PROGRAMME NATIONAL
Les Enveloppes Fluides et l'Environnement

Demande de financement

La demande de financement doit parvenir **par courrier électronique à : pascale.ebner@cnrs-dir.fr**.
L'envoyer en format DOC, en document attaché, nommé, avec les informations minimum suivantes : LEFE-ACTION-nom du responsable scientifique
N.B. Un exemplaire (imprimé recto-verso, simplement agrafé) signé par le directeur de laboratoire doit parvenir par courrier postal à Pascale Ebner - INSU - BP 287-16 - 75766 Paris cedex 16

ACTION (S) CONCERNÉE(S) (cocher la ou les cases dont relève le projet)
☐ Chimie Atmosphérique (CHAT)
☐ Evolution et variabilité du climat à échelle globale (EVE)
x Cycles biogéochimiques, Environnement et Ressources (CYBER)
☐ Interactions et Dynamique de l'Océan et de l'Atmosphère (IDAO)
☐ Assimilation de données

Ce projet est-il aussi soumis au Programme National de Télédétection Spatiale (PNTS) :
☐ Oui - x Non

Ce projet est-il aussi soumis à d'autres programmes nationaux ?
Si oui, indiquez lesquels (PNTS, ECCO, TOSCA…) :

Statut du Projet (nouveau, déjà engagé) : première soumission en 2006

BOUM Project
Biogeochemistry from the **O**ligotrophic to the **U**ltra-oligotrophic **M**editerranean

Scientist responsible for the project:

MOUTIN Thierry, Maître de Conférences

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ABSTRACT

Project summary, expected results, time schedule, detailed budget, equipment,

CYBER label request,

Scientists involved in the project

Pages

Project summary, expected results, time schedule:

Project summary:

The functioning and productivity of oligotrophic systems, and the balance between production and mineralization in these areas, is still the subject of much debate. Once thought to be biological deserts, recent research has shown that these systems could largely contribute to the total oceanic organic carbon export. It is important to thoroughly understand these vast ecosystems in order to characterise them and predict any modifications that could occur due to environmental changes. This project aims to give a longitudinal description of the biogeochemistry and biological diversity of the Mediterranean Sea and produce a detailed study of the biological production and its subsequent fate in 3 sites. These distinct ecosystems represent, on a regional scale, the main oceanographic features found in contrasting environments in the oligotrophic ocean as a whole. We will determine whether organic matter production, re-mineralization and export processes are different at these sites, and if so, determine how these differences are related to the structure of the planktonic community. Comparing different sites, along a longitudinal gradient of differing nutrient availability, should provide us with a new insight for identifying and understanding the fundamental interactions between marine biogeochemistry and ecosystems. We will focus on several current issues of interest regarding the coupling/uncoupling between carbon and nutrient (N,P,Si,Fe) delivery and removal processes in the surface layer. These processes control the planktonic community structure and function and ultimately, the ability of the ocean to biologically sequester carbon. We shall describe the functioning of each distinct ecosystem under physical conditions as stationary as possible, and finally, using experimental and modelling approaches, examine the response of each system to perturbations, i.e. pulsed nutrient additions.

Expected results

BOUM project is organized on three main objectives involving collaboration between physicists, biologists and biogeochemists owing specialities from marine optics to modelisation. The final goal is to obtain a better representation of the interactions between planktonic organisms and the cycle of biogenic elements (C, N P, Si, Fe), considering scales from single process to the whole Mediterranean Sea. The main objectives of this research are as follows:

1) To give a longitudinal description of the biogeochemistry and biological diversity of the Mediterranean Sea during the strongest stratified period.

The longitudinal description of short duration (few hours) stations from Gibraltar to the Eastern Mediterranean will provide a physical and biogeochemical data set which will be implemented into the CYBER and SESAME (Southern European Seas: Assessing and Modelling Ecosystems changes) data bases.

This will provide an accurate estimate of nutrient availability during pronounced oligotrophic conditions, enabling the quantification of mineral, organic, dissolved and particulate pools of biogenic elements over the whole water column and the penetration of anthropogenic CO_2. Such data will be used to validate multi-element biogeochemical models for the Mediterranean. A detailed description of the planktonic communities will be assessed by investigating biological diversity (using classical taxonomic tools and molecular biology). In this way, the key roles of some of the functional groups in the biogeochemical cycles, such as nitrogen fixing organisms, will be acknowledged.

2) To study in 3 contrasting oligotrophic environments, the production and fate of organic matter with particular emphasis on the processes which drive the divergence of the stoichiometric ratios of the biogenic elements in the organic matter found in the surrounding water and exported materials

High frequency acquisition of physical, optical, biogeochemical and biological variables will be assessed over long duration (5 day) stations that represent contrasting oligotrophic conditions. This sampling strategy will make it possible to identify the impact of small scale dynamics on primary productivity, by providing new estimates for the vertical diffusion coefficients. The importance of nitrogen fixation in new nitrogen input and the control of this flux by phosphate/iron availability will be assessed (*in situ* and mesocosm studies). We will study primary and secondary production as well as mineralisation and export of organic matter by measuring biogenic element (C,N,P,Si) fluxes and focusing on the major species responsible for these fluxes. Finally, physiological characteristics of microorganisms will be studied at the scale of species or group of species that play key roles in biogenic element fluxes. The results should provide a better understanding of the fundamental interactions between biogeochemical cycles and the structure of the planktonic food web in oligotrophic marine environments.

3) To obtain a satisfactory representation of the main biogeochemical fluxes (C, N, P, Si) and the dynamics of the planktonic trophic network, both *in situ* and by using microcosm experiments

The data obtained during the BOUM program will be rapidly available to the international scientific community. A multi-scale modelling approach is proposed for the initial phase of the project. Firstly, considering the Mediterranean scale as a whole, and in relation to the EU SESAME project, the data will be used to validate biogeochemical models (circulation-ecosystems models), whose overall objectives are to predict the impact of environmental climate changes on the Mediterranean marine ecosystems. Modelling on this scale will also make it possible to evaluate the distribution of anthropogenic CO_2. Secondly modelling on a finer scale is expected to give a better representation of certain processes (diazotrophy, phytoplankton – bacteria competition for a limiting nutrient, multi-limitation) in the biogeochemical model ECO3M. This model is multi-element and involves functional groups. The objective is to obtain a better representation of the carbon fluxes and associated biogenic elements in the surface layer of oligotrophic marine environments.

Calendar:
September 2007: Pre-cruise meeting (Marseille)
Mars 2008: Pre-cruise meeting of the enlarge steering comitee (Marseille)
August-September 2008: Cruise (detailed description in Annexe 2)
May-June 2009: Post-cruise meeting of the whole BOUM community (Marseille)
Oct-Nov 2009: workshop (10 people max)
Feb-March 2010: workshop (10 personnes max)
June-July 2010: workshop (10 personnes max)
2010: request for a special session at the OSM

Key words:
Biogeochemical cycles, Oligotrophy, Primary production, Mediterranean Sea

Project duration: 4 years.

Detailed budget required from the LEFE program and other funding (€ HT)**:**

<table>
<tr><th></th><th>Year 2007</th><th>Year 2008</th><th>Year 2009</th><th>Year 2010</th></tr>
<tr><td colspan="5">LEFE</td></tr>
<tr><td>Functionning</td><td></td><td>6 000</td><td>10 000</td><td>15 000</td></tr>
<tr><td>Travel</td><td>17 000</td><td>4 000*</td><td>21 000</td><td>8 000</td></tr>
<tr><td>Analyses</td><td></td><td>99 000</td><td>10 000</td><td></td></tr>
<tr><td>low cost equipement</td><td>10 500</td><td>12 200</td><td></td><td></td></tr>
<tr><td>high cost Equipment</td><td>62 920</td><td></td><td></td><td></td></tr>
<tr><td>Total LEFE</td><td>90 420</td><td>113 050</td><td>19 000</td><td>23 000</td></tr>
<tr><td colspan="5">Others fundings</td></tr>
<tr><td>European project
SESAME</td><td colspan="2">146 600 (obtained)
<i>120 000 consumable</i>
<i>26 600 travel and other costs</i></td><td colspan="2">20 000 (obained)
<i>20 000 travel and other costs</i></td></tr>
<tr><td>PAI AURORA</td><td>4 360
(requested)</td><td>4 140
(requested)</td><td></td><td></td></tr>
<tr><td>ANR**</td><td></td><td>4 260
(obtained)</td><td></td><td></td></tr>
<tr><td>PNTS</td><td></td><td></td><td></td><td></td></tr>
<tr><td>CARBOCEAN</td><td></td><td>4 000
(obtained)
<i>(consumable)</i></td><td>1 000
(obtained)
<i>(consumable)</i></td><td></td></tr>
<tr><td>Total other fundings</td><td colspan="2">154 260</td><td>21 000</td><td></td></tr>
</table>

* specific request for cruise travels (see detail p. 31)

** ANR Internal waves for the VMP5500 acquisition, ANR BOOM for the study of coccolithophores (equipment and analyses), ANR POTES for hyper bar measurements (equipment and analyses). Not detailed but aquired.

National Means required :
Research Vessel « Pourquoi Pas ? » or « l'Atalante »

High cost equipment required (annex 4):
See details in paragraph 5.5.

Long term observation services from which the project will utilise data:
In the thesis proposals of P. Raimbault and I. Biégala, some sampling is scheduled for the SOMLIT (Marseille) and DYFAMED sites. The BOUM cruise is part of the european program SESAME (WP1) which will gather all the available mediterranean data, particularly those provided by the french observatories.

PhD proposals for CYBER:

The CYBER label is required for 7 PhD proposals and one post doctoral project.

PhD proposals

- Impact of the small scale dynamics on the productivity in oligotrophic areas: data analysis from the BOUM cruise. Supervisors: P. Bouruet-Aubertot (LOCEAN) & L. Prieur (LOV)

- Quantification of the nitrogen fixation in the Mediterranean Sea – importance in new production evaluation and in the nitrogen biogeochemical budget. Supervisor: P. Raimbault

- Diversity and distribution of pico and nanoplanktonic diazotrophs in the Mediterranean Sea using molecular tools. Supervisor: I. Biegala (IRD)

- Competition between autotrophs and heterotrophs for phosphate in the Mediterranean Sea and the consequences on the biogeochemical carbon cycle, BOUM cruise. Supervisors: T. Moutin (LOB), P. Lebaron (LOBB)

- Modelling the coupling/decoupling between carbon and associated biogenic element cycles in the photic zone of contrasting oligotrophic sites in the Mediterranean Sea, BOUM cruise. Supervisors: M. Baklouti (LOB), F. Diaz (LOB)

- Metabolic balance of oligotrophic oceanic areas

- Co-limitation C-P: Control and regulation of degrading activities of heterotrophic prokaryotes in oligotrophic oceans in relation to the functional structure of communities. Supervisor: F. Van Wambeke (LMGEM)

post doctoral proposal

- Study of the biogeochemical factors controlling N_2 fixation in the Mediterranean Sea – Impact of athmospheric inputs on N_2 fixation.

Scientists involved in the project

NOM	LABORATOIRE	FONCTION	Participation au projet		% Participation à d'autres programmes (INSU, EUROPE, ANR)
			%	Expertise	
BAKLOUTI	LOB	CR Modelisation	35		LATEX 15
BIEGALA / Doctorant BM fixation N_2	IRD	CR Biol. (N_2 fixing organisms)	25		ANR BOA 20 EC2CO NC 30
CARLOTTI	LOB	DR Biol. (Macro-zooplancton)	20		
BOURUET-AUBERTOT	LOCEAN	MCF Phys. (Micro-turbulence)	20		ANR ONDES INT 40 TWIST 30
CATALA	LOBB	AI Biol. Cytometry (Pico Het)	25		MEDEA 20 ANR BIODIV 20
CHRISTAKI	ELICO	Pr Biol. (Micro-zooplancton)	20		BIOPROPHI 10 CPER 10
COURTIES	LOBB	IR Cytometry (Pico aut.)	20		MEDINA 20
DEVENON	IFREMER	Pr Phys.	5		
DIAZ	LOB	MCF Modelisation	20		BIOPRHOFI 20 LATEX 40 GOLPHYZ 20
DOLAN	LOV	DR Hervivorous Biodiv.	20		ANR 70
FUDA / ROUGIER	COM/LOB	Phys.-Instrument	20		
GARCZAREK	SB-Roscoff	CR Biol. Cyanobacteria	25		ANR 5 MARINE GENOMICS 25
GOYET	Univ-Perpignan	Pr Chemie CO2 anthropogenic.	20		CARBOCEAN 30
GOUTX	LMGEM	DR Biol. (Lipids)	10		
GUIEU / BLAIN	LOV/LOB	CR/Pr. Biogéoch. Iron	25		ANR BOA 20 DUNE 40 CEL PIEGE 5
DE VARGAS	SB-Roscoff	CR Biol. Coccolithophores	25		
GUIGUE	LMGEM	AI Biogéoch. OM	20		
GUILLOU	*SB-Roscoff*	*CR Biol. Picoplankton*	*20*		ANR 20 MELINA 10
JEANTHON	SB-Roscoff	Dr. Biol. Bacteria AAnP	25		MALINA 15 EC2CO 40
LEBARON	LOBB	Pr Biol. Heterotrophes	5		MEDEA 5 MEDINA 10 ANR BIODIV 25
LEBLANC	LOB	CR Biogéoch. Si cycle	25		CASOAR 25
LEBLOND	LOV	IE Biogéoch. sed. trapp	30		
LEFEVRE/Doctorant bilan O_2/CO_2	LMGEM	CR Biogeoch. $\Delta O_2/\Delta CO_2$	20		ANR POTES 20 POMME 20 BALTEX 10 PECHE 20 KEOPS 5
LEGENDRE	LOV	Pr Biogeoch. C cycle	5		
LOISEL	ELICO	MCF Marine optics	20		
ORIOL	LOBB	AI Biogeoch. Nutrients	20		MEDEA (CYBER) 20%, RIOMAR (EC2CO) 20%, Plumland (LEFE) 8%, Drake (clivar) 10%
MAREC	INSU	IE Instrumentation	20		
MOUTIN	LOB	MCF Biogeoch. C,P cycles	80		
OBERNOSTERER	LOBB	CR Biogeoch. Bacteria	5		MEDEA 60 ANR BIODIV 25

PICHERAL / GORSKY	LOV	IE Biogeoch. PVM	25		
PRIEUR	LOV	DR Phys.	25		
PUJO PAY	LOBB	CR Biogeoch. (OM, Nutrients)	25		MEDEA (CYBER) 15%, RIOMAR (EC2CO) 10%, Plumland (LEFE) 15%, Drake (clivar) 10%
QUEGUINER	LOB	Pr Biogeoch. C,Si Cycles	25		
RAIMBAULT	LOB	Dr Biogeoch. C,N Cycles	25		
RAS	LOV	IE Pigments HPLC	10		
Post Doctorant / RIDAME	LOCEAN-Paris	MCF Biogeoch. N_2 Fixation	40		
RIMMELIN/BARTHAUX	LOB/LOB	IR/IE SN (nano methods)	25		
ROY BARMAN	LSCE-Paris	CR Biogeoch. Geoch. Tracers			
TAMBURINI	LMGEM	CR Biogeoch. Deep min.	20		
TANAKA	LOB	CR Biogéoch. Micro-zoo P	30		PAME IPY 20
THIBAULT-BOTHA / STEEMANN	LOB/LOV	MCF/Macro-zoo (process)	25		
VAN WAMBEKE	LMGEM	Dr Biogeoch. Bact., C-P cycles	50		PECHE 15 BIOSOPE 10
VAULOT	SB-Roscoff	DR Biol. Picoplankton	5		
WEINBAUER	LOV	CR Biol. Virus	20		15
Collaborations internationales					
BERHENFELD	Etats-Unis	Searcher Marine optics	25		
LEWIS	Etats-Unis	Searcher Phys. et Marine opt.	5		
MARANON	Espagne	Searcher Biogeoch. DOC	25		
NEDOMA	Tchéquie		25		
PSARRA	Grèce	Searcher Biogeoch. Prim. P.	30		
THINGSTAD	Norvège	Pr Biogeoch. & Modelisation	10		
VAN MOOY	Etats-Unis	Searcher Biogeoch. P Cycle	25		
Doctorant (Physique turbulence)	LOCEAN/LOV	Phys. Micro-scale	100		
Doctorant (Production cycles N-C)	LOB	Biogeoch.	50		
Doctorant (Production cycles P-C)	LOB/LOBB	Biogeoch..	100		
Doctorant (Modélisation)	LOB	Biogeoch..	100		
Doctorant (Minéralisation)	LMGEM	Biogeoch..	100		
		TOTAL full time equivalent	15.5		
		including international collaborations	1.5		

in blue, non embarquing scientists

Total full time equivalent per year: 2007 (7.7), 2008 (15.5), 2009 (7.7), 2010 (5.2)

SCIENTIFIC PROPOSAL

1. Scientific interest:

General context:

Introduction:
Inputs, losses, dynamics and the chemical forms of macro- and micronutrients determine nutrient availability and influence both autotrophic and heterotrophic organisms in the ocean (Bruland et al., 2001). Metabolic processes, population and community dynamics and food web structures are all strongly dependent on nutrient availability. For example, a nutrient may be required for the functioning of a specific enzyme and/or metabolic pathway and thus exert considerable control on the species composition of marine communities. The marine osmotrophs (Karp-Boss et al., 1996) have specific characteristics enabling them to take up nutrients from the surrounding water and thus the concentrations in nutrients will strongly influence the composition of the species. This is particularly important where nutrient concentrations are low compared to the needs of the organisms especially in oligotrophic waters. Despite the complexity of the planktonic foodweb in the pelagic ecosystem, the ultimate constraints for autotrophic primary production are the abiotic factors: light and inorganic nutrients (De Baar, 1994). Indisputably, light variation is an important factor controlling the growth rate of algae. Light conditions at the sea surface may be approximated with simple calculations (Kirk, 1994), and changes in light conditions with depth may be seen as a consequence of biological activity in the open ocean. Assessing nutrient availability appears to be a key factor in understanding and eventually predicting the biological response (in terms of trophic structure and diversity, and/or in terms of carbon fluxes) in the upper photic zone (Moutin & Raimbault, 2002).

Reciprocally, planktonic organisms modify nutrient availability. Organisms can exhaust nutrients or introduce new nutrients. For example, the N_2-fixing organisms di-azotrophes, quantitatively introduce “new” nitrogen (Capone, 2001) which could account for 50% of the total allochtonous nitrogen input into the ocean (Gruber and Sarmiento, 1997). Organisms can also produce organic ligands able to alter nutrient availability. Most of the iron in the iron-depleted environment is taken up in the organic form (Achilles et al., 2003). Organisms are responsible for the major nutrient fluxes in oligotrophic surface waters through organic matter recycling processes, remineralisation, excretion and sloppy feeding (Jumars et al. 1989). The continuous transfer and transformation from inorganic to organic substrates and back again, explains why biological processes drive almost all biogeochemical cycles. A better understanding of marine life requires the simultaneous investigation of biogeochemistry and marine food web structures. Whilst our understanding of the links between biological, physical and chemical factors that influence nutrient uptake and remineralisation in the ocean, is improving, it is not sufficient for producing realistic predictive models. A holistic approach on the impact of macro- and micronutrients on food web structure and function in different ocean regimes is therefore necessary (IMBER, 2005).

Understanding the transformation of organic matter in marine food webs is also essential for assessing the global carbon cycle and subsequently evaluating the impact of the anthropogenic CO_2 input on climate change. Assessing the export of carbon from the photic zone to the deep layer is central in the debate on the oceans ability to absorb the anthropogenic CO_2 excess (Longhurst, 1991). Oceanic oligotrophic areas represent more than 50 % of the global ocean and about 40 % of the total oceanic production (Antoine et al., 1996). However, the functioning and productivity of oligotrophic systems and particularly the balance between production and mineralization in these areas, is still the subject of much debate (Karl et al., 2003; Williams et al., in press). The role of oligotrophic areas in overall export is probably not very important, as the greatest proportion of photosynthesised carbon is recycled in the surface layer and rapidly re-exchanged with the atmosphere (Fig. 1). Nevertheless, recycling may lead to the accumulation of dissolved organic carbon (DOC) (Thingstad et al., 1997) and needs to be considered since DOC accumulation and export in oligotrophic areas, is potentially more important than transport via sinking particles (Copin-Montegut & Avril, 1993; Carlson *et al.*, 1994; Avril, 2002). In the context of global warming, an increase in stratification has been predicted and thus the role of DOC in carbon export may change.

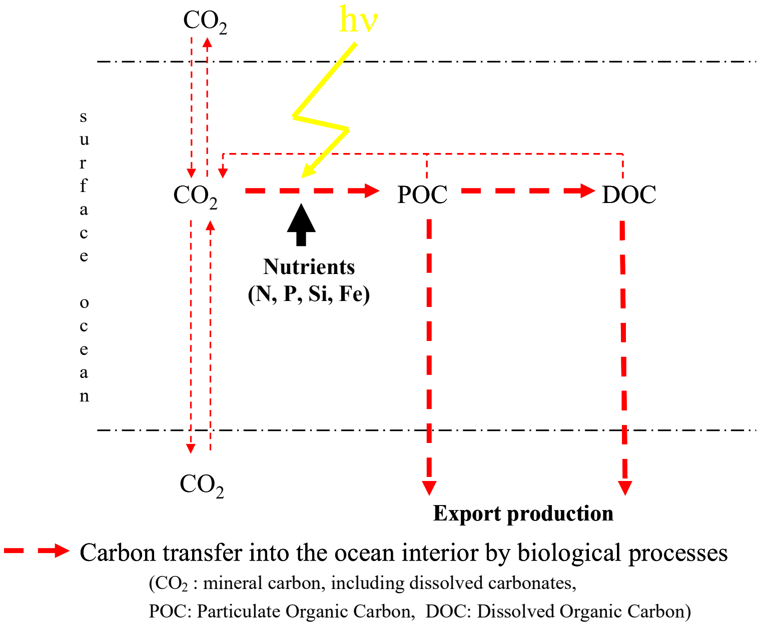

Fig. 1. Schematic representation of the major carbon fluxes in the photic zone of the ocean and the nutrients control of primary production (Moutin, 2000).

The Mediterranean Sea and the oligotrophic ocean (an overview of previous cruises):

Oligotrophic marine areas are characterized by a more or less, pronounced thermal stratification of the water column, which delimits (1) a warm surface mixed layer with high light intensity but depleted in nutrients and (2) a sub-superficial layer with low light levels and more nutrients. Tropical areas, as well as large anticyclonic gyres, the Sargasso Sea and the Mediterranean Sea, have long been considered as typical oligotrophic systems (Herbland & Voituriez, 1977). The depth where nitrate concentration approaches zero is around 10 m in the Alboran Sea, during the stratified period, and can reach more than 150 m in the Levantine basin of the Mediterranean Sea (Fig. 2). This is related to hydrological conditions and to, two major external sources of nutrients, the Rhône river input and the entry of the nutrient rich Atlantic surface waters, in the Western part of the Mediterranean Sea. The great depth of the Levantine Basin nitracline is only found elsewhere in ultra-oligotrophic conditions, for example, in the centre of the South Pacific gyre. It can be said that the Mediterranean Sea presents, on a regional scale, the main oceanographic features of contrasting environments in the oligotrophic ocean.

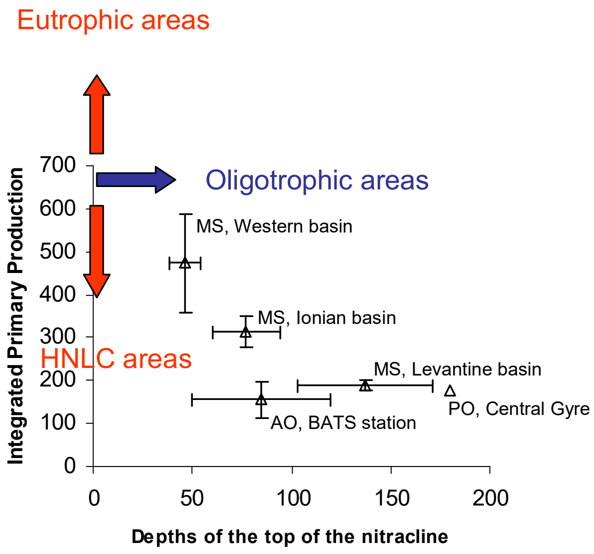

Fig. 2. Integrated Primary Production vs depth of the top of the nitracline.
MS = Mediterranean Sea: data from Moutin & Raimbault (2002), MINOS cruise (Western basin : 7 stations, Ionian basin: 8 stations, Levantin basin: 4 stations).
PO = Pacific Ocean: Unpublished data from the central station in the Gyre during the BIOSOPE cruise.
AO = Atlantic Ocean: data from Steinberg et al. (2001) at BATS station.

The distribution of primary production, particulate carbon export, from the photic zone to deeper layer and nutrient concentrations were investigated in the first trans-Mediterranean cruise during May-June 1996 (**MINOS** cruise : *Mediterranean INvestigation of Oligotrophic Systems*). A decrease in integrated primary production, particulate carbon export and nutrient availability towards the eastern side of the Mediterranean Sea was observed, while integrated chlorophyll *a* remained constant. Integrated primary production reached 150 mgC m^{-2} d^{-1} in the Levantine basin, a value considered as a limit for primary production rates under strong oligotrophic conditions (Moutin & Raimbault, 2002).

It has long been suspected that photosynthetic production is limited by phosphate availability, in the Mediterranean Sea. Bioassays have shown that phosphate enrichments stimulate photosynthesis (Berland et al., 1980; Diaz et al. 2001). However, not only primary production, but also bacterial production may be controlled by phosphate availability. During the **PROSOPE (PROductivité des Systèmes Océaniques PElagiques)** and **TMC (Trans Mediterranean Cruise)** of September and June 1999, it was demonstrated that phosphate limitation on bacterial production, already observed in several locations (Thingstad 1998; Zohary and Robarts, 1998), was a general feature of the western and eastern Mediterranean Sea (Van Wambeke et al, 2002). Dissolved inorganic phosphate concentrations in the upper photic zone were shown to decrease from west to east reaching levels well below 1 nM (Moutin et al, 2002). *Synechococcus spp.*, the most abundant phytoplankton in surface waters of the Mediterranean Sea during summer (Vaulot et al. 1996), were shown to have specific advantages concerning dissolved inorganic phosphate uptake that may explain their abundance in P depleted environments (Moutin et al, 2002). Adaptation to P limitation has also been demonstrated among higher trophic levels. For instance mixotrophic nanoflagellates obtain organic phosphate from their bacterial ingested prey using phagotrophy, stopping as soon as inorganic phosphate becomes available (Christaki et al. 1999). The study of tintinnid during the **PROSOPE** cruise showed that the diversity of this group reflects resource diversity rather than competitive interactions or predation (Dolan et al., 2002). Recent work has shown an increase in copepod egg abundance following phosphate addition to surface waters in the eastern MS (**CYCLOPS**), implying that they may be coupled to lower trophic levels through interactions which are not usually taken into account (Thingstad et al., 2005). **These mechanisms are not fundamentally new, however there are very few studies particularily concerning the**

consequences for the oceanic carbon cycle when there is a possible short cut of primary production between nutrients and exported carbon. In the past, research on marine food webs tended to focus on either, the phytoplankton and microbial food web, or on zooplankton, fish and top predators. Marine food webs must be considered as integrated systems because perturbations at any point in these systems can propagate both up and down through the trophic levels.

Nutrient limitation of organic production has been largely studied in the MS and **although there is a consensus on the major control exerted by phosphate availability, nitrogen is scarce as well and the availability of silicic acid may play a central role in controlling the export of production** (Leblanc et al., 2003). Biological diversity may reflect multiple organic production limitations, thus a multi-element approach is necessary to increase our understanding of marine food webs. Nutrient dynamics and its role in the variability of the stoechiometry of organic matter pools will be the central aspect of our biogeochemical study.

There is abundant evidence of the uncoupling between nitrogen and phosphate cycles in the MS. (1) The Nitrate:Phosphate ratio is higher in the deep Mediterranean waters than in other oceans (2) The highest Nitrate:Phosphate ratios, which are higher than the Redfield ratio, are found in the sub-surface waters of both the western (Mc Gill, 1961, Mc Gill,1965, Raimbault and Coste, 1990) and the eastern Mediterranean (Krom *et al*. 1991, Moutin & Raimbault, 2002). (3) The high N:P ratios in the particulate fraction (Krom et al., 2005). (4) The phosphacline is deeper than the nitracline in the eastern part of the MS (Moutin & Raimbault, 2002). **At the present time, there is no definitive explanation for this particular feature of the MS, also observed in the Sargasso Sea**. Two largely different processes have been proposed to explain the typical NO_3:PO_4 ratios observed in deep waters. Firstly, there is the biological process of nitrogen (N_2) fixation (Bethoux & Copin-Montegut, 1986; Bonin et al., 1989; Sachs & Repeta, 1999, Kerhervé et al., 2001; Pantoja et al., 2002) which may lead to nitrogen accumulation in deep waters, and secondly, chemical processes such as phosphate adsorption onto iron rich particles which lead to further P depletion in the Mediterranean Sea (Krom *et al*., 1991). As phosphate removal by adsorption from the water column did not represent a significant sink for phosphate in the MS (Herut et al., 1999; Ridame et al. 2003), **nitrogen fixation appears to be the key factor in explaining the high NO_3:PO_4 ratios. Never-the-less very few measurements are available.** Nitrogen fixation rates have been measured recently at the **DYFAMED** station in the western Mediterranean (**MELISSA** program). Whilst these rates were typically low, this biological process supplies significant new nitrogen which can balance the nitrogen biogeochemical budget and explain the high nitrate/phosphate ratio in deep waters (Garcia et al., 2006). Indeed, the role of N_2 fixation in the marine nitrogen cycle has been undergoing increasing scrutiny and re-evaluation over the last decade, leading to increased estimates of its role in supporting oceanic new production (Karl et al., 2002). The discovery of marine diazotrophs, other than *Trichodesmium* spp. (Zehr et al., 2001; Montoya et al. 2004), has given a new dimension to the significance of nitrogen fixation in the ocean. **If significant amounts of new nitrogen are introduced by small organisms, previously thought to recycle nitrogen, our conception of the functioning of oligotrophic systems needs to be revised** (Garcia et al., en révision). δ^{15}N data from fossilised chlorophyll (**MINOS** cruise) provides geochemical evidence for extensive nitrogen fixation in the eastern Mediterranean (Sachs & Repeta, 1999). Thus, it becomes of great interest to describe and quantify the nitrogen input by nitrogen fixation as well as understanding the organisms responsible for this biogeochemical function.

As the quantity of dissolved atmospheric nitrogen is inexhaustable, it is important to understand the control of N_2 fixation. It seems that phosphate or iron availabilities are key factors in controlling these fluxes on a global ocean scale (Falkowski 1997, Karl et al. 2002). If nitrogen availability by nitrogen fixation is important in the Mediterranean where low phosphate availability is thought to be the key factor controlling this flux, then the control of new production, initially defined as the fraction of production associated with new nutrients (generally nitrate), should be defined starting from new phosphate (Dugdale and Goering, 1967). **It is important to further our understanding of the phosphate cycle in surface waters, which is sparsely studied**, **(Benitez-Nelson, 2000 ; Karl, ASLO meeting 2006) in order to improve our understanding of oceanic production**

particularly in oligotrophic areas. The chemical element phosphorous ^{31}P only exists in the water in the form of phosphate, organic or mineral, particulate or dissolved, and is non reducable under natural conditions. Thus the many complex reactions of oxydoreduction found in the nitrogen cycles are not found in the phosphate cycle (Moutin, 2000), so it is possible to envisage coupling with production and the establishment of a budget from a different angle.

The Mediterranean Sea has a wide range of oligotrophic conditions suitable for studying the transformation of organic matter in marine food webs during low new nutrient availability and provides a case study for observing the links between the C, N, P, Si and Fe-cycles. Comparisons between different systems along a longitudinal gradient of trophic status will provide new insights for identifying and understanding fundamental interactions between marine biogeochemistry and ecosystems.

General objectives: (A x.x, supplement in Annex 3)

OBJECTIVE 1: DESCRIPTION OF THE BIOGEOCHEMICAL AND BIOLOGICAL DIVERSITY IN THE MEDITERRANEAN SEA

The main objective of the BOUM cruise is to give a description of the biogeochemical parameters and biological diversity along two gradients (1) North - South from the mouth of the Rhone to the centre of the Western basin and (2) West-east from Gibraltar to the coast of Syria, during a marked period of stratification.

Objective 1.1: Biogeochemical description

The classic variables temperature, salinity, dissolved oxygen concentration, along with alkalinity, pigments, organic and mineral carbon and other biogenic elements (C, N, P, Si) pools, will be measured as well as other specific variables at selected depths (nitrogen fixation, diazotroph diversity, recycling times for dissolved phosphate...). The aim is to describe the variations in the biogeochemical characteristics (nutrient availability in the photic layer, depth of the nutricline, ratios of dissolved/particulate and organic/mineral in the biogenic elements pools, drawdown of CO_2 of anthropic origin, phytoplankton biomasses and diversity, nitrogen fixation rates...) starting at the two principal sources of nutritive elements: the entry of Atlantic surface water from Gibraltar and the contributions of the Rhone river. This biogeochemical description will extend to the Levantin basin of the Mediterranean where oligotrophic conditions are the most extreme (not sampled during the PROSOPE cruise of 1999). **Our recently acquired techniques for biogeochemical measurements in ultra-oligotrophic waters should enable us to significantly improve our understanding of the biogeochemistry of the Mediterranean Sea**. We do not have a good insight into the spatial distribution of certain biogeochemical variables, such as nitrogen fixation, or absolute phosphate concentrations in surface waters, which have a predominant role in controlling planktonic production. Recent studies infer the importance of the Mediterranean Sea in the drawdown of anthropogenic carbon (Alvarez et al. 2005, Aït-Ait-Ameur & Goyet, 2005) from data in/off the Gulf of Cadiz**. It is necessary to quantify the drawdown of the anthropogenic carbon in the whole Mediterranean Sea** (**A 1.1**) and to evaluate its transport to the Atlantic Ocean through the Strait of Gibraltar.

Specific question: *What is the actual longitudinal distribution of carbon and nutrients in the Mediterranean Sea?*

Collaboration within the SESAME program

Within the framework of the European program SESAME, 10 Oceanographical Ships (Countries of origin: Bulgaria, Spain, France, Greece, Israel, Italy, Romania, Russia, Tunisia, Turkey) will simultaneously, on a survey study in the Mediterranean Sea and the Black Sea (Fig. 3). A quasi synoptic biogeochemical description of the biogeochemical parameters for all the Mediterranean is anticipated. This description will be compared with previous data and used as the basis for future work observing biogeochemical changes, in particular the nutrient availability in the Mediterranean Sea. It is envisaged that within the framework of WP2, in the SESAME program, that two oceanographical cruises will be conducted, one during March-April 2008 and the other during August-September of the same year. This has the objective of obtaining a physical and biogeochemical "snap-shot" of the oceanographical variables of the Mediterranean and Black Sea over 2 contrasting seasons, the spring bloom period and the period of marked stratification. French participation involves the acquisition of biogeochemical parameters and is envisaged to take place in March-April 2008 during the ARCHIMED cruise whose principal objective is to study contaminants and during the BOUM cruise in August-September 2008, whose objective is to study production and subsequent fate in contrasting oligotrophic environments. These two cruises, the European stage of SESAME, are under the responsibility of Thierry Moutin. Discussions concerning this subject were carried out with Daniel Cossa of the IFREMER in order to establish a common strategy for sampling the Western Mediterranean.

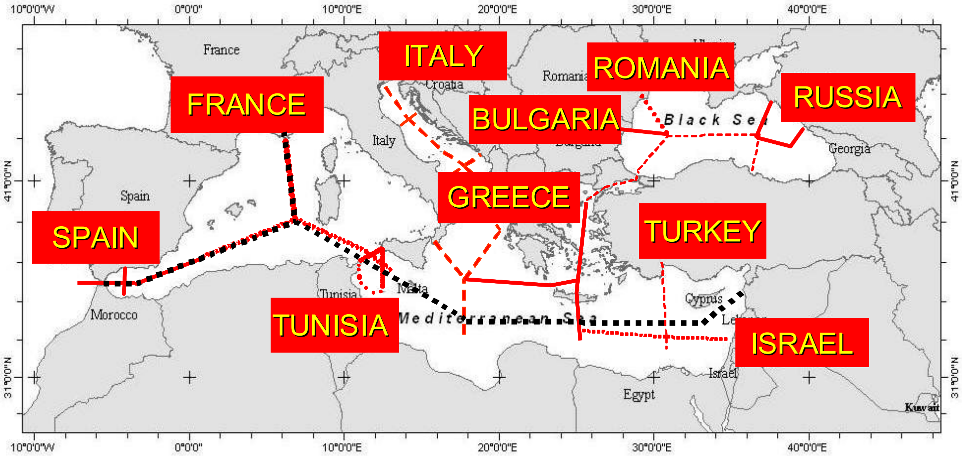

Fig. 3. Map of the transect locations and the countries responsible as set out in the European program SESAME and the approximate routes of the BOUM cruises (in black dotted line).

The priority of each variable was discussed within the SESAME community. It is the result of a compromise between feasibility by the various partners and the specifics of each cruise. Priority one variables are: temperature, salinity, fluorescence, concentration of dissolved oxygen and nutrients (Nitrate, Nitrite, Phosphate and Silicate) and chlorophyll *a* concentrations. Priority two variables are: particulate organic carbon, particulate organic nitrogen and phosphorus, dissolved organic carbon, dissolved organic nitrogen and phosphorus, $pC0_2$, pH and alkalinity, dissolved mineral carbon, bacterial biomass, density and phytoplankton diversity, size fractionated pigments and zooplankton biomass and diversity. 3rd priority variables are bacterial production, primary production, zooplankton and the tracer radioelements of water masses whose analysis is envisaged in some transects in the Eastern Mediterranean Sea.

Objective 1.2. Biological diversity

A major objective will be to produce an overall description of the planktonic community structure in Mediterranean pelagic ecosystems, providing a "functional" structure for specific analysis. A detailed description of the biological diversity is essential as it is hypothesised that biodiversity increases the functional redundancy of marine ecosystems. Such redundancy may play an important role in an ecosystems ability to withstand natural and anthropogenic disturbances (Fonseca and Ganade, 2001). The need to describe a more "operational" functional structure should not replace having a "complete" description of the species composition. Variation in species composition probably remains the most effective tool in identifying natural and / or anthropogenic perturbations.

Special attention will be given to studying the diversity of diazotrophs (**A 1.2**). Recent studies have shown that a large number of pico and nanoplanktonic prokaryotes are able to fix -nitrogen. This function was previously attributed to the *Trichodesmium sp*., a large bloom forming microplanktonic cyanobacteria (Zehr et al. 2001) and to *Richelia*, a heterocystous endosymbiont found in certain species of diatoms (Gomez et al., 2005). Surprisingly, *Trichodesmium sp*. have never been observed in the Mediterranean Sea, where di-nitrogen fixation rates are suspected to be high (Gruber and Sarmiento 1997, Karl et al. 2002). **It is likely that, the recently discovered small sized diazotrophs (Zehr et al. 2001, Montoya et al. 2004) are responsible for this important biochemical activity**. Recent data aquired thanks to the use of molecular tools revealed the existence of new very small diazotrophic cyanobacteria, belonging to the picoplankton size fraction (<3 µm), (Biegala et al, submitted, in prep). Only heterotrophic bacteria were previously thought to exert nitrogen fixation in this size fraction. Although these every small cyanobacteria were discovered in the south west Pacific, il should be interesting to investigate their presence in Mediterranean(**A 1.2**). Nevertheless, the relatively high accumulation of biogenic silica associated with the deep chlorophyll maximum, observed during the PROSOPE cruise in the Ionian Sea (Leblanc, pers. com.), indicates that **we should not dismiss the role of diatoms, associated with Richelia** (**A 1.3**), as possible key players in nitrogen fixation. If predominant, a new link between the nitrogen and silicone biogeochemical cycles must be considered.

Picoplankton (i.e. cells passing through 3 µm filters) are at the base of all marine food webs. They are composed of prokaryotes (*Bacteria and Archaea*) and eukaryotes. Among both groups, organisms can be either autotrophic (e.g. essentially relying on photosynthesis) or heterotrophic (relying on organic material) or possibly both (mixotrophs). The marine cyanobacteria *Prochlorococcus* and *Synechococcus* dominate the prokaryotic photosynthetic biomass particularly in oligotrophic areas of the world's oceans (Partensky et al. 1999a, Partensky et al. 1999b). **The vast distribution of these two genera, suggest that they possess efficient strategies for responding to environmental stresses. However, the fine-tuning of these responses is still largely unknown** (**A 1.4.**). Recent reports suggest that aerobic anoxygenic phototrophic bacteria (AAnPB) are abundant in pelagic marine systems (Kolber *et al*., 2000; 2001), and may be more abundant in oligotrophic areas (preliminary results from the BIOSOPE cruise, in the south pacific gyre, suggest that they can represent up to 20% of the total heterotrophic bacteria: work in progress P. Lebaron). **Given the large potential impact that AAnPB may have on marine carbon cycling and the recent conflicting reports concerning their abundance in the world's ocean (Schwalbach & Furhman, 2005), further work is necessary in order to determine the ecological significance of these photoheterotrophs** (**A 1.5**). Among the autotrophs, eukaryotes often dominate over prokaryotes in terms of biomass and production (Worden et al. 2004). In recent years, molecular methods have revealed a wide diversity among picoeukaryotes, in particular, the heterotrophs. However, **we still have very little information concerning the dominant groups and their ecology in most oceanic waters** (**A 1.6**) with the exception of coastal waters (Not et al. 2004).

Coccolithophores, calcifying haptophytes (Thierstein and Young 2004) are the key organisms responsible for the transfer of materials in oligotrophic areas (de Vargas et al. in press). Their diversity is maximal in these conditions. Their adapted strategies enable them to capture light from all depths, even those below the deep chlorophyll maximum. Their micro-skeleton enables the aggregation of organic particulate matter, which could be the major conveyor of the biological pump (Klaas and Archer 2002).

The residence time of water in the Mediterranean Sea is about a hundred years (Lacombe, 1990) which is less than that for other vast oceans. Thus, the effects of acidification related to the global increase in anthropogenic CO_2 should be observed more quickly in the Mediterranean Sea (C. Lee conference, Marseille 2006). **It is therefore particularly important to study coccolithophore diversity (A 1.7) as their calcification is highly dependant on pH.**

A pilot study in the Bay of Villefranche showed that the abundance of cyanophages increased along with abundance of cyanobacteria (the spring phytoplankton bloom of 2005, unpublished data). This suggests a co-variation in the diversity of the hosts and phages, and could explain certain mechanisms of cyanobacteria control. Despite the importance of this control, the data on viral diversity in oligotrophic areas, in particular in the Mediterranean Sea, are sparse (**A 1.8**).

Diversity in low trophic levels, i.e. primary producers, is often assumed to drive diversity in the higher trophic levels. This means that resource diversity may underly consumer diversity. However, this relationship remains hypothetical as it has rarely been shown. In fact, some have claimed that there is no such relationship in plankton (Irigoien et al. 2004), a claim that has been challenged (Dolan 2005). Among the consumers of phytoplankton, microzooplankton generally have the dominant role. They are composed predominantly of protists capable of growth rates equal to that of their prey. Thus, microzooplankton communities (**A 1.9**) are potentially as dynamic as their phytoplankton prey.

Specific questions: *Which community structures currently characterize the Mediterranean's pelagic ecosystems during oligotrophic conditions at the end of the stratified period? Which dominating species or group is responsible for specific functions (nitrogen fixation, dissolved organic phosphate utilization, bacterial production, silification rates, grazing)?*

OBJECTIVE 2. PRODUCTION AND FATE OF ORGANIC MATTER IN CONTRASTING OLIGOTROPHIC ENVIRONMENTS

The major objective is to study how production, mineralisation and export of organic matter is depending on the community structure in contrasted oligotrophic areas. We will quantify the flow of material (biogenic elements) through each ecosystem and particularly focus on the coupling/uncoupling between carbon and nutrient supply and removal processes.

Objective 2.1. Characterization of nutrient availability

Measurements of nutrient (N, P, Si, Fe) concentrations and/or availabilities

Nutrient availbility in the photic zone controls primary production in the organic matter, and thus exerts a strong influence on the species composition of the food web. It is dependant on several inputs, recycling and export fluxes. Nutrient concentration in the upper layer represents the equilibrium state between all those fluxes and is the first indicator of nutrient availability. **Nevertheless, as nutrient concentrations are very low in marine oligotrophic habitats, these measurements are very difficult and represent a challenge**. Nanomolar methods for nitrate and nitrite concentrations will be used. Continuous profiling of nitrate using an *in situ* ultraviolet sensor (Johnson & Coletti, 2002) will enable us, for the first time, to study small time scale variability in the nitracline. We will study dissolved inorganic phosphate availability, probably the principle factor controlling planktonic populations especially the diazotrophes (**A 2.1**), and the N:P balance in the euphotic layer (**A 2.2**) using methods recently adapted to ultra oligotrophic conditions (Rimmelin & Moutin, 2005, Tanaka et al., submitted, Thingstad & Mantoura 2005). Silicic acid availability (**A 2.3**) may also play a central role in controlling the export production (Leblanc et al., 2003). High dissolved iron concentrations have previously been observed in the western Mediterranean Sea at the end of the stratified period (Guieu *et al.* 2002, Bonnet & Guieu *submitted*, Sarthou & Jeandel, 2001). However, no data on iron availability (**A 2.4**), which may exert a strong control on N_2 fixation rates (Kustka et al., 2002), are yet available for the eastern Mediterranean Sea.

Identification and quantification of the major external biogeochemical fluxes related to nutrient (N, P, Si, Fe) availability

New production, supported by external sources of nutrients into the photic zone, may be quite low in oligotrophic habitats but is non - the- less a crucial fraction of total autotrophic production. Indeed, over a long term basis, there is an essential equilibrium state between New Production and export from the photic layer, to maintain the organic production in that layer.

-Input by hydrological process

Nutrient flux at the bottom of the photic layer is the result of permanent and/or intermittent advective or diffusive effects. It may be calculated as the product of an eddy diffusion coefficient by a nutrient concentration gradient. **This flux is not well established in oligotrophic areas and is a current subject of study** (Lévy 2003, McGillicudy & Robinson 1997, Balter et al. 2005, and comments from Levy 2005). We will study sites where low potential advection occurs to minimize lateral input, for example in the centres of anticyclonic gyres. Nevertheless, advection will be evaluated. Vertical nutrient gradients are easy to measure but this is not the case for eddy diffusion coefficients (K_z). These can however, be measured using specific methods based on correlations between vertical velocity fluctuations and temperature or salinity (Lewis et al. 1986). Unfortunately, these methods are expensive and take a long time to perform. Hence the eddy diffusivity is most often estimated using indirect methods based on fine-scale measurements (CTD and ADCP). One method currently used, relies on the evaluation of the Richardson number ratio of the vertical gradient of density per mass unit on the squared vertical shear of the horizontal current. This number is involved in Kelvin Helmotz instabilities that are probably responsible for the major vertical nutrient flux in stratified areas (Gregg 1987, Large et al. 1994, Kantha & Clayson 1994). An accurate measurement of this number can be obtained using a CTD SBE 911 coupled to a LADCP mounted on the rosette. Another method using the ratio of vertical temperature or salinity gradient variances, over a small to medium scale (10 cm and 5 m for example), could also be used. Simultaneous microstructure measurements that resolve the dissipative scales will be performed using a full ocean depth un-tethered profiling system, “VMP5500”. This profiler is equipped with microsensors for temperature and shear that enable two independant accurate estimates of Kz. These two estimates will be used to test the relevance of the different indirect methods based on classical CTD and ADCP measurements. This validation of indirect estimates of Kz will then be used as a guide to estimate Kz when microstructure measurements are not carried out.

-Atmospheric input – N_2 fixation

Aeolian dust transport represents, on a global scale, the dominant source of iron, an essential micronutrient for phytoplankton growth, to the ocean (Duce and Tindale 1991; Gao et al, 2001; Jickells et al., 2005). Moreover, it has been shown that the Saharan aerosols can also represent a significant source of phosphate (Ridame & Guieu 2002). Consequently, Saharan dust inputs could stimulate *in situ* biological productivity in oligotrophic ecosystems, such as the Mediterranean Sea and alter community structure, distribution of nutrients and finally the net sequestration of atmospheric CO_2. Considering the short duration of our survey (4 or 5 days at each station) and the low frequency of atmospheric nutrient input by aerosols, these inputs will probably be negligible and will not be considered. **We will only focus on nitrogen input by nitrogen fixation, which may be a major source of new nitrogen in the eastern and western Mediterranean Sea (A 2.5), and study the influence of atmospheric dust deposits on the food web and their potential impact on nitrogen fixation, using microcosm experiments (A 2.6).** Pantoja et al. (2002) estimated that up to 20% of the nitrogen in the western basin and up to 90% in the eastern basin may be derived from biological N_2 fixation, but there is very little data available for the eastern Mediterranean Sea.

Specific questions: *Is nitrogen fixation a major source of new nitrogen for primary production in the photic layer? Do direct measurements of eddy diffusion coefficients reasonably agree with previous estimations?*

Objective 2.2. Organic matter production and food web structure

Because of light/energy requirements, marine organic matter is almost entirely produced in the upper photic zone of the ocean. Variable primary production fuelled by physical processes acting over a wide range of scales, interact with predation to define the species composition of planktonic populations (Gargett & Marra, 2002). The basic principle of the conceptual food web structure, which has not been critically challenged since its original description (Johannes, 1965; Thingstad et al. 1999), is to consider two trophic strategies (Fig. 4). Osmotrophy refering to organisms that feed by taking up dissolved nutrients and phagotrophy refering to organisms that feed by eating particulate matter. Osmotrophs include both the heterotrophic bacteria and autotrophic phytoplankton, while the predatory food chain includes both the protozoa, mesozooplankton and higher predators (all heterotrophs).

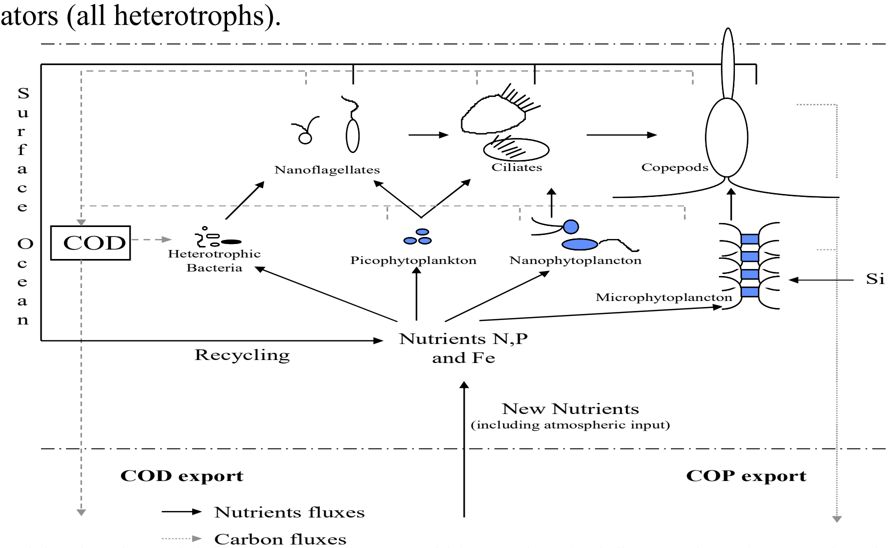

Fig. 4. Idealized food web model for the photic zone and biogeochemical fluxes of nutrients and carbon (redrawn from Thingstad et al. 1998 & 2005)

Biogeochemical fluxes in relation to osmotrophic production

The objectives are to measure fluxes of biogenic elements (including carbon) and to determine parameters that will help to represent these fluxes (P vs I parameters, K_s, V_{max}, affinity constants…). Classical approaches using stable (^{15}N, ^{13}C) and unstable (^{14}C, ^{33}P, ^{32}Si) isotopes for measuring biogeochemical fluxes will be used, along with new techniques that can help to address by which species (or group of species) a specific element has been taken up. **We will study the dissolved organic carbon primary production (A 2.7), recently acknowledged to significantly contribute to the total primary production in oligotrophic areas** (Marañón et al., 2005). We will quantify **nitrification** as well as the impact of dissolved organic nitrogen excretion on the measurement of "new" nitrate uptake (**A 2.8**). **New methods concerning both dominant species separation (Lebaron et al 2001, Servais et al 2003) and low detection limits of chemical analyses (Rimmelin & Moutin, 2005; Duhamel et al., in press.) will allow to define new specific uptake parameters.** We will characterize the dissolved inorganic phosphate uptake in a group of species at a specific level (**A 2.9**) and define the biochemical fate (**A 2.10**). This will give us a detailed view of the overall P-requirements for plankton growth (Van Mooy, 2003). The silicification process in marine diatoms (**A 2.11**) will be investigated using a new labelling technique (Shimizu et al., 2001; Leblanc & Hutchins, 2005) that enables us, for the first time, to discriminate between active and non-active siliceous biomass.

Phagotroph production (secondary production)

The planktonic food web structure influences the fate of primary production in the ocean and has consequences for the CO_2 transfer process. It is generally understood that when small eukaryote or prokaryote bacteria dominate the microbial community, the grazers of picoplankton are small protozoa which do not produce rapidly sinking faecal pellets. **Despite their omnipresence and their pivotal role in the energy flow in marine waters on a global scale, the physiological ecology of these organisms is poorly understood** (A 2.12). Several grazing steps are necessary to enable primary production to be incorporated into the upper trophic levels. Therefore, most of the carbon fixed by phytoplankton is respired and remineralised by the microbial community in the surface mixed layer and there is little or no net uptake of CO_2 from the atmosphere to the sea. In contrast, when large phytoplankton cells dominate, they can either be deposited to the bottom or be grazed by copepods and other mesozooplankton which produce rapidly sinking faecal pellets. However, Mesozooplankton, i.e. copepods have recently been coupled to lower trophic levels in the eastern Mediterranean Sea (Thingstad et al. 2005) and their contribution to the carbon cycle of oligotrophic areas needs to be reconsidered (**A 2.13**). **The upper meso- and macrozooplankton, largely under-sampled by classical techniques, may also significantly contribute to the vertical transport of organic matter** (**A 2.14**).

Biogeochemical process from optical measurements

The use of optical measurements for inferring biogeochemical process on a diel scale is now well acknowledged (Siegel et al., 1989; Claustre et al., 1999). **The technique for deducing high frequency particulate organic carbon from attenuation measurements (diffusion) has revealed higher estimates for primary production in the oligotrophic environment (Claustre et al., in prep.).** Most of these studies have been performed using the attenuation coefficient of suspended particles in the red part of the spectrum (equivalent to the particulate scattering coefficient, b_p), combined with chlorophyll-fluorescence measurements. The simultaneous measurements of b_p, b_{bp} (sub-micrometer particles) and of the Chl-fluorescence signal may be used to analyse the diel scale of the particulate organic matter (Loisel et al., 2002; Behrenfeld et al., 2005) and to understand how the diel variations of sub-micrometer and mostly non-living particles, as revealed by b_{bp}, behave compared to those of the larger and mostly authotrophic particles, as revealed by b_p. (**A 2.15**). Over the diel cycle and between ocean regions of changing nutrient stress, physiological separation of photosynthetic processes (initial charge separation and carbon fixation.) can cause a severe decoupling between variable fluorescence and carbon fixation patterns. **The understanding and characterization of such decoupling (A 2.16) constitutes the basis for relating high-resolution (even space-based) fluorescence data to carbon cycling in the sea.**

General questions: What are the characteristics of the dominant species concerning photosynthesis, nutrient uptake and phagotrophy?

Objective 2.3. Organic matter mineralization and food web structure

The question of specific mineralization of each element (carbon and nutrients) is central because differential mineralization may lead to decoupling of biogeochemical cycles within the water column (Raimbault et al., 1999; Karl et al., 2001, Leblanc et al. 2003). It is generally believed that phosphate is more rapidly recycled than nitrogen which is more rapidly recycled than silicic acid. **Such parallel recycling flux measurements are scarce and the latter assumption is essentially derived from indirect measurements.** The study of organic matter degradation will focus on the relationship between mineralization and bacterial diversity (**A 2.17**). **Hence, examining the relationship between diversity and functionality within the bacterial community (ectoenzymatic activity, uptake of specific organic compounds representative of a chemical family) is a major challenge for understanding the impact of prokaryotic heterotrophic processes on mineralization of organic matter along the water column**. We will also study the factors controlling bacterial production and the consequences of such controls on heterotrophic activity (**A 2.18**), the hydrolysis rate of Dissolved Organic Phosphate (**A 2.19**) and the effects of hydrostatic pressure on particles and dissolved organic matter mineralization (**A 2.20**).

General questions: *What are the changes in prokaryotic heterotrophic activity and community structure in relation to horizontal (west-east) and vertical (surface to depth) nutrient gradients and the composition of the dissolved organic matter?*

Objective 2.4. Organic matter export (particulate and dissolved matter)

The structure of pelagic ecosystems influences the size and type of downward flux of biogenic carbon in the sea (Legendre and Rassoulzadegan, 1996). This vertical flux is composed mainly of large particles such as fecal pellets, hard parts of zooplankton, amorphous aggregates, marine snow (Fowler and Knauer, 1986; Silver and Gowing, 1991), and senescent diatoms, particularly in the aftermath of blooms (Billet et al., 1983). Fecal pellets can sink quickly: 20-900 m d^{-1} for copepods (Lorenzen, 1983; Welschmeyer and Lorenzen, 1985), and up to 2700 m d^{-1} for large gelatinous zooplankton (Bruland and Silver, 1981; Madin and Purcell, 1992). High sinking velocities can lead to the efficient export of biogenic carbon. The downward flux of biogenic carbon can therefore be largely dominated by carbon of zooplankton origin (Thibault *et al*., 1999).

We will study the C/N/P/Si stoichiometry of settling particulate matter (**A 2.21**) **because despite its biogeochemical relevance, there are few direct measurements available** (Geider & La Roche, 2002). We will also examine the role of larger particulate matter responsible for the marine snow rarely observed in oligotrophic areas (**A 2.22**) and quantify instantaneous DOC export.

Objective 2.5. Oxygen budget

The balance between photosynthesis and respiration in the microbial community defines the metabolic state of the studied ecosystem (Net autotrophic or heterotrophic) and its capacity to export carbon. **The budget on a total oceanic scale is uncertain and the area of greatest**

uncertainty lies paradoxically in the surface layer of the ocean (Williams, ASLO meeting 2006). The originality of the approach used will consist of comparing the variations in oxygen concentration over different time scales, *in situ* and in incubation bottles, using a new system called a "Productivity Autosampler" (A 2.23). We will also use the more classical technique for assessing the daily budget (Gross and Net Community Production, community respiration), using the nychthemeral variations in oxygen concentration. This will yield information on the respiration rates of the autotrophic and heterotrophic communities (Pringault et al. L&O method, submitted) enabling us to determine the impact of the microbial community on the carbon cycle.

Objective 2.6. Responses in the microbial food web to pulse nutrient addition (microcosms studies)

Phosphate addition to the P-starved and ultraoligotrophic surface water of the Cyprus Gyre, Eastern Mediterranean in a Lagrangian experiment caused unexpected ecosystem responses (Thingstad et al. 2005). The system exhibited a decline in chlorophyll and an increase in bacterial production and copepod egg abundance. The results were supported by those obtained from an on-board microcosm experiment during the Lagrangian experiment (Zohary et al. 2005). Although N and P co-limitation hindered phytoplankton growth, they explain that P may have been transferred through the microbial food web to copepod via two, not mutually exclusive, pathways: (1) bypass of the phytoplankton compartment by P uptake in heterotrophic bacteria and (2) tunneling, whereby P luxury consumption rapidly shifts the stoichiometric composition of copepod prey (Thingstad et al. 2005). These mechanisms are not fundamentally new for limnologists but have been explored to a very limited extent in marine systems. It is thus necessary to test whether these mechanisms exist and how pulsed P addition is used by the microbial food web and mesozooplankton in different P limited regions. Since the result from the on-board microcosm experiment was fairly similar to that from the *in situ* experiment (Thingstad et al. 2005; Zohary et al. 2005), microcosm experiments (**A 2.24**) will allow testing the above mechanisms. In addition, microcosm enrichment experiments will be conducted on board using trace-metal clean techniques to address in particular the role of Fe and dust on N_2 fixation at a selected number of sites on water sampled in the surface mixed layer.

Studies driven at the scale of hour to year in oligotrophic environments have shown that most of the nutrient inputs from the deep to the surface layers were attributed to punctual pulses (Dickey et al 2001). **However the consequences of such pulse of nutrient have been scarcely studied**. Atmospheric input are also punctual and thus, it is particularly interesting to study simultaneously the in situ ecosystem in its most probable steady state as well at its response after a nutrient input.

Specific questions: *What are the consequences of pulsed nutrient addition on the microbial food web and mesozooplankton in marine waters with different oligotrophic status? What are the respective role of Iron and phosphate on the control of the* N_2 *fixation rates?*

OBJECTIVE 3. REPRESENTATION OF THE MAJOR BIOGEOCHEMICAL FLUXES AND OF THE DYNAMICS OF THE PLANKTONIC TROPHIC NETWORK

The pluridisciplinary field data gathered during the BOUM cruises will be available to the international community as soon as possible (data bases CYBER and SESAME) in order to help represent the interactions between climate, biogeochemical cycles and marine ecosystems. **A multi-scale modelling approach is proposed from the beginning of the BOUM project.** Its objective is to complement the observations with the analyzed data in order to understand the interactions between the biogeochemical cycles of the biogenic elements and the dynamics of the planktonic trophic network.

Three scales of modelling are proposed.

Objectve 3.1. Mediterranean scale studies

All the data that will be gathered during the cruises carried out in the European program SESAME, within the framework of WP2 (Fig. 3), will provide a unique data set for the Mediterranean. A great deal of effort will be done to use similar analyse protocols, similar strategies of sampling (horizontal and vertical), similar priority parameters. The intercalibration of analyses is already in progress for the nutrients. The SESAME data base, containing hydrological, chemical and biological information, will be used for the implementation and adjustment of parameters and for evaluating the ecological models (WP4 and WP6 of SESAME). This data set will also be used to validate a biogeochemical model for the whole Mediterranean Sea (Vichi et al, 2006).
The distribution of the anthropogenic carbon will also be estimated at the same scale by using the 3D model developed by the GHER (University of Liege in Belgium), currently available on a workstation Bi-opteron of the University of Perpignan (France), (**A 1.1**).

Objective 3.2. Process scale studies

We plan to work using finer scales adapted to studying the physical and biogeochemical processes through (i) the zero-dimensional (0D) modelling of microcosm experiments specifically designed to study a particular biogeochemical process (diazotrophy, phytoplankton-bacteria competition,...) in order to add to or improve its representation in the Eco3M biogeochemical model (Baklouti et al., 2006a) and (ii) the 1D vertical modelling of the physical and biogeochemical processes at the long duration stations. In order to reinforce the complementary approaches of modelling and experimentation, a common strategy has been developed for use in the BOUM project. Thus, the stations have been positioned in regions where the horizontal variability of the hydrological and biogeochemical properties is supposed to be low over the duration of sampling so making it possible to consider a 1DV modelling approach. Moreover, the majority of the model's needs, in terms of state variables and parameters, will be acquired through *in situ* measurements or by controlled activities i.e. sampling and microcosms during the cruises.

- controlled experiments (0D)

Experiments and modelling on a microcosm scale are important for several reasons: on one hand, to acquire certain model parameters (characteristics of nutrient assimilation, photosynthetic parameters, respiration rates, mineralisation...) and on the other hand, to test or develop new models for processes where current formula are not validated or represented. It is also important that these microcosm experiments are undertaken during the cruises (or field studies) with *in situ* water samples in order to obtain parameters which are representative of the studied ecosystem. The processes which have been chosen for detailed study are those thought to be important in the operation of the studied systems, and also those that are required to improve or validate the formulation of these important processes in the current model.
These processes are:

- Diazotrophy: Existing models for diazotrophy rely on empirical laws deduced from macroscopic observations. (e.g. Bissett and Al, 1999; Fennel and Al, 2002; Moore and Al, 2002) In order to improve such models the underlying mechanisms of diazotrophy need to be understood, especially the interaction between N_2 fixation and nutrient uptake (other nitrogen sources, phosphate, iron).

- Competition between phytoplankton-bacteria for nutritive resources.
This competition is particularly crucial insofar that it induces, especially during times of phosphate input, changes in the way that matter is transferred towards the higher levels (bypass, tunnelling)

when compared to those classically observed (Thingstad et al.. 2005). Modelling the experiments outlined in objective 4 will therefore make it possible to test the models capacity to reproduce the response of the trophic network to pulse phosphate additions and help with the interpretation of these results.

- The multi-limitation of the plankton growth by nitrogen and phosphate

In situations where several nutrients are likely to limit phytoplankton growth, current models tend to use empirical laws which force growth by the strongest of these limitations (law of Liebig) or by the product of these limitations. The basis of these laws still remains to be proven (e.g. Davidson and Gurney, 1999) and work towards a multi-limitation approach, complimented by specific experiments should make it possible to move forward and gain a better understanding in this field. The fine modelling of processes will make it possible to feed the 1DV model and will be applied to each long duration station.

In situ experiments at long duration station (1DV)

The major objective of 1DV modelling is to represent fluxes of biogenic elements in the surface layer and provide an accurate budget on the fate of the organic carbon produced (Re-mineralisation, export). The use of a multi-element model (not redfieldien) will potentially make it possible to illustrate the decoupling of the biogenic element cycles in the oligotrophic ocean. The objective is to understand the production and fate of organic matter in the oligotrophic food web, and their role in the global carbon cycle. It is not to describe in detail the complex relationships which exist between the organisms. This approach is to extract those criteria which appear essential in providing a satisfactory representation of the marine pelagic ecosystem and biogenic element fluxes (**A 3.1**). The complexity of the trophic relationships will be simplified by focusing on the groups of species having similar biogeochemical functions (i.e. functional groups). The relevance of the 1DV modelling will depend on *in situ* measurements carried out at the long duration stations as the process modelling described above will depend on the microcosm experiments. Indeed, a certain number of parameters which will be measured are essential in achieving a realistic representation of the dynamics of the pelagic ecosystem and thus a precise estimate of the carbon budget. These parameters include all the parameters related to phytoplankton photosynthesis and to nutrient uptake by the plankton. In the physics-biogeochemical coupled models, the parameterization of turbulence is frequently a source of error, because it is based on empirical laws, and because these laws are not then re-calibrated for each site studied (typical values found in the literature are used to parameterize these laws). Here, it will be possible to use realistic vertical profiles in the hydrodynamic 1DV model to accurately estimate the coefficients of turbulent vertical diffusion - K_Z- (cf obj 2.1). **The use of such realistic profiles is an essential step in representing the mixing processes in the surface layer and the nutrient fluxes at the base of the euphotic layer**.

The biogeochemical model implemented by default in the numerical platform ECO3M (Baklouti and Al, 2006a) gives a mechanistic or semi-mechanistic representation of the key processes of phytoplanktonic dynamics (photosynthesis, photoacclimation, nutrient absorption, DOM release, autotrophic respiration). The majority of these processes have been validated using microcosm experiments (Pawlowski, 2004) performed on a functional group of diatoms subjected to nitrate and light stress (Baklouti and Al, 2006b). The 1DV model, used in its Mediterranean version, relies on a similar representation of these processes, but includes several functional groups as well as biogenic elements other than nitrogen and carbon. This type of model enables us to obtain a representation of the biogeochemical cycles of the principal biogenic elements (C, N, P, Si and O) as well as representing organisms which play a key role ("functional" groups) in the dynamics of organic carbon production and transfer. Moreover, this model will be improved by the input of new processes studied during the first part of the process modelling (cf point 2.) and by the explicit representation of the bacterial and micro-zooplankton compartments (microbial). The whole data set collected from the long duration stations, and in particular the data obtained in a quasi continuous manner such as oxygen and nitrate fluxes and stocks, will offer the possibility of validating the coupled model which up to this point has never been carried out due to the strong mismatch between experiments and modelling.

Specific question: Is it possible to reproduce, with only one uniquely structured model, the principal physical and biogeochemical characteristics of the different oligotrophic areas studied? If so, the model should enable us to identify the origin of the processes and their different functions. In particular, this approach will enable us to answer an important question in the BOUM project: are the differences in functioning between the sites attributable to one or more production process of organic matter, remineralisation and export? If not with which process can they be connected?

2. Research plan

The BOUM project is based upon the preparation, the realisation and the analysis of results from a 35 days long oceanographic cruise in the Mediterranean Sea.
The detailed research schedule of the cruise is detailed in annex 2 (simplified form for R/V requirement).

3. Time schedule

September 2007: Pre-cruise meeting (Marseille)
Mars 2008: Pre-cruise meeting of the enlarge steering comitee (Marseille)
August-September 2008: Cruise (detailed description in Annex 2)
May-June 2009: Post-cruise meeting of the whole BOUM community (Marseille)
Oct-Nov 2009: workshop (10 people max)
Feb-March 2010: workshop (10 personnes max)
June-July 2010: workshop (10 personnes max)
2010: asking for a special session at the OSM

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OTHER REFERENCES IN ANNEX 3

Material/staff requested and available for the realisation of the project

1. Staff and Laboratories

A summary table is available on page 5

2. Equipement available for the projet

ISUS Nitrate	Université Dalhousie/Satlantic Inc.
Spectromètre de masse	Laboratoire d'Océanographie et de Biogéochimie
Flow cytometer (FACSAria) Tri cellulaire	Laboratoire d'océanographie Biologique de Banyuls
Flow cytometer (FACSCalibur)	Laboratoire d'océanographie Biologique de Banyuls
APO (Oxygen Productivity Auto Sampler	Laboratoire Microbiologie Géochimie Ecologie Marines
Autoanalyser Bran&Luebbe (Nutrient analyser)	Laboratoire d'océanographie Biologique de Banyuls
CHN LECO 900	Laboratoire d'Océanographie de Villefranche
laser OPC	Laboratoire d'Océanographie et de Biogéochimie
TCO2/TA potentiometric system	Université de Perpignan
pCO2 system	Université de Perpignan
TRIOS radiometer (350 nm to 850 nm)	ELICO, FRE 2816-CNRS
Radiometer TRIOS (350 nm to 900 nm every 3 nm)	ELICO, FRE 2816-CNRS
Wetlabs optical package mounted on a specific frame	ELICO, FRE 2816-CNRS
PVM	Laboratoire d'Océanographie de Villefranche
Plankton nets	Laboratoire d'Océanographie de Villefranche
Zooscan	Laboratoire d'Océanographie de Villefranche
HPLC	Woods Hole Oceanographic Institution
Hybridrization oven	Institut de Recherche pour le Développement
Microscopes	Laboratoire d'Océanographie et de Biogéochimie
Epifluorescence microscope	Laboratoire Microbiologie Géochimie Ecologie Marines
PCR	Laboratoire Microbiologie Géochimie Ecologie Marines
Bacterial cell culture	Laboratoire Microbiologie Géochimie Ecologie Marines
Electrophoresis	Institut de Recherche pour le Développement
CHN LECO 900	Laboratoire d'Océanographie de Villefranche
Flow injection analyser for the determination of DFe on board.	Laboratoire d'Océanographie de Villefranche
8 GoFlo bottles for ultra-clean sampling	Laboratoire d'Océanographie de Villefranche
Teflon pump for surface sampling	Laboratoire d'Océanographie de Villefranche
Flow cytometer (virus)	Laboratoire d'Océanographie de Villefranche
TOC-V analyser	Laboratoire d'océanographie Biologique de Banyuls
Autoanalyser Bran&Luebbe (Nutrient analyser)	Laboratoire d'océanographie Biologique de Banyuls

Scintillation counter (in lab)	Laboratoire d'Océanographie et de Biogéochimie
Spectrophotometer Spectronic 401	Laboratoire d'Océanographie et de Biogéochimie
Autoanalyser (Nutrient analyser)	Laboratoire d'Océanographie et de Biogéochimie
1 Spectrofluorometer Kontron SFM 25	Laboratoire Microbiologie Géochimie Ecologie Marines
2 pressure piloted generator (PGG)	Laboratoire Microbiologie Géochimie Ecologie Marines
1 High Pressure Sampler System with 10 independent sampling bottles	Laboratoire Microbiologie Géochimie Ecologie Marines
4 Temperature controlled insulated aluminium containers for incubations	Laboratoire Microbiologie Géochimie Ecologie Marines
1 centrifuge	Laboratoire d'Océanographie et de Biogéochimie
1 micro-centrifuge	Laboratoire Microbiologie Géochimie Ecologie Marines
Laboratory Optical Particles Counter	Laboratoire d'Océanographie et de Biogéochimie
Bongo Net tows	Laboratoire d'Océanographie et de Biogéochimie

3. Material requested outside of the LEFE project

Annex 2 shows the simplified form requesting the oceanographic research vessel

Travel costs for materials and scientific staff related to the oceanographic cruise, the financial request will be made to the DT INSU (soutien à la mer).

4. Label of phD or post doc fellowships

Already mentioned page 5.

5. Budget **(in the frame of LEFE)**

5.1 functionning

Modelling : **6 000 € HT**
Cost of a special issue : **15 000 € HT**
Cost of other publications : **10 000 € HT**

5.2 travels and subsistence : post by post for the whole duration of the project

September 2007: Pre- cruise meeting of the whole staff (Marseille): **17 000 € HT**.
March 2008: Pre- cruise meeting of the enlarged steering committee (Marseille): **4 000 € HT**.
August-September 2008: cruise (detailed description Annex 2)

specific line for « soutien à la mer » cost, total cost : 128 500 € HT
Detail :
Estimated cost for 25 travels to Marseille (France) + 2 days (per diem) for each participant :
20 500 € HT
Estimated cost for 37 travels to Latakia (Syrie) + 2 days (per diem) for each participant :
66 000 € HT
Estimated cost for conveying material: **30 000 € HT**
transport of laboratory containers
12 000 € HT
Remark: other solutions for deboarding at Chypre or in Egypt are still studied.

May-June 2009: Meeting post- cruise of the whole staff (Marseille): **17 000 € HT**.
Oct-Nov 2009 : dedicated workshop (10 pers max) : **4 000 € HT**
Feb-Mars 2010 : dedicated workshop (10 pers max) : **4 000 € HT**
June-July 2010 : dedicated workshop (10 pers max) : **4 000 € HT**

2010 : Organisation of a special session at Ocean Sciences Meeting : **15 000 € HT**

5.3 cost of analyses : see annexe 2

5.4 specific equipment : in detail and to justify the whole project

Budget for the construction of mesocosms: **10 500 € HT**
1 hybridization oven for phylogenetic analyses: **3 200 € HT**

5.5 "High cost" equipment requested :

Scientific equipment necessary for the oceanographic cruise BOUM and requested in 2006, which are already founded are the OPC laser (Optical Particule Counter, request F Carlotti), the series of 6 APO (Oxygen Productivity Auto Sampler, request D. Lefevre), and a CHN analyzer LECO 900 (request A. Sciandra).

The ISUS nitrate analyzer is provided by the Satlantic society (M Lewis). The BOUM project encourage the joint proposal of two recently recruited CNRS researchers, K Leblanc and T Tanaka, requesting a spectrofluorometer Shimadzu (24000 euros HT) and the G. Gorsky's proposal asking for 38920 euros HT to finish the construction of a mini PVM (Particule Video Profiler) adaptable to the CTD rosette.

5.6 Total requested from LEFE

245 470 **€ HT** for 4 years

6. Other funds (already obtained or requested outside of the framework of LEFE, including european projects). To detail and justify for whole project

Integrated Project SESAME
PAI AURORA (travel and per diem for exchanges with norvegian researchers)
ANR Internal waves, ANR BOOM, ANR POTES

ANNEXS JOINED TO THIS PROPOSAL

LEFE Annex 1 : Detailed **budget for analyses**

LEFE Annex 2 : Simplfied form requesting the research vessel

LEFE Annex 3 : Scientific proposal : specific contributions and list of references cited (not from the BOUM community)

LEFE Annex 4 : High cost equipment

LEFE Annex 5 :
Letter of support (J. Hall, IMBER and E. Papathanassiou, SESAME)

LEFE Annex 1 : Detailed analysis expected budget

POSTE	Nombres de mesures	Prix unitaire (€)	MONTANT total (K€)
PHYSICS+OPTICS			
Physics			
CTD variables (T,S,O2,Fluo…)	197	20	3.94
Horizontal Current on vertical profile (LADCP)			1.00
Kz estimations (VMP5500)			4.60
Optics			
PAR Downwelling irradiance			1.00
Dissolved absorption	288	5	1.44
Particulate absorption	288	5	1.44
In water upwelling radiance and irradiance (Radiometric package)			2.00
Backscattering, attenuation, fluorescence, and CTD (Optical package)			3.00
Variable fluorescence-based photochemical quantum efficiency (Fv/Fm)	480		NASA acquired
Functional absorption cross section of photosystem II (σ_{PSII})	480		NASA acquired
Electron transport rates on the acceptor side of PSII (τ_{PSII})	480		NASA acquired
Fluorescence-based photosynthesis-irradiance relationships	480		NASA acquired
BIOGEOCHEMISTRY			
Stocks			
Dissolved			
TCO2	1137	2.5	2.84
TA	1137	2.5	2.84
O2	1137	3	3.41
NO3	1148	2	2.30
NO3 nanomolaire (upper photic zone)	162	2	0.32
NO3 (ISIS)			
NO2	1098	2	2.20
NO2 nanomolaire (upper photic zone)	162	2	0.32
NH4	644	3	1.93
PO4	1148	2	2.30
PO4 nanomolar (MAGIC)	344	4	1.38
Silicates	1148	2	2.30
DFe	134	30	4.02

Fe L	32	30	0.96
DOC HTCO	1100	7	7.70
DON	1100	2.5	2.75
DOP	1100	2.5	2.75
Excess N or P (APA technic)	6	60	0.36
Free Amino acids	36	15	0.54
Combined Amino acids	36	15	0.54
Dissolved lipids	81	12	0.97
Particulate			
POC	1050	3	3.15
PON	1050	2.5	2.63
POP	1050	2.5	2.63
POP (0.2, 0.6, 2 µm)	24	10.5	0.25
BSi	954	1.25	1.19
L-Si	954	1.25	1.19
Pigments (HPLC)	480	11	5.28
Chlorophyll a (Fluo)	686	2	1.37
Phospholipids	516		NSF asked
C, N, P, Mg, Fe, elemental analysis	207	2.5	0.52
Fluxes			
Primary production (C uptake 24h dusk-to-dusk)	384	15	5.76
Particulate primary production	120	7	0.84
Dissolved primary production	120	7	0.84
Primary production by size class (C uptake 0.2, 0.6, 2 µm)	24	25	0.60
O2 production (O_2PA)			0.50
O2 production (in situ) NCP,GPP,R	36	100	3.60
N_2 fixation and C uptake, 13C/15N	162	10	1.62
N_2 fixation and C uptake by size class, 13C/15N	72	30	2.16
N_2 fixation and C uptake, 13C/15N (microcosmes)	92	15	1.38
N_2 fixation and C uptake by size class, 13C/15N (microcosmes)	0	30	0.00
N and C uptake, 13C/15N	114	15	1.71
Ammonium regeneration, 15N	114	15	1.71
Nitrate regeneration or nitrification, 15N	114	15	1.71
DON release, 13C/15N	114	15	1.71
PO4 turnover time	342	15	5.13
PO4 affinity (0.2, 0.6, 2 µm)	24	18	0.43
Si uptake	102	34	3.47

Si dissolution	60	25	1.50
Bacterial production	350	12	4.20
Bacterial production (hpss)	12	67	ANR
Bacterial production (0.2, 0.6 µm)	216	15	3.24
APA	144	2	0.29
Luxury P uptake	6	20	0.12
Assimilation AA	36	15	0.54
Assimilation fatty acids	36	15	0.54
Dark assimilation HCO3-	36	15	**ANR**
Dark assimilation hco3-, hpss	12	76	**ANR**
Lipase hydrolysis rate (3H oleate)	81	15	1.22
Lipase hydrolysis rate (muf-palmitate)	81	6	0.49
Phosphatase hydrolysis rate (muf-P)	114	6	0.68
Aminopeptidase hydrolysis rate	72	6	**ANR**
Aminopeptidase hydrolysis rate, hpss	12	6	**ANR**
ATP Hydrolysis rate (33P et 14C)	114	20	2.28
Phospholipid synthesis rates	516		NSF asked
Mass flux, C,N,P,LSi,BSi,Ca, COP, Al, Fe (Sediment trapps)	48	77.5	3.72
Natural abundance of ^{15}N ($\delta^{15}N$) and ^{13}C ($\delta^{13}C$)	162	15	2.43
BIOLOGY-DIVERSITY			
Molecular stress "indicators"	194	43	8.34
Prochlorococcus diversity	90	27	2.43
AAPB diversity (culture approach)	90	30	2.70
AAPB diversity (molecular approach on raw sample)	9	500	4.50
AAPB quantitative PCR	90	9	0.81
Total Eukaryotes diversity (ARN 18S clone sequencing on raw samples)	36	500	
Abundance of Eukariotic specific groups (Probes)	220	50	
Abundance of Eukariotic specific groups (Quantitative PCR)	76	21	1.60
Photosynthetic Eukaryotes diversity (Molecular biology on sorted samples)	12	250	3.00
Abundance of heterotrophic Eukaryotic specific groups (Probes)	30	50	1.50
Micro phytoplankton diversity	102	10	1.02
PDMPO	60	9	0.54
Flow cytometry analysis (bacteria+pico-nano-plancton)	1143	2	2.29
Heterotrophic prokaryotic community structure SSCP	25	30	0.75
Heterotrophic prokaryotic Clone library and sequencing	6	1200	7.20
DIP uptake kinetic parameters for specific groups	12	120	1.44
Small Diazotrophs quantification and distribution TSA Fish	202	40	8.08
Small Diazotrophs species richness (clone library)	3	700	2.10

Ciliates biomass	60	20	1.20
Ciliates growth rate	6	160	0.96
Ciliates grazing rate	6	160	0.96
Nanoflagellates biomass	180	7.5	1.35
Nanoflagellates growth rate	6	60	0.36
Nanoflagellates grazing rate	6	60	0.36
Tintinnids abundance	66	20	1.32
Tintinnids diversity	66	40	2.64
Zooplankton and marine snow Mini PVM	195	20	3.90
Zooplankton and marine snow PVM	60	20	1.20
Zooplankton biomass (Net tows)	60	50	3.00
Zooplankton biomass (OPC)	159	30	4.77
Zooplankton biomass (CHN)	60	10	0.60
Zooplankton diversity (Binocular treatment)	60	50	3.00
Zooplankton grazing rate (gut fluorescence)	60	40	2.40
Zooplankton eggs production rate	12	35	0.42
Zooplankton fecal pellets production rate	60	40	2.40
Zooplankton respiration rate	12	40	0.48
Zooplankton excretion rate	12	40	0.48
Viral abundance	480	1.5	0.72
Viral production (dilution approach)	60	15	0.90
Viral diversity (PFGE,DGGE)	20	20	0.40
Bacterial diversity (CARD FISH)	69	25	1.73
Functional bacterial diversity (MICRO-CARD FISH with Leucine)	77	40	3.08
Functional bacterial diversity (MICRO-CARD FISH with fatty acids)	18	40	0.72
Functional bacterial diversity (MICRO-CARD FISH with Polymer)	18	40	0.72
Functional bacterial diversity (MICRO-CARD FISH with HCO3)	18	40	0.72
phosphatase positive cells (ELF P)	174	10	1.74
		Total :euro =	224 059

LEFE Annex 2 : Simplified form for french R/V request

RÉCAPITULATIF -1-	**DOSSIER DE DEMANDE : BOUM** **DATE :** 2007

TITRE DE LA CAMPAGNE : BOUM

Année : 2008
Durée *(sur zone) :* 35 jours
Escale éventuelle (à justifier)

Période *(si impératif) :* septembre

Zone : Mer Méditerranée orientale et occidentale

Pays dont les eaux territoriales sont concernées :

Pays dont la zone économique est concernée :

	Chef de projet	**Chefs de mission**
Nom Prénom :	MOUTIN Thierry	(1) MOUTIN Thierry (2) VAN WAMBEKE France
Organisme :	COM-OSU	COM-OSU
Laboratoire :	LOB-CNRS	LMGEM-CNRS
Adresse :	Campus de Luminy, case 901 F-13288 Marseille Cedex 09	Campus de Luminy, case 901 F-13288 Marseille Cedex 09
Tél.:	04 91 82 95 60	04 91 82 90 49
Fax :	04 91 82 19 91	04 91 82 90 51
E-mail :	moutin@com.univ-mrs.fr	wambeke@com.univ-mrs.fr

Travaux : prélèvements hydrologiques, déploiement de mouillage dérivants.

Traitement des données et Besoins informatiques :

Navire : Pourquoi Pas ou Atalante

Engin(s) : Non

Gros équipements : Non

Nécessité d'une campagne pour récupération d'engin ? Non

Scientific teams
LOB Marseille, LMGEM Marseille, LOV Villefranche sur mer, SBR Roscoff, LOBB Banuyls, IRD, Université Perpignan, ULCO-ELICO Wimereux, INSU/CNRS, LOCEAN Paris

Foreign partners :

Vigo University, Espagne
HCMR grèce
HIASCR, République Tchèque
MEG-DBUB, University of Bergen, Norvège
WHOI, Etats-Unis
Oregon state University
Dalhousie University/Satlantic Inc., Canada

Cette proposition se rattache au(x) programme(s) (nationaux ou internationaux) avec comité scientifique :
(L'avis des comités scientifiques sera sollicité)
European program SESAME

Thème scientifique : Marine biogeochemistry

Responsable d'unité : Bernard Quéguiner
DATE : **SIGNATURE :**
Nombre de dossiers présentés pour 2007 au sein de l'Unité :

DOCUMENT N° 2	**DOSSIER DE DEMANDE : BOUM** **DATE :** 2007

METHODS AND STRATEGY

- **Méthodologie détaillée et liste précise des travaux pour atteindre les résultats escomptés - stratégie,**
- **Déroulement général de la mission, durée totale, *temps sur zone*, période souhaitée, escale éventuelle et raisons de ce souhait, *carte de situation générale***
- **Calendrier journalier prévisionnel des travaux (tableau J1 à Jx) précisant : travaux, trajets, stations, profils, les différentes zones d'études, ... documents cartographiques obligatoires. Pour les études de sismique et les levés sonars il est indispensable d'indiquer le nombre de milles nautiques des profils.**
- ***Pour les trajets, estimer les durées avec les vitesses suivantes : 10 nœuds pour le Suroît et Nadir, 11 nœuds pour Thalassa et L'Atalante***

The general thermohaline circulation (Fig. 1) is at the origin of the wide range of oligotrophic conditions observed in the Mediterranean Sea. The "**BOUM**" cruise (**B**iogeochemistry from the **O**ligotrophic to the **U**ltra oligotrophic **M**editerranean Sea) will focus on 2 transects East-West and North-South, and three selected stations (for process studies) sampled during 5 days (Fig. 2). They represent a unique context to understand at a regional scale, the link between biogenic biogeochemical cycles of C, N, P, Si, Fe and the planktonic community structures of highly contrasted oligotrophic marine waters. The two transects will include "core parameters" stations from the Rhone River mouth to the centre occidental Mediterranean Sea and from Gibraltar to the Levantine basin. They will be performed to describe the relevant biogeochemical parameters from surface to bottom in the whole Mediterranean Sea.

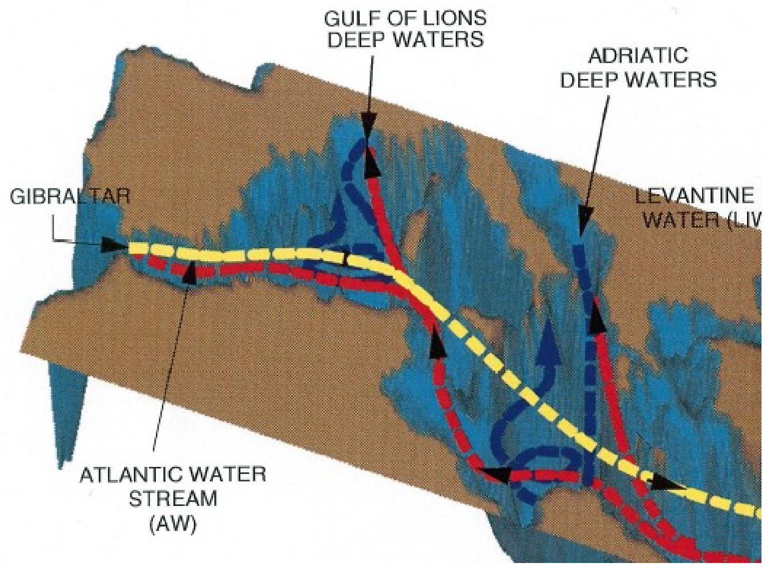


Figure 1: The schematic of the thermohaline circulation in the Mediterranean Sea with the major conveyor belt systems indicated by dashed lines with different colour. The yellow indicates the AW stream which is the surface manifestation of the zonal conveyor belt of the Mediterranean. The red indicates the mid-depth LIW recirculation branch of the zonal thermohaline circulation. The blue lines indicate the meridional cells induced by deep waters. LIW branching from the zonal conveyor belt connects meridional and zonal conveyor belts. (Pinardi & Masetti, 2000).

1- General strategy at each station:

1-1 Short duration stations

During transect to reach or between two long duration stations, 42 short "core parameters" stations of 3-h duration will be performed. Only one CTD profile with sampling from surface to bottom will be done for core parameter analyses (temperature, salinity, dissolved O_2, dissolved CO_2, alkalinity, inorganic nutrients, chlorophyll, particulate organic C, N and P (POC, PON, POP), particulate silica, dissolved organic C, N and P (DOC, DON, DOP). In &addition, two layers will be sampled (mixed layer, top of nutricline) for controlled in-board incubations. The distances between all short duration stations will be around 60 nmiles which corresponds to approximately 5 hours of ship time.

Niskin bottles (12 L) available for sampling:

- Deep cast O-bottom) : 21 Niskin bottles (OPC, mini PVM and Nitrate sensor occupies one bottle space each)

1-2 Long duration stations

To achieve our process study at each of the three long duration stations (1 in the central Western basin, 1 in the central Ionian basin and 1 in the central Levantin basin), a precise localisation and physical characterisation in low currents areas is needed. It will be performed by using all methodologies available at the present time. For each station, the successions of operations will be as follows:

1) Horizontal survey.

We will consider satellite imagery (Taupier Letage, expert) and prevision from MERCATOR to define areas were a rapid survey (maximum 12h) using thermosalinograph and ADCP data will allow to find precisely the location of the sites with low potential advection. During this survey, only continuous surface sampling will be possible.

2) physical and chemical characterisation of the area

The hydrological / chemical context will be appreciated by the exploration of 16 sub-stations in a 9 square miles geographic area around the position of the long duration station. In each sub station, profiles 0-500 m will be performed for CTD, O_2, fluorescence, and nitrate (ISUS). Each of the 16 stations will be occupied for 30 minutes and separated by one hour maximum of ship time. No water sampling by Niskin bottles will be possible during that survey.

3) 96-h process study

The stations sampled during that period of time will be at the centre of the sampling grid before the drifting of the mooring line and near the drifting mooring the following days (Lagrangian strategy). The sequence of operations will be as follows:

For a sun rise at 6:00

Day A

- 3:00-4:00 (3 hours before dusk): CTD casts 0-200m et 0-500m and sampling for production measurements (0-200 m)
- 4:15-5:00 PVM + Net
- 5:00 - 6:00 Drifted mooring deployment (in situ production measurements line ($C,N,P,Si,\Delta CO_2,\Delta O_2$) + O_2 Productivity Autosampler (O_2PA) + drifting sediment traps at 3 depths). + VMP5500 deployment
- 6:00-7:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 7:00-8:00 CTD cast 0-500m Physics + large water volumes for microcosms experiments (one depth)
- 8:00-9:00 In situ pumps
- 9:00-10:00 CTD cast 0-500m Physics+large water volumes for microcosms experiments (one depth) + optical grape
- 10:00-11:00 VMP5500 recovery

- 11:00-12:00 CTD cast 0-500m Physics+large water volumes for microcosms experiments (one depth)
- 12:00:13:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 13:00-14:00 PVM + Net
- 14:00-17:00 CTD cast 0-bottom coupled to HPSS
- 18:00-19:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 19:00-21:00 In situ pumps
- 21:00-22:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 22:00-24:00 In situ pumps
- 24:00-1:00 CTD cast 0-500m Physics+Cycle + Optical grape

Day B

- 1:00:2:00 PVM + Net
- 3:00-4:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 4:00-5:00 PVM + Net + VMP5500 deployment
- 5:00-6:00 in situ production line recovery (Zodiac)
- 6:00-7:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 9:00-10:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 10:00-11:00 VMP5500 recovery
- 12:00:13:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 13:00-14:00 PVM + Net
- 15:00-16:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 18:00-19:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 21:00-22:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 24:00-1:00 CTD cast 0-500m Physics+Cycle + Optical grape

Day C

- 1:00:1:45 PVM + Net
- 1:45-3:00 (Three hours before dusk): CTD casts 0-200m et 0-500m and sampling for production measurements (0-200 m)
- 3:00-4:00 CTD cast 0-500m Physics+Cycle + Optical grapp
- 4:00-5:00 PVM + Net + VMP5500 deployment
- 5:00 6:00 In situ production line deployment (Zodiac)
- 6:00-7:00 CTD cast 0-500m Physics+Cycle + Optical grapp
- 9:00-10:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 10:00-11:00 VMP5500 recovery
- 12:00:13:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 13:00-14:00 PVM + Net
- 15:00-16:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 18:00-19:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 21:00-22:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 24:00-1:00 CTD cast 0-500m Physics+Cycle + Optical grape

Day D

- 1:00:2:00 PVM + Net
- 3:00-4:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 4:00-5:00 PVM + Net + VMP5500 deployment
- 5:00-6:00 in situ production line recovery (Zodiac)
- 6:00-7:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 9:00-10:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 10:00-11:00 VMP5500 recovery
- 12:00:13:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 13:00-14:00 PVM + Net
- 15:00-16:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 18:00-19:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 19:00-21:00 Sediment traps and O_2PA lines recovery
- 21:00-22:00 CTD cast 0-500m Physics+Cycle + Optical grape
- 22:00-1:00 CTD cast 0-bottom coupled to HPSS

Day E

- 1:00-4:00 CTD cast 0-bottom Core Parameter
- 4:00 Departure to another station

Remark:
This time schedule will provide a continuous 84h of CTD cast every 6 hours (15 CTD cast) and 75h of CTD cast every 3 hours (26 CTD cast)

Niskin bottles (12 L) available for sampling:

- CTD cast 0-500m Physics+Cycle: 17 Niskin bottles (optical grape occupies 4 bottle spaces, OPC 1, miniPVM 1, Nitrate sensor 1)
- Deep cast: 21 Niskin bottles (OPC 1, mini PVM 1, Nitrate sensor 1)
- Deep cast with High Pressure Serial Sampler (HPSS): 13 Niskin bottles (hyperbar sampler 4*2=8, OPC 1, mini PVM 1, Nitrate sensor 1)

4) 24h physical and chemical characterisation

In case of large physical variations occurs during the 4-5 days time survey, we will repeat the sampling grid at the end the measurements.

Three steps will be necessary in order to quantify the nutrients input by hydrological processes in the 3 contrasting locations.

1/Precise localisation of a site and estimation of the horizontal advection
-A few days before arriving on station: examination of SST and Sea color satellite images together with temperature and current fields from Mercator products in order to determine the zone to be studied.
-Arriving on the area of the site: perform a short horizontal survey to observe the SST, SSS and the currents. Choose the site location.
-Arriving on the site: Perform some vertical casts around the location to control the depths of the photic zone and of the pycnocline, and detail currents that will be analysed after the cruise to evaluate horizontal advection of each parameter.

2/ Estimation of the turbulent activity
For each CTD profile, measure at 24 Hz each variable (T, S, O_2, NO_3) and calculate the vertical profile of the horizontal current (LADCP) at a vertical resolution of 2 m. The data treatment will give average profiles and TS anomalies with a high vertical resolution, the amplitudes of internal waves and eventually density inversions, which marks turbulent activity.

3/ Atmosphere ocean physical interactions
Water, heat and moment surface fluxes, will be calculated with considering the bulk method in order to evaluate the atmospheric forcing on the mixed layer activity and its temporal variability.

2- List of parameters and methods

Hydrodynamics and optics :

Parameter	Method
Temperature, salinity	• CTD SBE 911
Horizontal Current on vertical profile	• LADCP 300 Khz (INSU) Rosette mounted
Horizontal Current	• ADCP (75 KHz, 300 KHz Ship mounted)
Kz	• VMP5500
SST and SSS	• Thermosalinograph SBE 21
Wind speed and direction, Air temperature and humidity, solar radiation.	• On board meteo station
PAR	• Downwelling irradiance measured on the Deck by a TRIOS radiometer (350 nm to 850 nm every 3 nm)
In water upwelling radiance and irradiance	• Radiometer TRIOS (350 nm to 900 nm every 3 nm) mounted on a specific frame.
Backscattering, attenuation, fluorescence, and CTD	• Wetlabs optical package mounted on a specific frame
Fo, Fm, Fv/Fm, sigma-PSII, tau-PSII, tau-PQ	• Custom-built bench-top fast repetition rate fluorometer (FRRf)

Biogeochemistry :

Stocks :

Parameter	Method
Inorganic nutrients concentration: Nitrate, nitrite, phosphate, orthosilicic acid Nitrate (ISUS in situ) Ammonium Dissolved iron concentration	• Visible Spectrophotometry • UV spectrophotometry • Fluorescence • Chemiluminescence
TCO_2	• potentiometry
TA	• potentiometry
Oxygen	• Winkler • SBE43 sensor
Dissolved Organic Matter: DOC, DON, DOP	• Visible spectrophotometry after digestion
Particulate Matter: COP, NOP, POP, BSi and LSi C, N, P, Mg, Fe, elemental analysis	• CHN • Visible spectrophotometry after filtration and digestion • X-Ray Fluorescence spectroscopy
TOC analysis	• High temperature catalytic oxidation
Carbohydrate analysis	• Liquid chromatography High Pressure Anionic Exchange Chromatography / Pulsed Amperometric Detection (HPAEC-PAD)
Lipid analysis	• Iatroscan Analyzer (Thin Layer Chromatography / Flame Ionisation Detection (TLC/FID)

Fluxes :

Parameter	Method
Primary production (carbon uptake)	• RA incorporation (^{14}C)
Nitrogen fixation	• Mass spectrometry, dual labelling $^{13}C/^{15}N$ procedure
Phosphate uptake (affinity, maximum uptake rate and Turnover time)	• RA incorporation (^{33}P)
Silicic acid uptake	• RA incorporation (^{32}Si)
NCP	• Δ Oxygen
GCP	• Δ Oxygen
DCR	• Δ Oxygen
Ectoenzymatic activity (lipase, protease, aminopeptidase, phosphatases	• Spectrofluorometry with substrate MUF- and MCA-fluorogenic substrates

Lipase activity	- Radiolabelling with 3H-triolein
Turnover of DOP	- Radiolabelling with 33P-ATP and 3H-ATP
Prokaryotic heterotrophic production	- Radiolabelling with 3Hleucine and TCA extraction of the protein precipitate
Turnover of amino acids	- Radiolabelling with 3H-amino acids

Biology :

Phytoplankton

Parameter	**Method**
Pigments	- HPLC
Species richness of diazotrophs	- Genetic comparison of nifH gene
Abundance of diazotrophs	- Cell specific fluorescent probe hybridization (TSA-FISH technique)
Diatom abundance	- Optical microscopy
Coccolithophore morphology	- Polarization Optic Microscopy, Scanning Electron Microscopy
Coccoliths weight	- SYRACO (Beaufort 2005)
Coccolithophore biodiversity	- LSU rDNA clone libraries, after high-GC genomic extraction and PCR amplification with haptophyte specific primers.
Coccolithophore abundance	- COD-FISH (Frada et al. 2006)
Pico-haptophyte diversity	- LSU rDNA analyses

Protistan

Parameter	**Method**
Abundance and biomass	- Optical and epifluorescence microscopy
Growth rates	- Dialysis Bags for population estimates
Grazing rates and prey selectivity	- Labelled preys

Mesozooplankton

Parameter	**Method**
Species richness	- Taxonomical determination
Abundance and biomass of zooplankton	- OPC: Optical plankton Counter, microscopic counts and dry weight
Fecal pellets production (bulk and individual production)	-
Respiration	- O_2 consumption, CO_2 release
Excretion	- Dissolved inorganic ammonium and phosphate meas.
Ingestion	- Chlorophyll though gut fluorescence
Egg production	-

Uper meso- and macrozooplankton

Parameter	**Method**
Abundance and size spectra	- Specific plankton nets and Zooscan
Taxonomic determination	Zooscan + experts (microscopy)
Diel migration	- Plankton net + Underwater Video Profiler
Vertical structure of assemblages	- Underwater Video Profiler

Picoplankton

Parameter	**Method**
Planktonic abundance (specific verification)	- Flow cytometry
Microplankton characterization	- Lugol fixed samples
Abundance of specific picoeucaryote groups - Prasinophyceae - Photosynthetic stramenopiles - Prymnesiophyceae	- FISH (fluorescent in situ hybridization)

- Alveolates group I and II	

Bacteria (including AAP)

Parameter	Method
Phylogenetic diversity	- FISH, SSCP, clone library, sequencing
Abundance of AAnPB	- Flow cytometry, image analysis, epifluorescence and quantitative PCR
Molecular diversity of AAnPB	- Cloning and sequencing pufM gene

Virus

Parameter	Method
Viral diversity	- Pulsed-field gel electrophoresis and DGGE

Coupling Biogeochemistry and biology

Parameter	Methods
Phosphate uptake at a group of species specific level	- Flow cytometry (cell sorting) and RA incorporation (^{33}P) - Flow cytometry (cell sorting) and RA incorporation (^{33}P) in combination with MICRO-FISH
Si deposition rates at a species specific level	- New fluorescent probe PDMPO and RA incorporation (^{32}Si)
Specific incorporation or organic molecules by individual phylogenetic groups of prokaryotes	- Micro-autoradiography coupled to FISH

Specific material

Parameter	Method
Marine snow vertical abundance	- Underwater Video Profiler
Marine snow size spectra (integrated every 5m)	Underwater Video Profiler
Diel marine snow dynamics	- Underwater Video Profiler
Vertical flux of calculated marine snow carbon	- Underwater Video Profiler
Effect of hydrostatic pressure on marine prokaryotes	- High Pressure Serial Sampler
Effect of hydrostatic pressure on degradation of sinking particles	- Particles Sinking Simulator
O_2 production	- O_2PA : oxygen productivity autosampler

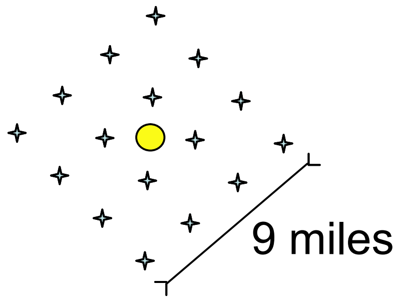
3- General development of BOUM cruise:

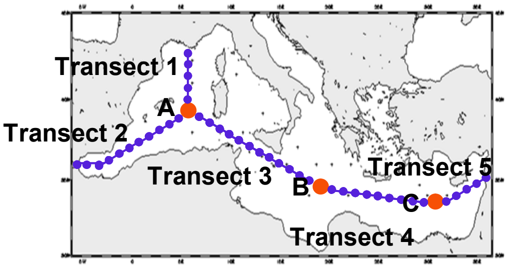
Total duration: 35 days
Period: August-September 2008

4- Provisional schedule and locations of stations:

Figure 2: General location of the Mediterranean Sea ecosystems under investigation in the sub-project (red dots) and short "core parameters" stations (blue dots).

A: Alboran Sea, B: central Western basin, C: central Ionian basin, D: central Levantine basin) and transects of short stations. Details concerning the sampling grid strategy (below) and position of the in situ mooring (yellow) deployed before the 96 h of process study.





Day	location	Time and operations
J1		Boarding
J2-J3		Transect 1 (5 short duration stations) Localisation of site A
J4 -J5	Site A (1st stay)	48h process study
J6-J7	From Site A to Gibraltar	transit
J8-J10	From Gibraltar to site A	Transect 2 (11 short duration stations)
J11	site A (2nt stay)	Sampling grid strategy
J12-J15	Site A	96 h process study
J16-J19	From site A to site B	Transect 3 (12 short duration stations) Localisation of site B
J20	Site B	Sampling grid strategy
J21 –J24	Site B	96 h process study
J25-J26	From site B to site C	Transect 4 (9 short duration stations) Localisation of site C
J27	Site C	Sampling grid strategy
J28-J31	Site C	96 h process study
J32-J33		Transect 5 (5 short duration stations)
J34		Transit
J35		Deboarding

DOCUMENT N° 3	**DOSSIER DE DEMANDE : BOUM** **DATE : 2007**

MOYENS A METTRE EN OEUVRE

- **Navire support, submersibles* et positionnement ;**
- **Équipements de base :** ***matériel mis en oeuvre par* GENAVIR*, par* l'I.F.R.T.P.*, ou par* l'IRD *et matériel propre de l'équipe demanderesse***

Quel matériel prévoyez-vous d'emprunter aux Parcs Nationaux (INSUE, IFREMER-GENAVIR, IRD, Météo-France, EPSHOM…)
- **Installations du navire qui doivent être utilisées**
- **Personnel spécialisé**
- **Besoins en équipements ou matériels complémentaires : quels types ? Quelles spécifications ?**
- **Est-il prévu d'embarquer des produits chimiques ou radioactifs (lesquels) ?**
- **Matériel fournis par des organismes extérieurs**
- **Évaluation du coût de fonctionnement à la charge de l'équipe scientifique**

The research vessel will be « Pourquoi Pas" or the "L'Atalante" with all basic equipment for hydrological work (Hull-mounted ADCP :75 KHz, 300 KHz)

Material that should be asked to the DT INSU:

Equipment DT INSU	quantity
CTDOD SBE 911+ rosette 24 + 24 bottles (With special frame for min DIG-UVP)	1
Fluorimeter	1
Transmissiometer	1
PAR surface and in situ	1
pinger	1
Winch and kevlar cable	1
Oranos cable (650 m) + associated equipment	1
teflon messengers for GoFlo bottles	
Container for iron chemistry (20 feet clean Container)	1
Driffting sediment traps. (3 traps+ argos + gonio)	1
LADCP 300 Khz (INSU) Rosette mounted	1
ADCP (75 KHz, 300 KHz Ship mounted)	
In situ chemical profiler	1
Milli Q water system	1
Packard scintillation counter	1

Equipment IFREMER	quantity
Container for Radio isotopes utilisation (^{14}C, ^{32}Si, ^{33}P, ^{3}H-leucine)	1

Laboratory containers provided by french laboratories : Container station biologique Roscoff

Material provided by other organisms:

ISUS Nitrate : Université Dalhousie/Satlantic Inc. CANADA
HPLC : Woods Hole Oceanographic Institution USA
Custom-built bench-top fast repetition rate fluorometer (FRRf) Oregon State University USA

DOCUMENT N° 4	**DOSSIER DE DEMANDE : BOUM** **DATE :** prévue pour janvier 2007

SAMPLE ANALYSIS AND DATA TREATMENT

- **Analyses et traitements des échantillons et des données effectués à bord (liste exhaustive, référence des méthodes de traitement)**
- **Analyses et traitements des échantillons et des données effectués à terre (liste exhaustive, référence des méthodes de traitement) - calendrier**
- **Archivage des données (lieux, supports, mise à disposition des utilisateurs)**
- **Stockage des échantillons (lieux et dispositifs pris pour leur conservation, leur maintenance et leur mise à disposition des utilisateurs)**
- **Prévision et calendrier de publication des résultats (distinguer rapports de données, articles scientifiques, thèses …)**

Data acquisition during the cruise

« Ship » data acquisition and storage (navigation, meteorology…) are the responsibility of the IFREMER crew

The acquisition of other scientific data is the responsibility of the scientific team during the cruise. A detailed description of methodologies acquired during the cruise will be established at the end of the cruise and is the responsibility of the scientific leader and L. Prieur who is responsible for the physics. This document will constitute a framework within which to expand the data base and its catalogue.

Data base access for the BOUM scientific team.

Scientific data, validated by the scientists, will be transferred as electronic data files to the CYBER data base, which is kept at Villefranche-Sur-Mer, and has restricted access via the internet. The responsible correspondants are Louis Prieur and Claudie Marec for hydrological data, France Van Wambeke for biological data and Cecile Guieu for biogeochemical data. In addition, data corresponding to the "SESAME" parameters will also be transferred to the **WP2 SESAME data bank, in MEDATALS format**. Data management will be the responsibility of Thierry Moutin. Technical aspects concerning the management of the CYBER data base and the transfer procedures to the SESAME data base will be realised by Marie-Paule Torre.

Data access

To start with access will be restricted to authorized BOUM community members and SESAME community members for SESAME parameters. Public access will be given after 2 years, following the CYBER rules (http://www.obs-vlfr.fr/proof/vt/infos/mini-charte.htm)

Calendar

Data type	Data acquisition period	Dead line for data collection in the data base	Public access to the data base
Measurements and analyses on board	Aug-Sept 2008	Sept 2009	Sept 2010
Post Cruise measurements (Traps)	March 2009	March 2010	March 2010

Possible exceptions

The delay necessary for some analyses, notably those of molecular biology, means that these deadlines may not be met. The CYBER committee will be informed in due time of such exceptions.

Publications

2009: First round of publications.
2010: publications in a special issue.

LEFE_Annexe 3 : Dossier scientifique : specific contributions and other references

A 1.1. *Penetration of anthropogenic carbon*

The sole time-series data available from the DYFAMED site indicate that over the past ten years, the temperature of these waters has increased and the capacity of these waters to absorb anthropogenic carbon has decreased (Touratier et al., 2005a). It is thus probable that this temporal trend observed in one area of the Mediterranean Sea is similar in other areas of the Mediterranean Sea. This has to be quantified accurately in order to determine the role of the Mediterranean Sea in the drawdown of anthropogenic carbon and consequently the role of its transport from the Mediterranean Sea to the Atlantic Ocean. It is particularly important to get data from the area of the LIW (Levantine Intermediate Water) formation in the eastern part of the Mediterranean Sea. This intermediate water plays a crucial role in the deep water formation in the western basin (Millot, 1999) and contributes to the water outflow through the Strait of Gibraltar. As a consequence, part of the carbon sequestered will be integrated in the global circulation.

The quantification of the anthropogenic distribution in the Mediterranean Sea will be computed using the simple and recent TrOCA approach (Touratier & Goyet, 2004a,b) that has been applied in the Atlantic ocean and the Gulf of Cadiz (Touratier et al., 2005b, Aït-Ameur et al., 2005) and the Antarctic ocean (Lo Monaco et al., 2005). We plan to measure TCO_2 and TA by potentiometry from discrete samples collected throughout the water column. The measurement technique is fully described in DOE (1994), and we will use reference material that we will buy from Dr. A. Dickson's lab (Scripps, CA, USA).

It is also planed to estimate the distribution of anthropogenic carbon using the 3D Mediterranean Sea model (with a resolution of 1/4° x 1/4°) developed by the GHER (University of Liège, Belgium) which is now running on a bi-opteron workstation at the University of Perpignan, France. All C_T measurements realized during the BOUM cruise, in combination with those of the DYFAMED times-series station and the German METEOR 51/2 cruise, will be used to estimate the four parameters (a to d) involve in the interpolation model developed by Goyet and Davis (1997), using a Singular Value Decomposition procedure: $C_T = a + b\theta + c\mathrm{AOU} + d\mathrm{S}$. Once parameters a to d are determined, it is possible to estimate C_T when the properties θ, O_2, and S are known. Similarly, using all A_T measurements from the BOUM cruise, we will determine the best linear relationship relying A_T to S: $A_T = e\mathrm{S} + f$. The next step is to compute the 3D fields of initial conditions for C_T and A_T on the grid model in order to run the 3D GHER model of the Mediterranean Sea. This will be realized using the two previous relationships for C_T and A_T and the MEDAR/MEDATLAS II 2002 database from which numerous measurements are available for the three required properties θ, O_2, and S.

All data that describe the carbonate system properties within the Mediterranean Sea will be gathered to perform the validation of the 3D model results. The model results will be use primarily to identify and understand the main meso-scale processes governing the sequestration of anthropogenic carbon. It is planed to perform simulations with a timescale varying from a year to a few decades.

A 1.2. *Diversity of diazotrophs*

The diversity of diazotrophs (photosynthetic or heterotrophic species either free living or associated with other organisms) will be assessed by measuring both their species richness and the abundance of each species using molecular techniques. The species richness will be investigated by the detection and the genetic comparison of nifH gene, which code for nitrogenase, the enzyme responsible for di-nitrogene fixation (Zehr et al. 2001). Clone library technique will be used to assess species richness. When the abundance of each diazotroph will be measured by whole cell specific fluorescent probe hybridization (TSA-FISH technique) approaches (Biegala et al. 2003). Diversity of diazotrophs will be investigated using different size fractionation at the 3 long duration stations through the water column, from sub-surface (-1 m), going through the chlorophyll maximum and down to below the euphotic layer. Changes in diazotrophs diversity will be also investigated during 4 days microcosms experiments. For each microcosm, water from each of the 3 long-duration stations will be enriched with different input in nutrients (N and/or P) and iron types. In addition, diazotroph activity will be measured in parallel to either in situ microcosm sampling (see section below).

A 1.3. *Diversity of diatoms*

The species present in the biogenic silica maximum observed in the Ionian Sea during the PROSOPE cruise were not identified. Diatoms are usually a minor contributor to phytoplankton abundance in very oligotrophic areas, but this deep biogenic silica indicates that some species could thrive at lower light levels where nutrients become more abundant. Whether diazotrophic diatoms such as *Rhizosolenia*, which hosts the cyanobacterium *Richelia*, may be found at deeper levels in the eastern MS is still unknown. Sample collection at several depths between the surface and the nutricline at the chosen study sites will allow a characterization of the diatom community structure and will reveal whether diazotrophic diatoms may be of importance in the N_2 fixation budget of the MS.

A 1.4. *Diversity of cyanobacteria*

Recently, the availability of numerous marine cyanobacteria genomes (11 *Prochlorococcus*, 12 *Synechococcus*) and of extended environmental gene sequences of both genera (Craig Venter databases) provides an unprecedented opportunity to characterize the expression of key genes involved in different biological processes (photosynthesis, carbon fixation, DNA repair, etc). This approach will allow us to study, directly in situ, physiological adaptation of cyanobacteria to environmental conditions (light, UV, temperature, CO2, nutrient deficiency, etc), and possible responses to environmental factors/stresses.

Laboratory studies have already allowed us to identify two molecular markers : a marker for UV stress using a gene coding for a DNA repair protein (*recA*, encoding the recombinase A) and the second one for growth rate status using the *ftsZ* gene, the product of which is a key factor for the initiation of cell division (Holtzendorff et al. 2002). Furthermore, a new marker indicating the carbon fixation level (*rbcL* coding for the RUBISCO) is actually under development in our laboratory (Wawrik et al. 2002). The use of real time PCR to follow the expression of these genes will allow circumventing the low amount of material present in sea water and to quickly analyze a large amount of samples as it is necessary in an oceanographic context. The expression of these genes will be studied over depth profiles and horizontal transects along the Mediterranean trophic gradient.

A 1.5. *Diversity of aerobic anoxygenic phototrophic bacteria (AAnPB)*

AAnPB are strict aerobes containing bacterial photosynthetic centers composed of bacteriochlorophyll that have been discovered in organic-rich marine environments. These bacteria are facultative phototrophs; they grow heterotrophically but can use light as additional source of energy. This implies that photosynthesis could be important for this group under conditions when the availability of dissolved organic carbon is limiting growth. It was therefore initially hypothetized that these bacteria may have a large ecological advantage and thus be more abundant in the opean ocean and oligotrophic areas. AAnPB genes which encode the pigment binding subunit of the bacteria photosynthetic reaction center (*pufLM* genes) have been found in estuarine and oceanic DNA samples collected around the world and their analysis has demonstrated the remarkable diversity of these bacteria (Béjà et al., 2002; Oz et al., 2005). Given the potential large impact that AAnPB may have on marine carbon cycling and some recent conflicting reports on their abundance in the world ocean (Schwalbach & Furhman, 2005), more work is needed to determine the ecological significance of these photoheterotrophs. Moreover, AAnPB may also play a significant role in the competition for phosphate in P-depleted environments.

The abundance of AAnPB will be assessed by flow cytometry (a specific protocol will be developed), image analysis and epifluorescence microscopy and quantitative PCR (Schwalbach & Furhman, 2005). The diversity of AAnPB will be assessed by cultural and molecular approaches. Different culture media will be used to isolate numerous pigmented strains in order to generate an extensive culture collection of these bacteria (Koblizek *et al*., 2003). These isolates will be genetically identified and characterized and examined for a suite of metabolic capacities. Molecular diversity will be assessed by cloning and sequencing *pufM* genes that are specific of AAnPB in pelagic areas (Oz *et al*., 2005). Taken together, results from these experiments will uncover the distribution and genetic and metabolic range of AAnPB.

A 1.6. *Eukaryotic picoplankton diversity*

Important eukaryotic groups will be quantified by Fluorescent in situ hybridization along the transect, and for different depths.

Photosynthetic picoeucaryotes. We will determine the dominant photosynthetic taxa at each station and depth and will try to establish the relationship between community composition and trophic status.

Marine Alveolates group I and II. These organisms are only known from environmental sequences and their nature is still elusive. We suspect that group II may correspond to parasites of larger phytoplankton such as Dinoflagellates. We will determine the abundance of the different clades of alveolates by FISH and will attempt to establish relationships with the microplankton community.

A 1.7. *Diversity and abundance of haptophytes and coccolithophores.*

The haptophytes are one of the main groups of marine microalgae and contain both calcifying (coccolithophores) and non-calcifying species (de Vargas and Probert 2004). The coccolithophores (~300 morphospecies) have evolved an astounding morphological diversity of calcified skeletons to flourish at different depths along the photic zone from surface waters to –200m. Through the process of intra-cellular biomineralization (or *coccolithogenesis*, Young et al. 1999), the coccolithophores control most of the open ocean carbonate chemistry and may be the major force for the oligotrophic biological pump their ballasting effect (Klaas and Archer 2002). Today, ocean acidification driven by anthropogenic CO2 threaten coccolithophore calcification and its buffering effect on the ecosystem. On the other hand, the non-calcifying haptophytes maybe of fundamental importance for primary production in oligotrophic oceans. Significant amount of the carotenoid pigment *19-hexanoyloxyfucoxanthin* and prymnesiophyceae cells were respectively detected by HPLC (Andersen et al. 1996) and FISH (Not, personal communication) in oligotrophic waters of the Pacific and Atlantic oceans. These naked haptophytes are tiny cells within the picoplanktonic fraction and may account for most of the primary production in many cases (more than the Synecococcus/Prochlorococcus production). This biodiversity of these pico-haptophytes is yet to be explored.

At the three main BOUM-station, we will analyze the morphological and genetic diversity of coccolithophores at 6 different depths, from surface waters to below the deep chlorophyll maximum. Different sorts of filters for comparative *SEM microscopy*, *Optic Microscopy*, *DNA extraction*, and *FISH analyses* will be prepared from 30 liters of water extracted from each depth. Polarization-optic microscopy will allow us to rapidly determine and quantify the coccolithophore flora at each station. Representative samples of the different flora along the transect and across depths will then be selected and thoroughly analyzed via SEM, and some of them will serve as template for LSU rDNA clone libraries. In addition, a new method recently developed in our laboratory (the COD-FISH, Frada et al. 2006) will be used to quantify the calcifying versus non-calcifying coccolithophores, a phenotypic status which can depend on the life-cycle stage. Coccoliths weights of key species will finally be assessed in collaboration with Luc Beaufort (CEREGE; Beaufort and Dollfus 2004; Beaufort 2005). For the analysis of pico-haptophyte diversity, we will select samples particularly rich in prymnesiophytes as detected in A.2.4 by FISH data, and build at least one LSU rDNA clone library per station using haptophyte specific primers.

A 1.8. *Diversity of viruses*

Viruses are on average 10 times more abundant than cellular organisms and they can cause significant mortality of bacterio- and phytoplankton (Fuhrman 1999; Suttle 2005). Metagenomic analyses suggest that viruses are very diverse and that most viruses in surface waters are bacteriophages, i.e. viruses infecting bacteria. Up to 7000 different types of viruses can be found in a 200 liter water sample and this is a conservative estimate, since rare viruses are not detected (Breitbart, Salamon et al. 2002). While cellular organisms have common genes such as the small subunit of rRNA, which can be used as phylogenetic marker, even subgroups of viruses, such as tailed bacteriophages, do not have a common gene. Thus, assessing diversity of viruses is difficult. Using pulsed-field gel electrophoresis, viruses from aquatic systems can be separated based on their genome size. Although several viruses can have the same genome size, this approach was surprisingly successful in describing viral diversity of systems or detecting changes with time and space. For specific groups such as algal viruses, cyanophages or podoviruses (a type of tailed phages infecting bacteria), primers have been developed and this allows genetic fingerprinting of their communities. In general, studies on viral diversity are still rare.

PFGE needs a relatively large volume of water, ca. 5-10 liters in oligotrophic systems. After prefiltration to remove cells, viruses are concentrated using ultrafiltration and –centrifugation and the genome is separated by size on agarose gels under pulsed electric fields (Wommack, Ravel et al. 1999). Primers are available for several groups of viruses and genetic fingerprinting techniques such as denaturing gradient gel electrophoresis (DGGE) have been applied to assess viral richness, i.e. the number of different viral types (Chen & Suttle 1995; Short & Suttle 2000). A benefit of this method is that bands can be excised from gels and sequenced to obtain a phylogenetic affiliation of the viruses.

Viral abundance can be detected by flow cytometry (Marie et al. 1999). Vital production and the impact of viruses for bacterial mortality can be studied using approaches such as transmission electron microscopy inspection of infected cells and a virus reduction approach (Weinbauer et al. 2002; Wilhelm et al. 2002).

A 1.9. *Resource and Consumer (tintinnid ciliate) diversity*

Among the microzooplankton are tintinnid ciliates which possess attributes making them ideal candidates to use as indicators of consumer diversity. Tintinnids are a monophyletic, species-rich group (> 600), all ecologically similar as consumers of phytoplankton. Species identifications are based on morphological characteristics of their shell or lorica. Thus tintinnids provide an opportunity to compare the diversity found in lower trophic levels (e.g., diatoms, cyanobacteria, eukaryotic picoplankton) to that of their consumers

Diversity in tininnid ciliates, members of the microzooplankton, will assessed using classical taxonomic criteria- morphology. In tintinnid ciliates, morphology is not only of taxonomic significance; morphology is also closely related to feeding ecology (Dolan et al. 2002). Diversity will be estimated in terms of taxa (species, evenness) as well as in terms of morphology: numbers of distinct size-classes and evenness (Dolan 2000; Dolan & Gallegos 2001). Spatial trends in tintinnid resources (phytoplankton) will be compared to tintinnid diversity, both taxonomic and morphological.

A. 2.1. *Dissolved Inorganic Phosphate availability:*

It is not always easy to identify the limiting nutrient (N or P?) and its magnitude (e.g. P-deficiency vs. P-limitation). Since PO4 is considered to be the most readily available form of P to osmotrophs, it would appear simple to measure this pool (i.e., PO4 availability). However the molybdenum blue reaction method, which is currently used as the ‘standard method’, measures not only orthophosphate but also has a potential ‘background’ such as from acid labile dissolved organic P and arsenate. This is why the measured phosphate is defined as soluble reactive phosphate (SRP). In addition, SRP concentrations are often below the detection limit of the standard method in euphotic layers during the stratified period. Recently Rimmelin and Moutin (2005) have improved the magnesium co-precipitation method, so-called MAGIC25, by which PO_4 in water can be detected down to 0.8 nmol L^{-1} without significant interference by SRP background. In addition, specific affinity for PO_4 uptake for osmotrophs, which is the slope of the specific uptake for PO_4 versus PO_4 concentration curve, is a very useful diagnostic tool for detecting P limitation among planktonic systems of different trophic status (Tanaka et al. *submitted*).

At each station, water samples will be collected from different depths from euphotic down to mesopelagic layers (ca. 1000 m). To compare chemically measured PO_4 concentration, biologically required PO_4 pool, and specific affinity for PO_4 uptake, measurements in concentration and turnover time of PO_4, and bacterial biomass and production will be done down to mesopelagic layers, while those of phytoplankton biomass and production are only down in euphotic layers.

A 2.2. *Longitudinal variation of N:P balance in the euphotic layer*

To understand ecosystem productivity, not only the question of which nutrient is limiting, but also the excess amount of the next potentially limiting nutrient, is of great importance. Alkaline phosphatase activity (APA) has long been used as a molecular indicator of P deficiency of osmotrophs. Thingstad and Mantoura (2005) showed that APA in the P-starved eastern Mediterranean surface waters is induced by excess NH_4 and reduced by excess PO_4 and that most of the APA reduction by excess PO_4 seems to be caused by repression of AP production, combined with inactivation of the existing enzymes. Using these characteristics, they established a new method for estimating excess N by titrating P-starved water samples with PO_4 until APA disappears and excess P by titrating P-pulsed water samples with NH_4 until APA is induced during a Lagrangian experiment (Thingstad and Mantoura 2005). However this method has not yet been applied to other parts of the Mediterranean.

We plan to estimate excess N or P using the APA titration technique at each station. Water samples are collected from surface, chlorophyll maximum depth, and bottom of the euphotic layer, and incubated with different concentrations and ratios of N and P.

A. 2.3. *Silicic acid availability:*

Recent studies have shown that silicic acid availibility could exert a control over diatom abundance and Si production in the Mediterranean Sea (Leblanc et al., 2003) where previous observations discarded the role of Si as a potential limiting factor for phytoplankton growth in this region (Crispi et al., 2002). Nonetheless, very low silicic acid surface concentrations (<1 µM) have been observed in different parts of the Mediterranean basin and are likely to limit diatom growth. The silicon cycle in the Mediterranean Sea remains poorly documented, and this project further aims at quantifying the various silicon pools (dissolved and particulate) at the chosen study sites.

A. 2.4. *Iron availability :*

Although low concentrations of dissolved iron were found at the DYFAMED site at the end of the bloom period (<0.13 nM: Sarthou & Jeandel, 0.2 nM: Bonnet & Guieu, *submitted*) that could have an impact on biological activity at this time (in particular by lowering the intensity of the bloom after most of the iron was depleted), high DFe encountered at the end of the stratification period (due to the accumulation of atmospheric Fe during this low productivity period) (Guieu *et al.* 2002, Bonnet & Guieu *submitted*, Sarthou & Jeandel, 2001) may only have a limited effect on biological activity. However, it has been suggested by Bonnet & Guieu, (*submitted*) that the occurrence of Saharan input during summer could stimulate N_2 fixation, not only because of (high) DFe concentrations in surface waters but by providing a source of new P (Ridame & Guieu, 2001). [DFe] distribution in Mediterranean Sea is poorly documented, in particular in the Eastern Basin. The accumulation in the surface mixed layer will highly depend on the intensity of the atmospheric flux that will happen during the summer prior to the cruise and we may also expect a situation where very low Saharan inputs occur leading to poorly Fe enriched surface waters that could affect the ecosystem functioning.

Dissolved iron distribution will be determined on deep profiles at each of the 4 sites with emphasis on the surface distribution.

A 2.5. *N_2 fixation*

Extensive nitrogen fixation in the Mediterranean Sea has been inferred from nutrient budgets (Bethoux and Copin –Montégut, 1986; Béthoux et al., 1992) and elevated N/P ratio in deep waters. $\delta^{15}N$ data in fossil chlorophyll provide geochemical evidence of extensive nitrogen fixation in the eastern Mediterranean in the past (Sachs and Repeta, 1999). Kerhervé et al. (2001), using $\delta^{15}N$ data in settling particles suggest that dissolved atmospheric N_2 may act as a significant new nitrogen source. Based on calculations of excess dissolved fixed nitrogen (N*) in the MS, Gruber and Sarmiento, 1997 suggested that N_2 fixation should be an essential key in explaining the high N/P ratio in Mediterranean waters. Recent work (Garcia et al., submitted) conducted at the DYFAMED site has revealed a significant nitrogen fixation throughout the year, in spite of absence of *Trichodesmium*. While nitrogen fixation rates were typically quite low relative to the apparent demand of the primary producers, this biological process supplies significant new nitrogen which can balance the nitrogen budget and could explain the high nitrate/phosphate ratio observed in deep water.

At the 3 long duration stations, N_2 fixation and CO_2 uptake rates will be measured throughout the water column with the tracers $^{15}N_2$ and $^{13}CO_2$, following the general procedure described in Montoya et al., (1996). Labelling with $^{15}N_2$ and $^{13}CO_2$ permitted the assessment of nitrogen fixation by diazotrophs and carbon fixation by whole plankton community. Both parameters will be measured on the total fraction and at specific depths, on different size fractions (according to those chosen for the diversity of diazotrophs, cf. objective 2). The natural abundance of ^{15}N ($\delta^{15}N$) and ^{13}C ($\delta^{13}C$) on particulate matter in the euphotic zone, and in settling particulate matter (from sediment traps) will also be measured.

A 2.6. *Atmospheric dust traces in the upper water column and their potential impact on the N_2 fixation rate*

Dust deposition from Saharan dust clouds or “red rains” in very oligotrophic regions of the MS may be a potential source of fertilisation for surface waters, inducing higher Fe concentrations. This type of dust deposition can be traced in the surface layer by measuring lithogenic silica (LSi) which is an important component of terrestrial dust. Higher surface concentrations of LSi in the eastern MS have previously been used to confirm a dust deposition flux in the surface layer (*Claustre et al., 2002*). The availability of phosphate and/or iron is suspected to limit the N_2 fixation (Berman-Frank et al., 2001; Sanudo-Wilhelmy et al., 2001; Mills et al., 2004, Moutin et al., 2005). Saharan dust inputs, by supplying both iron and phosphate, may stimulate *in situ* nitrogen fixation in oligotrophic ecosystems. Lenes et al. (2001) and Walsh & Steidinger (2001) reported a large *Trichodesmium* bloom in response to a Saharan dust event on the West Florida shelf. More recently, Mills et al., (2004) demonstrated for the first time that the addition of Saharan dust in surface waters of the Eastern Tropical Atlantic (bioassay experiment) enhanced significantly nitrogen fixation rate by diazotrophs. The Mediterranean Sea is strongly impacted by Saharan dust input (Loye-Pilot and Martin 1996; Guerzoni et al. 1999). We can hypothesize that Saharan dust deposition could promote N_2 fixation in the Mediterranean Sea particularly during stratification period, ie when the atmosphere may be a significant source of allochtonous nutrients to the surface waters.

Vertical profiles of lithogenic silica will be conducted at the study sites to investigate the occurrence of dust deposition events. These measurements will be coupled to satellite imagery showing the aerosol index over the MS (TOMS,
http://toms.gsfc.nasa.gov/aerosols/aerosols.html).

A 2.7. *Dissolved vs particulate primary production:*

The extracellular release of recently fixed photosynthate is a major process contributing to the production of dissolved organic carbon (DOC) in marine ecosystems. This source of DOC is particularly important for the trophic ecology of plankton, since the released compounds are susceptible to rapid uptake by heterotrophic bacteria (Cole et al. 1982), thus creating a link between primary and bacterial production that is essential for the cycling of matter through the microbial loop and the microbial food web (Ducklow & Carlson, 1992). Despite its widely acknowledged ecological and biogeochemical significance, DOC production is not routinely measured in most surveys of productivity in the sea, which typically include determinations of particulate organic carbon (POC) production only. For this reason, our knowledge of the dynamics of microbial DOC production in marine ecosystems is still limited. As is the case with many other marine regions, dissolved primary production has not been well studied in the Mediterranean Sea. The measurements collected during the BOUM cruise along a biogeochemical gradient will allow us 1) to gain deeper insight into the mechanisms responsible for the extracellular release of recent photosynthate, and 2) to determine quantitatively what is the relative significance of DOC production and how does this importance change with the productivity of the ecosystem and its trophic structure.

During each process study, dissolved and particulate primary production will be measured daily by ^{14}C uptake experiments. Measurements will also be taken during intermediate stations in between process study sites. In every case, experiments will be conducted at 5-6 depths throughout the euphotic layer. In addition to end-point experiments, several 24-h time series experiments will be conducted, in order to check the linearity in production rates and also to help ascertain the underlying mechanisms involved in the release of dissolved photosynthate. This experimental approach has been succesfully applied before both in eutrophic (Marañón et al 2004) and oligotrophic (Marañón et al 2005) marine waters.

A 2.8. *Assimilation and regeneration of nitrogen: Quantification on new production in oligotrophic system*

The original definition of new primary production rests on the assumption that nitrogenous substrate taken up to fuel algal growth is coming into contact with phytoplankton for the first time that year. Therefore, should the generation of nitrate from ammonium by nitrification turn out to be significant in surface waters then nitrate uptake can no longer be simply ascribed to new production. The results confirm previous limited observations (Raimbault et al., 1999) suggesting that a major fraction of nitrate uptake in oligotrophic regions, previously ascribed to new production, may actually involve "recycled" nitrate. In samples from oligotrophic waters (Raimbault et al., 1999, Diaz and Raimbault, 2000), $^{15}NO_3$ loss as $DO^{15}N$ represented up to 20% of net NO_3^- uptake. Then estimation of new production needs to take into account nitrification as well as DON release, processes that are still poorly quantified in oligotrophic waters, leading to inaccurate *f* ratio estimation and export production rates.

A 2.9. *Dissolved inorganic phosphate uptake at a group of species specific level*

Micro-organisms in the 0.6-2 µm size fraction, probably *Synechococcus*, were found to have, in addition to high affinity for orthophosphate, significantly higher maximum PO4 uptake rates than heterotrophic bacteria or eukaryotic algae (Moutin et al. 2002). These specific advantages concerning orthophosphate uptake at low (< 5nM) as well as at relatively high (5-25 nM) concentrations may explain both general *Synechococcus* abundance in the Mediterranean sea during the stratified period, and transient blooms of this species in the open ocean where episodic orthophosphate nano-pulse events are likely to occur. Nevertheless, phosphate addition to surface waters of the ultraoligotrophic phosphate starved eastern Mediterranean in a Lagrangian experiment, caused a decline in chlorophyll and an increase in bacterial production (Thingstad et al. 2005) that may only be explained by specific advantage of "large" heterotrophic bacteria concerning PO4 uptake. These apparent contradictory results reinforce the need to define PO4 uptake characteristics at the species level.

Aerobic Anoxygenic Photosynthetic (AAnPB) bacteria may also contribute to this phosphate uptake. Although the ecological importance and role of these bacteria are unknown, there is recent evidence that they could represent a significant proportion of total heterotrophic bacteria (up to 20% in oligotrophic waters), primarily due to their large biovolume (Lami et al in prep). Their potential role in PO4 uptake should therefore also be considered.

We propose to couple ^{33}P incorporation and cell sorting techniques to identify the major biological players in PO4 uptake (Lebaron et al 2001, Servais et al 2003). Cell sorting will allow us to discriminate ^{33}P incorporation into autotrophic versus heterotrophic cells of different size classes. Furthermore, we propose to describe the phylogenetic diversity of the sorted cells using single cell (e.g. FISH) and molecular analysis. To determine ^{33}P uptake at the single cell level we propose FISH in combination with microautoradiography (MICRO-FISH) of sorted cells. Additionally, *in situ* bioassay experiments based on PO4 additions could be performed and, based on MICRO-FISH, the phylogenetic groups that are stimulated can be identified. The role of different groups like SAR11, CFB,. in PO4 uptake could be investigated in this way.

A 2.10. *Biochemical fate of PO4 incorporation*

Phosphorus has long been recognized as an essential element for planktonic growth, yet there is very little quantitative information on the specific biochemical functions phosphorus serves in the typical planktonic cell. During several cruises to station ALOHA in the North Pacific subtropical gyre, the synthesis of the phosphorus-containing biochemicals RNA, DNA and lipids accounted for an average of 50%, 20% and 20% of the total incorporation of phosphate respectively (Van Mooy, 2003). However, at any given time, these rates can vary significantly from the average value. So while each of these rates reflect planktonic growth in general, the preferential synthesis of one of these groups molecules over another reflect a complexity that is not normally observed in measurements of total PO4 incorporation. By making these observations within the coordinated framework proposed in the BOUM project, we will gain a more detailed view of the overall chemical requirements for planktonic growth. For example, enhanced RNA synthesis is a signal of protein synthesis, which is a very nitrogen intensive process. While in contrast, the synthesis of lipids demands comparatively less nitrogen, but more carbon. In addition, a transect through the Mediterranean Sea as part of the BOUM project would be an ideal place to study the impact of phosphate limitation on the synthesis of RNA, DNA and lipids; this is in contrast to the North Pacific subtropical gyre, where phosphate concentrations and turnover times are an order of magnitude higher (Björkman et al. 2000; Moutin et al. 2002). We hypothesize that under phosphate-limiting conditions, and greater fraction of total phosphate incorporation will be used for DNA synthesis; parallel research in pure cultures has revealed a number of adaptations that can allow plankton devote significantly less phosphate to RNA and lipid synthesis when dissolved phosphate is scarce in the environment.

Incubations spiked with radiolabeled phosphate will be conducted in the upper 250m at stations along the transect. These incubations will be terminated by filtration on 0.2 micrometer membranes. The membranes will be frozen in liquid nitrogen and then shipped to my laboratory at Woods Hole Oceanographic Institution after the cruise. There quantitative extractions for RNA, DNA and lipids will be conducted. The quantity of radiolabeled phosphate in these incubations will then be quantified by liquid scintillation counting, the rates of RNA, DNA and lipid synthesis will be determined.

A 2.11. *Silicic production and diversity of diatoms*

A new labelling technique was developped in order to trace the silicification process in marine diatoms, and offers new insights in the biological Si cycle by allowing for the first time a discrimination between active and non-active siliceous biomass (Shimizu et al., 2001; Leblanc & Hutchins, 2005). This new fluorescent probe (PDMPO) is incorporated into the diatom siliceous wall, also called frustule, during its formation, and fluoresces under UV-light wherever new biogenic silica is formed. This new molecular tool, coupled with the isotopic measurement of Si uptake rate (using the radioactive isotope ^{32}Si), will allow the determination of the relative contribution of each species within a mix of diatoms to total Si production. A full characterization of the Si cycle in surface waters (dissolved and particulate silicon pools, as well as Si production and export fluxes) will allow quantification of the relative contribution of diatoms to primary production and to the sinking particle flux.

A 2.12. *Ciliates production*

Protozooplankton is an ubiquitous and abundant component of the microzooplankton community in a large number of aquatic systems. This group is mainly represented by heterotrophic Nan flagellates and ciliates which are generally the major grazers in oligotrophic systems where pico- and nanoplankton dominate biomass and primary production (Kuipers and Witte, 2000, Burkill et al., 1995, Karayanni et al., 2005). Food availability appears to exert the major impact on the productivity of ciliates however the standing stocks of protozoa are little informative because due to strong top-down control by copepods (e.g. Christaki & Van Wambeke, 1995). Thus, evaluating protozooplankton growth rates is a key parameter in order to asses a better understanding of the functioning and productivity of the oligotrophic systems. There are still innumerable problems for fixing, identifying and estimating their grazing and production rates. In the few existing in situ studies dealing with ciliate production rates the most popular method is the size fractionation method (Wright & Coffin 1984) with more or less long incubations in polycarbonate bottles. The major problem of this approach is the decrease of the abundance of protists during the incubations due to manipulation and bottle effect (Gifforrd & Caron 2000). In situ incubation of water in dialysis bags allows diffusion of dissolved organic material and minimise bottle effect (Verity 1986). We will assess: (i) Protistan (nanoflagellates and ciliates) abundance and biomass (optical and epifluorescence microscopy), ii) Protistan growth rates using two alternative experimental methods a) size franctionation incubation in bottlres b) size franctionation incubation in dialysis bags. This approach will be completed by the estimation of protistan carbon demand and their potential grazing rates using a theoretical approach (Fenchel, 1987).

A 2.13. *Mesozooplankton production*

The role of the zooplankton component in the pelagic ecosystem is often limited to the study of the main (often larger) copepods, while small species like *Oithona*, *Oncea* and gelatinous zooplankton (ctenophores, siphonophores, salps) displaying a large range of functional response could play an important role in oligotrophic regions. Mesozooplankton, i.e. copepods are important contributor to the Nitrogen pool through dissolved excretion. $P0_4$ excretion might also be important in this P-limited region. Experiment using enriched P-medium can highlight the potential limiting role of this nutrient in the development of local population. The way how copepods may be coupled to lower trophic levels in such conditions should be revisited (Thingstad et al. 2005).

Species richness (taxonomical determination), abundance and biomass of zooplankton (OPC: Optical plankton Counter, microscopic counts and dry weight).

On going measurement of plankton size spectrum will be performed between stations using the boat flow-through system.

Fecal pellets production (bulk and individual production), respiration (O_2 consumption, CO_2 release), excretion (colorimetric methods for NH_4, PO_4), ingestion (chlorophyll though gut fluorescence) of main zooplankton contributors (copepods and main gelatinous organisms)

A 2.14. *Uper meso- and macrozooplankton*

The mesozooplankton size class is comprised between 200 and 2000 µm. The dominant group herein is the copepod group. However, the proportion of zooplanktonic organisms above 1 mm under-sampled or damaged in the net samples is unknown. In oligotrophic zones, the role of planktonic predators and especially those belonging to the gelatinous compartment is important. In addition numerous ‘gelatinous filter-feeder taxa’ play an important role in the oligotrophic geochemistry. In fact the retention and the packaging efficiency of appendicularians and dolioles and their ability to feed on detritic particles make them suitable for the colonization of extreme environments such as the oligotrophic or the deep sea. The lack of information on these groups is due also to the fragility of these organisms and due to the vanishing taxonomic expertise. Appendicularians are known to play a major role in the formation of marine snow. In several oligotrophic zones the vertical migration of zooplankton is perturbed and often inexistent. The up-down control in thess cases is exerted mostly through the gelatinous 'low energy' predators.

The problems inherent to net sampling are: 1) depths or space integration, 2) avoidance and sub-sampling and 3) damage of the organisms. A new plankton net is in construction in LOV. It will allow the acquisition of more than 10 samples during its oblique ascent. The problems described above will be reduced by discrete sampling, the study of optimal porosity and the decreased of noise of the gear as well as through the optimisation of samples handling and preservation. The samples will be analysed using the Zooscan system described at the site www.zooscan.com. The Underwater Video Profiler is described at www.obs-vlfr.fr/LOV/ZooPart/UVP/ and here below.

A 2.15. *Diel variations of submicrometer and mostly non-living particles*

Among the different particulate components, small-sized, mostly non-living submicrometer, particles are believed to be generally a dominant source of the particulate backscattering coefficient, b_{bp}, in surface waters of the open ocean under conditions of no phytoplankton bloom [Morel & Ahn, 1991; Stramski & Kiefer, 1991]. While scattering (b_p) is most sensitive to micron-sized particles, backscattering of spherical particles is relatively more responsive to submicrometer particles. Therefore simultaneous measurements of b_p, b_{bp}, and of the Chl-fluorescence signal may be used to analyse the diel scale of the particulate organic matter, and of its different components especially to know how the diel variations of submicrometer and mostly non-living particles, as revealed by b_{bp}, behave compared to those of larger and mostly authotrophic particles as revealed by b_p?

In contrast to b_p, b_{bp} may be assessed from space (Loisel & Stramski, 2000). Based on this principle, we have shown in the Mediterranean Sea (Loisel et al., 2001), and over the global ocean (Loisel et al., 2002) some remarkable temporal patterns between b_{bp} and Chl. These patterns can be caused by the net accumulation of small, mainly non-living particles in the summer stratified layer, but also by the variation in the chlorophyll-to-carbon (Chl/C) ratio in surface phytoplankton population undergoing photoacclimation [Loisel et al., 2002]. A recent study stated that most of the b_{bp} seasonal variability are due to the C/Chl variability [Behrenfeld et al., 2005]. The different measurements in the BOUM cruise will then allow us to examine these different assumptions at the diel scale for futures interpretations of the remotely sensed b_{bp}.

A conductivity-temperature and depth (CTD) profiler (Sea-Bird, Inc.) and an optical package (WET Labs, Inc.) will be deployed every 3 hours. The optical package included a chlorophyll fluorometer, a beam transmissometer (at wavelength λ=650 nm), and two ECO-VSF (at λ=532 nm and 650 nm, respectively).

3 radiometers for measurements of downwelling irradiances (in water and above water-reference) between 350 and 700 nm will also be deployed around noon.

Water samples will be collected for measurement of absorption by dissolved et particulate material.

A 2.16. *Decoupling of electron flow in phytoplankton thylakoid membranes and photosynthetic carbon fixation*

Measurements of phytoplankton variable fluorescence provide information on the initial steps of photochemical charge separation and electron transport in thylakoid membranes. For ecological and biogeochemical issues, carbon fixation [and more specifically, net primary production (NPP)] is the photosynthetic parameter of greatest relevance. While variable fluorescence data are related to NPP, there are many biochemical steps between initial charge separation and carbon fixation. Over the diel cycle and between ocean regions of changing nutrient stress, this physiological separation of photosynthetic processes can cause a severe decoupling between variable fluorescence and carbon fixation patterns. The understanding and characterization of such decouplings constitutes the basis for relating high-resolution (even space-based) fluorescence data to carbon cycling in the sea. It also provides detailed insights toward interpreting spatial and temporal variability in photosynthetic rates as measured in the field using carbon isotopes. Parameters of particular importance include initial and maximal fluorescence (Fo, Fm), photochemical efficiencies (Fv/Fm), functional absorption cross-sections (sigma-PSII), and electron turnover times downstream of photosystem II (tau-PSII, tau-PQ).

Variable fluorescence measurements will be made using a custom-built bench-top fast repetition rate fluorometer (FRRf). The instrument employs a measurement protocol that allows single-turnover and multiple-turnover determinations of Fo, Fm, and Fv/Fm, single-turnover sigma-PSII and tau-PSII, and multiple-turnover tau-PQ. Measurements will be conducted continuously during the entire cruise using a flow-through of clean seawater. Vertical profiles of variable fluorescence parameters will also be collected using discrete samples (<500 ml) collected from the rosette. The FRRf can also measure changes in variable fluorescence parameters with changes in background illumination. This capability allows assessment of 'photosynthesis-irradiance' relationships at the level of electron transport.

A 2.17. *Organic matter mineralization and bacterial diversity*

At the base of microbial loop, heterotrophic bacteria play the key role of re-mineralizing detrital and dissolved organic matter. The importance of chemical composition of organic matter on bacterial metabolisms and dynamics is well known. Organic matter in the ocean is mainly under the form of polymeric substrates and ecto-enzymatic hydrolysis by specific enzymes is a preliminary step to assimilation of most substrates. Ectoenzymes are specific of chemical forms and are generally inducible. After hydrolysis, small organic forms are directly assimilated into bacteria cells for bacterial production. It has been recognized that the preliminary hydrolysis of bio-polymers by ectoenzymatic activity is often a rate-limiting step for bacterial degradation of organic matter. In addition, at a given environmental condition corresponds a given taxonomic composition of heterotrophic procaryotes. Molecular phylogenetic methods have provided means for identifying types of organisms that are present in microbial communities without cultivation. Fluorescence *in situ* hybridisation (FISH) with r-RNA-targeted oligonucleotides probes selectively visualizes prokaryotic cells with defined phylogenetic affiliation. The recently developed catalyzed reporter deposition FISH (CARD-FISH) method allows the use of oligonucleotide probes labeled with horseradish peroxidase, resulting in a sensitivity comparable to that of polynucleotide probes (Pernthaler et al. 2002). There is also increasing methodological development dealing on functional diversity aspects; notably microautoradiography coupled to in situ-hybridization (MICRO-FISH, Cottrell & Kirchman, 2000) which has also shown that cell specific bacterial production encompasses a large range. By MICRO-FISH technique, a variety of substrates have been tested which are not used in the same proportion by all phylogenetic groups (Elifantz et al, in press). In addition, ectoenzymes are expressed in some specific strains only (Martinez et al., 1996). Due to their species-specific heterotrophic activities, free-living procaryotes are able to modify the biochemical composition of organic matter. Hence, examining relationships between diversity and functionality (ectoenzymatic activity, uptake of specific organic compounds representative of a chemical family) within bacterial community is a major challenge for understanding the impact of prokaryotic heterotrophic processes on mineralization of organic matter along the water column. But there is scarce information in the Mediterranean, particularly in the East, and scarce information on transformation of organic matter in deep waters. Recent studies showed that the relative abundance of free-living *Archaea* increases throughout the water column and becomes about equal to the abundance of *Eubacteria* in deep-sea waters (Karner et al. 2001).

We propose to measure simultaneously 3 families of ectoenzymatic activities (lipase, protease, phosphatase) and the assimilation of their by-products (amino acids, fatty acids, and see A 3.19 for the fate of the DOP hydrolyzed) in combination with MICRO-FISH technique to target phylogenetic groups mostly responsible of heterotrophic activity. This will be made in the upper layer, in the twilight zone and in deep water layers (Van Wambeke et al 2002, Bourguet et al 2003, Cottrell & Kirchman, 2000). The chemo-autotrophic potential of *Archaea* will be determined by measuring dark incorporation of radiolabelled bicarbonate. The chemical composition of organic matter (dissolved lipids, aminoacids, DOP, DOC, DON) could provide information on the quality of substrates available for bacteria from the surface to the deep water column in relation to their heterotrophic activities.

A 2.18. Factors controlling bacterial production and consequence on heterotrophic activities.

Two main tools are generally used to detect P stress within bacteria and phytoplankton: phosphatase alkaline activities and enrichment experiments. Interpretation of the enrichment experiments are not always very clear and often, co-limitation of bacterial production is suggested, notably C - P (Carlson and Caron 2001, Sala et al 2002, Van Wambeke et al. 2002). The second approach is based on the measurements of the activity of the ectoenzyme alkaline phosphatase. Bacteria develop alkaline phosphatase (AP) activities on exogenous substrates to fulfil their phosphorus requirement for growth when available phosphate becomes limiting. AP activities have generally been studied with fluorogenic substrate but only from the point of view of kinetics, or in the frame of global biogeochemical approach (measurements in the bulk sea water). However, by this method it is impossible to target organisms responsible of the activity. Knowing these enzymatic activities at the cell level appears necessary to evaluate the impact of phosphate limitation on aquatic ecosystem diversity. Localization of phosphatase active bacteria and phytoplankton is now possible at the cellular level by the use of a new fluorogenic substrate: the ELF-97 (Molecular Probes) that precipitates and fluoresces yellow-green after cleavage by alkaline phosphatase (Nedoma et al 2003). Such approaches will be made in situ and on surface sea-water amended with different combinations of nutrient amendments (N, P, labile C). Determination of the main phylogenetic groups responsible of heterotrophic activity (Cottrell and Kirchman 2000) as well as quantification of cells-exhibiting phosphatases activity, will give information about these potential co-limitation processes.

We will measure bacterial production (^{3}H leucine technique) and estimate major phylogenetic groups involved in bacterial production (microautoradiography coupled to hybridization in situ). Alkaline phosphatase activity will be determined for the whole water samples with the fluorogenic substrate MUF-P and at the single cell level with ELF-97. We will also determine the effects of nutrients amendments on bacterial production, respiration, diversity of phylogenetic groups, and the participation of the major phylogenetic groups to bacterial production.

A 2.19. Dissolved organic phosphate hydrolysis

There are 2 major groups of enzymes involved in terminal step of hydrolysis of dissolved organic phosphate (DOP) and thus responsible for inorganic phosphate (PO4) regeneration: alkaline phosphatases (AP), and 5'-nucleotidases (5NP). AP are localized both in algae and bacteria, they are synthesized in response to P-deficiency (or P-stress), which means they can be used in P-deficiency diagnosis both at system and cellular level, and their activity can be relatively easily measured with fluorogenic substrates, e.g. MUF-P (system level) or ELF-97 phosphate (single-cell level, Nedoma et al., 2003b). The use of fluorogenic substrates like MUF-P, although giving semi-quantitative information by the use of kinetic parameters derived from enzymatic kinetics (Vm Km), do not allow realistic estimates of DOP hydrolysis rates. The other group, 5NP, are mainly of bacterial origin, they are mostly constitutive, which means they have no diagnostic value in P-deficiency assessment, and there is no fluorogenic substrate suitable for their activity estimation - measurement of their activity requires using radiolabelled nucleotide, mostly 32P-labelled ATP (Ammerman & Azam, 1991). As nucleotides (eg. ATP) are hydrolysed *both* by AP and 5NP, they can be used as universal substrates to measure DOP turnover. In freshwater, DOP turnover is always shorter when measured with nucleotide (32P-ATP, hydrolyzed by AP+5NP) than with non-nucleotide (MUF-P, 32P-glucose-6-phopshate hydrolysed by AP only) (Nedoma et al. 2003a).

However, DOP turnover rate provides only relative measure of the regeneration process intensity (e.g. in the units of % h^{-1}). To get flux (e.g. in the units of nmol l^{-1} h^{-1}), it is necessary to multiply turnover rate by the size of the relevant DOP pool, i.e by the concentration of that fraction of DOP, which can by enzymatically hydrolyzed yielding orthophosphate (enzymatically hydrolysable dissolved organic phosphate, EHDOP). The most suitable method of EHDOP estimation available is enzymatic: filtration of the sample, incubation of the natural DOP in the 0.2 µm filtrate with pure AP from *E. Coli* at optimum hydrolysis conditions, and chemical measurement of the PO4 liberated (Labry, 2001).

Although it is recognized that procaryotes in the deep strata are limited by bioavailability of C, surprisingly some relative high values of alkaline phosphatase have been detected in deep Indian Ocean waters (Hoppe 1999). This activity should not be related to the necessity for phosphate uptake but rather on the organic C compounds which simultaneously produced during hydrolysis and which could probably not be taken up prior to the hydrolytic detachment of phosphate. These enzymes may be important for the slow, but steady regeneration of phosphate and organic C in mesopelagic waters. Besides depth profiles of AP activities, parallel measurement and comparison of turnover rates ^{33}P and ^{3}H from ^{33}P- and ^{3}H-labelled ATP, respectively, could provide indirect information about relation between bacterial P- and C-demand and thus potential P- or C-limitation of bacteria in surface and deep waters.

We will measure turnover rates of orthophosphate and organic moieties of a model radiolabelled DOP substrate (^{33}P-ATP, ^{3}H-ATP) hydrolyzed by natural extracellular enzymes, and an enzymatic estimation of EHDOP (biodegradable DOP) pool.

A 2.20. Effects of hydrostatic pressure on organic matter mineralization

Most of the organic material produced in the photic zone is recycled in the upper layer of the ocean. However, part of this production is exported downwards through the water column both by sinking particulate matter and by diffusion of the dissolved organic matter. During its transit towards the seafloor, most particulate organic carbon (POC) is returned to inorganic forms. Mineralization of the exported material (and subsequent production of CO_2, a greenhouse gases) depends on the prokaryotic activities in mesopelagic and deep water masses. Attached and free-living bacteria both contribute to the oceanic carbon cycle. The fate of biogenic particles during their fall depends on processes leading to mineralization of the organic matrix, and on processes leading to the dissolution of the mineral matrix. Regeneration is now known to play a major role in oceanic Si cycling but the mechanisms that regulate the process are relatively well identified. Bidle & Azam (1999) demonstrated that bacteria accelerate diatom silica dissolution by colonizing and enzymatically degrading the organic matrix of diatom frustules. During a sinking particle fall experiment with axenic diatom detritus, Tamburini et al. (submitted) have shown that the degradation of the organic matrix of diatom frustules by aminopeptidase activities of bacteria were lower during sinking particles fall than those measure at atmospheric pressure conditions. This should help us to understand both the role of mesopelagic and bathypelagic procaryotes in the mineralization of organic matter and their contribution to regeneration to the euphotic zone of mineral ballast (e.g. silica and carbonates). This information is essential for improving models to describe organic matter mineralization by procaryotes and the transfer of carbon and energy to the dark ocean and for exploring the consequences of these processes in the global carbon cycle.

During the BOUM cruise, both sinking particles experiments and measurement of deep-sea prokaryotic activities (ectoenzymatic activity, prokaryotic – *Archaea* and *Bacteria* – production) maintained in *in situ* pressure conditions by will be performed. Moreover an emphasis on the phosphatase activity (and the responsible from) will be made. Finally, the prokaryotic structure (deep vs surface, attached to particle vs free-living) will be done. We will use high pressure serial sampler and particle sinking simulator able to maintain and increase hydrostatic pressure, respectively.

A 2.21. *C/N/P stoichiometry of settling particulate matter*

The degree to which the C/N/P stoechiometry of marine particulate matter can deviate from the Redfield ratio of 106/16/1 is critical to our understanding of the role of phytoplankton in biogeochemistry (Falkowski, 2000). The Redfield ratio is used in oceanography for calculation of export production, and for nutrient-based production calculations, as well as in models of ocean productivity. The evolution of the N:P ratio can marks the transition between N- and P-limitation of phytoplankton growth and can be associated with nitrate- and phosphate-replete conditions (Geider & La Roche, 2002). These ratios can reflect a physiological or biochemical constraint on the elemental composition of primary production. Large variations of C/N/P ratios compared to Redfield have been reported (see Geider & La Roche, 2002). They are clearly related to variations in phytoplankton species dominance. Despite its relevance for modelisation, the stoechiometric composition of material exported to depth is not well constrained by observations (Geider & La Roche, 2002). Comparison between C/N/P stoechiometry of particulate matter in the water column and in the settling particulate matter will allow examining whether one element is preferentially mineralized than another one before particles sink out of surface waters.

Sediment traps will be deployed at 3 depths between the bottom of the euphotic layer and the bottom of the Surface Mediterranean Water. The C/N/P and Si ratios will be measured and compared to the ratios in the water column.

A 2.22. *Large Particulate Matter – Marine snow*

Marine snow is an important pool of atmospheric carbon storage in the ocean’s interior. Processes of aggregation and of dissolution are continuous. Processes of re-aggregation are often concentrated in distinct layers in the mesopelagic depths. Data on the distribution and the dynamics of the marine snow in the oligotrophic sea are scarce. In order to estimate correctly the carbon cycle in the Mediterranean, it is important to determine the proportion of the superficial biological activity trapped and transported to the deep layers in the form of large particles. According to Jackson et al. (1999), by volume the particles > 1 mm are the most important particulate pool of organic carbon in the oceans.

The Underwater Video Profiler (UVP) is used extensively since 1991. The instrument is specifically well adapted to the oligotrophic situation and is measuring fragile objects in an undisturbed and precisely determined water volume. The UVP is acquiring and treating automatically reliable data on the marine snow abundance, size structure, vertical distribution as well as on the macrozooplankton vertical stratification. These data can be transformed into carbon values and vertical fluxes can be assessed down to 1000 m depth. The UVP has its ovn CTD, turbidity meter and fluorometer mounted on the frame. Therefore it is possible to relate the vertical distribution of the POC to the physical structures. One 0-1000 m deployment of the UVP lasts 40 min deck to deck. We are constructing a new model that could be mounted on a rosette instead of one bottle.

A 2.23. *Oxygen budget*

We propose to couple in these low advective environments the O_2 budget within the mixed layer and subsurface layer from 4 approaches: data derived from O_2 field measurements (obtained on the SBE 43 sensor of the CTD, Minas et al 1986), data derived from high frequency O_2 field measurement on immerged optodes (AANDERAA sensors) at selected depths, and rate measurements of net community production derived from *in situ* auto sampler (Langdon et al 1995) deployed at 6 depths which are designed to collect water at the depth of their immersion. After deployment, the oxygen concentration is continuously monitored in the closed chamber which is regularly opened and closed again for pre-selected periods, allowing measurements of respiration (dark period) and net community production (light period). Finally, the more classical approaches deriving net community production from O_2 and TCO_2 variations during bottle incubations will be also carried out (Williams & Jenkinson 1982, Goyet & Hacker 1992, Johnson 1993). This more traditional technique will allow complementary estimates on the coupling between O_2 and CO_2 fluxes. Photosynthetic quotient and respiratory quotients are representatives of the ecosystem functioning.

The originality consists in comparison of different time scales of O_2 variations to reconcile *in situ* observations which integrate the whole community processes compared to discrete bottle incubations. In addition, the study of the kinetic of O_2 change during the day obtained in the PA chamber will provide information on autotrophic *versus* heterotrophic respiration.

A 2.24. Microcosm experiments design

We plan to perform on-board microcosm experiments at each station. Water samples are collected from one or two different depths (e.g. surface and chlorophyll maximum) and pre-filtered through 200 µm mesh to eliminate mesozooplankton. This is because the “microcosm”-size incubator (ca. 10 L) will result in different numbers of mesozooplankton among the microcosms and also create stress to mesozooplankton. A 2x2 factorial design is adapted (i.e. control, PO_4, NH_4, PO_4+NH_4 treatments) in triplicate. Key parameters, such as concentrations of dissolved and particulate nutrients, PO_4 turnover time, alkaline phosphatase activity, luxury P uptake, biomass of osmotrophs and phagotrophs, primary and bacterial carbon production, and copepod egg production, will be measured during incubation. Using the X-Ray Fluorescence spectroscopy (XRF), elemental analysis will be performed for biological materials (content and composition of C, N, P, Mg, Fe, etc). Since the XRF is a quite new instrument and not destructive, it is possible to use the same filter samples for further analyses (e.g. POC, PON).

Five additional treatments (in triplicate over 48h) will be achieved using trace-metal clean techniques to address in particular the role of Fe and dust on N_2 fixation: control, +P, +Fe, +Saharan dust, + anthropogenic dust. These nutrient-addition bioassays are designed to investigate which nutrient (P or Fe) limits N_2 fixation in MS. Additions of dust will be made to assess the potential impact of dust deposition on the primary and diazotrophic production. Labelling with $^{15}N_2$ and $^{13}CO_2$ will allow the assessment of nitrogen fixation by diazotrophs and carbon fixation by the whole plankton community. Changes in diazotrophs diversity will be also investigated during the incubation time. Other key parameters such as total dissolved iron, organic iron, phosphate concentrations (MAGIC method), chlorophyll-a will be followed over 48h.

A 3.1. Modelisation

The multi-element multi-functional group (mNmFG) biogeochemical mNmFG model will be implemented in the modular tool Eco3M (Ecological Mechanistic and Modular Modelling) developed in the Centre d’Océanologie de Marseille through cross-institution collaborations between LOB and IRD. This new numerical tool has been devised to handle mNmFG models (Baklouti et al. submitted) with the main objective of being wholly modular as regards the model state variables and the associated functions for biogeochemical processes. New variables or processes can therefore be added or removed with great simplicity. In addition, a numerical library of functions of biogeochemical processes is already available in Eco3M and many of these parameterizations rely on mechanistic basis.

The structure of the mNmFG will be close to the idealized food web structure proposed by Thingstad et al. (1998, 2005) though we aim at deriving simpler models relying on the same basis through microcosm experiments.

Due to the sampling strategy that will be undertaken during the BOUM cruise (drifting mooring), we will consider in a first approximation that all samples will concern the same water column and then focus on the vertical exchanges into this water mass. In this context, the mNmFG model will be coupled with a 1-D version of the physical model Symphonie to try to catch the vertical fluxes of nutrients as well as living and detritic organic matter (dissolved and particulate) measured at each experimental site. In practice, starting from arbitrary initial conditions for the state variables and thanks to experimental measurements concerning the vertical diffusion and the biogeochemical kinetics, simulations will be undertaken until reaching a cyclic steady-state (corresponding to light-dark variations). This steady-state solution will be compared with organic (phytoplankton, DOM, POM, microzooplankton) and inorganic (nutrients, DIM, PIM) concentrations collected every six hours.

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