CNR-ISMAR
Istituto di Scienze Marine

Cruise Report

OCEAN CERTAIN 2015

18- 31 August 2015

Edited by Mireno Borghini

Contents

Cruise Details

Name	Ocean Certain 2015
Data	18-31 August 2015
Study Area	Tyrrhenian Sea Sardinian Channel Sicily Channel *Alagerian Basin*
Project Responsable	Japoco Chiggiato
Head of Mission	Mireno Borghini
Chief Scientist	Japoco Chiggiato
Partecipant Institutes	CNR-ISMAR
Research Vessel	MinervaUno
Departure Port	Mahon
Arrival Port	Naples

Scientific Staff

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Scientific Objectives

The main aim of the OC cruise in the western Mediterranean Sea will be to investigate, with a multidisciplinary approach, the food web and the connected biological pump structure and functioning under different environmental conditions, including global changes scenarios (+ THC and CO_2 fluxes)
The biological C pump (export of fixed CO2 from the euphotic zone to deep water layers) includes passive flux of sinking organic particles (POM), the active flux of DOM and POM mediated by vertical migration of zooplankton and vertical transport of DOM by physical process.
Therefore the composition of plankton assemblages is a major biological factor that governs the functioning of pelagic food-webs and consequently affects the biological C pump. Both trophic state and physical events (e.g. eddies, heat-waves, etc.) may have important consequences on composition and functioning of the pelagic microbial loop thus influencing the efficiency of material and energy transfer to the deeper water layers and to higher trophic levels.

1 Long term monitoring THC West Med
This cruise is aimed at continuing the climatic monitoring at the large scale and on the long term of the Western Mediterranean. Recent data collected in the deep western basin have evidenced an abrupt change, with the appearance and spreading in the whole basin of a new deep water, significantly warmer and saltier, which has substantially substituted the resident deep water. This new deep water has been formed during intense formation events in winter 04/05 and 05/06 in the north-western Mediterranean. This event has been called Western Mediterranean Transition (WMT), in analogy with the EMT. The proposed plan aims to continue the monitoring of the event and to study the causes (remote or local) that have triggered it, as well as of the repercussion it is likely to have. The study of this anomaly implies a basin-wide cruise plan
2 budget and box modeling carbonate system
At a steady-state, we may hypothesize a balancing between the losses of dissolved substances and the inputs coming from the Atlantic, the Eastern Mediterranean Sea, the atmosphere and the continents, minus the losses towards the deep sediments. Such budget calculations allow an estimate of fluxes on the basin scale and of the transfer processes across the interfaces between ocean, atmosphere, continents, sediments and ecosystems. Further, biogeochemical budgets are key elements in the analysis of the ecosystem functioning. Generally the distribution of biogeochemical properties in the different water masses is controlled by the interactions between physical and biogeochemical processes and external sources, as well as by their ages allowing the accumulation of inorganic nutrients.
The MEDOCC series campaigns (that have been carried out by CNR-ISMAR and CNR-IAMC at the western basin scale in 2005, 2006, 2007 and 2008) are carried out along multidisciplinary sections closing portions of the basin from coast to coast. It is a box-model approach that allows the estimate of budgets of mass, salt, heat and biogeochemical properties. The closed transects are carried out also in the strait regions (channels of Corsica and Sicily).
The total contemporary carbon budget for the Western Mediterranean will be computed by the combination of the DIC field with an estimate of the underlying circulation (horizontal geostrophic and diapycnal velocities, eddy fluxes, the air-sea exchange of heat and freshwater), diagnosed by means of a box inverse model.
3 The first objective is to study the relative contribution to the pelagic carbon flux of different heterotrophic and autotrophic planktonic components (virus, pico-, nano-, micro and mesoplankton) and on the ecosystem functioning. This approach will be applied in ca. 30 stations

selected along coastal vs open sea transects plus a repetition of the Balearic Island - Sardinia transect in order to be able to evaluate the impact on the biological pump of Mistral windstorms (if happening) and/or meteorological heat waves (if happening). Also, we plan to sample a target eddy usually produced in summer by (baroclinic) instabilities of boundary currents. Water samples will be compared in term of: i) patterns of abundance, structure of planktonic communities (virus, pico-, nano-, microzooplankton, mesozooplankton); (ii) ecosystems functioning measured as prokaryote C production, and total respiration rates (microbial and mesozooplakton activity of the Electron Transport System - ETS); iii) and C transfer along planktonic food webs by measuring biomass ratios of the different components.

4 The natural stable carbon isotopic composition (δ 13C) of marine suspended particulate organic matter (POM) and dissolved organic matter (DOM) can potentially provide important insights into marine carbon cycle, biological processes and environmental changes. Another objective proposed during this cruise is to study concentration of POC, PN, DOC and stable isotope δ13C signature of POC and DOC, along vertical profiles and in particular in areas interested by coastal inputs and physical mixing (eddies etc.). In addition samples will be collected for δ 13C of lipid biomarker (phospholipid fatty acids) for the identification of microorganisms participating in POM and DOM cycling. All this data will be coupled with values of cDOM, CO2 aq, nutrients and microbial C production/respiration rates.

5 Zooplankton plays a prominent role in marine pelagic systems due to their trophic position and their role in organic matter transfer from phytoplankton to upper level consumers. It is the major primary consumers and exhibit diversity of traits, ecological strategies and plays a key role in biological carbon binding activity in the pelagic marine ecosystem. Objective of this cruise will be to assess how trophic, and physical factor affect the structure of the mesozooplankton food web by using measures of stable isotopes of Carbon and Nitrogen in food sources and zooplankton consumers.

6 An adequate representation of the diversity of planktonic community is necessary to generate realistic predictions on the functioning of the C pump and in relation to global change scenarios. In the case of microbes, metagenomic and metatrascriptomic methods are the most powerful approach for providing insights into their potential metabolisms and biogeochemical roles. In the case of upper trophic levels, in several cases and in particular for metazoan propagules (eggs and larvae of fishes and invertebrates) specimens cannot be properly identified. However the correct identification of propagules is crucial for providing insights on the health and functioning of marine ecosystems. Metagenetic studies of zooplanton are extremely rare and completely lacking in the Mediterranean Sea.

During this cruise we aim to characterize microbial communities and identify microbial zooplankton diversity (including eggs and larvae of fishes and invertebrates) by applying cutting edge mass-parallel sequencing technology.

7 Another objective will be to investigate the phage-host interactions in the deep Mediterranean Sea, and to understand the role in the C cycling. There are up to 1030 viruses in the ocean, and they play a significant role in influencing microbial communities, ecosystem functioning and the global biogeochemial cycles. The phage-host interactions in the deep ocean are, however, only poorly understood. During the cruise, we will attempt to isolate bacterial strains and their phages from the bathypelagic Mediterranean Sea, characterize the bacterial strains and use plaque assays to isolate the phages. The viral genomes of the isolates will be sequenced and then analysed with the appropriate bioinformatics tools to gain information about the infection ability and gene properties. The results will be compared with the metagenome data from the same water samples.

Cruise Plan

On board there were several groups working together, with different instruments. Both are listed below.

Parameters	*Working Group*
Salinity	CNR-ISMAR and CNRS
Temperature	CNR-ISMAR
Beam Attenuation	CNR-ISMAR
Fluorescence	CNR-ISMAR
Dissolved Oxygen	CNR-ISMAR
Dissolved Inorganic Nutrients (NO2, NO3, PO4, NH4, SiO3)	CNR-ISMAR
Light (including UV) – CANCELLED by coordinator	
Total Organic Carbon	
Total Organic Nitrogen	ISMAR CNR
pH	
Alkalinity	
Dissolved Barium (by external partner)	
Clorophyll-a – DEU-IMST	
Bacterial abundance and biomass	
Bacterial processes	
Ciliates/flagellates	
Phytoplankton – DEU-IMST	
microzoo (TBD internally)	
Zooplankton abundance e biomass	
Virus	
POM (POC +PN)	
DOM (DOC)	
cDOM	

Tabella 1. Cruise measurements

Instruments	
Small-Volume Sampling	General Oceanic 24-place rosette with 12 L bottles
CTD	SBE 911 plus
Fluorimeter	AQUA^{traka} MK III
Salinometer	Portasal Guideline
Dissolved oxygen	Winkler titration with Titrino SBE 43
Nutrients	Only samples frozen on board
Vessel Mounted ADCP system	RDI OS 150 kHz
Lowered ADCP	RDI WH 300 kHz

Cruise Maps

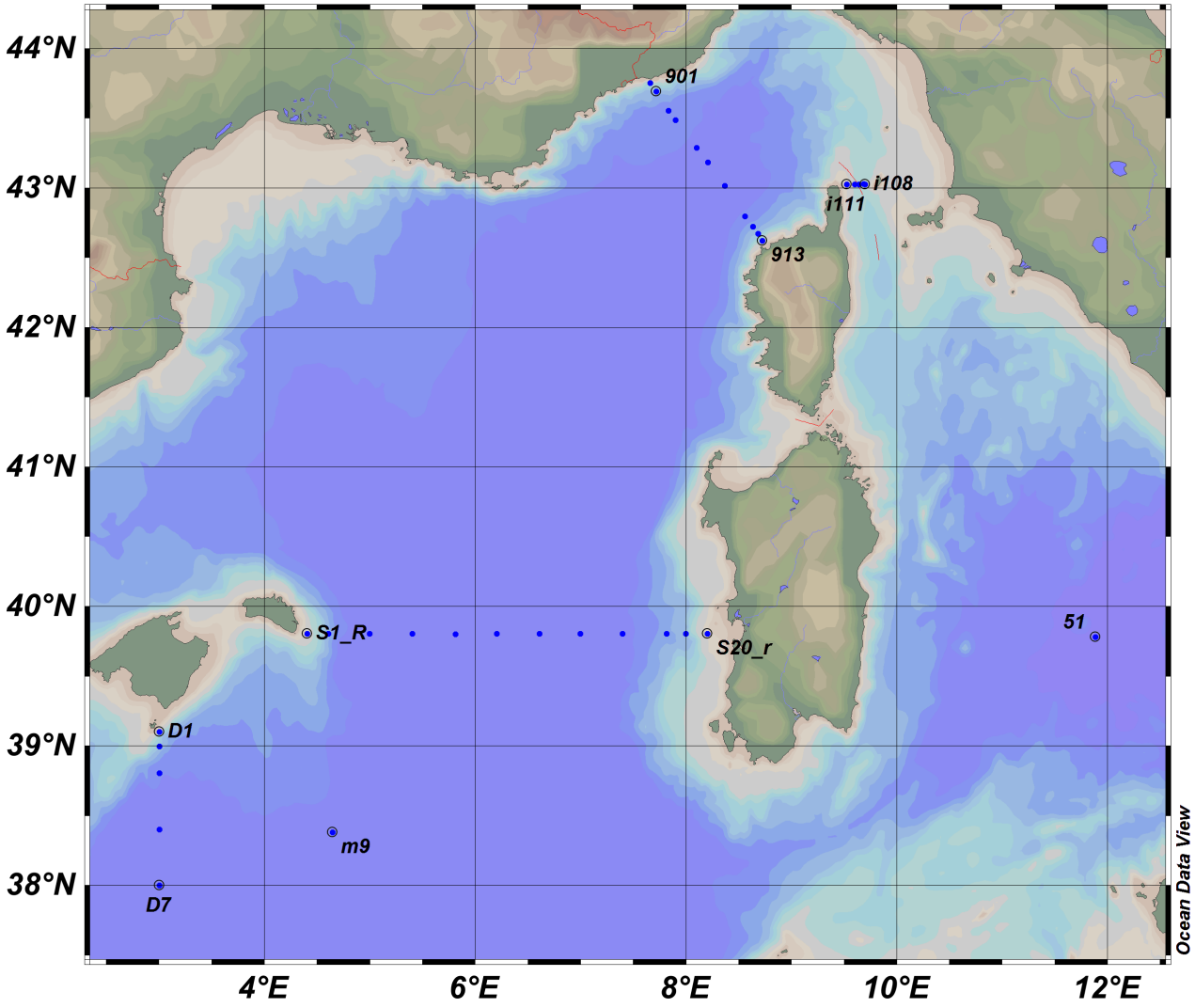
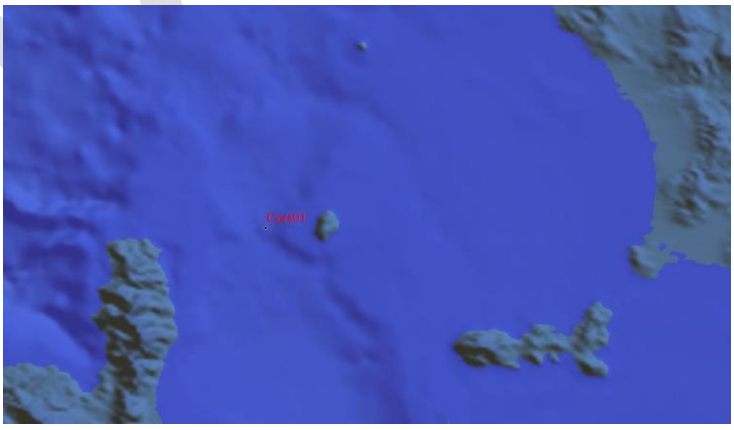


Figura 1. Map of the casts

Figura 2. Map of the moorings



Cruise Stations

STATIONS	DATE	LAT	LONG	DEPTH
51	2015-08-9T20:20:24.000	11.88333	39.78083	3450
900	2015-08-28T12:46	7.65833	43.75333	336
901	2015-08-28T08:26	7.7165	43.6915	1112
903	2015-08-28T03:32	7.83467	43.55483	2361
904	2015-08-28T15:57	7.89967	43.48867	2455
906	2015-08-26T22:14	8.09983	43.28967	2535
907_3	2015-08-27T05:26	8.20633	43.18567	2567
908	2015-08-27T07:35	8.36683	43.01667	2641
908_b	2015-08-27T10:12	8.36683	43.0165	2641
910	2015-08-27T13:56	8.55717	42.79683	2336
911	2015-08-27T16:23	8.63233	42.72483	1717
912	2015-08-27T18:13	8.6815	42.67383	1111
913	2015-08-23T12:55	8.72483	42.62117	260
cors_moor	2015-08-24T08:11	9.68533	43.02867	441
D1	2015-08-19T13:23	3.00433	39.09967	111
D2	2015-08-19T14:24	3.00433	38.997	1232
D3	2015-08-19T18:48	3.00417	38.805	2480
D5	2015-08-19T23:19	3.00417	38.40233	2708
D7	2015-08-20T04:47	3.00317	38.00033	2800
i108	2015-08-24T13:15	9.69967	43.02483	446
i109	2015-08-24T12:12	9.64117	43.02483	365
i110	2015-08-24T11:08	9.599	43.026	234
i111	2015-08-24T10:07	9.52517	43.02667	128
m9	2015-08-20T18:53	4.647	38.38133	2720
S10_R	2015-08-21T22:35	6.20133	39.80317	2846
S12_R	2015-08-22T01:17	6.61	39.80333	2851
S14_R	2015-08-22T03:53	6.9975	39.80317	500
S16_R	2015-08-22T06:30	7.39533	39.80367	500
S18_r	2015-08-22T12:53	7.81633	39.80333	2519
S19_r	2015-08-22T14:26	7.99767	39.80333	500
S1_R	2015-08-21T06:55	4.40383	39.80333	100
S20_r	2015-08-22T17:54	8.20333	39.80317	103
S2_R	2015-08-21T08:20	4.60717	39.80333	500
S4_R	2015-08-21T12:28	4.99583	39.80317	2708
S6_R	2015-08-21T15:30	5.404	39.80317	2820
S8_R	2015-08-21T18:03	5.81317	39.80283	500

Sampling strategy

The CTD stations have been chosen both on historical basis, following the box-model approach, and depending on the weather and the needs of the cruise. CTD, LADCP and fluorescence measurements were performed. Thanks to CTD casts we have hydrological information about the area, sample bottles have been taken in order to determine dissolved oxygen and salinity (on board) and then nutrients (just sampled and frozen). In order to achieve information about the spatial variability of this parameters, a high-resolution sampling has been applied at standard depths as we can see in the table below For a better sampling of the biological and chemical parameters, extra sampling depths were defined in the water column by analyzing the CTD profile during the acquisition, if it was needed.

Level	*Standard depth (m)*
1	0
2	25
3	50
4	75
5	100
6	200
7	300
8	400
9	500
10	750
11	1000
12	1250
13	1500
14	1750
15	2000
16	2250
17	2500
18	2750
19	3000
20	3250
21	3500

Tabella 2. Standard depth

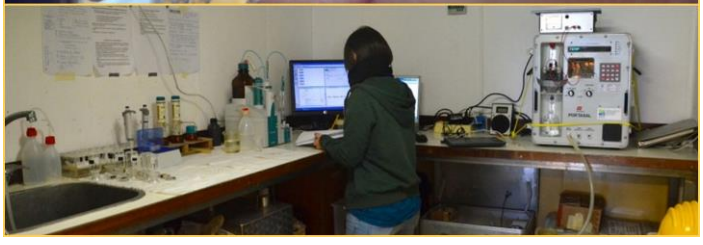
Onboard Operations

CTD Casts

At every station, pressure (P), salinity (S), potential temperature (θ) dissolved oxygen concentration (DO) and fluorescence were measured with a CTD-rosette system consisting of a CTD SBE 911 plus, and a General Oceanics rosette with 24 Niskin Bottles (12 liters each).
Temperature measurements were performed with a SBE-3/F thermometer, with a resolution of 0.00015 °C/bit at -1 °C or 0.00018 °C/bit at 31 °C, and conductivity measurements were performed with a SBE-4C sensor, with a resolution of 3 x 10-4 S/m. Dissolved oxygen was measured with a SBE-43 sensor (resolution 4.3 µM). 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. The data were processed on board, and the coarse errors were corrected thanks to the sampling and analyzing on board o f oxygen and salinity of the deepest station.

The rosette is equipped with a sonar altimeter which intercept the bottom 70-50 meters before getting to it. The altimeter is used just for safety, to avoid the rosette to touch the bottom, and for more precision in measuring depth.

Oxygen and salinity determination



Salinity samples were collected, stored and analyzed with a Guildline Portasal Salinometer, standardized with IPSO standard 34.999 PSu Water and at controlled temperature.
Also dissolved oxygen samples were collected and analyzed with the Winkler method,using a computer controlled potentiometric end-point titration procedure. Samples were taken from the Niskins bottle with the recommended precautions and following the Winkler procedure. For example oxygen was sampled before everything else, paying attention not to make the water gurgle, to prevent any biological activity and gas exchange with the

atmosphere. The oxygen content was "fixed" immediately after collection, adding manganese (II) and alkaline iodide with semi-automatic dispensers. The bottles need to be shaken vigorously for about 30 seconds to bring each oxygen molecule in contact with the reagents. After fixation time is given to every sample for sedimentation. The samples need to stay for almost three hours in the dark, avoiding any change of temperature. Before titration the precipitated hydroxides are dissolved with sulfuric acid and the titration is carried out with a standardized thiosulfate solution using a Dosimat Methrom, software tiamo 2.0.

LADCP

Two Lowered Acoustic Doppler Current Profilers (LADCP) are used to measure velocity and magnitude of the currents during the CTD cast. They are two RDI Workhorse 300 kHz ADCP, a master facing down and a slave facing up. For data post-processing we used the LDEO LADCP (version 10.16) software.

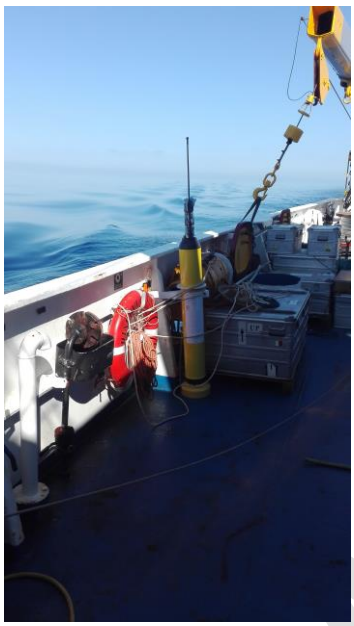
Inorganic nutrients

Seawater samples for nutrient measurements were collected at standard depths of every cast, within oxygen and salinity samples. Nutrient samples were stored at –20°C and nitrate, orthosilicate and ortophosphate concentrations will be determined later in the laboratory, using a hybrid Brän–Luebbe AutoAnalyzer following classical methods (Grasshoff et al., 1983) with slight modifications.

Vessel Mounted ADCPs

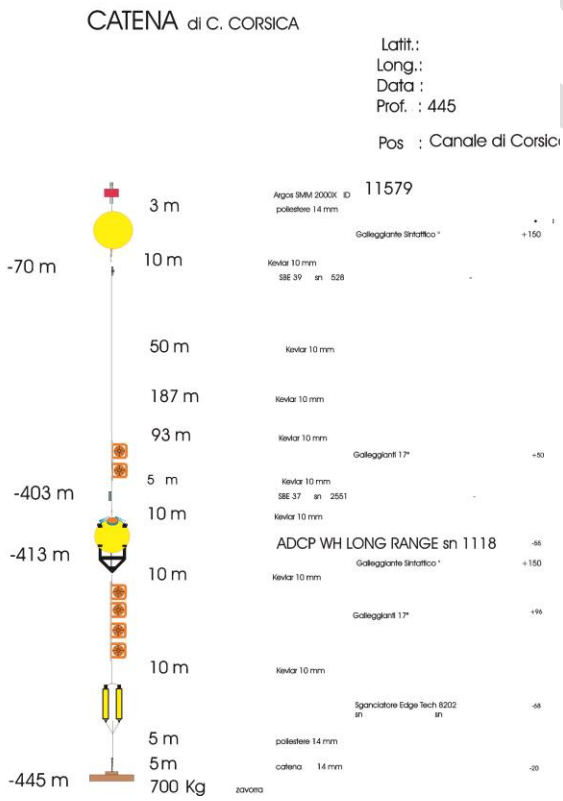
The hydrographic data set has been integrated with direct current measurements. During the whole campaign two VM-ADCPs (RDI Ocean Surveyor, 150 KHz,) works along the whole ship track. The depth range of the two current profilers is about 150 m (OS150). Data acquisition is carried out using the RDI VMDAS 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 in the value of sound velocity in water and in alignment of the instrument with respect to the axis of the ship.



Profiling Floats

Two Apex ,is deployed by ISMAR-CNR for OGS.



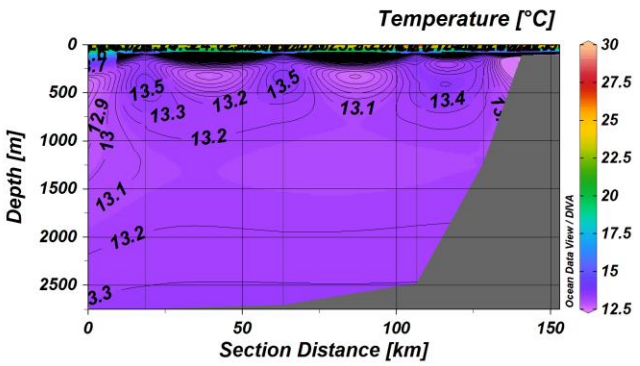
Moorings Recovery and deployment

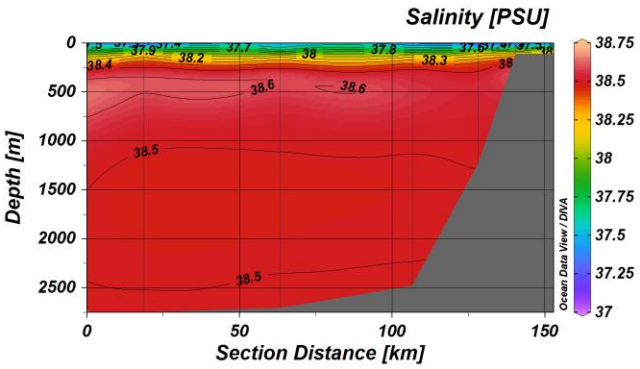
During the cruise three moorings were recovered and deployed again in their original position
Mooring C.Corsica

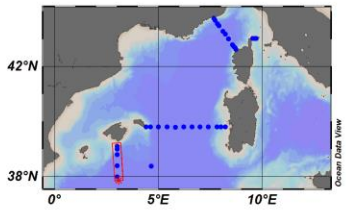
Preliminary Results

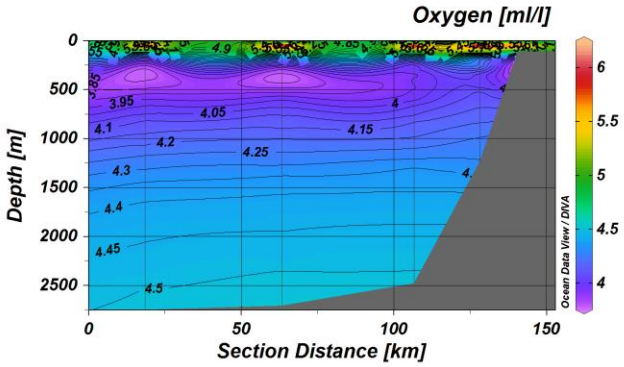
Hydrology

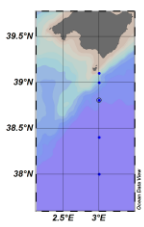
The D transect

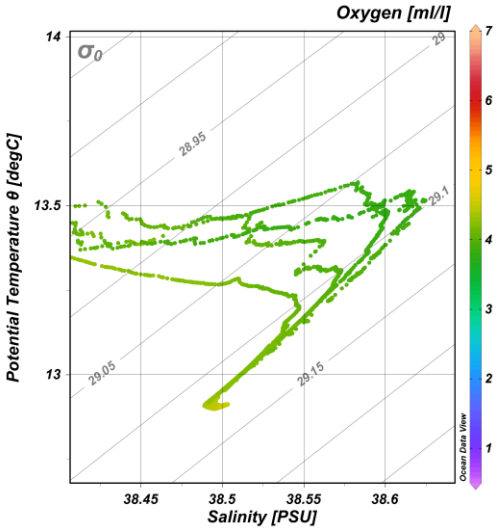












The S transect _500 m

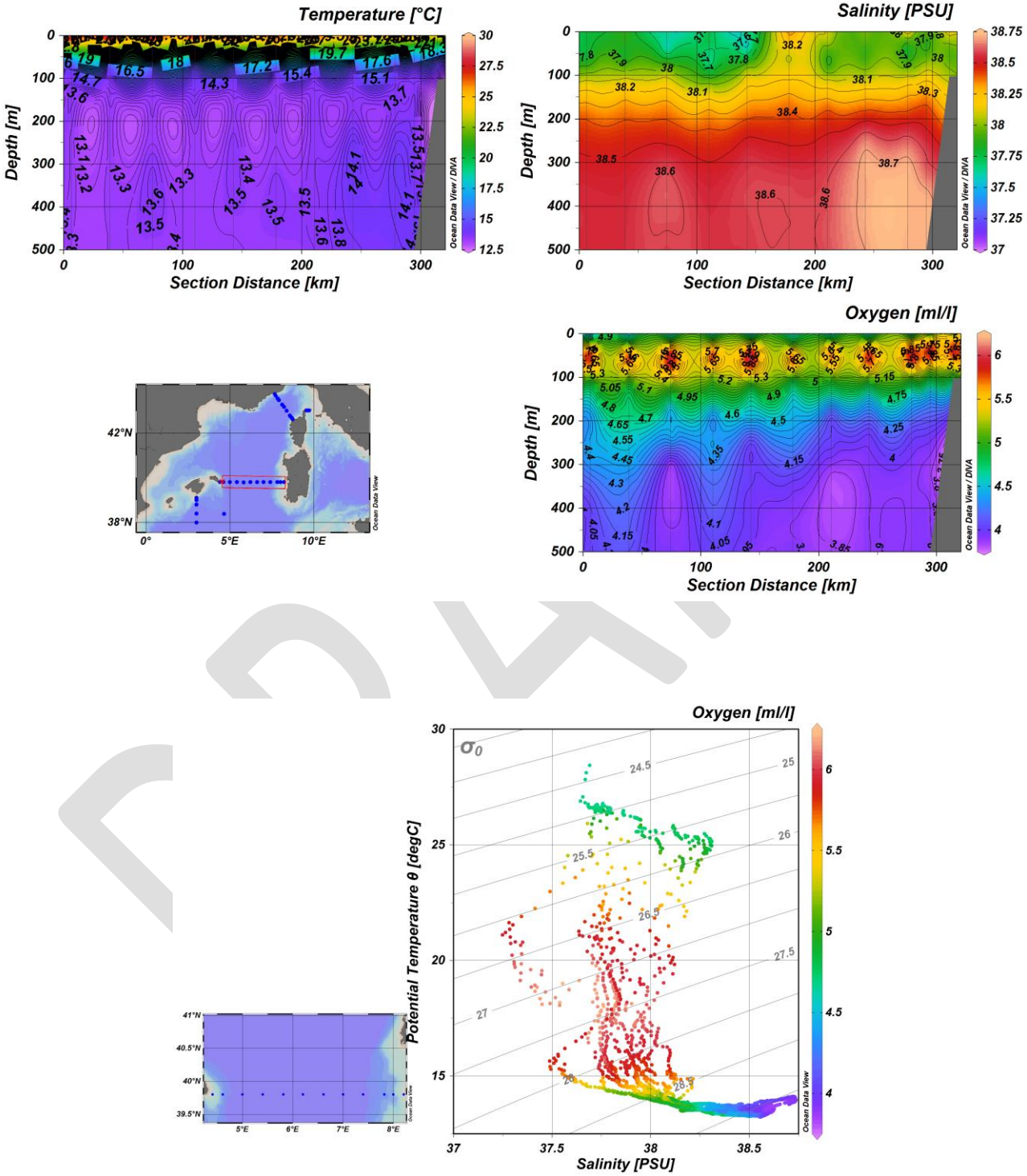


Figura 3. TS diagram of the S transect_500

The 900 transect

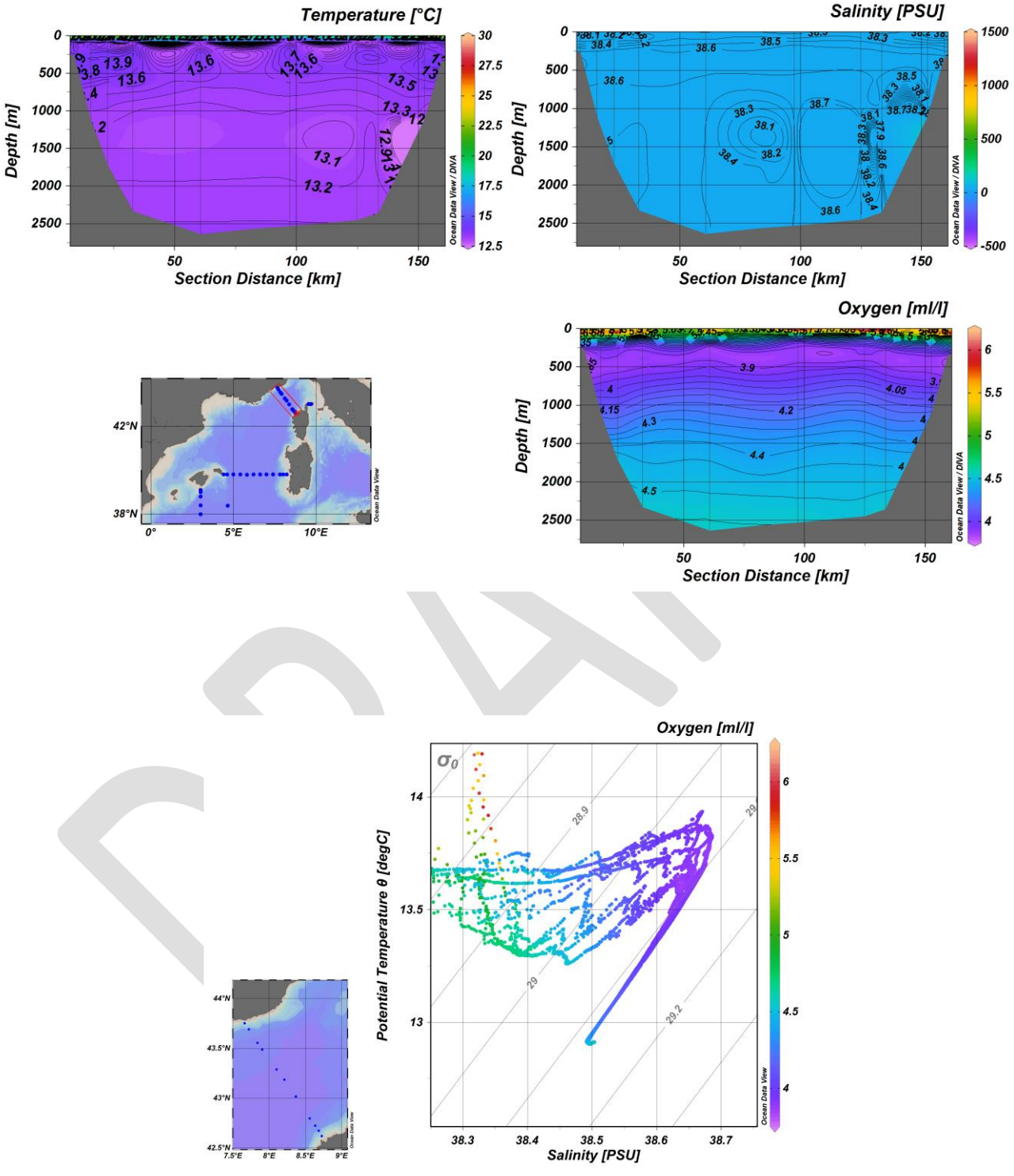
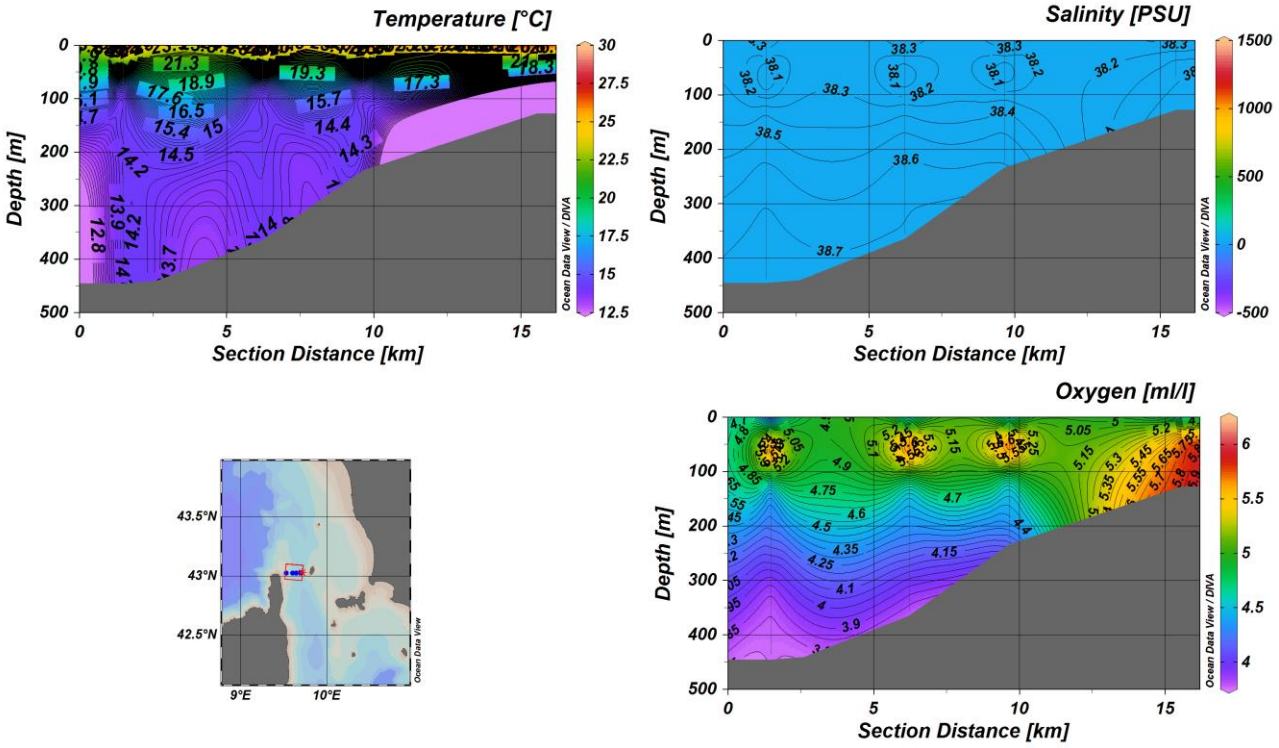


Figura. TS diagram of the transect

The 100 transect



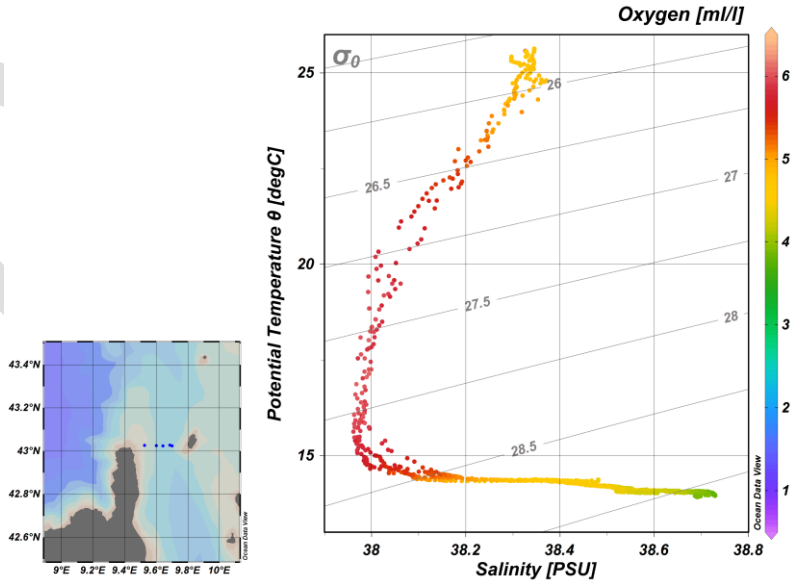
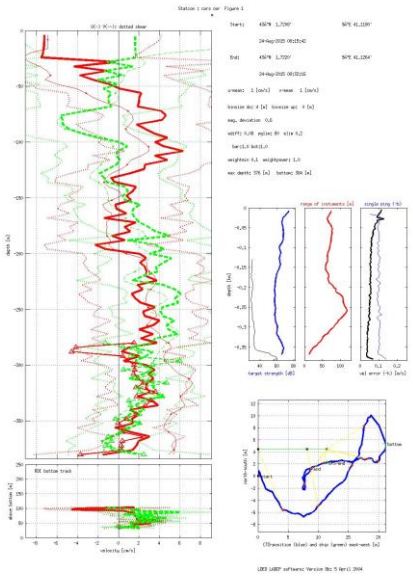


Figura. TS diagram of the transect

Currents from LADCP

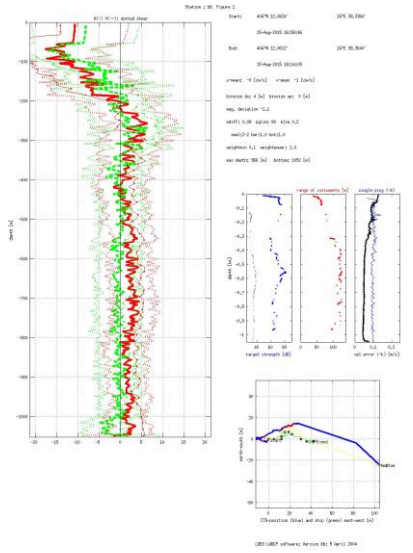
Station cors_moor



Profiles of the measured velocities in cm/s at the station.

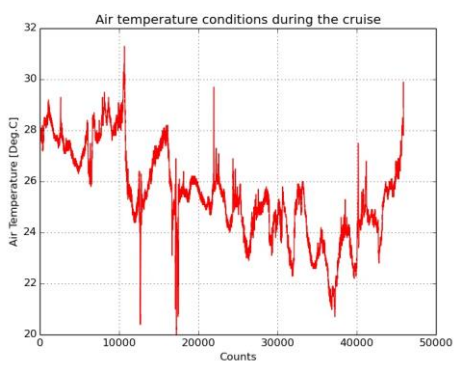
Currents from LADCP

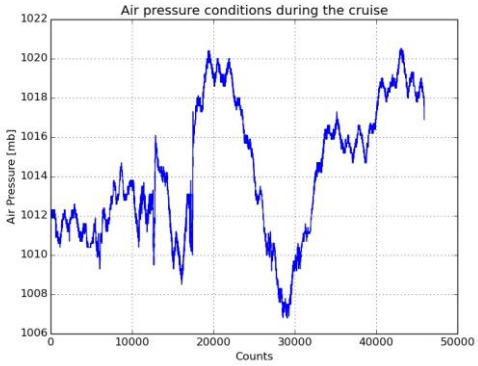
Station b6

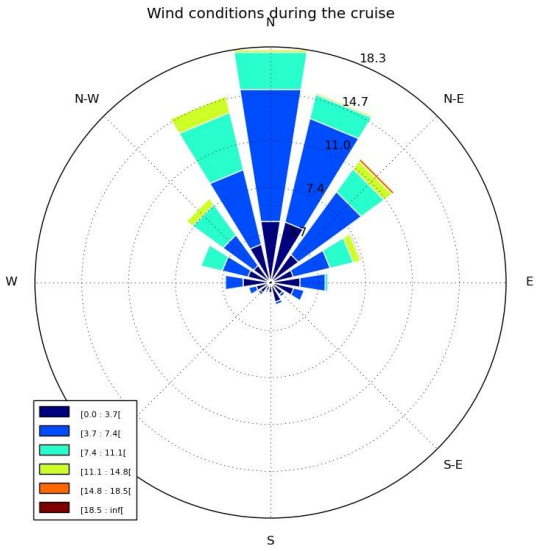


Profiles of the measured velocities in cm/s at the station.

Weather conditions







Evolution of the weather conditions during the cruise (air temperature, air pressure, wind rose).

CHEMICAL AND BIOGEOCHEMICAL WATER MASSES CHARACTERIZATION

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Objectives

- To provide the basic chemical characterization of the different water masses identified in the area by the analysis of inorganic nutrients (NO_3, NO_2, PO_4, NH_4, SiO_2) and dissolved oxygen (DO).
- To study the inorganic carbon system and air-sea CO_2 fluxes in different areas of the Mediterranean basin. The measurements of pH_T and total alkalinity (TA) allow the calculation of all the other parameters of the inorganic carbon system (CO_2, DIC, etc…).
- To study the distribution and characteristics of dissolved organic matter by the analysis of dissolved organic carbon (DOC) and its stable isotopes (δ^{13}C-DOC), dissolved organic nitrogen (DON) and chromophoric dissolved organic matter (CDOM).
- To study dissolved barium distribution (Ba) to get new insights on organic matter production and remineralisation.
- To measure carbon and nitrogen stable isotopes in the particulate organic carbon fraction (δ^{13}C-POC; δ^{15}N-PN) to get new insights on nitrogen sources and to study the trophic web in collaboration with the other groups.

The samples collected are summarized in the following table

Station	Nut.	DO	Ba	pH	TA	DIC	DOC DON	CDOM 13C-DOC	POC 13C-POC	PON 15N-PON
D1	5	5	5	5	5					
D2	12	12	12	12	12		12	7	5	5
D3				1						
D5	16	16	16	16	16	10				
D7	15	15	15	15	15		15	7	5	5
M9	1			1			1			
S2_R	10			10	10		10		5	5
S4_R	7			7	7					
S6_R				1						
S8_R	10			10	10		10		5	5
S10_R				1						
S12_R	10			10	10					
S16_R	9			9	9		9		5	5
S17_R	1						1			
S18_R				1						
S19_R	10			10	10		10		5	5

S20_R	6			6	6		6		3	3
913	6	6	6	6	6		5	5	4	4
M. Corsica	1			1	1					
111	4	4	4	4	4					
110				1						
109	9	9	9	9	9					
108	10	10	10	10	10					
906	13	13	13	13	13		13	10	5	5
907				1						
908	16	16	16	16	16		16	11	5	5
910	15	15	15	15	15					
911				1						
912	10	10	10	10	10		10	8	5	5
903	15	15	15	15	15					
901	11	11	11	11	11		11	7	5	5
900	8	8	8	8	9		8	5	5	5
51	10	10	10	10	10		10	10		
TOT	**240**	**175**	**175**	**246**	**239**	**10**	**147**	**70**	**62**	**62**

Methods

The samples to determine dissolved oxygen (DO), were drawn from the Niskin bottles into 60 ml BOD bottles and the analysis were performed on board using an automated potentiometric Winkler titration system. The precision measured on samples analyzed in triplicate was 0.5 µmol/l (0.01 ml/l) (expressed as standard deviation).

The samples to measure pH on the "total hydrogen ion scale " (pH_T) were drawn soon after the ones for DO analysis, directly into cylindrical 10 cm path length spectrophotometric cells. pH_T was measured on board within 24 h from the collection, according to the spectrophotometric method using m-cresol purple as indicator (Clayton and Byrne,1993; Dickson et al., 2007). The analytical precision was estimated to be ± 0.002 pH_T units, determined by the triplicate analysis of samples, accuracy was monitored by the use of stabilised SW certified for At and DIC.

The samples to measure total alkalinity (TA) and dissolved inorganic carbon (DIC) were collected in 300 ml borosilicate bottles, poisoned with mercuric chloride, tightly closed and stored at temperatures between +4 °C and +25 °C until the analysis.

Total alkalinity will be analyzed by potentiometric titration in an open cell with a difference derivative readout (Hernandez-Ayon et al., 1999) . The typical precision is sd= 2.0 µmol kg^{-1} (n = 6) and the accuracy will be checked by the titration of reference seawater certified for At value. DIC will be analyzed by GEOMAR (Kiel, De) by columetric titration.

The samples to determine dissolved inorganic nutrients were filtered, collected in 20 ml HDPE vials and frozen at -20°C immediately after collection. The analysis for the determination of nitrate plus nitrite, ammonium, nitrite, reactive phosphorus and reactive silicon will be performed using a Flow-Solution III autoanalyser (OI-Analytical), following standard colorimetric methods in line with those reported by Grasshoff et al. (1999).

The samples to measure the dissolved organic carbon (DOC) and dissolved organic Nitrogen (DON) concentration were filtered, collected in 20 ml HDPE vial, previously conditioned with an acid treatment, and immediately frozen. The analysis will be performed by HTCO technique. The samples to measure chromophoric organic

matter (C-DOM) and δ^{13}C-DOC where collected in the same way and stored frozen in precombusted glass vials. The analysis will be performed by the specrtrofluorimetric technique and by GC-MS in collaboration with ISPRA (Chioggia).

The samples to be analysed for Barium concentration (Ba) were collected in 15 ml HDPE vials, poisoned with 15µl of ultrapure HNO_3, tightly closed and stored at room temperature. The analysis will be performed in collaboration with MIO (Marine Oceanographic Institute, Fr).

Two different samples were collected for particulate organic carbon concentration and its isotopic abundance (POC, δ^{13}C-POC) and for particulate organic nitrogen (PN, 15N-PN). For each sample 5 to 10 L were filtered on a precombusted GF/F filter that was immedialtely frozen at -20°C. The analysis will be performed by a mass spectrometer coupled to an elemental analyser in collaboration with ISPRA (Chioggia).

VENUS3 OCEAN CERTAIN
Diociaiuti

Cruise Report Leg 2: Microzooplankton

Objective

- Quantitative and qualitative analysis of microzooplankton (20-200 µm) communities in the different basins of the Mediterranean Sea from mesopelagic layer to the surface, to determine the influence of this functional group on the carbon flux between different levels in the trophic web.
- Characterization of eukaryotic diversity in the deep sea performing metagenomic analysis.

Methods

For microscope analysis of microzooplankton, different volumes of sea water (table 2) were prefiltered on 200µm, filtrated on 10µm , resuspended in 200ml, fixed with formalin 4% and stored in the dark at 4 °C.

	surf	50m	DCM	100m	200m	500m	>1000m
microscopy	5L	5L	5L	5L	10L	10L	15L
genetic	5L	5L	5L	5L	10L	10L	15L

Table 2 (filtered volumes for each depth)

The same volumes were collected for the metagenomic analysis, organisms were concentrated on 2µm polycarbonate filters and preserved at -20 °C.

Samples were collected during 3 transects, at 17 different stations (listed in table 1). A total of 73 samples were collected for the microscopic analysis and 47 for the metagenomics and will be

analyzed in the laboratory of the University of Trieste by inverted microscope and metagenomics techniques.

BIOLOGICAL STATIONS		
Transect	*Station*	*Depths (mt)*
Palma-Algeri	D2	0-50-DCM(75)-200-500
	D7	0-50-DCM(80)-200-500-2797
	M9	2721
Menorca-Sardeña	S2R	0-50-DCM(93)-200-500
	S8R	0-DCM(70)-100-200-500
	S16R	0-50-DCM(90)-200-500
	S17R	2764
	S19R	0-50-DCM(?)-200-500
	S20R	0-50-DCM(65)-100
Corsega-Liguria	913	0-50-DCM(80)-200
	906	0-DCM(40)-100-200-500-int1-2535
	908	0-DCM(?)-100-200-500-int1-2640
	912	0-50-DCM(76)-200-500
	901	0-50-DCM(85)-200-500
	900	0-50-DCM(85)-200
	904	2450
Study case	51	3471

Table 1 (list of samples collected)

CAMPAGNA VENUS 3 04-17 Agosto 2015
RAPPORTO ATTIVITA'

Cognome (partecipante/i)	Pansera
Nome (partecipante/i)	Marco
Attività	Rete trofica MESOZOOPLANCTON e MICRONECTON
Laboratorio	Ecologia dello Zooplancton - ISMAR Venezia
Ente di appartenenza	Consiglio Nazionale delle Ricerche, Istituto di Scienze Marine

Piano di campionamento

Durante la campagna Ocean Certain, svoltasi dal 18 al 30 Agosto 2015, a bordo della N/O Minerva Uno, sono stati effettuati dalla nostra U.O. campionamenti di meso e macrozooplancton in 17 stazioni, nei transetti: Baleari-Algeria, Sardegna-Baleari e Corsica-Liguria, Corsica-Capraia .

La strategia di campionamento ha previsto 3 retinate nelle stazioni biologiche dalla profondità di 500 metri o, in caso di profondità inferiore, da 10 metri sopra il fondo fino alla superfice. In alcune stazioni considerate non biologiche è stato effettuato una singola retinata.

Campionamento

I campioni sono stati raccolti con la rete da mesozooplancton IOSN (Indian Standard Ocean Net) con bocca di 1 m^2 e rete con vuoto di maglia di 200 µm, su cui è stato montato un flussimetro della Hydro-Bios Kiel per la misura dei metri cubi filtrati di acqua di mare.

Ogni operazione di campionamento, effettuato dal portale laterale della nave, ha avuto una durata di circa 30 minuti ed ha comportato la calata in mare della rete sino alla massima profondità ed il successivo recupero, con profilo verticale, sino alla superficie con una velocità costante di 1 metro al secondo.

Fuori acqua e prima del ritiro a bordo, il retino è stato accuratamente sciacquato con un getto di acqua salata per raccogliere gli organismi, eventualmente rimasti sulle pareti, nel bicchiere di raccolta.

Pretrattamento e conservazione dei campioni

Nelle stazioni biologiche le 3 retinate effettuate sono state trattate e conservate per:
- analisi dell'abbondanza e della diversità con metodologia classica al microscopio,
- analisi della diversità con tecniche di metagenomica,
- analisi dei tassi di respirazione per mezzo dell'ETS activity,
- analisi degli isotopi stabili del carbonio e dell'azoto.

Per l'analisi quali/quantitativa al microscopio il campione è stato conservato e fissato in acqua di mare e formalina tamponata con tetraborato di sodio ad una concentrazione

finale del 4% in bottiglie da 250ml; per l'analisi della diversità con tecniche di metagenomica il campione è stato fissato con etanolo (96%) in falcon da 50ml; per l'ETS activity il campione è stato filtrato su garza da 200 μm, messo in falcon da 15 ml e conservato in azoto liquido; per l'analisi degli isotopi stabili, una volta a bordo, il campione è stato messo per un ora in un bidone da 10 litri con acqua di mare filtrata, per la gut clearance, e successivamente filtrato e conservato in falcon da 50ml a -20C°.

Nelle stazioni non biologiche in cui è stata fatta una sola retinata, il campione è stato conservato per l'analisi quali/quantitativa al microscopio e per l'analisi con tecniche di metagenomica.

Nelle 4 stazioni biologiche (900, 901,906,912) del transetto Corsica-Liguria, sono stati filtrati volumi d'acqua da 4 a 6 litri su filtri GF/F muffolati, per l'analisi dei fosfolipidi nelle quote superfice, DCM e 500 metri, nella stazione 900 al posto della quota 500m è stata filtrata l'acqua prelevata a 200m.

Campagna Ocean Certain

STRUMENTO UTILIZZATO: "**INDIAN OCEAN STANDARD NET"**

CORREDATO DI FLUSSIMETRO **"HYDRO BIOS KIEL"**

Stazione n.	Retinate n.	Data	Ora UTC inizio retinata
D02	3	19-08-15	15-27
D05	1	20-08-15	01-29
D07	3	20-08-15	06-35
M09	1	20-08-15	09-00
S02-bis	3	21-08-15	08-50
S08-bis	3	21-08-15	18-40
S16-bis	3	22-08-15	07-00
S19-bis	3	22-08-15	14-54
S20-bis	3	22-08-15	17-55
913	1	23-08-15	13-20
Mooring-Corsica	1	24-08-15	08-37
i110	1	24-08-15	10-00
906	3	27-08-15	00-00
908	3	27-08-15	09-12
912	3	27-08-15	19-02
901	3	28-08-15	09-11
900	3	28-08-15	12-20

Biodiversity and abundance of planktonic Bacteria and viruses

Gian Marco Luna, Elena Lara, Laura Perini National Research Council – Institute of Marine Sciences (CNR-ISMAR), Venezia

Stefano Amalfitano (CNR-IRSA), Roma

Objectives

- Investigate and analyze the abundance and diversity of planktonic Bacteria and viruses in epi-, meso- and infrapelagic waters of the Western Mediterranean sea;

- Describe, in selected samples, the abundance of Bacteria and viruses, and the metagenome, metavirome and metatranscriptome of deep microbial assemblages.

Methods

Seawater samples were collected at each "biological" station (see Table 1) from 3 to 5 depths (from 0 to 500 meters). In the biological and/or deep stations the bottom water was also sampled. An extra station (51) was also sampled as "study case" due to its water masses characteristics.

Table 1. List of "biological", deep and "study case" stations sampled in the first leg of the cruise.

BIOLOGICAL STATIONS		
Transect	*Station*	*Depths (mt)*
	D2	0-50-DCM(75)-200-500
Palma-Algeri	D7	0-50-DCM(80)-200-500-2797
	M9	2721
Menorca-Sardegna	S2R	0-50-DCM(93)-200-500
	S8R	0-DCM(70)-100-200-500
	S16R	0-50-DCM(90)-200-500
	S17R	2764
	S19R	0-50-DCM(?)-200-500
	S20R	0-50-DCM(65)-100
Corsica-Liguria	913	0-50-DCM(80)-200
	906	0-DCM(40)-100-200-500-2535
	908	0-DCM(?)-100-200-500-2640
	912	0-50-DCM(76)-200-500
	901	0-50-DCM(85)-200-500
	900	0-50-DCM(85)-200
	904	2450
Study case	51	3471

For abundance analyses, water was collected without prefiltrations, while for the other analyses a

200 and 20 µm prefiltration step was added. In the biological stations with depths exceeding 1000 mt, we also sampled the bottom water layer (Table 1). In selected deep samples (> 1000 mt), not included among the "biological" ones, 65 liters of bottom water were collected.

Bacterial abundance and diversity

For analyses on the 0-500 mt layers, 1.8 mL (in duplicate) of each water sample were fixed with 100 µl of formaldehyde, vortexed and immediately stored at 4°C. All samples were immediately analyzed on board by the flow cytometer Apogee A50-micro. From 5 to 10 liters of prefiltered seawater were filtered onto 0.22 µm Cellulose Nitrate filters (Sartorius). Filters were put into sterile Eppendorf tubes and stored at -20°C until return to the laboratory. Diversity will be described using amplicon-based Next Generation Sequencing of the 16S gene, in order to assess the patterns of α- and β-diversity and community composition.

In deep water samples, about 2 liters of prefiltered bottom water were filtered through a Sterivex 0.22 µm filter within 20 minutes from sampling. The sterivex was then filled with RNA later (Ambion) and immediately stored at -20°C. Sterivex filters will be used for the analysis of the metatranscriptome. For shotgun metagenomic analyses, 60 liters of prefiltered water were filtered through a 0.22 µm Cellulose Nitrate filters (Sartorius), put into sterile Eppendorf tubes and stored at -20°C.

Viral abundance and diversity

For analyses on the 0-500 mt layers, 2 mL of each water sample (in duplicate) were fixed with 40 µl of glutaraldehyde, stored at 4°C in the dark for 10 minutes and then stored at -20°C. Viral concentrates were obtained with tangential flow filtration using a 30KDa VIVAFLOW cartridge (Sartorius) starting from 4 liters of 0.22 µm filtered water to a final volume of 30 ml. Viral concentrates were stored in the dark at 4°C.

In deep water samples, for metavirome analyses, 6 mL of 10 g/L of iron chloride were added to 60 liters of bottom 0.22 µm filtered water in order to precipitate the viruses. Samples were incubated at room temperature for one hour in the dark or at 4°C overnight. After incubation, the 60 liters were filtered onto a 1 µm Nucleopore membrane circles, put into sterile falcon tubes and stored at 4°C. Viral concentrates were obtained with tangential flow filtration using a 30KDa VIVAFLOW cartridge (Sartorius) starting from 5 liters of 0.22 µm filtered water to a final volume of 35 ml. Viral concentrates were stored in the dark at 4°C.

For each deep station a viral decay rate experiment was performed. Fifty mL of 0.22 µm filtered water (in triplicate) were collected and incubated in the dark at *in situ* temperature. Aliquots of 2 mL were collected from each replicate at 0, 3, 6, 12, 18 and 24 h, fixed with 40 µl of glutaraldehyde, vortexed, incubated in the dark at 4°C for 10 minutes and then stored at -20°C.

In the "biological" stations with depths over 1000 mt (see Table 1), a viral production experiment was also performed. One liter of prefiltered water was filtered with 0.8 µm Nucleopore membrane circles. A bacterial concentrate was obtained from this filtered water with a tangential flow

filtration using a 0.22 µm VIVAFLOW cartridge (Sartorius) to a final volume of 100 mL. A volume of 200 mL of virus-free seawater was added to the bacterial concentrate and mixed. This solution was then aliquoted in 6 sterile 50 mL falcon tubes. In 3 of the tubes, 1 µg/mL of mytomicine C was added. Tubes were incubated in the dark at *in situ* temperature. Aliquots of 2 mL were collected from each replicate at 0, 4, 8, 12 h, fixed with 40 µl of glutaraldehyde, vortexed, incubated in the dark at 4°C for 10 minutes and then stored at -20°C. All bacterial samples were immediately analyzed to assess the cell abundance and the experiment dynamics during the incubations.

Report Venus3-Ocean Certain cruise 2015- second leg

Microbial heterotrophic production and respiration

Lucia Bongiorni, Federica Fiorentino-National Research Council – Institute of Marine Sciences (CNR-ISMAR), Venezia

Objectives

- Investigate carbon flux between different levels in the planktonic food web.
(in particular nanoplankton 2-20µ size, abundance, biomass, microbial heterotrophic Carbon production and respiration) in the epi-, infra- and mesopelagic waters of the Western Mediterranean Sea;

- Invetigate in selected deep-water samples, the microbial C flux (heterotrophic C production and respiration).

Methods

Seawater samples were collected at each "biological" station (see Table 2) from 5 water layers (from 0 to 500 meters). In the biological and/or other selected deep stations bottom water was also sampled. Stations along the transect Baleares-Sardinia (investigated during the first leg of the cruise) were sampled for the second time to investigate possible changes due to metereological events (case study) The same analyses described for 0-500 mt samples were performed in these stations.

Table 2. List of "biological", deep and "study case" stations sampled in the second leg of the cruise.

BIOLOGICAL STATIONS		
Transect	*Station*	*Depths (mt)*
Palma-Algeri	D2	0-50-DCM(75)-200-500
	D7	0-50-DCM(80)-200-500-2797
	M9	2721
Menorca-Sardinia (case study)	S2R	0-50-DCM(93)-200-500
	S8R	0-DCM(70)-100-200-500
	S16R	0-50-DCM(90)-200-500
	S17R	2764
	S19R	0-50-DCM(?)-200-500

	S20R	0-50-DCM(65)-100
Corsica-Liguria	913	0-50-DCM(80)-200
	906	0-DCM(40)-100-200-500-int1-2535
	908	0-DCM(?)-100-200-500-int1-2640
	912	0-50-DCM(76)-200-500
	901	0-50-DCM(85)-200-500
	900	0-50-DCM(85)-200
	904	2450
Case study	51	3471

Microbial heterotrophic Carbon production

Secondary production was performed in both 0-500 m and deep samples. For all the analysis, water was collected with a 200 µm mesh prefiltrations and put into 1 liters dark bottles. For each prefiltered water sample at each depth, 1.7 mL of water (in triplicate) was put into a sterile 2 mL eppendorf and incubated by adding standardize concentration of aminoacids. A 1.7 ml water aliquot was also used as blank, and 90 µl of Trichloroacetic acid TCA (final concentration 5%) was added. Each eppendorf was then vortexed and incubated at *in situ* temperature for one hour in the dark. After the incubation, 90 µl of TCA was added to the sample tubes. Samples were then stored at 4°C in the dark.

Microbial respiration

Samples for microbial respiration were collected at five water depths between 0 and 500 m and in selected deep stations. For each water depth, 10 liters of prefiltered water was collected and filtered through a 47 mm diameter GF/F filter. After filtration, the filters were put into sterile 2 mL cryovials and stored in liquid nitrogen.

Nanoplankton abundance and biomass

Samples for Nanoplankton abundance and biomass were collected in all the biological, deep and case studies stations (0-500 mt and deep). One liter of prefiltered water was collected for each depth. An aliquot of 240 mL of water was put into a 250 mL dark bottle and fixed with 10 mL of 25% glutaraldehyde. Bottles were then stored at 4°C.

We collected phytoplankton and pigment samples during the survey. The first leg is made by Exp. Biologist Janset KANKUS and second leg made by Marine Biologist Vahit ALAN. The second leg was started 18 August and finished 30 August. Also, during the second leg we collected phytoplankton and pigment samples from 13 stations. The stations are D2, D7, S2, S8, S16, S19, S20, 913, 906, 908, 912, 901 and 900. Also, usually 4 phytoplankton samples stored from different depth (200 m, 100 m or 50 m, DCM, and surface water) for each depth. 5 pigments samples was filtered from (500 m, 200 m, 100 m or 50 m, DCM and surface water) for each depth.

About stations;
D2; The station was completed. Cryo numbers from 69 to 73.
D7; The station was completed. Cryo numbers from 74 to 78.
S2; The station was completed. Cryo numbers from 79 to 83.
S8; The station was completed. Cryo numbers from 84 to 88.
S16; The station was complete. Cryo numbers from 89 to 93.
S19; The station was completed. Cryo numbers from 94 to 98.
S20; The station was completed. Cryo numbers from 99 to 101.
913; The station was completed without 50 m because of the France Coast Guards. Cryo numbers from 102 to 105 (cryo 104 did not stored).
906; The station was completed. Cryo numbers from 106 to 110.
908; The station was completed. Cryo numbers from 111 to 115.
912; The station was completed. Cryo numbers from 116 to 120.
901; The station was completed. Cryo numbers from 121 to 125.
900; The station was completed without 50 m layer phytoplankton sample because of sample bottles was finished. Cryo numbers from 126 to 129.

Totally, 128 pigment samples and 100 phytoplankton samples are collected by us.

By, Vahit ALAN

VENUS3 OCEAN CERTAIN
Cruise Report Leg 1: CFCs

Group description

The CFC group is collecting ocean water samples for the purpose of chlorofluorocarbon (CFC) analysis. CFCs are human-made chemical tracers in the ocean water. They are used to track ocean currents, date water masses and measure anthropogenic carbon uptake by the ocean. This is important for understanding the global carbon cycle and tracking where the emitted carbon in the atmosphere ends up.
The CFC group is represented by Lilo Henke from the University of Exeter, UK.

Method

At each station listed below, 500ml water was collected at each depth in glass bottles. It is important that the CFC samples are the first to be collected from the niskins to prevent contamination by the atmospheric air. The bottle caps were then sealed with two types of water resistant insulating tape and stored upside down in a Ziploc bag containing some of the sample water. The sealed bottles are stored at 4°C. Due to lack of space on the vessel analysis cannot be done on board.

Stations

TRANSECT	STATION	DEPTH	No. SAMPLES
Palma-Algieri	d2	1239	8
Palma-Algieri	d5	2709	14
Palma-Algieri	d7	2804	14
N/A	M9	2722	7
Corsica Channel	109	367	8
Corsica Channel	108	447	9
Ventimiglia-Calvi	906	2535	11
Ventimiglia-Calvi	908	2642	14
Ventimiglia-Calvi	910	2332	14
Ventimiglia-Calvi	903	2362	16
N/A	51	3471	11
TOTAL	11		126

Investigating the formation of H_2O_2 in seawater below the mixed layer (part 2).

Continuation of work conducted on leg 1

H_2O_2 depth profiles have been completed at all deep (>2000 m) stations in order to ensure that data is available at all stations where bacterial abundance and respiration data will also be available. Additional data points were also collected in surface waters at varying times of day, to determine the balance between solar and bacterial sources of peroxide in the surface ocean, and (whenever spare Niskin bottles were available) using triplicate Niskin bottles to assess the reproducibility of measurements. These independent triplicate measurements will also contribute to an assessment of any artefacts in the method used for peroxide analysis caused by, for example, contaminants on the Niskin bottle rosette.

Methods

H_2O_2 concentrations were determined using luminol chemiluminescence on a custom made flow injection system. Water for analysis was taken straight from Niskin bottles to the laboratory and analysed within one hour of collection to produce realistic depth profiles with no significant artefacts from oxidation of the peroxide present in situ. Additional samples were collected at intervals throughout the day directly from surface waters using a rinsed LDPE bucket.

Preliminary Results

On the second leg, H_2O_2 concentration depth profiles were made at over 20 stations. Additional surface samples were collected at all deep stations and at some additional 'short' CTDs in coastal waters when the ship was moored due to bad weather.

Triplicate measurements from different Niskin bottles were very similar with standard deviations of approximately 1% or less which strongly suggests that the peroxide measurements were not subject to any contamination from the Niskin Rossette. The quality of the data is also supported by the reproducibility of the observed relationship between peroxide and depth at every deep station studied.

The work is conducted as a component of GEOMAR's contribution to Ocean Certain Work Package 2.

Dr Mark Hopwood
Ocean Certain postdoc, GEOMAR
30 August 2015

Acknowledgements

The authors are deeply indebted to the Captain and the crew of the CNR R/V Minervauno for continuous support during the whole measurement phase