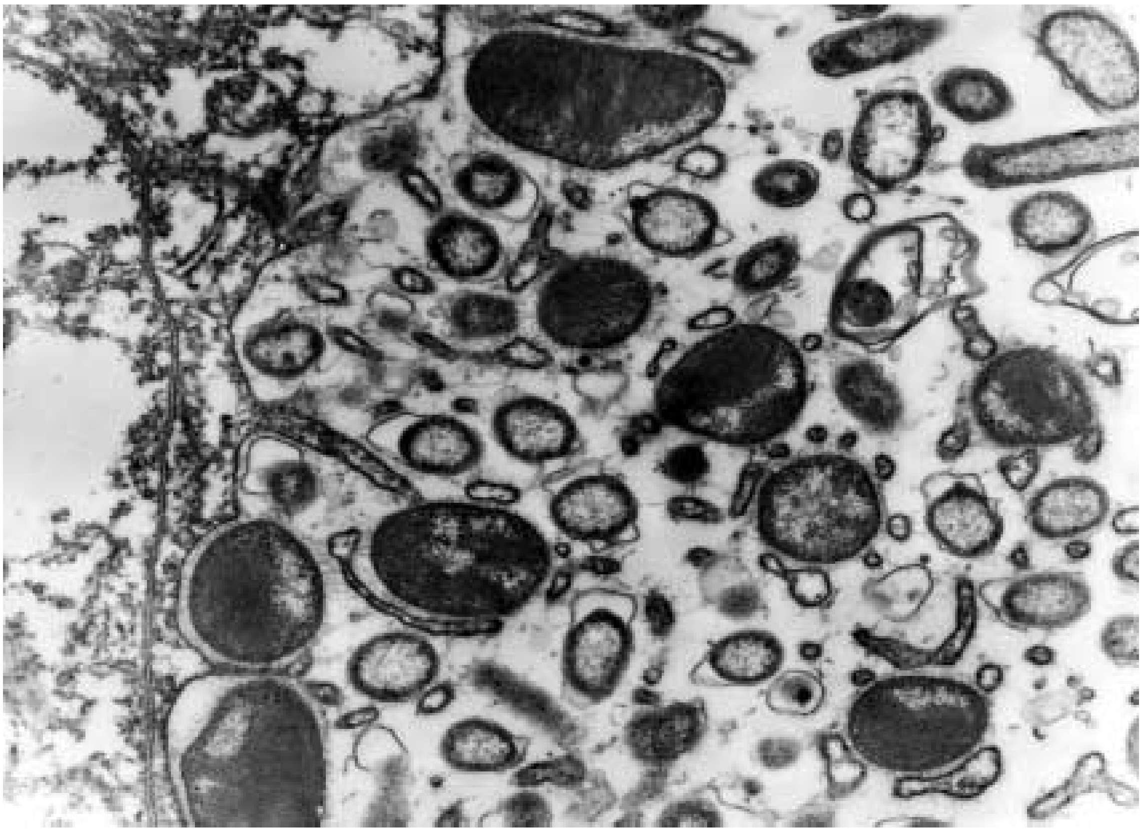

Fig. 2. Mytilid *Idas modiolaeformis*. Transmission electron micrograph of endosymbiotic bacteria on transversal sections of the gill : dual symbiosis with both large methylotrophic bacteria with specific internal membranes and small sulfur-oxidizing bacteria

+11.6‰ and 15.7‰ (Van Dover and Fry, 1989; Kennicutt *et al.*, 1992). The values found for the Kazan MV species range from -1.0‰ to 1.4‰ for *Myrtea sp*., from 0.2‰ to 0.4‰ for the *I. modiolaeformis,* from -1.6‰ to 1.9‰ for *I. perplexum,* and from -0.8‰ to 0.2‰ for the tube worm *Lamellibrachia sp.*

The chemosynthetic macrofauna is located at sites where fluids are expelled (Olu *et al.*, 1997; Sibuet and Olu, 1998), but there is, up to now, no statistical evidence of any direct relationship between methane concentrations and living biomasses : this will need more integrated investigations. With the exception of the mytilid, such relationship appears however necessary indirect for Kazan MV seep species where no methylotrophic-like symbionts were observed.

Within Mediterranean mud volcanoe fields, it has been evidenced that concentrations of methane increase with depth in the uppermost meter of the mud breccias. In most cases this appears to be associated with the depth zone of sulfate reduction (De Lange and Brumsack, 1998) and results in the generation of inorganic carbon and hydrogen sulfide which can be used as a substrate by the sulfide oxidizing symbionts of the macrofauna.

All the result are in agreement with the hypothesis that the worm, the vesicomyid and the lucinid bivalves are relying, through their sulfur-oxidizing-type symbionts, on the sulfide issue from microbial sulfate reduction processes in the superficial sediment when the mytilid symbionts could use either sulfides from the sediment or the methane expelled in the seeps.

Research needs on biodiversity of deep-sea ecosystems

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Our knowledge of deep-sea ecosystems has been limited historically by its vast area and the cost and difficulty of access. Nonetheless, there are compelling reasons to improve on current levels of understanding in the most expansive habitat on Earth. From a pragmatic perspective, increasing encroachment by human activities from fishing, oil exploration, mining of seabed resources, and even pollution by deliberate dumping (sludge, radioactive waste, dredge material) creates significant potential for human impact (Thiel, 2003) at greater and greater depths with ever-accelerating technology developments. From a scientific perspective, deep-sea ecosystems represent the largest ecosystem on Earth, one of the most pristine natural "laboratories" in which to test and understand biodiversity questions (e.g. maintenance, resilience), and very possibly one of the most species-rich habitats on the planet (Grassle and Maciolek, 1992). Given the cost and limited scientific resources that can be directed towards deep-sea ecosystem studies, it is necessary to identify and prioritize ecological questions that remain unresolved for these environments. This paper, largely based on Snelgrove and Smith (2002), is strongly biased towards biodiversity and community ecology of sedimentary systems. Hard substrate systems such as hydrothermal vents and volcanoes are discussed in the papers of Metaxas, Desbruyères and Fiala-Médioni (this volume). The summary below also focuses on macrofaunal organisms (organisms such as small polychaete worms, crustaceans and bivalves too small to be identified in photographs but that are retained on a 300 micron sieve). Megafauna (organisms that can be identified in bottom photographs) are discussed in the papers of Billett, Company *et al.*, and Moranta (this volume), whereas meiofauna (organisms that pass through a 300 micron sieve but are retained on a 44 micron sieve) are discussed below in the papers of Danovaro and Gambi and Lampadariou *et al.* (this volume). In a general sense the discussion below is organized into research priorities based on sampling needs and experimental needs. Although these two categories are interrelated, they require different approaches in many cases. This summary deliberately reflects broad research recommendations for biodiversity research in deep-sea ecosystems rather than a specific focus on the Mediterranean Sea.

SAMPLING NEEDS

Descriptions of broad-scale patterns in deep-sea communities have generated considerable interest. Depth (e.g. Rex, 1983) and latitudinal patterns (e.g. Rex *et al.*, 1993) in diversity have been described for different taxa and different geographic areas but are not always consistent. Whether this variation reflects geographic variability or differences in the responses of different taxonomic groups needs to be assessed. For example, Lambshead *et al.* (2000) have argued that nematodes are more diverse at higher latitudes, the reverse of patterns described for macrofaunal groups by Rex *et al.* (1993). Differences in evolutionary history among groups (e.g. Wilson, 1998) add an additional complication. The main problem is that funding has rarely allowed studies of multiple taxonomic groups in a single study and geographic area. Collaborative studies among ecologists working on different groups (megafauna to microbes) could rectify this shortcoming.

Another issue that has generated great interest is the estimation of species numbers in deep-sea regions and in the deep sea overall. Estimates for macrofauna vary hugely from 10^7 (Grassle and Maciolek, 1992) to 500,000 (May 1992) to 5 million (Poore and Wilson, 1993). For meiofauna, uncertainty is even greater; Lambshead (1993) estimated 30 million nematode species though Lambshead and Boucher (2003) offer a much more conservative perspective on nematode diversity. Diversity estimates in the deep sea are limited by many assumptions that are made in extrapolation, and by the small portion of the deep-sea floor that has been sampled. Paterson (1993) estimated that a total of ~2 km^2 of ocean floor has been sampled globally for macrofauna and Lambshead (1993) estimated that ~ 5 m^2 has been sampled for meiofauna. Huge areas of the oceans, particularly in the South Pacific and Indian Oceans, present vast unknowns. Even the Mediterranean, despite the presence of many research institutes in the region, has been sampled quantitatively in only a few small areas below 1000 m for macrofauna (see Tselepides, this volume) or meiofauna (see Danovaro and Gambi, this volume). For megafauna there are only a handful of quantitative samples below 1000 m (see Company, this volume). Given that there are ~10^5 species described for marine sediments (Snelgrove *et al.*, 1997), ~ 95 to 99.9% of fauna may be undescribed. Even among described species there is increasing evidence that some marine "cosmopolitan" species may be sibling species (Knowlton, 1993). We may therefore be underestimating species richness even in the best known deep-sea habitats. Molecular biology has elucidated previously unknown patterns in deep-sea species and genetic diversity (France and Kocher, 1996; Chase *et al.*, 1998), indicating further challenges to evaluation of diversity. Clearly, there is much work remaining to characterize patterns and scales of species diversity on the deep-sea floor. Existing data indicate higher within-habitat diversity in deep-sea sediments than in most shelf habitats that have been sampled (but see dissenting view in Gray *et al.*, 1998), although the existing database is very poor. Scaling up from alpha diversity is extremely difficult with such limited data, and considerably more data are needed to elucidate the contributions of shelf and deep-sea communities to the global species pool. Inclusion of features such as canyons and seamounts also creates additional potential species pools that must be considered.

The controversy and debate (e.g. May, 1992; Gray *et al.*, 1998) sparked by Grassle and Maciolek's (1992) study point to several obvious research needs in understanding deep-sea diversity. First, sampling efforts that employ the same high spatial intensity and taxonomic quality of their study (Fig. 1) are needed elsewhere in the deep sea if we are to understand broad-scale patterns in deep-sea species diversity. As coverage of previously unsampled areas is achieved, we should be able to determine whether the proportion of undescribed species is closer to 50% or 0.5% on a global basis. Another useful, though difficult goal, is to sample an area of the deep sea well enough for species *versus* abundance curves to approach asymptotes at multiple scales (e.g. km scale to hundreds of kms). *Thus, the rates at which species turn over spatially for different taxonomic groups remain one of the greatest unknowns in efforts to inventory and develop conservation strategies for deep-sea ecosystems.* This approach would provide another means to estimate total deep-sea species richness or richness in a specific basin (e.g. the Mediterranean). In terms of taxa, there is a pressing need to improve estimates for meiofaunal species number; Lambshead's (1993) estimate of 10^8 species of nematodes swamps macrofaunal estimates, but its validity must be tested. Bacteria and *Archaea* also represent unknown, but potentially enormous, species pools (e.g. the spectacular diversity observed in planktonic microbes by Giovannoni *et al.*, 1990). Because most marine taxa are more species-rich in the benthos than in

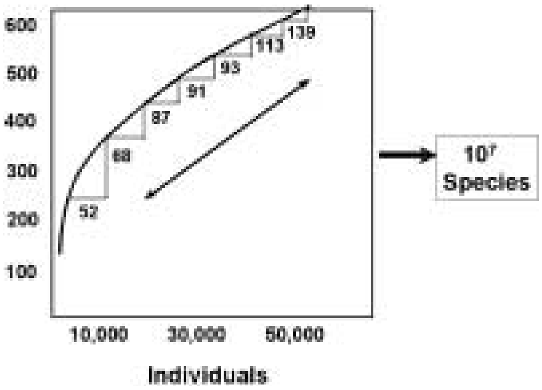

Fig. 1. From Grassle and Maciolek, 1992.

the water column, and microbial habitat complexity in sediments is likely very high, the unknown microbial world in deep-sea sediments is probably very diverse (see Fuhrman and Davis, 1997). Spatial extrapolations from areas other than the North Atlantic are needed to produce better estimates of global species numbers and patterns. *We must therefore generate better estimates of alpha (local) diversity and better understanding of beta (patch) diversity as we try to scale to gamma (regional) diversity.*

Better evaluation of diversity patterns will also lead to greater understanding of diversity maintenance in the deep sea because current broad-scale comparisons and hypotheses are based on limited data (e.g. Rex *et al.*, 1993; Poore and Wilson, 1993). Generalisations are often refuted by conflicting data from areas that are poorly sampled (e.g. South Pacific), or because a different taxonomic group was examined. In the latter case, there is some evidence that meiofaunal taxa may be less impacted by physical disturbance than macrofaunal groups (e.g. Thistle *et al.*, 1985). Given the differences in the life habits and dispersal potential between macrofauna and meiofauna, it is quite conceivable that processes regulating diversity differ between the groups. *It is for this reason that there is a pressing need for more taxonomically comprehensive studies within a single locale.* When such studies are paired with improved models and carbon export data (Falkowski *et al.*, 1998), they will clarify the enigmatic relationships between production, depth, latitude, and deep-sea diversity.

EXPERIMENTAL NEEDS

Regulation of communities (Biodiversity)

From an experimental perspective, repeated access to the deep-sea environment is critical to evaluate the importance of habitat heterogeneity. Improved access is needed not only to sample more natural patch types, but to provide better time-series coverage. For most experimental patches, data exist for only 1-2 time points, and for natural patches the time issue is largely unknown. *In summary, we need better sampling of episodic events from multiple areas globally, including the Mediterranean.* Underwater observatories that provide real time, continuous data could achieve some aspects of this sampling, but deep-sea observatories have not yet developed to fill this niche (though efforts are underway – see <www.neptunecanada.ca>).

A variety of disturbance processes operate in the otherwise physically benign (with some exceptions), food-limited deep-sea environment and may help to create conditions that favour high diversity (Snelgrove and Smith, 2002). The high alpha diversity in the deep sea appears to result from an array of disequilibrium processes, including disturbance, succession, and response to different types of patchiness of the seafloor. Limited small-scale experiments support the idea that small-scale patches support a relatively specialized fauna, though many of these studies indicate lower diversity in patches than in surrounding sediments. Moreover, it is very difficult to scale up from experiments that focus on small scales. Pairing these experimental approaches with broad-scale sampling programmes offers a way forward. For example, sampling efforts suggest that deep-sea areas subjected to strong environmental forcing, such as intense currents, hydrogen sulphide, or oxygen stress, are likely to have reduced macrofaunal diversity (e.g. Levin and Gage, 1998). There does not appear to be any simple relationship between productivity and deep-sea diversity (e.g. Snelgrove *et al.*, 2000), but it is hypothesised that if highly productive areas and environments with overriding environmental (e.g. high currents, hypoxia) or geological (e.g. isolation, anoxia) controls are excluded, a positive relationship between amount and variability of production and diversity may emerge (e.g. Lambshead *et al.*, 2000; see also Danavaro and Gambi, this volume). This may prove to be similar to the parabolic relationship proposed for terrestrial systems (Rosenzweig and Abramsky, 1993), but available data are inadequate for all size groups to fully test this idea. It appears likely that multiple factors are important and no single process or characteristic regulates deep-sea diversity. These multiple variables also confound interpretation of pattern and potential regulatory process. The implication is that *confounding variables, such as depth or total productivity, must be removed statistically or experimentally in order to evaluate other potentially important variables.*

Regulation of populations (larval ecology)

A second area where research is needed is in larval supply in deep-sea environments, and the mechanisms by which species arrive in specific habitats. This is an issue that straddles sampling and experimental needs, but represents a major unknown for deep-sea communities. Colonization studies (e.g. Grassle, 1977; Desbruyeres *et al.*, 1985; Snelgrove *et al.*, 1992, 1996) suggest that scavenging and opportunistic species in particular actively respond to elevated food resources, but what factors moderate this response? *To what extent is post-settlement movement a factor? How seasonal is larval settlement? Is deep-sea species recruitment limited or does post-settlement mortality play a major role? Do species disperse over scales of meters or thousands of kilometers?* These questions can be resolved best using a combined sampling and experimental approach. For example, in situ larval samplers can provide critical data on timing and abundance of larval supply, whereas caging experiments (akin to those used in intertidal studies) can elucidate issues related to post-settlement mortality. Unfortunately, manipulation of sedimentary habitats is extraordinarily difficult because removal of specific groups of organisms usually disturbs the sediments and alters basic geochemistry. Baited traps and selective poisoning offer one approach to “removing” certain groups. It is also feasible to build on caging experiments by excluding pelagic species completely or selectively, allowing in situ tests of their influence.

Ecosystem processes in deep-sea sediments

Within the last decade there has been increasing interest in the role that living organisms play in the regulation of key ecosystem processes (e.g. Daily, 1997), and sedimentary ecosystems are no exception (Snelgrove *et al.* ,1997). Deep-sea sediments play a critical role in recycling carbon (e.g. Heip *et al.*, 2001), though the specific role of biodiversity (as opposed to functional groups such as bacteria – Giuliano, this volume) is poorly known. Sedimentary fauna also play key food web roles for species that are fished commercially and can influence the burial and mobility of contaminants in sediments (e.g. Van Dover *et al.*, 1992). The role of biodiversity in completing ecosystem processes in deep-sea sediments is a virtually untapped area of research.

Establishing conservation criteria for deep-sea communities

Deep-sea sediments are vulnerable to multiple threats effects related to fishing, mining, waste disposal and accidental spills, and global climate change (e.g. Thiel, 2003). Fishing removes top predators that could play a regulatory role in deep-sea systems through cropping (Dayton and Hessler, 1972) and disturbance (Grassle and Sanders, 1973). An additional concern is that fishing gear can destroy bottom habitat – particularly biotic structure – with poorly known long-term consequences (e.g. Koslow *et al.*, 2000). Dumping in the deep sea has included munitions and chemical weapons (Schriever *et al.*, 1997), radioactive wastes (Thiel, 2003), sewage sludge (Bothner *et al.*, 1994), dredge spoil containing contaminants and discards associated with the military, shipping or oil and gas exploitation (Schriever *et al.*, 1997). A future threat may be deep-sea disposal of carbon dioxide (Herzog *et al.*, 2000) to reduce atmospheric levels with unknown consequences to deep-sea organisms.

Because deep-sea organisms usually grow slowly, take years to reach maturity, and produce comparatively few offspring (see Gage and Tyler, 1991; Merritt and Haedrich, 1997), they are potentially much more vulnerable than their shallow-water counterparts. Thus, deep-sea communities are expected to have low resilience to increasing disturbances and long recovery trajectories (Fig. 2). It is because of these attributes that Merritt and Haedrich (1997) suggested that deep-sea fisheries may never be sustainable. An additional complication for conservation of species is that most deep-sea habitat is in international waters (e.g. Butler *et al.*, 2001), making conservation efforts even more complex. This does not mean that conservation should be ignored.

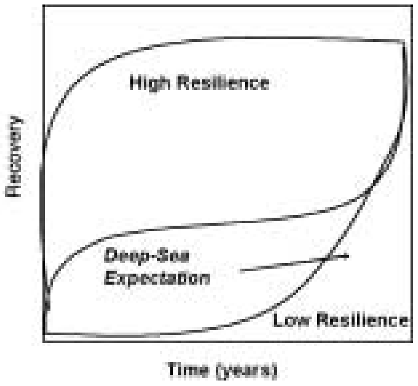

Fig. 2.

Some specific questions include: *Which species are quickest to recover, and which are most vulnerable? How do recovery trajectories* (Fig. 3) *relate to reproductive strategy and autoecology? What aspects of disturbance create the slowest recoveries (e.g. exposure of anoxic sediments) and why? Will adults or larvae lead the recovery, and is that dependent on the scale of the disturbance? What are the spatial scales of* *species distributions in terms of recovery – are most species truly cosmopolitan and will this distribution allow recovery regardless of the spatial scale of disturbance?* Some of the variables that could be monitored include sediment geochemistry (e.g. hydrogen sulfide, ammonium, labile carbon) and sediment geotechnical properties (e.g. compaction, ease of resuspension). These questions could be addressed for microbial, meiofaunal, macrofaunal and megafaunal communities

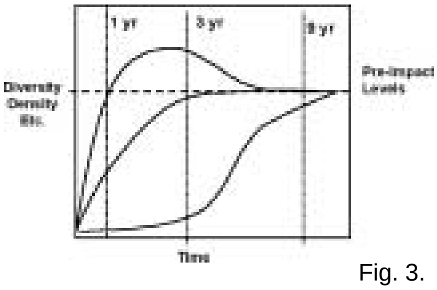

Fig. 3.

THE MEDITERRANEAN AS A UNIQUE OPPORTUNITY

The Mediterranean is somewhat unique among deep-sea habitats in that there is a historical framework and a large number of interested and qualified scientists (and laboratories) in close proximity. Within the Mediterranean, there is a range in surface productivity (e.g. Eastern versus western basins), levels of human disturbance, proximity to the Atlantic, and other variables that may play a role in the regulation of deep-sea communities. It is also the only warm deep-sea basin in the world. Existing data (see executive summary) suggest that the different basins of the Mediterranean support different faunas, as well as biogeographic linkages to the Atlantic (e.g. endemism, rates of invasion etc.). Clearly there is strong potential for interdisciplinary studies that are needed to advance deep-sea research. Moreover, ships can access deep-sea habitat in the Mediterranean without extensive steam time, and put into any number of ports for personnel change etc. *All of the questions outlined above have relevance to the ecology and conservation of Mediterranean fauna, and these geographic attributes make it an ideal laboratory in which to test key ideas in deep-sea ecology.*

SUMMARY

Any understanding of global biodiversity regulation must include a basic understanding of the Earth's most extensive habitat in area – the deep sea. Despite unique characteristics, it is likely that any lessons learned regarding regulation of deep-sea diversity will be very useful in the management of other ecosystems. The deep sea offers one of the few remaining habitats on Earth largely unaltered by human activity. It is therefore one of the last "laboratories" in which we can evaluate the dynamics of biodiversity in a truly natural ecosystem. The proportion of the deep sea that remains unsampled is staggering, but it also means that there is tremendous potential for discovery of many new species and potentially fundamentally new processes such as the chemosynthetic communities discovered at hydrothermal vents less than 30 years ago.

Long-term change in deep-sea environments: examples from the Atlantic Ocean

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In order to understand deep-sea ecosystems we must know about their variability in both space and time (Rogers *et al.*, 2002). Only then will we be able to plan the sustainable use of deep-sea resources (Thiel, 2003). In the past many deep-sea studies have been carried out without setting samples in their spatial and temporal context, mainly because of the difficulties in sampling such a remote and hostile environment. This has led to the impression that deep-sea environments are immutable with time. However, recent programmes in the Mediterranean (Danavaro *et al.*, 2002), the Atlantic Ocean (Billett *et al.*, 2001) and the Pacific Ocean (Smith and Druffle, 1998) have demonstrated that deep-sea ecosystems may change radically with time. I will review what is known about long-term change in the deep sea, drawing upon examples from the Atlantic Ocean. I will also review new approaches for 1) the rapid characterization of habitats, 2) precision sampling of the seabed, and 3) seabed monitoring.

LONG-TERM CHANGE IN THE DEEP SEA

In recent years there has been a dramatic change in the abundance of animals living on the abyssal seabed over a vast area of the NE Atlantic (Billett *et al.*, 2001). Increases in the abundances of actinarians, polychaete worms, ophiuroids, pycnogonids, tunicates and holothurians have been noted. Figure 1 shows the increase in the abundance of these animals as determined from trawl catches. In particular, one holothurian (sea cucumber), *Amperima rosea*, has increased in abundance from about one individual per hectare (before 1996) to more than 6,000 individuals per hectare (1996-present), as determined from time-lapse photography. Abundances in trawl

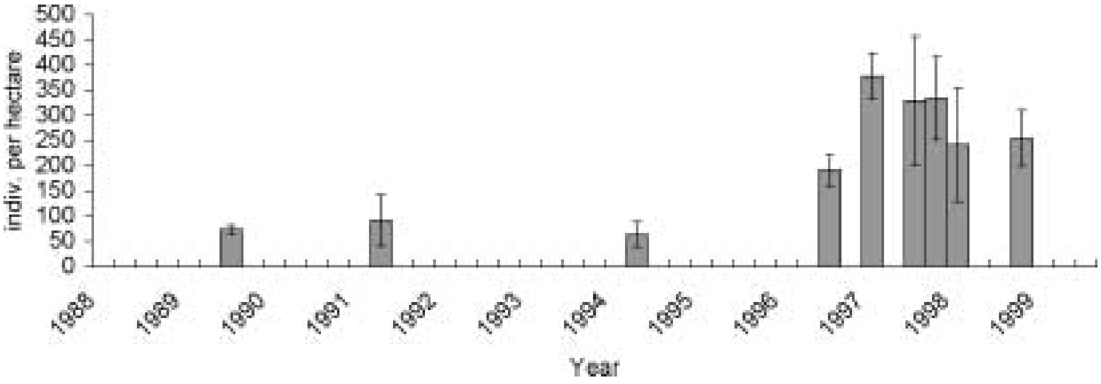

Fig. 1. Abundance of megafauna on the Porcupine Abyssal Plain, NE Atlantic.

catches are much lower because of the effect of the bow wave in front of the trawl. The changes in abundance have had a significant effect on the rate at which organic matter was reworked by megafauna at the sediment surface (Bett *et al.*, 2001). This has affected the biogeochemistry of the sediments and the species dominance of certain infaunal groups.

Changes in the large animals are also reflected in the smaller infauna. Work on the foraminiferan meiofauna shows that while diversity and species richness are unaffected the dominant species have changed radically with time, coinciding with the "*Amperima* Event". The foraminiferans have high local diversity, but most species appear to be very rare (Fig. 2). The functional role of these "rare" species is unclear. However, it is known that the foraminiferan species, *Trochammina*, which was very rare prior to the "*Amperima* Event", is now the dominant form (Fig. 2). This suggests that we have still much to learn about how seabed communities change with time. It also raises questions about the degree of functional redundancy in biodiversity in natural deep-sea systems.

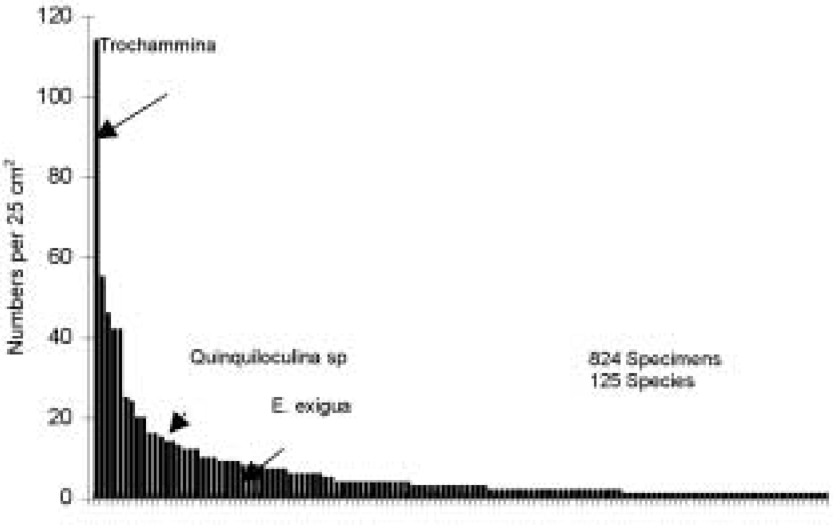

Fig. 2. Live Foraminifera species by rank order for the Porcupine Abyssal Plain site (from one 5cm diameter core, top 1cm).

Recent work has indicated that an important driving factor for change in the deep sea is the composition of organic matter deposited on the seafloor. Changes in the type of organic matter may be affected by 1) the type of phytoplankton forming the organic matter at the surface and 2) the zooplankton that feed on the phytoplankton and create the larger particles that sink to the seabed. The "*Amperima* Event" may be driven by changes in the flux of cyanobacteria to the seafloor (Wigham *et al.*, under review). Carotenoid pigments, characteristic of cyanobacteria are critical for reproductive success in *Amperima* (Hudson *et al.*, under review). In food-limited environments such as the deep sea, and perhaps particularly in oligotrophic areas, subtle changes in the chemistry of the flux to the seabed may lead to ecosystem changes in the deep sea. Biophysical variability in the surface waters may drive natural variability on the deep seafloor.

In planning future work in the deep sea it is important to study natural intra- and inter-annual variability. Significant differences in surface primary production occur over the various abyssal basins of the Mediterranean (Tyler, 2003). It would be useful to compare these different environments through the creation of long-term time series stations.

THE RAPID CHARACTERIZATION OF HABITATS

With regard to setting samples within a spatial context, the use of sidescan sonar in combination with seabed photography is providing a useful technique for rapid, wide-area seabed surveys (Bett, 2001; Masson, 2001). While some areas of the Mediterranean are now well understood, owing to large-scale European research programmes, much of the seafloor in the Mediterranean remains to be characterized. While bathymetric charts provide the basic road maps of the deep Mediterranean, remote sensing methods must be used to generate "land use" or habitat maps. At present knowledge of the extent of habitats in the deep European marine estate is wholly inadequate.

The long term vision for the mapping of the Mediterranean margin should include Autonomous Underwater Vehicles (AUVs) which can be fitted with sidescan sonars and imaging systems (McPhail, 2002). Such systems are becoming standard tools within industry working on the continental margin and need to be taken up more widely by the scientific community and Government-sponsored environmental surveys. Recent work using sidescan sonar has shown that subtle changes in backscatter can be related to faunal communities, opening up the possibility of using the technique for habitat characterization of sedimentary habitats. New improved seabed classification systems are available (Blondel, 2002).

TECHNOLOGY FOR PRECISION SAMPLING

In addition to new technology for remote sensing of the deep seafloor, Remotely Operated Vehicles (ROVs) are available at a number of European institutions for deep-water exploration, particularly of environments that are inaccessible to more conventional sampling gears, such as canyon systems. It is now possible to collect samples with precision from a wide variety of ecosystems and to relate the samples to their immediate surrounding environment.

In addition, ROVs are providing new opportunities for experimentation in deep water. As biodiversity research in shallow water enters a new phase of testing hypotheses on the role of biodiversity in ecosystem functioning, so now it is possible to plan similar experiments in deep-sea ecosystems. A number of ROV systems exist in European institutions, but we need to coordinate their use in a more dynamic way. The Mediterranean offers a good setting for the greater use of ROV systems. In addition the high quality images generated by ROVs and submersibles can be used for public outreach and education.

SEABED MONITORING

In the Northeast Atlantic, one approach to studying the temporal variability has been to set camera systems on the deep sea floor timed to take photographs at regular intervals (e.g. every four hours). The Southampton Oceanography Centre's *Bathysnap* system records an area of $2m^2$ and can be left on the seabed for a year or more. By overlaying a perspective grid, processes and features on the seafloor can be measured (Bett, 2003).

The time-lapse images have been used to compare two quite different areas in the Northeast Atlantic. The Porcupine Abyssal Plain (48° 50'N, 16° 30'W, 4,850 m) water depth, is a highly seasonal area. The overlying surface waters experience large changes in the productivity of surface phytoplankton and there are concomitant large changes in the flux of organic matter to the seafloor in spring and summer (Lampitt *et al.*, 2001). The seabed may be covered in a carpet of phytodetritus for several months (Billett *et al.*, 1983; Lampitt, 1985). Oligotrophic areas in the Mediterranean, however, will be more similar to observations on the Madeira Abyssal Plain (31° 06'N, 21° 11'W, 4,994 m). Here large seasonal changes are not apparent and there is little change in the appearance of the seabed with time. The contrast between these two sites shows how different these natural systems can be. The differences are related to the amount of organic matter, or phytodetritus, deposited from the sea surface. Photographic methods provide a powerful tool of monitoring changes with time.

Many other landers are now available for seabed monitoring and experimentation. Systems may be used for sediment biogeochemical studies (Tengberg *et al.*, 1995; Viollier *et al.*, 2003) or for behavioural studies (Priede and Bagley, 2000; Bailey *et al.*, 2003). These systems should be used more widely in observational sampling programmes in the deep sea.