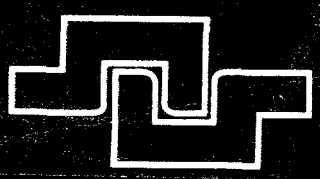
NATO

Advanced Study Institute

Molecular Ecology of Aquatic Microbes

Il Ciocco, Castelvecchio Pascoli, Tuscany
28 August - 9 September 1994



Molecular ecology of aquatic microbes

Programme

Abstracts of invited lectures

Titles of offered papers

Titles of posters

Addresses of lecturers

Addresses of participants

NOTES

Monday
29 August 1994

Theme 1: Introduction - the opportunities offered to aquatic ecology by molecular biology

0900 *Ian Joint*
Why aquatic ecology needs molecular biology - a personal view

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1030 Coffee

1100 *Paul Falkowski*
The molecular basis of phytoplankton photosynthesis

1230 Lunch

Offered papers

1430 R J Geider, *Control of light saturated photosynthesis: concentration and activity of RUBISCO*

1450 T Anning, *Expression of RUBISCO in natural populations of estuarine phytoplankton*

1510 M J Merrett, *Do marine phytoplankton assemblages possess extracellular carbonic anhydrase?*

1530 Ena Urbach, *Genetic diversity of Prochlorococcus marinus: field populations and cutures*

1600 Coffee

1630 V Rousseau, *Biogeochemistry of Phaeocystis colonies and aggregates*

1650 E Marañon, *Changes in the physiological state of phytoplankton across a coastal upwelling front*

1700 Set up posters

1930 Dinner

Tuesday
30 August 1994

Theme 2: The role of aquatic microbes in biogeochemical cycles

0900 *Farooq Azam*
The microbial loop - is it real?

1030 Coffee

1100 *Henry Blackburn*
The role and regulation of microbes in sediment nitrogen cycle

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1230 Lunch

1430 *Bess Ward*
Functional and taxonomic probes for bacteria in the nitrogen cycle.

1600 Coffee

Poster Session

1630 - 1830

1930 Dinner

Wednesday 31 August 1994

Theme 3: Characterisation of the microbial community

0900 *Bo Riemann*
The role of mixotrophy in aquatic environments

1030 Coffee

Offered papers

1100 I Head, *Achromatium oxaliferum: a molecular ecological approach*
1120 J Stein, *Genomic characterisation of marine archaeoplankton*
1140 W D Hiorns, *Detection of autotrophic ammonia oxidising bacteria using oligonucleotide probes targetted at 16S rRNA genes*
1200 F Rassoulzadegan, *Perspectives on molecular ecology of aquatic microzooplankton (protozooplankton): trophic interactions*

1230 Lunch

1430 *Colin Reynolds*
Successional changes in plankton vegetation: species, structure, scale.

1600 Coffee

Offered papers

1630 R P Hirt, *The use of small subunit rRNA sequences to study ciliate molecular diversity*
1650 Ó S Andrésson, *Mixed sample PCR and sequencing for rapid genetic analysis of aquatic microbial communities*
1710 M A Voytek, *The use of molecular probes to detect and enumerate nitrifying bacteria in natural systems*
1730 R Powell, *Identification of marine Archae from deep-sea sediments*

1930 Dinner

Thursday 1 September 1994

Theme 3: Characterisation of the microbial community (cont.)

0900 *Linda Medlin*
Can molecular techniques change our species concept?

1030 Coffee

Offered papers

1100 L J Kerkhof, *Assessing microbial population dynamics by RFLP analysis of 16S rRNA clonal libraries*

1120 E Berdalet, *What can we learn about plankton ecology by estimating RNA and DNA?*

1140 D J Scanlan, *Interrogation of natural phytoplankton assemblages for their phosphate status: a molecular approach*

1200 G. Smerdon *Copepod developmental genes*

1230 Lunch

Theme 4: The effect of the environment on aquatic microbes

1430 *Jean Houmard*
How do cyanobacteria perceive their environment.

1600 Coffee

Discussion groups

1630 - 1800

1930 Dinner

Friday
2 September 1994

Theme 4: The effect of the environment on aquatic microbes (cont.)

0900 *Nick Mann*
How do cells express nutrient limitation at the molecular level.

1030 Coffee

Offered papers

1100 M Heldal, *Osmotic and nutritional status of bacteria in aquatic communities*
1120 J W Ammerman, *Enzymology on the run: continuous underway measurement of microbial enzyme activity in aquatic environments*
1140 S C Cary, *A molecular analysis of bacterial symbiont transmission in marine invertebrates*
1200 M Cooper, *The role of autoinducer mediated signalling in the starvation and nitrogen cycling of nitrifying bacteria*

1230 Lunch

1430 *Ricardo Guerrero*
The problem of excess and/or limitation of the habitat conditions - do natural assemblages exist?

1600 Coffee

Discussion groups

1630 - 1800

1930 Dinner

Saturday
3 September 1994

Theme 4: The effect of the environment on aquatic microbes (cont.)

0900 *Melvin Simon*
Signal transduction mechanisms in microorganisms

1030 Coffee

Discussion groups

1100-1230

1230 Lunch

Afternoon free for individual discussions

1930 Dinner

Sunday
4 September 1994

Free day

An excursion will be arranged to Florence

Monday
5 September 1994

Theme 5: Targeting specific biological processes.

0900 *Stephen Giovanonni*
Molecular genetic studies of bacterial population dynamics in the open ocean

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1030 Coffee

1100 *Gunnar Bratbak*
Viruses - the new players in the game; their ecological role and could they mediate genetic exchange by transduction?

1230 Lunch

Practical session

1430 Presentations by representatives from some major molecular biology companies of their products and an opportunity to discuss individual problems.

1600 Coffee

1630 Practical session continues.

1930 Dinner

Tuesday
6 September 1994

Practical session (cont.)

0900 Round table discussions on problems of transferring laboratory techniques to field populations

1030 Coffee

1100 Round table discussions continue

1230 Lunch

Theme 5: Targeting specific biological processes.

1430 *Wolfgang Löffelhardt*
Molecular analysis of plastid evolution.

1600 Coffee

1630 *Paul Kemp*
Can we estimate bacterial growth rates from nucleic acid content?

1930 Dinner

Wednesday 7 September 1994

Theme 5: Targeting specific biological processes (cont.)

0900 *Daniel Vaulot*
Cell cycle analysis - prospects for estimating phytoplankton productivity.

1030 Coffee

Discussion groups or offered papers

1100 - 1230

1230 Lunch

1430 *Aaron Kaplan*
The cyanobacterial inorganic carbon concentration mechanism: mutants and CO_2-dependent gene expression.

1600 Coffee break

Discussion groups or offered papers

1630 - 1800

1930 Dinner

Thursday
8 September 1994

Theme 5: Targeting specific biological processes (cont.)

0900	*Jonathan Zehr* **Nitrogen fixation in the sea: why only Trichodesmium?**
1030	Coffee
1100	*Colin Murrell* **Molecular ecology of methane oxidising bacteria**
1230	Lunch
1430	*Noel Carr* **Microbial cultures and natural populations**
1600	Coffee
1630	Final discussion session and close of meeting
1930	Dinner

Abstracts of invited lectures

Why aquatic ecology needs molecular biology

Ian Joint

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There is an overriding need in aquatic microbiology to measure the metabolic rates of microbes involved in biogeochemical cycles. We have very few methods that allow us to investigate specific processes or even to identify the microbes responsible. At best, we are able to measure bulk processes but are not able to quantify the contribution of different organisms. For example, one of the most used (and some say, abused) methods in microbial ecology is the measurement of primary production by following the incorporation of ^{14}C. This gives a measure of the photosynthetic rate of the algae and, making some assumptions, we get a measure somewhere between gross and net production. Yet, this is an average value for all of the phytoplankton present in the sample bottle; some species will have generation times of a few hours but others may be very slow growers with doubling times of days or weeks. With our current methods, we have difficulty in saying anything useful about the generation time of the individuals within an assemblage. We can identify and quantify the phytoplankton present but we cannot ascribe a rate to these individuals.

The situation with processes mediated by bacteria is even worse. The techniques that were so successful in classic microbiology have largely failed and, apart from a few exceptions, there are no methods to distinguish different functional groups. We are not even sure the epifluorescent images which we count are growing or moribund organisms. The bacteria involved in major biogeochemical processes are elusive and most cannot be cultured; progress has, therefore, been slow in identifying and quantifying the important rates.

All these problems stem from our inability to target individual organisms or functional groups. I will discuss some of the key questions that I hope can be solved using molecular techniques.

The molecular ecology of phytoplankton photosynthesis

Paul G Falkowski,
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The basic features of the photosynthetic apparatus of oxygenic organisms are highly conserved, and over the past two decades, significant progress has been made in elucidating many of these features using biophysical, molecular genetic and structural biological techniques. We have developed sensitive biophysical tools for oceanographic research, especially variable fluorescence techniques, which allow non-destructive and real-time analysis of variations in the quantum efficiency, effective absorption cross section, and electron transport of the photosynthetic apparatus. Application of these techniques to natural phytoplankton communities reveals large variations in quantum efficiency, which can be related to the availability (flux) of nutrient. Based on laboratory analogue experiments, we can interpret the variations in the fluorescence parameters in terms of molecular modifications within the photosystems. We have extensively explored how iron limitation leads to a decrease in energy transfer from the antennae systems of PSII reaction centres, how nitrogen limitation leads to a differential decrease in chloroplast encoded proteins relative to those encoded in the nuclear genome, and how growth irradiance affects the ratio of membrane-bound electron transport components to stromal enzymes. Our field and laboratory results predict that if the regeneration of nutrients by the microbial loop in the oligotrophic ocean was sufficient to meet phytoplankton demands, then the quantum efficiency of the photosynthetic apparatus should be relatively high and constant. This is not observed. We argue, therefore, that phytoplankton photosynthesis is physiologically limited by nutrient availability, and cells cannot be growing at their relative maximum specific rates.

The Microbial Loop: Is It Real?

Farooq Azam,
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Studies during the past 15 years have lead to a dramatic change in our ideas of the role of bacteria, protozoa and viruses in the ocean's ecosystems. It is now generally thought that the microbial loop is a major biological/biochemical force in the pelagic ocean which substantially influences the variability in the ocean's biogeochemical state. I will examine whether this conclusion is justified. I will then address the problem of the structure and dynamics of the microbial loop and how the microbial loop fits into the overall structure of the pelagic marine ecosystems. Finally, I will propose specific ways in which the study of aquatic microbes at the molecular level might fruitfully address some fundamental issues of the structure and dynamics of the pelagic ecosystems.

The role and regulation of microbes in sediment nitrogen cycle

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Microbial activity in sediment is regulated principally by the quantity and quality of organic matter (POM) reaching the sediment. If there is no sedimentation of POM, there is no N-cycle. The C:N ratio of the POM is very important, as is the distribution of POM within the sediment. If the POM is transported (bioturbation) below the sediment surface, the mineralised nitrogen (ammonium) may have a different fate from that produced close to the sediment surface. Much depends on the diffusibility of the products of anoxic respiration (reduced iron, manganese and sulfur). The interrelationship between these factors are explored in simulations. Particular attention is given to coupled nitrification-denitrification reactions.

Functional and Taxonomic Probes for Bacteria in the Nitrogen Cycle

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Nitrification and denitrification are mediated in the environment by bacteria which appear to have incompatible physiologies: obligate aerobic chemoautotrophs vs. facultative anaerobic heterotrophs. Nevertheless, the two processes often occur together or at least in proximity that cannot be distinguished by normal sampling scales. Hybridization and oligonucleotide probes and PCR assays offer specific and sensitive ways to investigate these processes, by focussing on the bacteria responsible for them. Initial development of such probes has shown that different approaches will be useful for the different groups: the narrow phytogenetic range of the autotrophic nitrifiers makes 16S rRNA probes useful for them, but not for the extremely diverse denitrifiers. Although bacteria capable of denitrification are phylogenetically diverse, the enzymology of the denitrification pathway appears to be more conserved, and this may provide a functional approach to probe production. The probes currently under development and in use will be described in terms of the advantages, disadvantages, constrains and power of each as applied to the nitrogen cycle in aquatic environments. Application of some of the probes will be discussed in results from several lakes and from the marine environment. In addition to new information on the distribution of bacterial processes in the environment, the diversity of denitrifying and nitrifying isolates from various environments is also addressed.

The role of mixotrophy in marine environments

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In nutrient-depleted environments, the competition for nutrients favours growth of small-sized heterotrophs like bacteria compared to the success of obligate autotrophs. Mixotroph protists represent an alternative strategy that connects the traditional food web with the microbial loop. Compared to a large number of reports on the ecological importance of mixotrophs in freshwater, only few studies have been made in seawater. A number of laboratory studies and some field studies have demonstrated a large number of flagellates and ciliates as mixotrophs and a number of species specific factors controlling the degree of phototrophy/phagotrophy. Both mixotrophic flagellates and ciliates are common and often important components in marine environments. Natural assemblages of marine mixotrophic flagellates can dominate the biomass of phototrophs and be responsible for the entire grazing on bacteria or protists. Results from a theoretical model suggest that there is an upper limit to the potential success of mixotrophic flagellates.

Successional change in the planktonic vegetation: species, structure, scales

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The paper will examine the mechanisms by which planktonic algae harvest their material requirements from a rarified and often hostile environment and how specific morphological adaptations provide dynamic advantages to species depending upon whether they are primarily invasive, acquisitive or attuning in their exploitation of the supply of resources. The manner in which the dynamics of selectively advantaged species contribute to the assembly of communities is highlighted, as is their relative vulnerability to external forcing. A model of ecological succession is briefly reviewed. While its logical outcome is an idealised steady-state condition with few species, external forcing promotes reversion to a less mature successional structure. The principle of intermediate disturbance will be argued to regulate community development and organisation in most planktonic assemblages.

How do cyanobacteria perceive their environment?

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Cyanobacteria have been recognized in almost all of the ecosystems that have been examined heretofore, and they probably represent the most highly diverse group of prokaryotes. Cyanobacteria are quite remarkable in their abilities to adapt to their environment, whether the changes concern nutrient availability, temperature, intensity or spectral quality of the light, for example. The environmental parameters have to be sensed before cells can answer and adapt to the various changes. A series of mechanisms are involved in the sensing and transduction of signals. Both the mechanisms and the connections that may exist between them have recently started to be elucidated. We shall describe and summarise the few examples which have been studied at a molecular level in cyanobacteria. A particular emphasis will be given to photoregulation of gene expression since light plays a major role in the metabolism of these photoautotrophic organisms.

How do cells express nutrient limitation at the molecular level

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Microbial growth in aquatic ecosystems is usually restricted by the availability of one or more essential nutrients. The response of the cell to the onset of nutrient limitation is initially characterized by the induction of relevant high affinity nutrient transport systems and, in the case of organic carbon-energy sources, may also include the elaboration of pathways for the utilization of alternative nutrients. As nutrient limitation becomes increasingly severe, more general processes of physiological and morphological adaptation occur. Finally, at the point where growth ceases, cells undergo a further series of adaptations that permit them to retain viability during prolonged periods of starvation. This chapter will outline the range of responses of cells to different nutrient limitations and to starvation (e.g. nucleoid condensation, decrease in cell size, increased proteolysis, phycobilisome breakdown, etc.) and attempt to interpret these changes in terms of their adaptive value. Where possible the molecular nature of high affinity transport systems will be described, with a particular emphasis being given to phosphate acquisition. The components of the transcriptional control mechanisms involved in adaptation to both specific nutrient limitations and general starvation (i.e. alternative σ factors, gearbox promoters, transcriptional activators *etc*) will be considered along with more short term responses such as protein modification.

The problem of excess and/or limitation of the habitat conditions - natural assemblages exist?

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Microbial populations rarely occur alone in nature, but rather interact with each other forming complex communities. A microbial community can be regarded as an assemblage of physiologically connected micro-organisms living together at a given place or habitat. The community is the highest biological unit in an ecological hierarchy. The study of the microbial communities of both Lake Cisó and the microbial mats at the Ebro Delta, in Catalonia, Spain, have shown that principles from general ecology can be applicable to micro-organisms. The structure of natural systems present in both habitats, as well as their changes with time and depth, were studied following different approaches: taxonomic and physiological analyses, trophic dynamics and structure of the food web, competition among bacteria, etc. Habitats where either an excess or a limitation of some natural condition occurs harbour different kinds of microbial assemblages, which can be regarded as a means for self-regulation of the participating populations. The excess of a toxic compound may be eliminated by the linked action of several microbial species that degrade or transform the toxic material. Limitation of nutrients triggers gene transfer processes that may yield new functions in some of the populations of the microbial assemblage. Assemblages between phototrophic bacteria (cyanobacteria and purple and green sulphur bacteria) are among the most stable and balanced microbial communities. The population structure of sulphur phototrophic bacteria in Lake Cisó is determined by light penetration and by the abundance of sulphide. Nowadays light and sulphide are two environmental factors found rarely together in the earth, except in habitats such as the metalimnion of lakes (as it is the case of Lake Cisó) and shallow water sediments, where light diminishes from top to bottom and sulphide increases from the bottom up. As a result, both competition and coexistence of bacteria take place. Simultaneous presence of green and purple sulphur bacteria has been observed on many occasions in karstic lakes. However, few studies exist analysing which environmental factors are important in determining whether purple or green sulphur bacteria should be more abundant at a given time of the year. Light quantity and light quality have been implicated in selection of purple or green sulphur bacteria indifferent lakes. This phenomenon has been studied in detail in Lake Cisó, where both groups of bacteria are present at different times of the year, thus providing a natural experiment where changes in environmental conditions provoke changes in the

relative abundance of the green and purple bacteria. Major factors for determining the abundance of these two groups of bacteria are: solar radiation, temperature, accumulated rainfall, and both oxygen and sulphide vertical distribution.

Signal transaction mechanism in microorganisms

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The ability to adapt to an ecological niche requires a variety of sensory mechanisms. Bacteria are equipped with systems that allow them to measure the physical and chemical parameters of their environment and to respond in an ecologically appropriate fashion. Thus, for example, there are clear mechanisms for sensing osmolarity, nutrient, temperature, light, pressure, and surfaces, as well as a variety of other characteristics, including the density of organisms in the environment. All of this information is integrated into a series of complex biochemical circuits. Even simple organisms are capable of a variety of responses ranging from modifications of biochemical pathways to motility, growth, adhesion and other adaptation.

Many sensory signalling mechanisms have similar characteristics, and these characteristics are mediated by a variety of proteins, including specific receptors, kinases, and methylases that amplify and transduce chemical information into metabolic changes, and effectors that modulate enzyme activity and mediate changes in gene expression and gene function. We will describe some of the circuitry that has been studied in a variety of microorganisms and show how the information processing systems are required by the organism in order for it to efficiently and successfully populate a specific environment.

Molecular genetic studies of bacterial population dynamics in the open ocean

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Bacterioplankton play important roles in nutrient regeneration and carbon flux in planktonic marine systems. Until recently, most ecological studies regarded bacterioplankton populations as a "black box" because standard bacteriological methods could not accurately resolve community composition. Knowledge of bacterioplankton community structure has advanced significantly with the application of ribosomal RNA gene cloning and sequencing methods to natural populations of bacteria. These studies have proven that numerous novel microbial taxa populate seawater. Many of these taxa branch deeply within phylogenetic trees and have only weak affiliations with known bacteria phyla. Of considerable importance to ecological studies has been the observation that many of the same taxa occur in surface samples from the Atlantic and Pacific Gyres. Thus, ecological studies which focus on the most abundant of these taxa using oligonucleotide probes may provide an important avenue for investigating bacterioplankton population dynamics. The hybridisation of taxon-specific oligonucleotide probes to rRNAs and rDNAs at the Bermuda Atlantic Time Series Station (BATS) in the Sargasso Sea has revealed pronounced spatial and temporal patterns in the distributions of major bacterioplankton groups.

Viruses - the new players in the game; their ecological role and could they mediate genetic exchange by transduction

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The abundance of viral like particles in marine ecosystems range from $<10^4$ ml^{-1} to $>10^8$ ml^{-1}. Their distribution in time and space parallels that of other biological parameters such as bacterial abundance and chl *a*. The abundance of viral particles change on a seasonal and a diel basis, but the change in viral abundance may also be much faster, resembling "one-step" growth curves. Few studies have been carried out, but viruses appear, at least in some cases, to have a significant impact on carbon and nutrient flow in microbial food webs. Virus have also been demonstrated to exert a species specific control of both bacteria and phytoplankton populations in natural waters. With model phage-host systems, transduction has been demonstrated to occur in aquatic environment. Virus infection and lysogenization of bacteria are known to give some pathogenic bacteria a competitive advantage and it is possible that similar mechanisms are important in natural waters.

Molecular analysis of plastid evolution

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The endosymbiotic origin of plastids from (cyanobacterial) endosymbionts is now generally accepted. The chloroplasts *sensu stricto*, the chlorophyll *b*-containing plastids of higher plants and green algae share common features of morphology, starch deposition, a two-membrane envelope and comparable gene content and organization of their genomes. On the other hand algae without chlorophyll *b* constitute a very interesting group comprising two to four envelope membranes, morphological peculiarities, storage of assimilatory starch outside of the plastid, and in general a complement of protein genes higher by about 50% than that of chloroplasts. There is still a controversy about details of the endosymbiotic plastid evolution: a monophyletic origin would mean that cyanobacteria are the photosynthetic prokaryotes ancestral to all plastid types. At present the results of phylogenetic analysis do not lend much support to a polyphyletic theory. Another point is, if there was a singular or multiple primary endosymbiotic event, *ie* are all types of plastids derived from a common (semiautonomous) endosymbiont. Here comparison of plastid genome organization might be more useful than phylogenetic analysis of single traits. Secondary endosymbiosis involving an eukaryotic alga as the photosynthetic invader of a heterotrophic host cell is invoked to explain the origin of the euglenoid and chromophyte plastids that are surrounded by three and four envelope membranes, respectively. The unusual targeting signals of nucleus-encoded plastid polypeptides with these algae and the presence in cryptomonads of the nucleomorph, most likely the vestigial nucleus of the eukaryotic endosymbiont, strengthen this attractive hypothesis.

Can we estimate bacterial growth rates from nucleic acid content?

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Present concepts regarding the importance of marine bacterial processes are based on techniques that are inherently unable to recognize the great taxonomic and functional diversity of marine bacteria. These methods provide community averages of such parameters as bacterial abundance, growth rate, production, or respiration. Furthermore, conventional methods rarely address the distribution of activity among individual cells, although the available data suggest that as few as 10-50% of cells may be metabolically active at a given moment. In other words, bacterial activity at any given time is likely to be attributable to a small and unknown fraction of the bacteria present. It is extremely difficult or impossible to develop a mechanistic understanding of bacterial responses to environmental conditions, when all bacteria are treated as functionally equivalent and equally active by the methods used to examine them. Consequently, marine microbial research tends to be descriptive rather than predictive.

Several studies have demonstrated a strong relationship between the quantity of ribosomal RNA (rRNA) in bacterial cells and their growth rate under laboratory conditions. It may be possible to use this relationship to provide information on the activity of natural bacterial communities, and in particular on the growth rate. A requisite of such applications is that the rRNA-growth rate relationship must be universal among bacteria, or alternately that the relationship can be determined and measured for specific bacterial taxa. Some currently available methods are capable of providing taxon-specific measurements of rRNA content. In addition, some of the methods used to measure bacterial rRNA content are cell-specific and can provide information on the distribution of activity among cells.

The rRNA-growth rate relationship has not been used to evaluate bacterial growth in field studies, although rRNA content has been measured in single cells and in bulk extracts of field samples taken from coastal environments. These measurements have been treated as probable indicators of bacterial activity, but have not yet been interpreted as estimators of growth rate. The primary obstacle to such interpretations is a lack of information on the biological and environmental factors that affect the rRNA-growth rate relationship.

I will describe the current information and hypotheses regarding the regulation of rRNA synthesis and degradation as a function of growth rate and environmental factors; *ie* the basic reasons why rRNA is found to be proportional to growth rate under controlled laboratory conditions, and the reasons why it may (or may not) prove to be proportional to growth in field populations. Field and laboratory data will be presented as examples of the utility of this approach, and of the questions remaining to be answered.

Cell cycle analysis: prospects for estimating phytoplankton productivity

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Phytoplankton cell cycle has been relatively little studied in comparison to that of other organisms such as *E. coli*, yeast or mammals. However, as expected in view of the very good conservation of the cell cycle machinery during evolution, at least for eukaryotes, phytoplanktonic organisms follow the general rules of cell cycle regulation. In particular the DNA synthesis phase (S) is always well delimited, preceded by the G_1 growth phase and followed by the G_2 prior to mistos (M). This applies also to dinoflagellates, that have a nuclear and chromosome structure that differ from that of other eukaryotes (permanently condensed DNA) and even to prokaryotes (*Prochlorococcus, Synechococcus*).

Phytoplankton specificity is to be found in the factors that regulate the cell cycle. Light and nutrients (nitrogen, silica for diatoms) are the most important for which the bulk of the studies have been undertaken. For these factors, it seems that the concept of "restriction point", developed for animal cells, applies very well. For example, the absence of light or nitrogen usually blocks cells in the G_1 phase. In some cases, however the situation is more complex. Silica depletion blocks diatom cell cycle at two different points, one just prior to DNA synthesis, the other just prior to division, during frustule formation. In *Synechococcus*, darkness blocks cells in G_2.

The recent development of the possibility to measure cell DNA of natural phytoplankton populations by flow cytometry and therefore to estimate the fraction of cells in the different cell cycle phases allows us to apply these concepts to solve two types of oceanographic questions.

1. Which nutrients limit specific cell populations? The classical paradigm that nitrogen is the key limiting factor in oceanic waters has been recently challenged. There is a definite need to evaluate this question at the cellular level. By adding a range of potentially limiting nutrients to seawater enclosed in bottles and by monitoring the change in the cell cycle distributions, one can determine which addition accelerate cell cycling of specific populations. This technique allowed to demonstrate that, in the northwestern Mediterranean Sea nitrogen does control the cell cycle of *Prochlorococcus* in nutrient-depleted surface waters during winter. In contrast in summer, phosphorus seems to be the key controlling factor for *Synechococcus* in the same region.

2. How fast are cells dividing?
One of the key variables to estimate primary productivity is the growth rate of phytoplankton populations. Its assessment is very difficult, however, because ubiquitous grazers remove cells as quickly as they are produced. The only direct method available to date is based on monitoring the fraction of cells in the terminal phase of the cell cycle. However, this method has been little used because it is very fastidious and only applies to species that have easily recognizable division phases. The use of flow cytometry makes its application much more easy. Recently, cell cycle data have been obtained on *Prochlorococcus* populations in the equatorial Pacific. These data demonstrate that: (1) the cell cycle of these prokaryotes is highly synchronized to the light-dark cycle and (2) that their maximum cell division rate reach one doubling per day. Using estimates of cell concentration and cell carbon content one can then easily compute the contribution of *Prochlorococcus* to gross production, that reach from 5 to 15% at the equator.

It is likely that in the near future, new methods based on the extraordinary development of the understanding of cell cycle at the cellular and molecular level that have occurred in the past ten years, will receive applications in biological oceanography.

The cyanobacterial inorganic carbon concentrating mechanism: mutants and CO_2-dependent gene expression.

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Cyanobacterial mutants impaired in the ability to grow under different levels of CO_2 are being used to elucidate the physiological and molecular functions involved in their inorganic carbon (Ci)-concentrating mechanism. The relevant *Synechococcus* sp. strain PCC7942 mutants so far obtained had defects mapped in the genomic region of *rbc* but none was directly impaired in Ci uptake. *Synechococcus* cultures were transformed with an inactivation plasmid library comprising small genomic fragments, flanked with a cartridge enclding kanamycin-resistance (kmR). Single crossover recombination events resulted in inactivation of various genes. Five different kmR, high-CO_2-requiring mutants were selected following exposure to low CO_2 conditions in the presence of ampicillin. The mutants exhibit impaired ability to transport bicarbonate but normal uptake of CO_2. This may indicate two different transport systems for the two Ci species. Since the interposon mutated genes are tagged with the kmR cartridge, the latter is being used to identify and map them. It is not yet known whether these genes are clustered but they are not located in the genomic region of *rbc*. Cyanobacteria adapt to the ambient level of CO_2 by a syndrome of cellular changes. In *Synechococcus* sp. strain PCC7942, transcripts originating from *cmpA* (encoding a 42 kDa polypeptide which accumulates following exposure to low CO_2) are only observed when the cells are exposed to low CO_2. Fusion of 700 or 380 bp upstream of the translation starting codon of *cmpA* with a promoter-less *cat* resulted in mutants capable of growing in the presence of chloramphenicol under low but not high-CO_2. On the other hand, fusion with the 150 bp near the translation start codon enabled growth under high and low CO_2. Quantitative analysis of the expression of *cat* in cells bearing different parts of the *cmpA* promoter fused to *cat* indicated the presence of enhancing and repressing elements in the promoter region of *cmpA*. In these studies we also used a high-CO_2-requiring mutant which does not transcribe the *cmpA* due to a mutation in another gene, *ccmN* (located upstream of *rbc*). Inactivation of *ccmN* modified the dependence of *cat* expression on the ambient level of CO_2.

Nitrogen-fixation in the sea: Why only *Trichodesmium?*

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The relative importance of different nutrients in limiting primary production in the sea continues to be the subject of debate, but it is clear that several nutrients are often in short supply in many regions of the oceans. Nitrogen-fixation capabilities should provide an ecological advantage to microorganisms in the oceanic environment, regardless of the primary nutrient limiting productivity at any particular time. *Trichodesmium* is a filamentous nonheterocystous nitrogen-fixing cyanobacterium which is a conspicuous component of tropical and subtropical oceans, and appears to play a major role in carbon and nitrogen-fixation in regions where it is found. It is not intuitively obvious which characteristics of *Trichodesmium* confer an ecological advantage such that it is the predominant organism to exploit nitrogen-fixation as a mechanism to obtain nitrogen in nitrogen-deficient oligotrophic oceans.

Furthermore, there still remains the question of whether *Trichodesmium* is truly one of only a few species to fix nitrogen, or whether other nitrogen fixing organisms exist in the open ocean, but have yet to be cultivated.

Molecular approaches have provided a way to examine both of these issues: what is the distribution of nitrogen fixing microorganisms in the marine environment, and what are the molecular and biochemical features that determine the ecological success of *Trichodesmium* in the open ocean environment?

Molecular ecology of methane-oxidising bacteria

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I will cover the background to the importance of methane-oxidising bacteria (methanotrophs) in the global cycling of carbon and their role as a sink for methane in freshwater and marine environments. Traditional microbial ecology of the methanotrophs will also be summarised and the gaps in our knowledge of the ecology of these organisms will be outlined *ie* important questions to be addressed including the diversity and activity of this apparently ubiquitous group of bacteria. Problems of cultivation of marine and freshwater methanotrophs will then be addressed.

The physiology and biochemistry of methanotrophs will be summarised as background for what we know about the molecular biology of methanogrophs *ie* the structure and regulation of the genes encoding the unique methane oxidation system that they contain. Their phylogeny and data on 16S rRNA gene sequences of these organisms will then set the scene for studies on the molecular ecology of methanotrophs in aquatic environments *eg* PCR amplification and identification of methane monooxygenase genes from the environment, 16S rRNA sequence retrieval, fluorescence microscopy using functional gene probes and group-specific 16S rRNA probes to identify and enumerate methanotrophs.

Microbial cultures and natural populations

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Most of our information regarding the growth rates, cell cycles and morphological variations of bacteria have been derived from laboratory cultures of organisms selected, deliberately or not, for metabolic versatility and rapid division times. Increasingly it has become apparent that in natural populations, perhaps especially in oligotrophic environments, a major proportion appear to be of unculturable organisms, whose growth rates are very low. Some of the ways in which bacteria grow in structured associations and deviations from normal life-cycles will be discussed. The extent to which programmed cell death, apoptosis, can be considered as operational in some bacteria will be examined in the context of understanding why natural populations appear as they do.

Titles of offered talks

J W Ammerman, Center of Marine Biotechnology, University of Maryland *Enzymology on the run: continuous underway measurement of microbial enzyme activity in aquatic environments*

Ó S Andrésson, Institute for Experimental Pathology, University of Iceland *Mixed sample PCR and sequencing for rapid genetic analysis of aquatic microbial communities*

T Anning, Plymouth Marine Laboratory *Expression of RUBISCO in natural populations of estuarine phytoplankton*

E Berdalet, Institut de Ciències del Mar, Barcelona *What can we learn about plankton ecology by estimating RNA and DNA?*

S C Cary, Department of Microbiology, Oregon State University *A molecular analysis of bacterial symbiont transmission in marine invertebrates*

M Cooper, Department of Molecular and Cell Biology, University of Aberdeen *The role of autoinducer mediated signalling in the starvation and nitrogen cycling of nitrifying bacteria*

R J Geider, College of Marine Studies, University of Delaware *Control of light saturated photosynthesis: concentration and activity of RUBISCO*

I Head, Fossil Fuels and Environmental Geochemistry, University of Newcastle *Achromatium oxaliferum: a molecular ecological approach*

M Heldal, Institutt for mikrobiologi, Universitetet i Bergen *Osmotic and nutritional status of bacteria in aquatic communities*

W D Hiorns, University of Liverpool, Department of Genetics and Microbiology *Detection of autotrophic ammonia oxidising bacteria using oligonucleotide probes targetted at 16S rRNA genes*

R P Hirt, Group of Microbiology, The Natural History Museum, London *The use of small subunit rRNA sequences to study ciliate molecular diversity*

L J Kerkhof, Institute of Marine and Coastal Sciences, Rutgers, the State University of New Jersey *Assessing microbial population dynamics by RFLP analysis of 16S rRNA clonal libraries*

E Marañon, Universidad de Oviedo *Changes in the physiological state of phytoplankton across a coastal upwelling front*

M J Merrett, School of Biological Sciences, University College of Swansea *Do marine phytoplankton assemblages possess extracellular carbonic anhydrase?*

R Powell, Recombinant DNA Group, University College Galway *Identification of marine Archae from deep-sea sediments*

F Rassoulzadegan, Station Zoologique, Villefranche-sur-Mer *Perspectives on molecular ecology of aquatic microzooplankton (protozooplankton): trophic interactions*

V Rousseau, Groupe de Microbiologie des Milieux Aquatiques (GMMA), University of Brussels *Biogeochemistry of Phaeocystis colonies and aggregates*

D J Scanlan, Dept of Biological Sciences, University of Warwick *Interrogation of natural phytoplankton assemblages for their phosphate status: a molecular approach*

Gary Smerdon, Plymouth Marine Laboratory, *The cloning of developmental genes from Calanus helgolandicus.*

J Stein, The Agouron Institute, La Jolla *Genomic characterisation of marine archaeoplankton*

Ena Urbach, Massachusetts Institute of Technology, Cambridge *Genetic diversity of Prochlorococcus marinus: field populations and cutures*

M A Voytek, Marine Sciences, University of California, Santa Cruz *The use of molecular probes to detect and enumerate nitrifying bacteria in natural systems*

Titles of posters

S M Allison, Department of Molecular and Cell Biology, University of Aberdeen *Confocal laser microscopy and 16S rRNA techniques for spatial and temporal studies of sulphate reducing bacteria in aerobic aquatic biofilms*

M Balode, Latvian Academy of Sciences, Institute of Biology *Population dynamics of harmful cyanobacteria in the Baltic Sea, Gulf of Riga*

J A Berges, Brookhaven National Laboratory *Enzyme indices of nitrate assimilation in marine phytoplankton*

C Brussaard, Netherlands Institute for Sea Research, Texel *The influence of plankton loss factors on the structure of a coastal ecosystem*

H B Büyükisik, Ege University, Dept of Hydrobiology Turkey *Growth kinetics and limiting factors on Thalassiosira gravida in Izmir Bay*

U Christaki, Station Marine d'Endoume, Marseille *Phytoplankton decaying in microcosms manipulated at the top predator. Role of the microbial loop and of mesozooplankton*

K E Cooksey and B. Wigglesworth-Cooksey, Department of Microbiology, Montana State University *Some molecular approaches to the study of interactions of organisms and their environment.*

P Cummins, Plymouth Marine Laboratory, Plymouth. *Molecular analysis of haloperoxidase genes in marine algae.*

M E Hatipoglu, Middle East Technical University, Institute of Marine Sciences, Turkey *Molecular ecology of Black Sea phytoplankton*

M LaMontagne, Marine Biological Laboratory, Woods Hole *Application of DNA hydridization to the study of denitrification in coastal sediments*

W.K.W. Li and J.F. Jellett, Biological Oceanography Division, Bedford Institute of Oceanography *Flow cytometric analysis of marine bacteria stained with TO-PRO*

S Y Maestrini, CREMA Houmeau, L'Houmeau *Nutritional features in the toxic dinoflagellate Dinophysis: the enigma continues*

P de Marco, Biological Sciences, University of Warwick *Molecular ecology of methansulphonic acid oxidising bacteria*

V Martin Jezequel, Station Biologique, Roscoff *Studies on amino acid metabolism in algae*

C L Moyer, Dept of Oceanography, University of Hawaii *Genetic diversity of bacterial 16S rRNA genes from a microbial mat at an active hydrothermal vent system, Loihi Seamount, Hawaii*

Y Nikolaev, Dept Molecular and Cell Biology, University of Aberdeen *Protection of E. coli cells against N-ethylmaleimide by activation of glutathione adduct gated potassium efflux system*

A Oren, The Hebrew University of Jerusalem *Interrelationships between the green alga Dunaiella and halophilic archae in hypersaline environments*

O Prasil, Institute of Microbiology, Academy of Sciences of Czech Republic *The influence of acceptor side of photosystem II activity on the regulation of photosynthetic yield in algae*

G Rocap, Massachusetts Institute of Technology, Cambridge *Application of constant denaturant capillary electrophoresis to assess genetic heterogeneity in picoplankton populations*

A Shalapyonok, Institute for the Biology of Southern Seas, Sevastopol *Microfluorometric investigation of the cell-to-cell heterogeneity in the natural microbial population: composition and functioning of light harvesting pigments in picocyanobacterial cells*

W Stolte, Netherlands Institute for Sea Research, Texel *Influence of nutrient regime on phytoplankton size within natural assemblages*

K R Timmermans, Netherlands Institute for Sea Research, Texel *Effect of low concentrations of trace metals on phytoplankton physiology*

A Vonshak, Ben-Gurion University of the Negev, Microalgal Biotechnology *The interaction of light and salinity stress*

W H Wilson, Dept of Biological Sciences, University of Warwick *Characterisation of viruses infecting marine phytoplankton*

Address lists

Names and addresses of organising committee and invited lecturers

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