ISMAR - Istituto di Scienze Marine
Sede di La Spezia

BIOFUN 2008

CRUISE REPORT

11th – 24th June 2008

Edited by F. Polonelli

Contents

Cruise Details

NAME	*BIOFUN 2008*
DATE	*11 – 24 JUNE 2008*
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Scientific framework

This report presents the preliminary results obtained during the BIOFUN 2008 cruise, carried out from 11th – 24th June 2008, on board of the Italian R/V URANIA in the Eastern Mediterranean Sea.

State of the Art

The Deep sea

The bathyal and abyssal ecosystems are the largest habitats on Earth, covering over 60% of the Earth's surface. Yet, only a very small fraction of the deep sea has been explored to date and the surface that has been physically sampled amounts to a few hectares. Consequently, exploration still plays a major role in deep-sea research, ensuing in the regular description of new species and even the discovery of new habitats such as hydrothermal vents or deep-water corals. What little we know indicates that the deep oceans are characterized by biodiversity levels among the highest on the planet (Grassle & Maciolek 1992; Rex et al., 2006), much of which remains un-described, especially in the case of small-size organisms and prokaryotes. This gap in knowledge has been recognised by the Census of Marine Life initiative, a 10-year long programme with the aim to describe the diversity, distribution and abundance of life in the oceans, and from which the EuroDEEP programme was initiated. The thorough description of the species inhabiting a community is the essential first step to understanding the ecosystem and its links to the global biosphere. The development of new molecular techniques coupled with traditional taxonomy provides the tools to describe not only new species, but also investigate evolutionary lineages, species migration pathways, interconnectivity between populations and detection of cryptic species.

The deep sea also contains important biological and non-renewable resources, which, with the decrease of fish stocks on the continental shelf and development of underwater technologies, are increasingly being targeted. The impact of such activities on the deep-sea habitat and its associated fauna is mostly unknown but the deep sea biodiversity is surely threatened because it's seen as the final destination of the cascade effects of anthropogenic changes, such as climate change, (Glover & Smith 2003 Environm Conserv 30:219-241). First investigations conducted so far on this topic have already indicated potentially dramatic effects of deep sea biodiversity loss (Hooper et al. 2005 Ecol Monogr 75:3-35) and recently, a major change in the community structure of the dominant epibenthic megafauna was documented in the northeast Pacific as a con sequence to climatic fluctuations induced by El Niño/La Niña (Smith et al., 2006).

The Mediterranean Deep sea

The Mediterranean Sea is characterised by a permanent homeothermia below 200-300 m depth, resulting in a lack of thermal barrier for the bathymetric distribution of the deep-sea fauna. The high temperature of the deep Mediterranean waters (13-14ºC) accelerates the decomposition of the sinking particles, resulting in a higher refractory index of the organic matter reaching the seafloor.

This is especially important in a poor sea with a strong oligotrophic gradient that increases from west to east.
In the deep Mediterranean Sea, the megafauna that occupies the higher levels of the trophic system include important commercial species such as the red shrimp *Aristeus antennatus* (Sardà et al., 2004a) and fishes (D'Onghia et al., 2004). Previous studies (see Sardà et al., 2004b for a review) have described the population structure and spatio-temporal variations of decapod crustaceans and fish in the deep Mediterranean (Sardà et al., 1994), but little is known on the trophic relationships that sustain these exploited populations. Furthermore, the composition and distribution of deep-water non-decapod invertebrate megafauna is one of the major biological unknowns in the Mediterranean (Ramirez-Llodra et al., 2008).
Describing the diversity, distribution and abundance of the fauna in the context of environmental conditions, hydrology and geology of the Mediterranean basins, and understanding their trophic structure in relation to the microbes, meiofauna macrofauna and megafauna studies is essential to understand the functioning of the Mediterranean deep-water benthic ecosystem.

Mediterranean deep hypersaline anoxic lakes

The world's deepest and most hypersaline lakes are unique environments created by the dissolution of evaporite from the Miocene period. They are located in the East-Mediterranean sea and represent a very hostile environment for the high level of salinity (5 to 10 times higher than that of seawater) of their strongly stratified bottom waters, called "brines".
In these anomalous conditions only very peculiar forms of microbial life are adapted to live, such as prokariotes, but a large fraction of the microorganisms inhabiting hypersaline anoxic lakes and are yet unknown.
Describing and characterizing these indigenous microbial communities is necessary to understand their role in biogeochemical processes.

With all this in mind, this cruise aims is to provide a contribution to the understanding of the actual deep Eastern Mediterranean sea ecosystem functioning in relation with the biodiversity of the deep-sea benthos (from prokaryote up to macrobenthic organisms).

Affiliation to research projects

The activities of this cruise have been performed under the support of the EURODEEP, program of the European Science Foundation who support BIOFUN, the leading project of this cruise, and the MIDDLE project, led by IAMC CNR who joined together in this cruise in a collaborative effort to study of the deep sea environment.

BIOFUN project aims to study the functioning of the deep sea under different physical chemical conditions and along longitudinal gradients. The exploration is done at different size classes from viruses to megafauna.

MIDDLE project focuses on the saline brines largely found in the Eastern Mediterranean basin. These peculiar deep sea environments, although known for a long time now, still have largely unknown aspects to be explored, especially the microbiology and molecular biology.

Cruise objectives

During this cruise we collected samples and measurements to investigate the diversity, spatial distribution, and abundance of deep-sea prokaryotes and macrofaunal species to describe biogeographic patterns of some dominant species across the Mediterranean Sea, and analyzing these in relation with geomorphological barriers and gradients of biotic (e.g. nutrient availability) and abiotic (e.g. thermohaline characteristics, sediment dynamics) variables.

Some deep-sea selected spots with different nutrient availability and sediment/slope conditions have been explored. Results of this cruise will be linked to those of other cruises currently running in the Atlantic within BIOFUN project in a broad European exploration of the deep sea in the Southern and Western borders of CE.

The main objectives of the cruise were:

1) To investigate the spatial patterns of diversity and activity of deep-sea prokaryotes (Eubacteria and Archaea) in terms of biomass, C production, metabolism and degradation rates in the deep Mediterranean sea with special attention in evaluating the role of viruses (viral shunt; Wilhelm & Suttle 1999 Bioscience 49:781-788) in the benthic ecosystem. This objective is perceived in collaboration with CNR Institute Marine Science of Ancona, NIOZ (The Netherlands, carbon flow analysis in the benthic food webs), NUI (Ireland, prokaryote diversity) and the Polytechnic University of Marche (Italy, meiofaunal diversity).

2) To study the macrofaunal biodiversity in relation with physical/environmental gradients across the Mediterranean (longitudinal, N/P, sediment/slope, sea mounts, water dynamics, hydrology). The results of this study are to be utilised as part of the size spectra analysis of deep sea fauna at different spatial scales (local, meso- and macro-scales), that will be carried out within BIOFUN project in conjunction with CSIC (Spain) for comparison of macro-fauna diversity and analysis of macrofaunal larvae, and within a collaboration with the University of Bari for intercalibration of macro-megafaunal census.

3) To explore biodiversity and molecular biology of prokaryotes living in anoxic brines in collaboration with CNR- Marine Coastal Environment Institute of Messina within the DHAL, MIDDLE project. The main objective of IAMC-CNR was to characterize the indigenous microbial communities inhabiting the deepest hypersaline anoxic lakes of the East-Mediterranean Sea.

Scientific Background

General description

Circulation

The Ionian Sea is one of the eastern basins. It is bordered by Italy, Greece, Libya and Tunisia and has a volume of 10.8 x 10^4 km^3. The basin is connected to the Cretan Sea through the Straits of Kithira (depth 160 m and width 33 km) and of Antikithira (depth 700 m and width 32 km), to the Levantine Basin through the Cretan Passage, to the Adriatic Sea through the Otranto Strait (depth 780 m and width 75 km) and to the Western Mediterranean through the Sicily Strait.
The thermohaline circulation of the eastern basin is composed of two cells. The first one is an internal cell, deep and vertical, which involves the Ionian and the Levantine Basins. This deep thermohaline cell, the "conveyor belt" of the Eastern Mediterranean, is maintained by a deep water source in the Adriatic Sea, with the Eastern Mediterranean Deep Water (EMDW) reaching the Levantine Basin with a renewal time of 126 years (Roether and Schlitzer 1991; Schlitzer et al., 1991; Roether et al., 1994). During the 90's also another deep water source located in the Aegean Sea was observed (Roether et al. 1996). The external cell comprises water exchanges between the eastern and the western basin and with the North Atlantic. The Atlantic Water (AW), which enters the Mediterranean through the Strait of Gibraltar, moves eastward, spreading through the entire Mediterranean Sea, after passing the Sicily Strait, occupying a layer of about 200 m depth. At the same time, the Levantine Intermediate Water (LIW), which forms mainly in the north-eastern Levantine Basin, moves westward, in a layer between 200 and 600 m depth, exiting the Mediterranean towards the North Atlantic, where it constitutes the well-known MOW (Mediterranean Outflow Water). In the Ionian Sea there are water and property exchanges with the Levantine Basin, in the East, and with the Aegean Basin, in the North. It is therefore considered a transition basin for all eastern water masses, where they are subject to important mixing and transformation processes along their pathway.
The main Ionian water masses are the Atlantic Water (AW), which moves eastward from the Sicily Strait in the surface layer, normally identified by a subsurface salinity minimum, between 30 m and 200 m depth. Below the AW, there is the Levantine Intermediate Water (LIW), which enters the Ionian Sea through the Cretan Passage, spreading westward from its formation site, the north-eastern Levantine Basin. The LIW is identified by its salinity maximum, between 200 and 600 m depth. The abyssal layer, below 1600 m, is occupied by the Eastern Mediterranean Deep Water (EMDW), colder and less saline, that forms mainly in the Adriatic Sea. In the layer comprised between 700 m and 1600 m, we find a transition water mass, with intermediate properties between the LIW and the EMDW. To these water masses, we have to add the Ionian Surface Water, ISW, which is clearly distinguishable from the AW in summer in the surface layer, being warmer and saltier than the AW.
The deep EMDW has well-defined core properties, because it is less influenced by the transformation processes. On the other hand, the distinguishing properties of the AW and the LIW has modified during their pathway, and depend on the distance from their formation sites.

Ecology

The Mediterranean Sea is divided into two basins the Western and Eastern Mediterranean, which are separated by the straits of Sicily. The two basins display important geological and biological differences. The Eastern Basin is generally very deep, (more than 80% of the Ionian and Levantine Seas being below 200 m) and characterized by abyssal plains around 3000 m depth and deep trenches (max. depth 5093 m) related to theMediterranean ridge system.Because of its geological activity (it is a contact zone between African, Eurasian and Arabian tectonic plates), it harbours interesting cases of unique biocenoses, such as mud volcanoes, seamounts or cold seeps. The Western Mediterranean is relatively featureless in comparison, although still not devoid of unique environments.

Biologically, the Western Mediterranean has higher primary production rates (about 350–450 mg Cm^{-2} d^{-1}; Moutin and Raimbault, 2002), than the Eastern basin in wich PP rates are very low (about 150 mg Cm^{-2} d^{-1}; Turley et al.,2000; Moutin and Raimbault, 2002), particularly in the Levantine sea. This strong oligotrophy is related to a depletion nutriments in the euphotic zone. This peculiarity in addiction to higher decomposition rates in the warm and well oxygenated intermediate and deep waters gives a very low organic matter supply to the deeper waters and the benthos. The strong grazing of phytoplankton by the zooplankton in the upper layers induces a consequent restriction of the vertical transport of particles to deep sea floor. All these characteristics result in a scarcity of marine organisms (macrofauna, meiofauna) as well as low levels of microbial activity and biomass at water depths between 2000 and 4000 m. Another important factor that affect the distribution of the Mediterranean deep-sea fauna is the homothermy that exists from around 200-300 m to the bottom. Temperature increases West to East from about 13° to 15.5°C and consequently no thermal boundaries exist in the deep-sea, thus, the distributional limits of the deep-sea faunas are governed by factors, such as salinity, grain-size distribution, pressure, food, hydrodynamics. The Mediterranean macrofauna, although still poorly known, is represented by about 7200 species (5.6 % of the world marine fauna) but despite the large number of species,a few of these there are true abyssal species and one of the most characteristics is their small average size. (most animals are between 0.5 and 10 mm).

Macrofaunal abundance and biomass decrease generally with depth, and most likely also along a west-east gradient; in this scenario there are some areas of local high productivity in wich macrofaunal biomass may increase, ***e.g*** close to submarine canyons. Macrofaunal density in the Mediterranean is about 1/10 of the densities reported for the Atlantic at comparable depths (Cosson *et al.,* 1997; Flach and Heip, 1996).

In the Western basin where the depth does not exceed 3000 m, the abyssal fauna is less abundant than in the deeper Eastern basin and macrofaunal biomass values are between 2.54 g/m^2 dry weight (at 250 m) and 0.05 g/m^2 (at 2000 m) (Stora *et al.,* 1999). Richness of species decreased also with depth (from 124 species at 250 m to 31 at 2000 m). In the more oligotrophic Cretan sea comparable values of macrofaunal biomasses 1 g/m^2 dry weight at 200 m depth and 0.06 g/m^2 at1570 m.are reported (Tselepides *et al.*2000) and the bathyal benthos appears to be composed of autochthonous – self-sustaining populations of eurybathic species.

In the Cretan sea species richness is considerably lower than in the NW Mediterranean, with around 35 species at 200 m and around 8 at 1570 m. So, for the Mediterranean fauna as a whole, the general tendency is an impoverishment in species from West to East.

The deep-water homothermy, the elevated salinity from about 38 to 39.5 psu, the stratification of the water layers and their thermohaline (barotropic) motions; with the system of currents above the Gibraltar and Sicily straits constitute a barrier for a large number of species.Probably for these reasons cold stenothermal species are not represented and several typically deep-sea taxonomic groups, *e.g.* hexactinellid sponges and holothurid elasipods, are very rare in the Mediterranean Sea.

Cruise Plan

During the cruise we investigated different deep sea environment of the Ionian sea - Aegean Sean along a longitudinal gradient from Sicily to Crete (Fig.1). The deepest area of this basin, the Matapan trench (>5000 m depth), is in Greek waters almost mid way along the transfer transect toward the shallower station of this cruise located in the south coast of Crete. This shallow sampling area, called Area2, includes two stations at about 1300 and 2500 m depth (Area2 and area 2b). A second area positioned in the centre of the basin,the ADIOS area, was sampled in three sites (Alfa Bravo Charlie) that were considered different in physical and morphological properties of the sea bed. The station 3 (4000 m depth), situated in the north-eastern part of the basin and the stations Km4 and Km3 located toward the italian coast are regularly sampled by CNR ISMAR SP with the aim to make these points permanent monitoring sites. Anoxic basins Atalante and Medea has been included in the sampling effort with samples collected at depths of about 3000-3500 m depth (Fig.2).

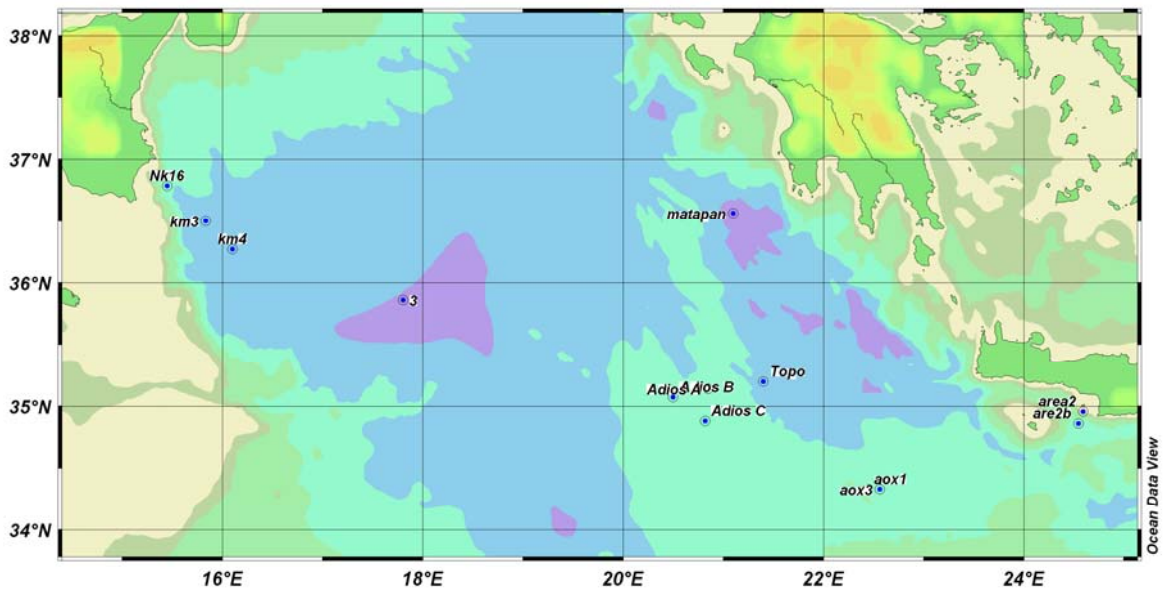


Fig.1- Map of the sampling area

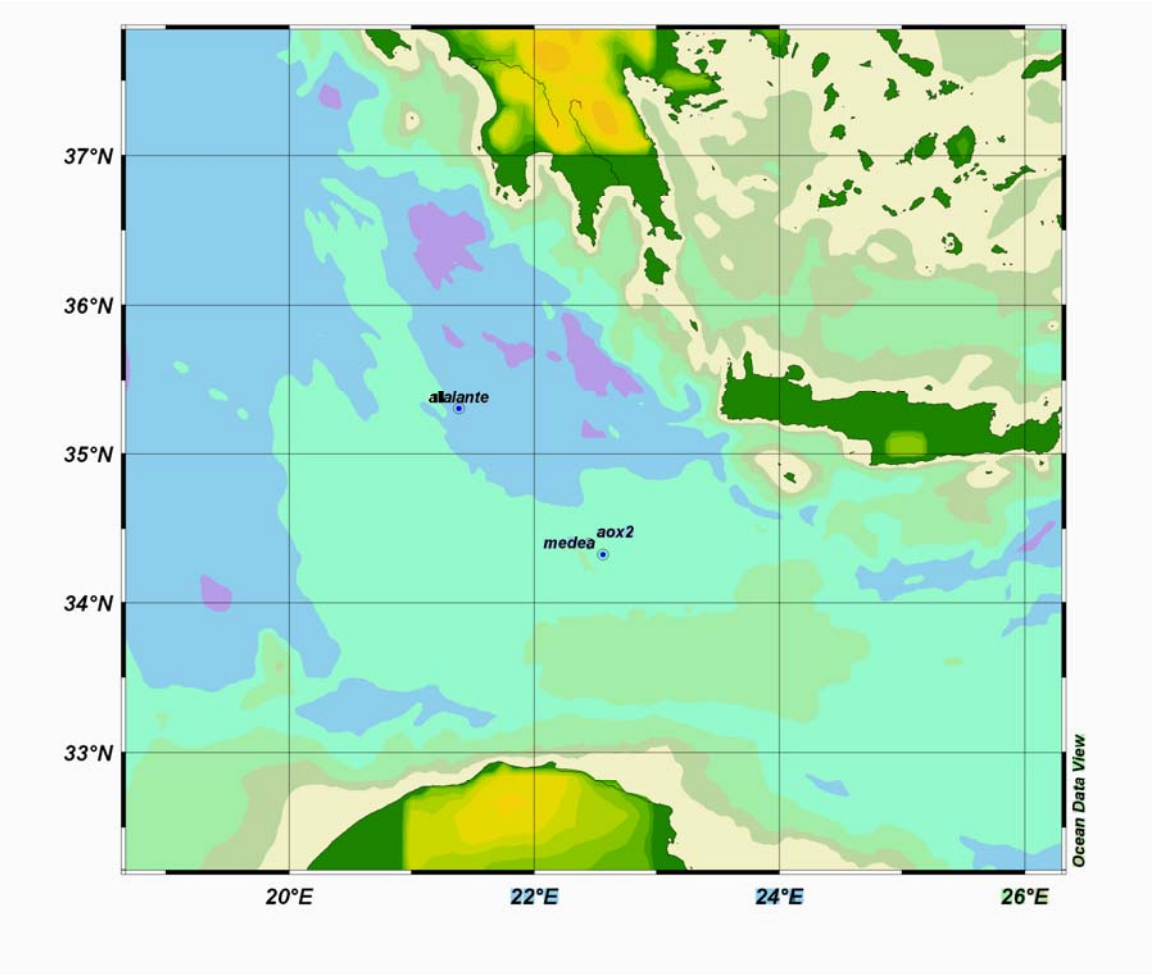


Fig.2 – Map of the anoxic basins investigated.

In table 1 we summarize the parameters that have been measured and the groups involved in the sampling operations.

Table1-Measured parameters.

Parameters/Instruments	Working Group
CTD/O2/Fluorescence/Trasmissometer/ rosette	CNR-ISMAR
Dissolved Oxygen	CNR-ISMAR
ADCP	CNR-ISMAR
LADCP	CNR-ISMAR
Meteo station on board	CNR-ISMAR

Parameters/Instruments	Working Group
XBT	CNR-ISMAR
Boxcorer	CNR-ISMAR, ANCONA; UNI. PDM
Sanders and Agassiz sledges	UNI. BA; CNR-ISMAR,LA SPEZIA; CSIC; SNG
Macrofauna	CNR-ISMAR, LA SPEZIA, CSIC, UNI. BA; SNG
Meiofauna	UNI. PDM; UNI. GHENT
Viral abundance and production	CNR-ISMAR, ANCONA; UNI. PDM
extracellular enzymatic activities	CNR-ISMAR, ANCONA; UNI. PDM
heterotrophic prokaryote production	CNR-ISMAR, ANCONA; UNI. PDM
Protozoa, foraminifera abundance	CNR-ISMAR, ANCONA; UNI. PDM
organic matter	CNR-ISMAR, ANCONA; UNI. PDM
Prokaryotic community structure	NUI
halophiles bacteria and archaea	CNR-IAMC

The geographic boundaries of the survey are 34 °N - 37 °N latitude and 15 °E - 25 °E longitude. We planned to spend 15 days at sea to complete the sampling program. The track of the cruise is shown in Figure 3 and in table 2 is shown the station list.

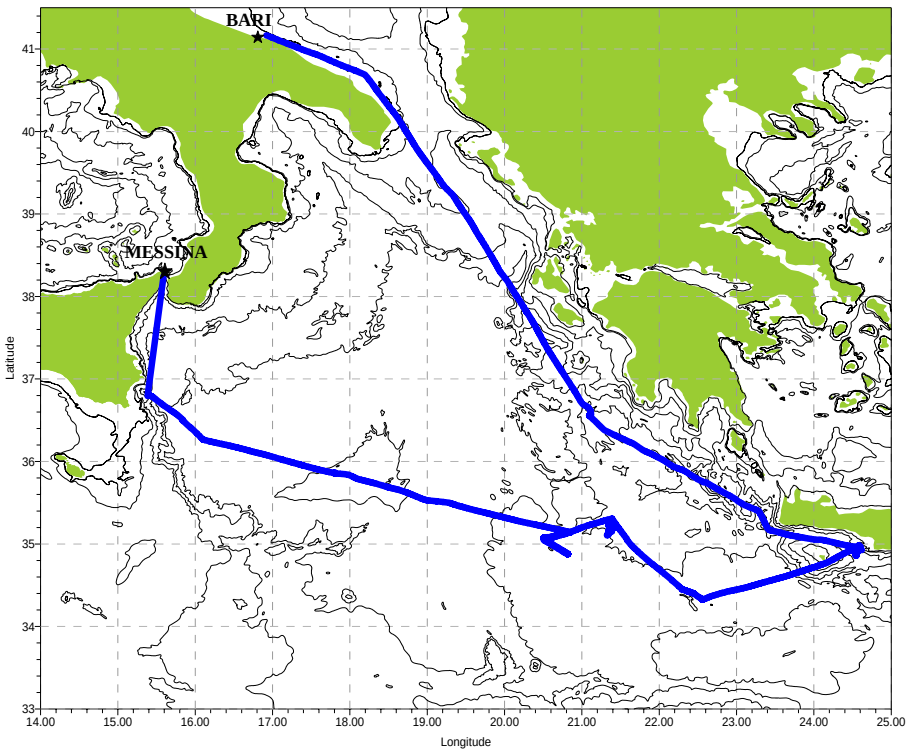


Fig.3 – Navigation track

Cruise Stations

Table 2- List of all stations with positions and description of the activities.

Station	Lon (°E)	Lat (°N)	Depth (m)	Operations
AREA 2	24°35.49'	34°57.16'	1200	CTD, boxcorer, multibeam, trawling, mooring
ADIOS A	20°50.90'	35°08.32'	3000	CTD, boxcorer
ADIOS B	20°30.45'	35°04.09'	3000	Boxcorer, trawling, mooring
ADIOS C	20°49.14'	34°52.71'	3000	CTD, boxcorer, trawling
ATALANTE	21°23.34'	35°18.18'	3400	CTD, boxcorer
TOPO	21°24.48'	35°11.82'	3400	boxcorer; trawling
AOX 1	22°18.00'	34°27.00'	3500	boxcorer
AOX 2	22°27.00'	34°24.00'	3500	CTD, boxcorer
AOX 3	22°34.00'	34°19.00'	3500	Boxcorer
MATAPAN	21°05.89'	36°33.58'	5000	CTD, boxcorer
3	17°48.18'	35°51.42'	4100	CTD, boxcorer
NK 16	15°27.00'	36°46.98'	2450	CTD
KM 3	15°50.04'	36°30.00'	3350	CTD
KM 4	16°06.00'	36°15.96'	3400	CTD

Description of Activities

Meteorological data

Meteorological data has been continuously collected by the ship meteo system and successively analyzed by CNR-ISMAR, La Spezia.

Laboratory: CNR-ISMAR, LA SPEZIA

CTD

Vertical profiles of hydrological properties of the water column has been recorded in every sampling station. To get high quality hydrological data, CTD casts has been performed just before sediment sampling or a long period after sediment sampling. Data collection in anoxic brines required special sensors that has been set on a spare probe dedicated to brines. Sensor data were checked for quality by chemical analysis on board. In all stations pressure (P), salinity (S), potential temperature (θ) and dissolved oxygen (DO) were measured with a CTD-rosette system consisting of a CTD SBE 911 plus and a General Oceanic rosette equipped with 24 Niskin Bottles of 12 liters each. Temperature measurements has been performed with a SBE 3/F thermometer with 10-3 °Celsius resolution; and conductivity measurements were performed with a SBE-4 sensor with a resolution of 3 x 10-4 S/m. Dissolved oxygen was measured by a SBE-13 sensor (resolution 4.3 µM). Water samples has been collected for the determination of dissolved oxygen by Winkler titration method to check sensor data against chemical analysis. The vertical profiles of all parameters were obtained by sampling the signals at 24 Hz, with the CTD/rosette going down at a speed of 1 m/s. Data were processed on board, and the coarse errors were corrected.

Laboratory: CNR-ISMAR, LA SPEZIA

XBT

Expendable bathythermographs has been regularly released along the tracks from Messina to sampling sites and back. Water temperature down to 1000 m has been recorded about every 10 nautical miles using the Deep Blue probe. Data have been transmitted to the S.Teresa Centre in almost real time by the web connection provided by the ship .

Laboratory: CNR-ISMAR, LA SPEZIA

Lowered ADCP

Velocity profiles of water currents has been recorded in every water sampling station by Lowered Acustic Doppler Current Profilers (LADCP), mounted on the Rosette. We used two upward and downward looking RDI Workhorse 300 kHz ADCPs. For the post processing of raw data the LDEO LADCP software, version 8.1 was used. It was not possible to use standard ADCP into the harsh environment of the brines so the system had to be disassembled before deployment in brines.

Laboratory: CNR-ISMAR, LA SPEZIA

Vessel-mounted ADCPs

The hydrographic data set has been integrated with direct current measurements. During the whole campaign two VM-ADCPs (RDI Ocean Surveyor, 75 KHz, and RDI Workhorse, 300 KHz) which operated during the whole campaign, along the whole ship track. The depth range of the two current profilers is about 700 m (OS75) and 150 m (WH300). Data acquisition is carried out using the RDI VMDAS software vers. 1.44. The ADCP data will be submitted to a post-processing with the CODAS3 Software System which allows to extract data, assign coordinates, edit and correct velocity data. Data will be corrected for errors in the value of sound velocity in water and misalignment of the instrument with respect to the axis of the ship.

Laboratory: CNR-ISMAR, LA SPEZIA

Multibeam survey

A brief Multibeam survey has been performed in the coastal station to roughly assess the quality of the seabed before trawling to avoid entanglements. The explored area was south of Crete from 1200 to about 2500 m depth. The ship mounted RESON Multibeam had been used. The 2D color rendering map was superimposed on the navigation system of the ship.

Sediment sampling

Sediment sampling was by box corer. A large oceanic box corer has been used down to 5000 m depth with very high percent of success. We deployed this instrument more than 30 times and we had only minor failures in sampling when hard bottoms over steep slope have been sampled. Cores had been subsampled for organic matter, micro, meio and macrofauna analysis. Remaining sediment has been sieved searching for macrofauna.Visual observation of the surface cores has been regularly performed looking for plastic fragments over the sediment's surface.

Laboratory: CNR-ISMAR, LA SPEZIA; CNR-ISMAR, ANCONA; UNI .DPM, UNI. GHENT; CSIC; SNC.

Organic matter composition

Each box-corer was sub-sampled using thin plexiglass liners of 5.5 cm internal diameter. Three replicates from independent deployment were selected. Sediment corers were sliced into different layers: 0-1, 1-3, 3-5, 5-10 and 10-15 cm and placed in Petri dishes. Samples were immediately frozen at -20°C and stored until the analysis in laboratory.

Laboratory: CNR –ISMAR, ANCONA; UNI. DPM

Macrofauna

Macrofauna sampling was by box corer and by trawling of bottom sledges Agassiz and Sanders. Sediments from the corer has regularly been gently sieved over 0.5 mm mesh seeking for macrofauna. A visual survey of mud's surface evidenced sometimes small animals of fragments that have been sampled before sieving. The Agassiz sledge has been used only in the coastal station trawling for 1 hour bottom time, then it got entangled and damages occurred preventing further use of it. A modified Sanders bottom sledge was necessarily used on further deep sea station. This sledge has the peculiar feature of sampling the seabed with the lower open-mouth part and collect suprabenthos about 1m from the seabed with two closing-mouth nets.

Laboratory: UNI.BA; CNR-ISMAR, LA SPEZIA; ICM – CSIC; SNC

Meiofauna and forams

Each box-corer was sub-sampled using thin plexiglass liners of 5.5 and 3.6 cm internal diameter. Three corers from independent deployments were immediately frozen at -20°C and stored until the analysis in laboratory.

Laboratory: UNI. DPM; UNI. GHENT

Extracellular enzymatic activities; Prokaryote heterotrophic production, abundance, biomass and diversity

Each box-corer was sub-sampled using thin plexiglass liners of 4.6 cm internal diameter. For the prokaryote production and enzymatic activities, the top 1cm of three liners was immediately used to perform analyses on board. Enzymatic activities were spectrometrically determined on board whereas prokaryote production was stored at +4°C. For prokaryote abundance, biomass and diversity, sediment samples were sliced into different layers: 0-1, 1-3, 3-5, 5-10 and 10-15 cm placed in sterile plastic tubes. Samples were immediately frozen at -20°C and stored until the analysis in laboratory.

Laboratory: CNR–ISMAR, ANCONA; UNI. DPM

Viral abundance and production

Each box-corer was sub-sampled using thin plexiglass liners of 4.6 cm internal diameter. For viral production, the top 1cm of three liners was immediately used to perform analyses on board. For viral abundance, sediment samples were sliced into different layers: 0-1, 1-3, 3-5, 5-10 and 10-15 cm placed in sterile plastic tubes. Samples were immediately frozen at -20°C and stored until the analysis in laboratory.

Laboratory: CNR – ISMAR, ANCONA; UNI. DPM

DNA extraction from prokaryotes

Prokaryotic assemblages were collected and concentrated by filtering 10-20 L volumes of seawater sampled at depths representative of the surface, mid-water and benthic water column.

Total community DNA will be extracted from these samples and prokaryotic assemblages will be reconstituted in sterile seawater medium and incubated with nutrient amendments under various conditions of temperature and pressure. Continuous cultures will be established and monitored by DGGE in order to investigate changes in community structure in response to various conditions. Attempts will be also made to identify, isolate and characterise microorganisms responsible for ecosystem functioning.

Laboratory: NUI

Baited traps

Baited traps had been deployed at sea in a temporary moorings in the coastal area South of Crete at 1300 m depth and in the Adios area (2900m depht). Shallow mooring was arranged with a long line reaching the surface and just a surface buoy for retrieval after about 36 hours. Deep Sea traps (fig. 4) has been deployed with an acoustic releaser from EG&G, deep sea gloss buoys and expendable ballast. Traps were filled with dead fishes and other baits to attract scavengers and deep sea megafauna.

Laboratory: CNR-ISMAR, LA SPEZIA; UNI. DPM

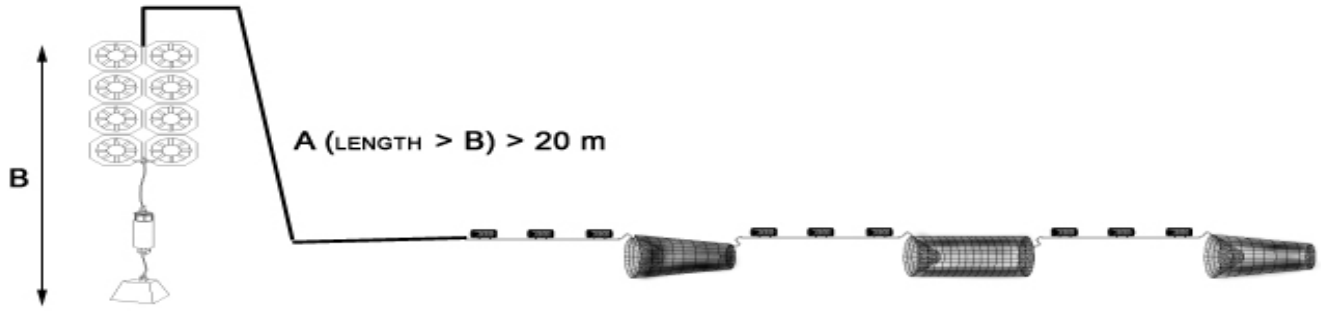


Fig 4. – Temporary Deep Sea mooring scheme.

Preliminary Results

Weather conditions

The diagrams in figure 5 show the sea and weather conditions during the cruise.

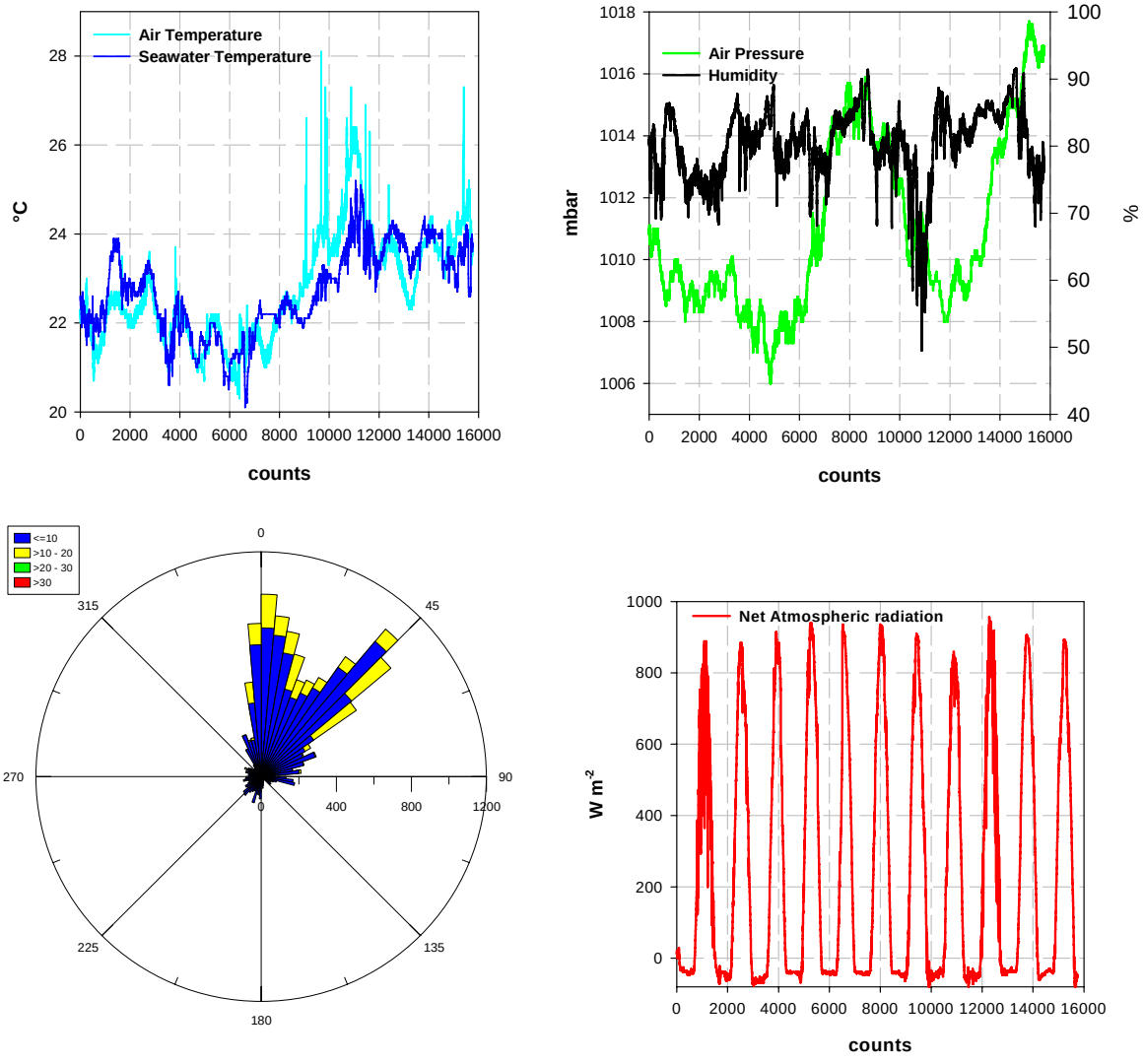


Figure 5- Evolution of the weather conditions between 11th and 24th June 2008 (air temperature, sea temperature, relative humidity, air pressure, wind rose, irradiance).

Hydrology

In the following some preliminary hydrological data and current measurements are presented (Figg.6-10).

CTD casts

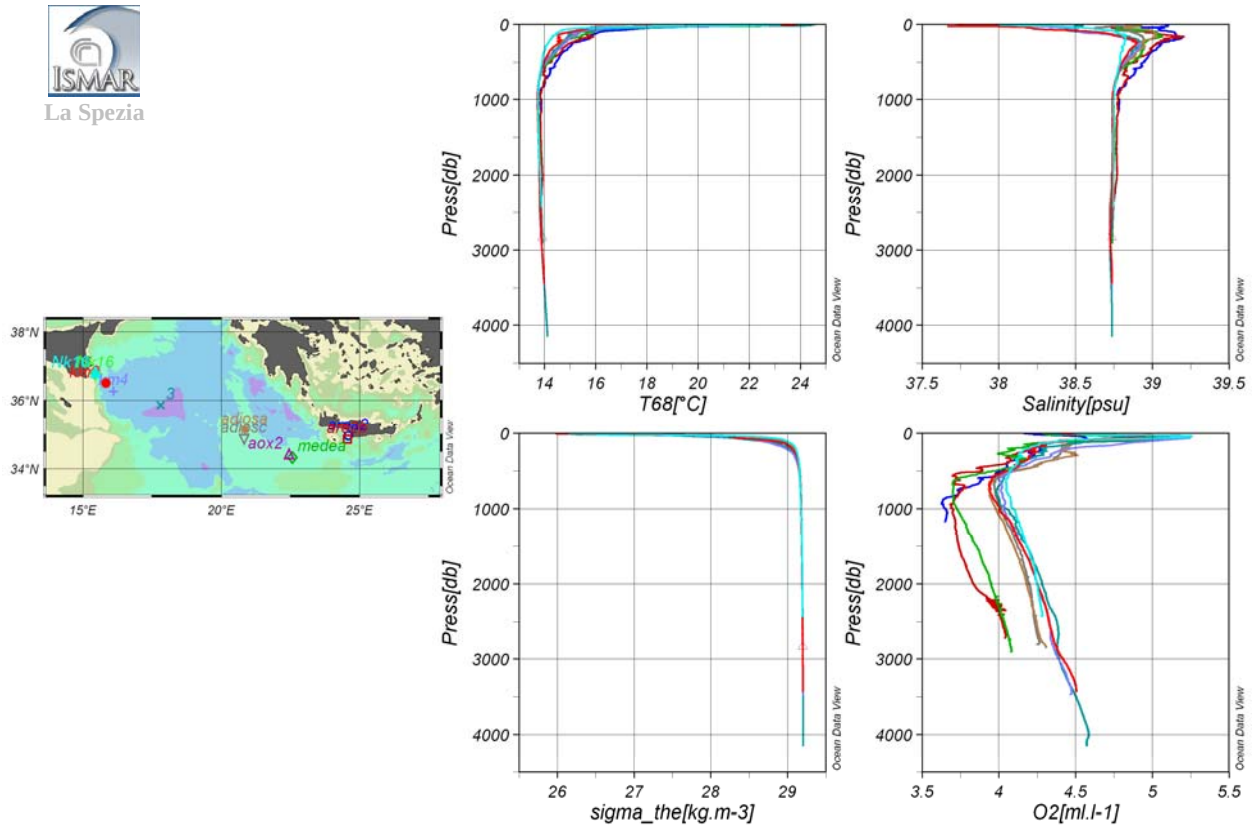
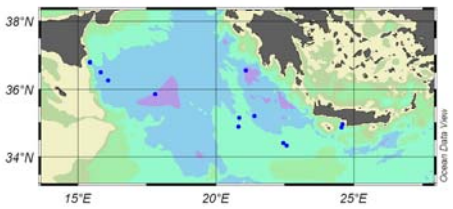


Fig.6 – CTD's profiles of Temperature [°C], Salinity [psu], Potential density [kg m^{-3}] and Dissolved Oxygen [ml l^{-1}].

Potential Temperature vs Salinity Diagrams



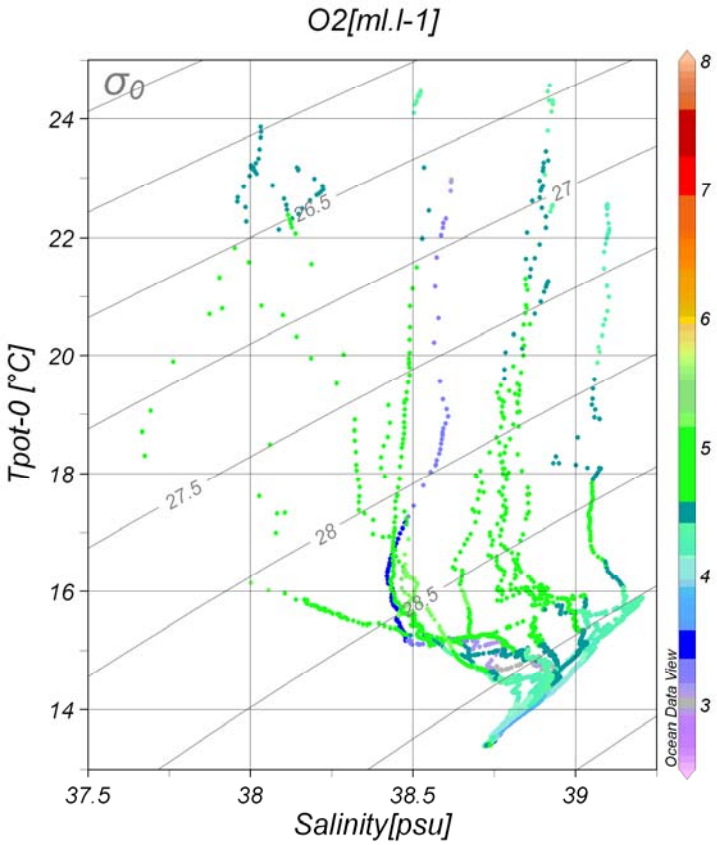


Fig. 7 – Theta-S diagram of all station in the whole water column. Colours indicate oxygen concentrations.

Lowered ADCP

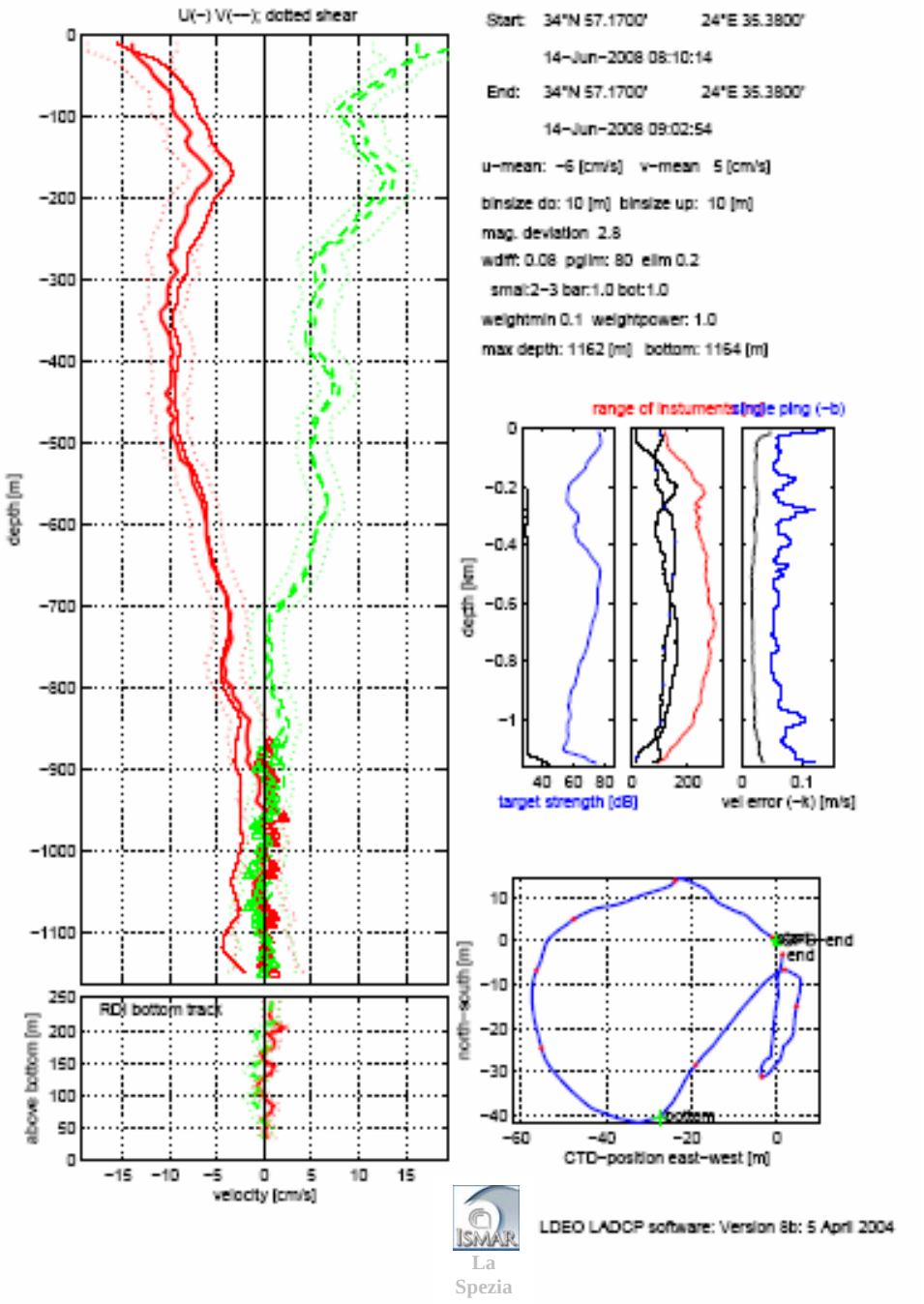


Fig 8. – Profile of L-ADCP in the station Area 2.

Vessel-mounted ADCPs

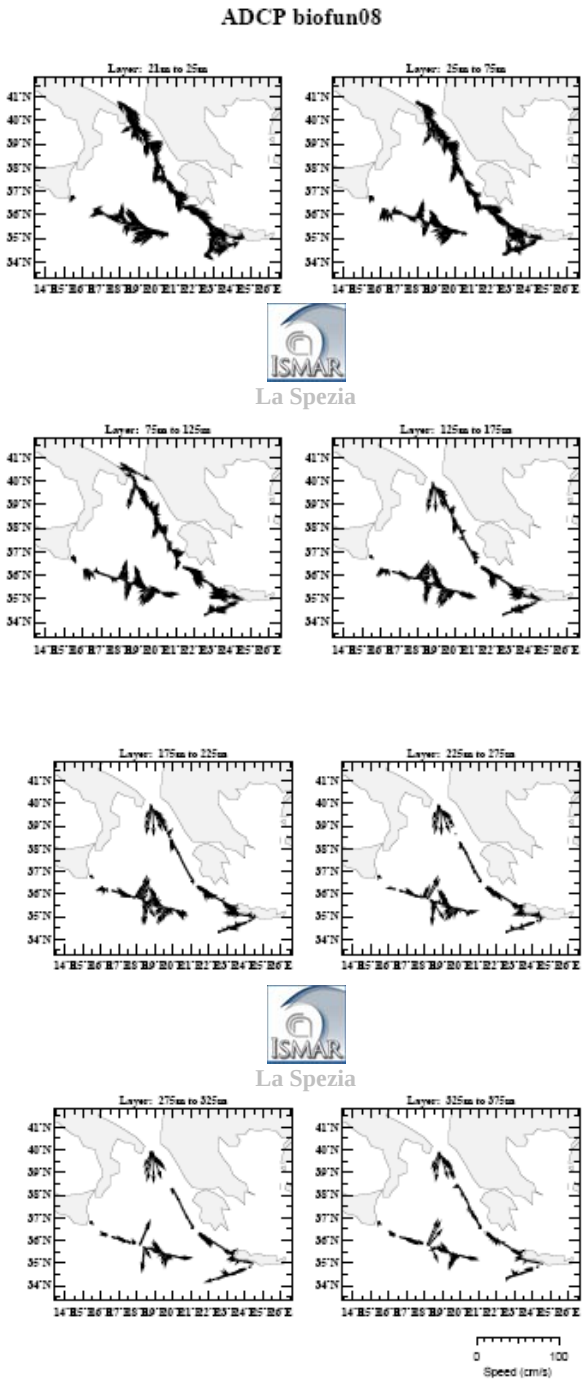


Fig 9. – Current measurements fromVessel mounted ADCPs at different dephts.

XBT

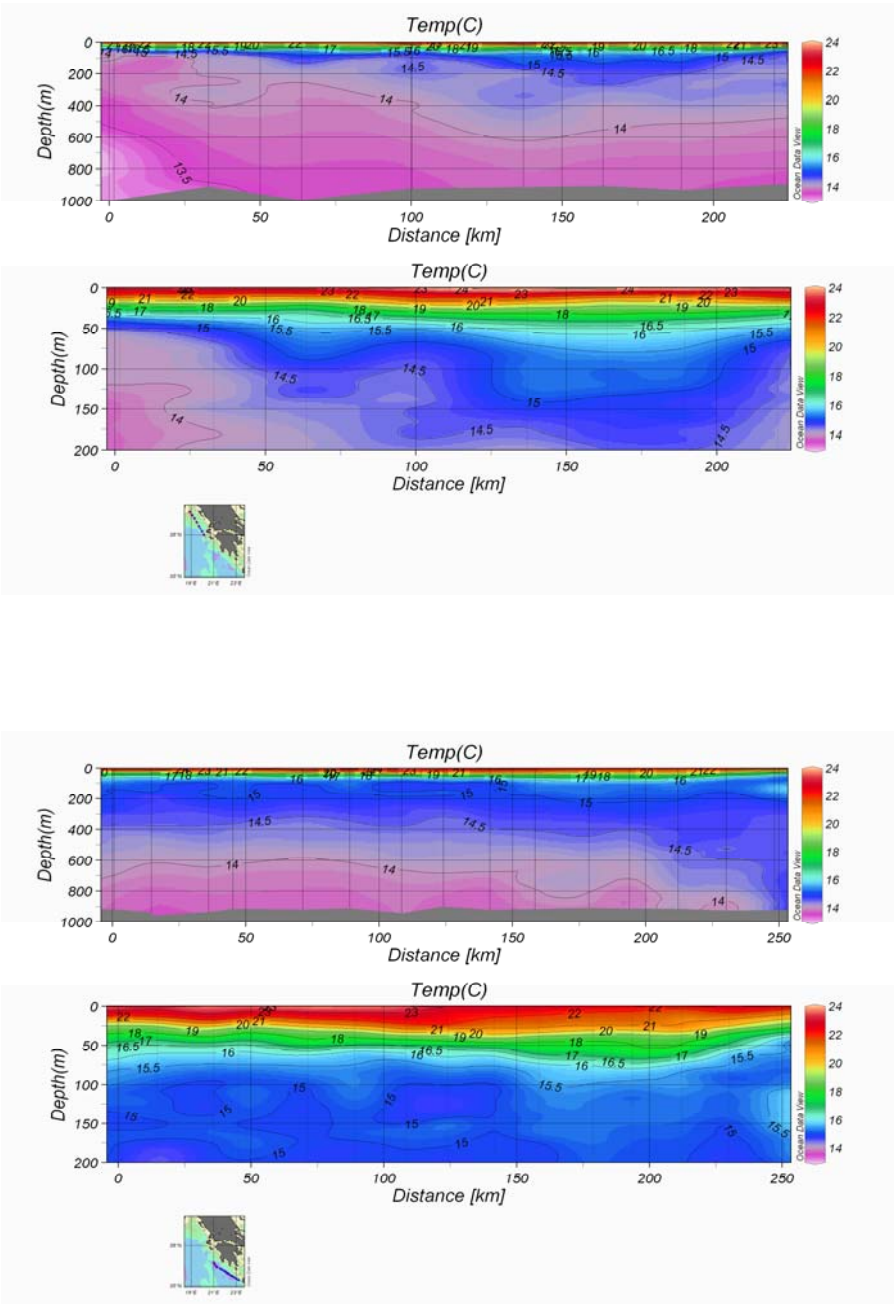


Fig.10- Distribution of Temperature (°C) along Section 1 (up) and Section 2 (down).

Anoxic basins Medee and Atalante

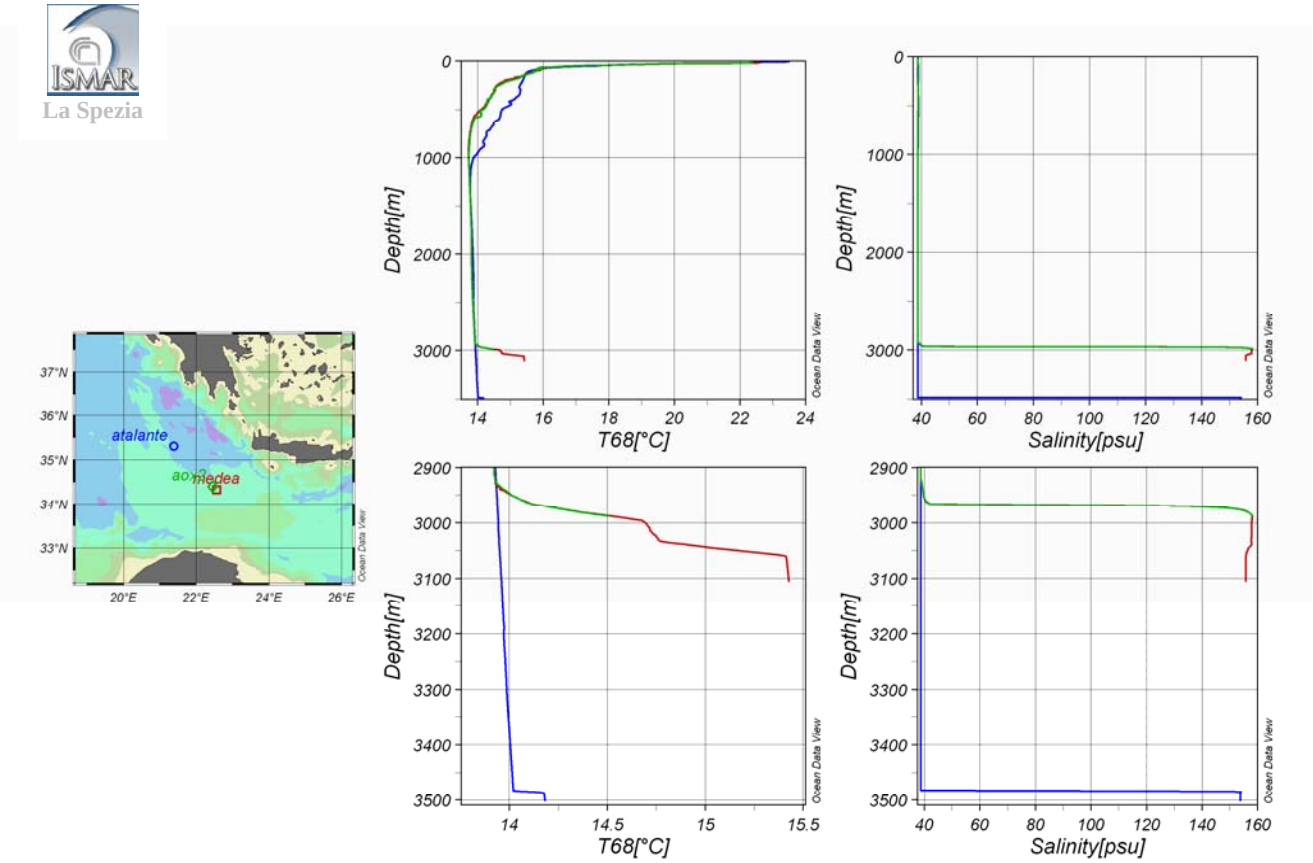


Fig.11 - Profiles of: Salinity [psu] and Temperature [°C] in the two anoxic basins: whole water column (up) ; "brine" (down).

Macrofauna

Sediment samples by box corer and by trawling of bottom sledges Agassiz and Sanders has been collected to charecterize and describe the diversity, distribution and abundance of the macrofauna of the Eastern deep-Mediterranean sea.
Samples were collected from:

MATAPAN. 1 boxcorer
AREA 2: 9 boxcorer, 3 trawling (1 sanders, 2 Agassiz)
AOX 1: 1 boxcorer
ATALANTE: 4 boxcorer
TOPO: 3 boxcorer, 3 trawling (3 Sanders)
ADIOS B: 3 boxcorer, 2 trawling (3 Sanders)
ADIOS C: 5 boxcorer (3 failure), 1 trawling (1 Sanders)
ADIOS A: 3 boxcorer, 1 trawling (1 Sanders)
St. 3: 2 boxcorer

Both sediments from the corer and trawls has regularly been gently sieved over 0.5 mm mesh seeking for macrofauna after a visual survey of mud's surface that sometimes evidenced small animals of fragments that have been sampled before sieving.

All samples were similar with large quantities of pteropod shells and scarce macrofauna.
All macrofauna found (mainly small decapod crustaceans, molluscs, fish juveniles and larvae, polychaetes, scaphopods and formaminifera) was preserved in small jars. A subsample of pteropod shells from each of the 3 nets of the Sanders sledge was also preserved from each trawl for further analyses in the laboratory.
Samples were preserved in 4% buffered formalin and then transferred to 90% alcohol. Some crustaceans and fish were frozen -20º, instead the phyllosoma larva was preserved in 96% ethanol.

Benthic communities: biological and biochemical analysis

Sediment samples were collected for biological and biochemical analyses to characterise the benthic communities in the eastern Mediterranean. Sediment samples were collected to analyse the benthic oxygen consumption, the biochemical composition of organic matter (chlorophyll *a*, phaeopigments, carbohydrates, lipids and proteins), extracellular enzymatic activities, heterotrophic prokaryote production, viral abundance and production, protozoa abundance, foraminifera abundance and diversity, meiofaunal abundance, biomass and diversity.
Each box-corer was sub-sampled using thin Plexiglas liners of 10, 5.5, 4,6 and 3.6 cm internal diameter (Table3). Chemical and biological analyses were carried out on three replicates (from independent deployments) at each sampling station. For the heterotrophic prokaryote production and enzymatic activities, the top 1 cm of three liners was immediately used to perform these analyses on board. Enzymatic activities were spectrometrically determined on board. For the benthic oxygen consumption, a microprocessor dissolved oxygen-meter was used on the sediment-water interface for six hours. For the organic matter and prokaryote diversity, sediment corers were sliced into different layers: 0-1, 1-3, 3-5, 5-10 and 10-15 cm and were immediately frozen and stored until the analysis at -20°C. For meiofauna and forams, three corers were immediately frozen at -20°C and stored until the analysis.

Table 3. Subsampling of box cores for biological analyses. (OM=Organic matter; EEA = extracellular enzymatic activities; MEIO=meiofauna; EXP = experiment on microspatial distribution of meiofaunal diversity; Repls = replicates).

Event number	OM	EEA, prokaryote and vira production	Prokaryote and viral abundance	O2 consumption	Protozoa	Forams	MEIO
26	3 repls	3 repls	3 repls	3 repls	3 repls	3 repls	3 repls
54	R1	R1, R2, R3	R1, R2, R3	R1	R1	R1	R1
55	R2				R2	R2	R2
56	R3				R3	R3	R3
59							EXP
60	3 repls	3 repls	3 repls	3 repls	3 repls	3 repls	3 repls
131	R1	R1, R2, R3	R1, R2, R3	R1	R1	R1	R1
132	R2, R3			R2, R3	R2, R3	R2, R3	R2, R3
133	R1	R1, R2, R3	R1, R2, R3	R1	R1	R1	R1
134	R2, R3			R2, R3	R2, R3	R2, R3	R2, R3
151	3 repls	3 repls	3 repls	3 repls	3 repls	3 repls	3 repls
159	R1	R1, R2, R3	R1, R2, R3	R1	R1	R1	R1
160	R2				R2	R2	R2
161	R3				R3	R3	R3
182	R1	R1, R2, R3	R1, R2, R3	R1	R1	R1	R1
183	R2				R2	R2	R2
184	R3				R3	R3	R3
210	R1	R1, R2, R3	R1, R2, R3	R1	R1	R1	R1
213	R2, R3			R2, R3	R2, R3	R2, R3	R2, R3
240	R1	R1, R2, R3	R1, R2, R3	R1	R1	R1	R1
241	R2				R2	R2	R2
242	R3				R3	R3	R3

IAMC Microbial communities of the deepest hypersaline anoxic lakes

During the Biofun 08 cruise, water and brine samples were collected from 2 basins, namely Medee and Atalante to characterize the indigenous microbial communities on several different aspects. Both dipendent- and indipendent-culturing methods were used. In addition samples for microbial activity mesuraments were collected and analyzed. Novel biologically active products of industrial and technological importance from the microbial communities of these extreme environments will be explored.
Deep-sea water samples were collected from a total of 5 stations in the two above metioned basins. The sampling effort changed according to the station.
Microbial diversity will be analysed by means of DNA and RNA-based analyses. Water samples were filtered immediately after collection through 0.22 µm filters (Sterivex, Millipore) and stored at -20°C. Molecular analysis, i.e. clone libraries, metagenomic libraries, SSCP, Real-Time PCR will be performed in laboratory.
To determine the abundance of bacteria and archaea in water and brine samples direct cell count and fluorescent in situ hybridization (FISH) will be performed.
Samples for cultivation of halophiles bacteria and archaea were collected by means of an oxygen-free system. Water and brine samples were inoculated into 10ml Hungate tubes. Basic anaerobic media and different carbon sources together with vitamine and trace-element solutions were added to the tubes, finally stored at 14°C.
Primary production was calculated through uptake of [14C]bicarbonate (100 mCi, 2mCiml^{-1}, Amersham, Buckinghamshire, UK), according to the protocol of Herndl et al. (2005), in 40-ml vials samples in duplicate with a formaldehyde-fixed blank. Samples have been incubated for 7 days in the dark at in situ temperatures (13 °C). After incubation samples will be filtered and placed in scintillation vials until count.

Deep-Sea Prokaryotic Community Structures

Samples were recovered at various depths from eight geographically distinct locations, representing a number of potentially contrasting marine environments in the Eastern Mediteranean. Water samples were collected from benthic (near bottom), mid-water column and surface (200-300m) depths.

Microbial biomass was collected and concentrated by filtering 10-20 L volumes of seawater using 0.2 µm filter cartridges (Sterivex, Millipore). The Filter cartridges were stored frozen (-20°C) with 2 ml of lysis buffer (for DNA isolations) or 2 ml of 40% glycerol (for viable cell preservation). A sub-sample (50 ml) of every sample was fixed by the addition of formaldehyde (0.4% final concentration) and stored at 5°C.

Prokaryotic abundance in these samples will be determined by epifluoresecent microscopy using Sybr-Gold® nucleic acid stain.

Total community DNA will be extracted from Sterivex filter columns and the16S small subunit rRNA genes amplified by PCR. Denaturing Gradient Gel Electrophoresis (DGGE) will then be used to seperate 16S rRNA genes and thereby characterise microbial community structures at different depths and locations. Dominant species will be identified by isolating and sequencing clonal DNA from relevant DGGE bands.

Enrichment incubations of deep water microbial communities will be established under different conditions in order to assess the influence of hydrostatic pressure, temperature and nutrient amendments on the structuring of marine prokarotic communities. Enrichment incubations will be monitored by DGGE analysis and bacterial and archaeal species of ecological relevance will be identified. Attempts will be made to isolate ‘keystone’ species for further characterisation.

Megafauna studies at bathyal (1200-1500 m) and abyssal (3000-3500 m)

Agassiz and Sanders trawls

We used an Agassiz and a Sanders Sled to sample the benthic macrofauna at the different stations. In total, we conducted 8 Sander sleds and 2 Agassiz trawls and sampled 10 sites in 5 areas:
AREA2 (~1200 m): 1 Sanders and 2 Agassiz.
TOPO (~3200-3500 m): 3 Sanders sleds
ADIOS-A (~2800 m): 1 Sanders sled
ADIOS-B (~2800 m): 2 Sanders sled
ADIOS-C (~2750 m): 1 Sanders sled

All samples had a similar composition, with mud containing large quantities of pteropod shells and scarce macrofauna. All sediment was sieved on a 1 mm pore sieving table and 500 μm sieve. All macrofauna found (mainly small decapod crustaceans, molluscs, juveniles and larvae of fish, polychaetes, scaphopods and foraminifera) was preserved in small jars. A subsample of pteropod shells from each net was also preserved for further analyses in laboratory. Unless differently stated, the samples were preserved in 4% buffered formalin and then transferred to 90% alcohol. Some crustaceans and fish were frozen -20º. The fillosoma larva was preserved in 96% ethanol.

Fauna collected

Generally large quantities of pteropod shells and scarce macrofauna as:

- small decapods
- polychaetes
-gastropods
- foraminifera
- scaphopods (*Dentalium*)
- *Batypteroids*
-small macrurids
- small crustaceans
-cephalopods,
-sipunculids,
-*Acanthephyra eximia*
-fish larvae
-a fillosoma larva.

All samples preserved in formalin are taken by CNR-ISMAR- La Spezia for identification.The frozen samples with crustaceans and small fish are taken by University of Bari. The fillosoma larva and white polycahetes are taken by ICM-CSIC for identification.

Fig.12- From the top: pteropods shells, crustaceans, white polychaetes, Phyllosoma larva.

Traps

The Deep sea trap deployed by CNR-ISMAR-La Spezia and DISMAR-UNIDPM (30 hours deployment) collected 12 *Chaceon mediterraneus* and a few decapod (shrimp) crustaceans (Fig. 12). The *C. mediterraneus* were measured, weighed and sexed (see Table 3) before they were frozen for toxicology analyses (CNR-ISMAR, ANCONA; UNIDPM).

Fig. 13- *Chaceon mediterraneus* and shrimps collected with the cages

Table 4. Size, weight and sex of *Chaceon mediterraneus*

Individual #	Size (mm)	Weight (g)	Sex
1	38	32	♂
2	39	32	♂
3	40	29	♂
4	48	48.5	♂
5	36	24	♀
6	49	61	♂
7	45	51.5	♂
8	43	40	♀
9	38	32	♂
0	36	26	♂
1	35	19	♀
2	31	16.5	♀Ovigerous

Nematofauna in deep-sea sediments

The overall aim of this study is to identify the role of meiofauna in the C flow through benthic deep-sea sediments of the Mediterranean in relation to their biodiversity.
Samples were taken using box-corer to know the local meiofauna and specially the nematofauna, the most abundant metazoan group in deep-sea sediments, from depths that varied from about 1000 m to about 5000 m.
Samples were collected from:

Matapan – 1 deployment
Area 2 – 4 deployments
TOPO – 1 deployment
ADIOS B - 3 deployments
ADIOS C – 1 deployment
ADIOS A - 3 deployments.

From each deployment three corers of 3,6 cm diameter were used to get the samples.
-One corer was taken for the analysis of meiofauna composition: the sediment was divided in slices from 0-1, 1-2, 2-3, 3-4, 4-5, 5-10, 10-15 and 15-20 cm depth. The overlying water that remains on top of the corer was also taken. All strata and the overlying water were fixed with formalin 4%.
-One corer was taken for the analysis of fatty acids, divided in slices from 0-1, 1-2, 2-3, 3-4, 4-5, 5-10, 10-15 and 15-20 cm depth and put in deep-freezer at -20°C.
-One corer was taken for the analysis of environmental variables, divided in slices from 0-1, 1-2, 2-3, 3-4, 4-5, 5-10, 10-15 and 15-20 cm depth and put in deep-freezer at -20°C.
The samples will be analyzed at Ghent University, department of Marine Biology by Tania Nara C. Bezerra (Meiofauna), Ellen Pape (Fatty acids) and Dirk Van Gansbeke (Environmental Variables), supervised by Prof. Ann Vanreusel.

Acknowledgements

The authors are deeply indebted to the Captain and the crew of the CNR R/V Urania for continuous support during the whole measurement phase, and to the NURC NATO Undersea Research Center of La Spezia for the possibility of periodically testing the CTD probe in the calibration bath.

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