Mediterranean Targeted Project (MTP) - MEDIPELAGOS Project
Contract MAS2-CT93-0063

Synthesis of Final Results

Coordinator:

F. Rassoulzadegan
Station Zoologique
B. P. 28
F-06230 Villefranche-Sur-Mer

Contract No.: MAS2-CT93-0063
Starting Date: 1 July 1993
Duration 30 months
EC Scientific Officer: E. Lipiatou

Partners:

Instituto de Ciencias del Mar (ES)	M. Estrada
Centre d'Estudos Avançats de Blanes (ES)	A. Cruzado
Dept. Microb., Univ. Bergen (NO)	F. Thingstad
Lab. Biotech., Univ. Trondheim (NO)	Y. Børsheim
Station Biol., Roscoff (FR)	D. Vaulot
Dept. Microbiol., Univ. Umea (SE)	A. Hagström

Mediterranean Pelagic Ecosystem Study: Plankton Dynamics

ABSTRACT

Key results of the Medipelagos project, a 30 month collaborative effort involving 7 institutions representing 4 European countries, are presented. Research efforts were focused on nutrient dynamics in the N. W. Mediterranean, an issue of importance not only in terms of the goal of MTP (a system model) but also of immediate relevance to management. The main issues addressed were: determining which of the macro-nutrients likely limits production in the N. W. Mediterranean in the spring and summer months, identifying the major pathways of nutrients within the pelagic food web, and examining the effects of rapid changes in nutrient concentrations. Results obtained, using different lines of evidence, strongly support the hypothesis of phosphorus limitation in the N. W. Mediterranean during summer months. In addition to phytoplankton, bacterial uptake is likely a major nutrient uptake pathway in this system. Excretion by grazers on bacteria and small phytoplankton and viral lysis of bacteria are probably responsible for most of the regeneration of phosphorus in the water column. Rapid changes in nutrient concentrations can lead to rapid changes in the structure of the pelagic food web. However, the food webs appear to be capable of quickly reverting back to their previous structure.

Key Words: Nutrients, Phosphorus, Nitrogen, Bacteria, Virus, Phytoplankton, Microzooplankton

INTRODUCTION

The long term goal of the Mediterranean Targeted Project (MTP) is the construction of a model of the system capable of predicting both short and long term changes. The pilot phase of MTP, 1993 - 1996, was specifically focused on four objectives as preliminary steps. The role of MEDIPELAGOS (Mediterranean Pelagic Ecosystem Study) was within the objective "to improve knowledge of key biological (pelagic and benthic) processes in the biogeochemical cycles and their controlling factors" (MTP General Technical Annex 1A).

The key biological process in aquatic escosystems is primary production. In most marine systems, primary production is limited by a nutrient in short supply, which is generally nitrogen (N), in contrast to freshwater systems, in which phosphorus (P) supply usually limits primary production (Hecky & Kilham 1988). However, in recent years evidence has accumulated suggesting that, similar to freshwater systems, the Mediterranean Sea could be P-limited, especially the eastern basins (e.g., Krom et al. 1991; Bethoux et al. 1992) and perhaps the western basin as well (Berland et al. 1988; Raimbault & Coste 1990). Investigating this question was the major motivation behind Medipelagos, a collaborative study of nutrient limitation and pathways in the North-western Mediterranean Sea. The project was designed to provide authoritative answers to questions of nutrient dynamics because correct characterisation of the key biological process in the ecosystem was clearly a prerequisite to MTP modelling efforts.

Medipelagos joined researchers from France, Norway, Spain and Sweden, representing 7 institutions: CNRS-URA 716, Villefranche-Sur-Mer, France, Institut de Ciències del Mar, Barcelona, Spain, Centre d'Estudis Avancats de Blanes, Spain, Department of Microbiology, University of Bergen, Norway, Laboratory of Biotechnology, University of Trondheim, Norway, CNRS-UPR-4601, Roscoff, France, Department of Microbiology, Umea University, Sweden. Over a 30 month period, through collaborative efforts in the form of co-ordinated research programs, workshops, group experiments, and oceanographic cruises, three main questions were addressed:

Which macro-nutrient N or P likely limits primary production?

What are the major pathways of nutrients within the pelagic food web?

What are the effects of rapid changes in nutrient concentrations (pulses)?

In addition to serving the long-term goals of the MTP, the aim of Medipelagos-understanding nutrient limitation in the Mediterranean, has an immediate utility in management decisions. Determining the nature and extent of nutrient limitation is fundamental to understanding the factors controlling the trophic state of an aquatic ecosystem. The clear blue waters of the Mediterranean are evidence, and a product of, its largely oligotrophic condition. Preservation of the trophic state of the Mediterranean, a preoccupation in management decisions in the areas of sewage treatment-disposal, tourism and fishing, requires knowledge of the identity of the limiting nutrient as well as nutrient dynamics in the system.

RESULTS

Which macro-nutrient N or P likely limits primary production?

Preliminary investigations demonstrated that the turnover time of P (as orthophosphate) in coastal surface waters was very short (a few hours) during summer stratification (Dolan et al. 1995). These observations suggested that P could be limiting the growth of phytoplankton and/or bacteria, the producers of particulate organic matter. We critically tested our initial assumption of P-limitation in the Mediterranean using a variety of independent techniques. Experiments were conducted with natural populations from surface layers during seasons when the water column is thermally stratified. The experimental site was on the French Mediterranean Coast, "Point B" the entry of the Bay of Villefranche, a standard oceanographic sampling site in an oligotrophic area, considered typical of the N.W. Mediterranean (Ferrier-Pages & Rassoulzadegan 1994b). We found:

1) Stimulation of DNA synthesis in the primary producer *Synechococcus* with P but not N additions (Vaulot et al. 1996).
2) Stimulation of bacterial growth and the degradation of dissolved organic carbon compounds with P but not N additions (Thingstad et al., submitted).
3) Rapid uptake of added orthophosphate in excess of growth requirements "luxury consumption") in both phytoplankton and bacterial size-fractions (Thingstad et al. submitted).
4) High alkaline phosphatase activity (Thingstad et al. submitted).
5) High short-term variability of bacterial exoenzyme activities (Karner & Rassoulzadegan 1995)

In our opinion, our findings unequivocally demonstrated that both phytoplankton and bacteria in our North-western Mediterranean test site, Villefranche Bay, experience a starvation for P.

What are the major pathways of nutrients?

Determining the relative importance of pathways involves estimating standing stocks as well as fluxes. Stock distributions of P in Villefranche Bay, determined using standard methods, mirrored those of other P-limited systems (such as lakes):

1) Most P is present in the form of dissolved organic phosphorus (Dolan et al. 1995; Thingstad et al. 1996).
2) The largest pool of particulate P is found in the size fraction corresponding to bacteria and small phytoplankton, 0.2 - 5 µm in size (Thingstad et al. 1996).

Fluxes of P into the particulate stocks via absorption of orthophosphate, which represents uptake by the producers of particulate organic matter, was examined using ^{32}P-labelled orthophosphate. Results showed:

1) During the summer, concentrations of dissolved inorganic P are undetectable. During this period, we found rapid P-turnover times, occasionally under 1 hour and P uptake to be dominated (>50 %) by bacteria (Thingstad et al. 1996, submitted).
2) Larger size-fractions (> 5 µm), increase their share in nutrient uptake at higher P concentrations (Dolan et al. 1995).

Such results support the hypothesis that temporarily high concentrations of nutrients, "nutrient pulses", as preferentially profiting larger phytoplankton cells.

As the dominant consumers of P were found to be bacterioplankton and small phytoplankton, a major flux of P from the particulate phase back into the dissolved phase (nutrient regeneration or recycling) should be the excretion of P by consumers of bacterioplankton and phytoplankton. Laboratory data was used to estimate excretion of phosphorus and ammonia by microflagellates and ciliates, organisms that ingest bacterioplankton and small phytoplankton. Other experiments examined the role of viruses in nutrient regeneration, as viral attacks also represent "consumption" of bacteria. The results showed:

1) Microflagellates and ciliates have high weight-specific excretion rates, higher than those of larger organisms, conforming to expectations based on theoretical size-metabolic rate relationships (Allali et al. 1994; Ferrier-Pages & Rassoulzadegan 1994; Dolan 1997)
2) When microflagellates and ciliates are growing rapidly, excretion of phosphorus compared to ammonia is high, suggesting that consumers of bacteria and small phytoplankton may be more efficient recyclers of orthophosphate (P) than ammonia (N) (Dolan 1997).
3) Viral attacks may be a significant mortality source for bacteria and be disproportionately important in the regeneration of P relative to N because bacteria are P-rich (Blackburn et al. 1996, Zweifel et al. 1996).

Consideration of the patterns established in P uptake and excretion, combined with recent observations on the role of dissolved organic carbon in carbon flux to Mediterranean deep waters, led to the development of a new conceptual model. The model postulates that dissolved organic phosphorus acts as a short-term phosphorus sink and the form of phosphorus exported from the surface layer with water column mixing (Thingstad & Rassoulzadegan 1995).

The Thingstad & Rassoulzadegan model has provided a conceptual basis for linking the observed P-limitation of bacteria to a lowered degradation of dissolved organic carbon in the Mediterranean (Thingstad et al. 1997). This adds a new element to our understanding of how carbon is sequestered, and it what form, from the atmosphere in the Mediterranean Sea (Figure 1).

The model, which links surface layer increases in dissolved organic carbon to those of dissolved organic phosphorus, illustrates the considerable importance of a previously ignored pathway- formation and consumption of dissolved organic phosphorus

What are the effects of rapid changes in nutrient concentrations (pulses)?

There is a general agreement that hydrographically stratified, nutrient-poor pelagic systems (such as most of the Mediterranean) develop rather complex "microbial food webs" with small algae at the base of the food web (picoplankton -> flagellates -> ciliates -> copepods -> fish larvae). In contrast, turbulent, nutrient-rich environments are characterised by simpler "classical food chains" based on primary production by large algae (diatoms -> copepods -> fish larvae). While the Mediterranean Sea is generally nutrient poor, it is far from homogeneous spatially or temporally. Marked increases in nutrient concentrations are often found in frontal areas and following water column mixing events associated with sustained periods of strong winds. Such temporally nutrient-rich systems often show changes in food web structure (Legendre & Rassoulzadegan 1995).

We examined the hypothesis that food web structures could be variable in the Mediterranean on relatively small scales of time and space. More specifically, the oceanographic cruises, "Fronts 93 & 95", were conducted to study differences in food web structures across a frontal zone in the Catalan Sea. Temporal variability was examined in the Ligurian Sea through "Dynaproc" cruises which investigated short-term effects of wind-driven water column mixing events. Laboratory studies with mesocosms were employed to examine experimentally the effects of sudden increases in nutrients on surface layer microbial communities. The mesocosm studies were carried out in Barcelona, Spain. Studies with clonal cultures were conducted in Roscoff, France.

For the oceanographic cruises, data analysis is far from complete. The three cruise programs represented over 60 total ship days and are expected to produce a very large amount of data. However, the last cruises were completed only as of June 1995 so that only a limited amount of data is available to date.

From the Oceanographic Cruise Programs FRONTS 93 & 95:

The Catalan front separates communities with different food web structures. In late spring, differences in the abundance of microbes were relatively small between frontal and non-frontal zone stations. For example, stocks of ciliate micro-zooplankton were quite similar among stations (Dolan & Marrasé 1995). However, differences in their activities were significant. Dimethylsulfoxide (DMSO) concentrations are tightly linked to bacterial activity and a marked gradient across the frontal zone was detected (Simo et al. 1995). In the frontal zone, a relatively high percentage of phytoplankton carbon circulated through the microbial food web. The reverse was true for the carbon processed by zooplankton (Calbet et al. submitted). These spatial changes can be related to the availability of nutrients in the lower part of the euphotic zone.

From the Oceanographic Cruise Program DYNAPROC :

Wind-mixing events which inject nutrients from deep layers into surface waters can yield changes in the phytoplankton communities. In particular, such events may profit diatoms but the algal community can revert to its previous composition within a few days.

From the Laboratory Studies:

The mesocosm studies, which were used to experimentally examine the responses of native microbial communities to nutrient additions, indicated that N and P-deficiencies markedly affect both the biochemical and microbial compositions of planktonic communities.

1) P levels affect mainly DNA concentration which indicates total community biomass (Berdalet et al. 1996).
2) Protein or chlorophyll concentrations are related to the availability of N (Berdalet et al. 1996).

Culture studies were employed to examine short-term changes in cell cycles in the prokaryotic alga *Prochlorococcus*.

1) In the Mediterranean strain, cell cycle arrest induced by phosphorus starvation could be reversed by the addition of phosphorus (Parpais et al. 1996).

Our results suggest that nutrient pulses can produce temporary increases in phytoplankton stocks. Nevertheless, microbial food web components appeared to vary only within narrow limits.

Additional MEDIPELAGOS benefits

In addition to providing answers to the three major questions posed, several technical advances, in the form of the refinements or developments of new techniques also resulted from MEDIPELAGOS. A new method was developed to measure pico and nano detritus (particles ranging in size from 0.2 - 20 µm) in the water column (Mostajir et al. 1995a); such small particles likely represent a food and nutrient resource for microbial communities and (Mostajir et al. 1995b). Analysis of nutrient-limitation effects on cell cycles was based on techniques of DNA analysis using flow cytometry and new techniques for flow cytometry were developed and refined for the analysis of picoalgae (Marie et al. 1996). A novel method for investigating selective predation on picoplankton, adding in target populations to natural samples, was investigated (Perez et al. 1996). An automatic phytoplankton culturing device which allows continuously fluctuating nutrient supplies was developed (Bernard et al. 1996). A procedure to microscopically distinguish living from dead bacteria in seawater was introduced (Zweifel & Hagström 1995). Improvements were made in fluorometric methods for estimating concentrations of DNA and RNA in seawater (Fara et al. 1996). Also, the innovative use of existing techniques was developed. For example, the proposal of the ratio of chlorophyll *a* to *in vivo* fluorescence as an ecological indicator (Estrada et al. 1995).

CONCLUSION

The overall results of Medipelagos strongly support the hypothesis that phosphorus most likely limits primary production in the North-western Mediterranean, from late spring through late autumn. We also found that planktonic microorganisms other than algae are important components of nutrient pathways. For example, bacteria compete with algae for nutrients while protists, who feed on bacteria and small phytoplankton, and viruses who lyse bacteria are probably the most important "recyclers" of nutrients. We hypothesise that the dynamics of P in the Mediterranean are intimately tied to those of carbon much like nitrogen and carbon dynamics in the Atlantic and Pacific Oceans.

In terms of scientific output, over 40 research reports, formally acknowledging the financial support of the European Community contract, have been produced. Over 30 papers have been published or are in press, as of November 1996, in standard peer-reviewed scientific journals. Medipelagos was also quite successful in terms of building international relationships. This can be seen in the significant number of MEDIPELAGOS publications and manuscripts in which authorship was shared by researchers representing partners of different countries.

A collaborative, European effort, Medipelagos provided important information needed to accurately characterise the Mediterranean ecosystem, vital to the long-term MTP goal of constructing a model capable of predicting change. It also produced findings of immediate significance for the management of the Mediterranean Sea by providing evidence of phosphorus rather than nitrogen limitation.

ACKNOWLEDGEMENTS

The work described here was supported by the Commission of the European Communities, Mediterranean Targeted Project, Contract No. MAS2-CT93-0063, MEDIPELAGOS.

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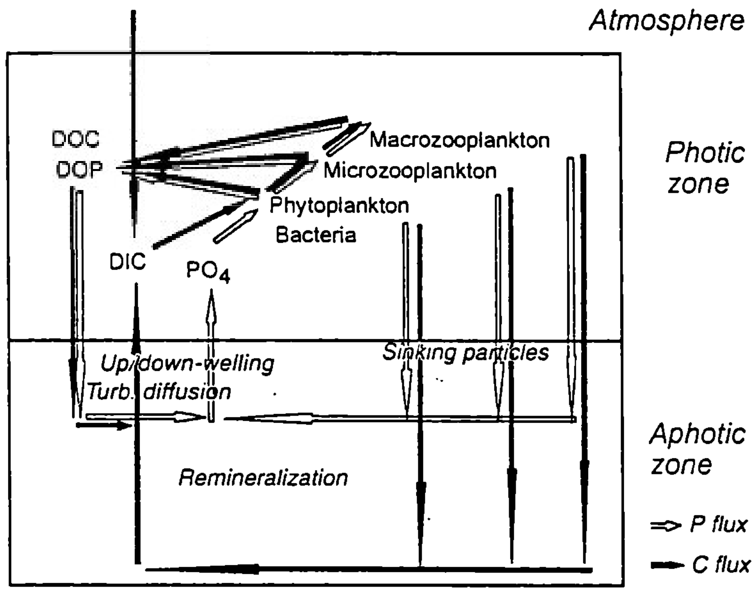

Illustration of a conceptual model suggesting an alternative functioning of the photic zone food web in phosphorus cycling: in addition to transforming imported 'new' phosphorus into sinking particles, it is also transformed into DOP re-exported by the processes of turbulent diffusion and downwelling. Spatial separation of C and P in deep waters is suggested to occur primarily for particle transport