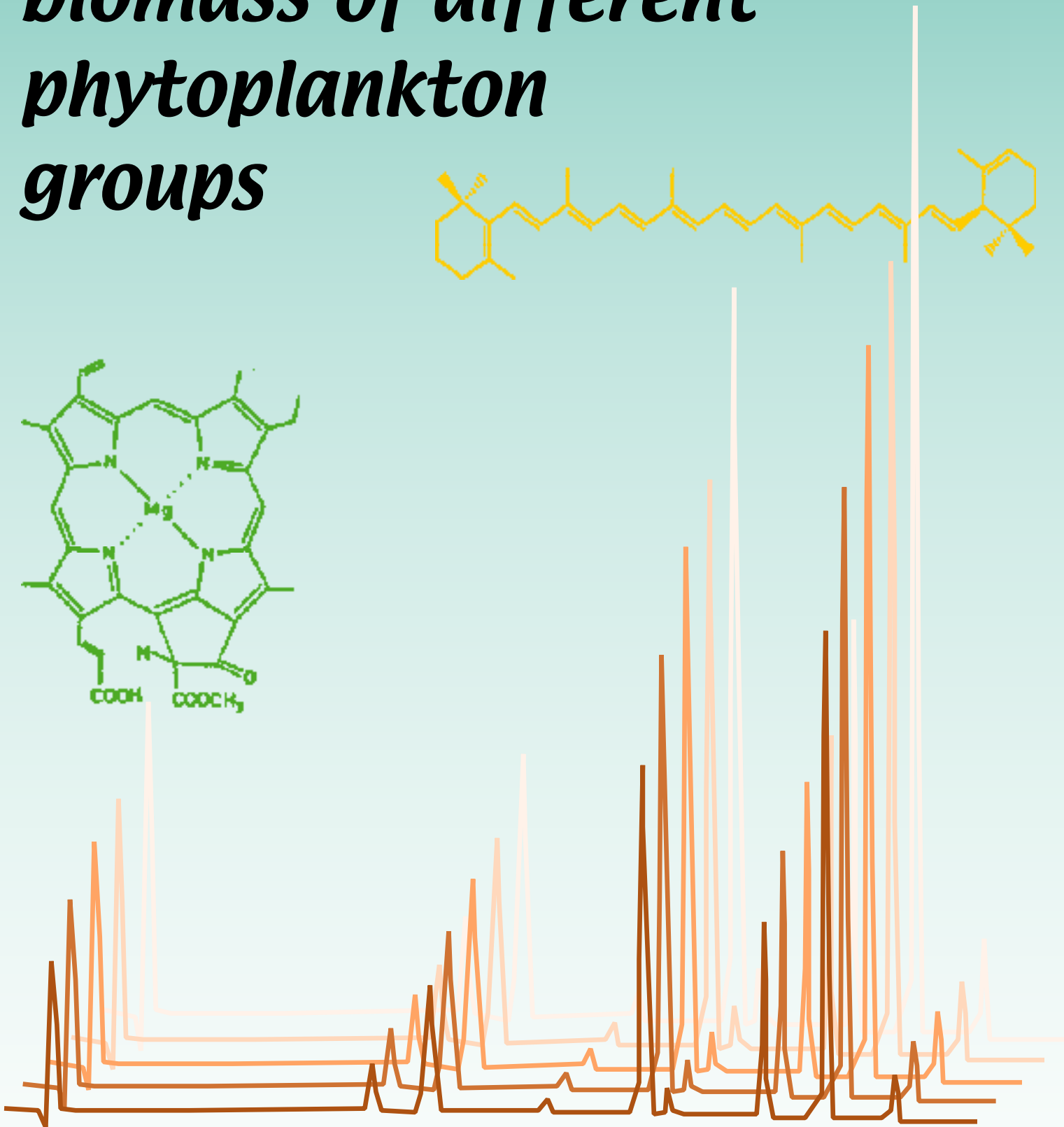
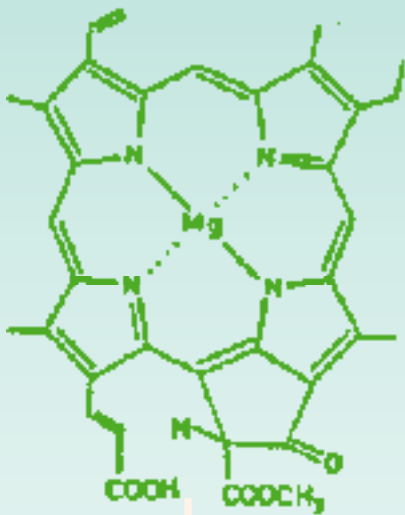


Pigments

as a tool to estimate the biomass of different phytoplankton groups



Barcelona, spring 2001

Schedule

Sunday 29		Monday 30		Tuesday 1	
9:15	CHECK - IN	9:00	<i>Session: Chemtax-1</i>	9:00	<i>Session: Pigment results-1</i>
9:30	WELCOME AND OPENING REMARKS	10:00	KEYNOTE LECTURE: SIMON WRIGHT	9:25	<i>Lochhead et al.</i>
10:00	<i>Session: Pigment methodology-1</i>	10:25	<i>Llewellyn & Cummings</i>	9:50	<i>Millán-Núñez et al.</i>
10:00	KEYNOTE LECTURE: MANUEL ZAPATA	10:55	Coffee break	10:15	<i>Obayashi & Suzuki</i>
11:00	Coffee break	11:20	<i>Session: Chemtax-2</i>	10:45	<i>Session: Pigment results-2</i>
11:30	<i>Session: Pigment methodology-2</i>	11:45	<i>Schlüter & Møhlenberg</i>	11:10	<i>Peeken</i>
11:55	<i>van Lenning et al.</i>	12:10	<i>Irigoyen et al.</i>	11:35	<i>Wulff & Aten-Åke</i>
12:20	<i>Ruecker et al.</i>	12:35	<i>Eser & Huber</i>	12:00	<i>Yacobi & Herut</i>
12:45	<i>Schaegerl et al.</i>	14:35	Lunch break	12:25	<i>Brunet</i>
14:45	<i>Session: Comparing approaches-1</i>	15:35	<i>Session: Practical Chemtax</i>	14:25	<i>Session: Other</i>
15:10	<i>Furuya & Ramaiah</i>	16:00	KEYNOTE LECTURE: DENIS MACKAY	14:50	<i>Charpy</i>
15:35	<i>Terzic et al.</i>	17:20	LAB SESSION: HARRY HIGGINS	15:15	<i>Wiltshire & Beutler</i>
16:00	<i>Díez et al.</i>	17:45	LAB SESSION: FRANCISCO RODRÍGUEZ	15:40	<i>Wieland</i>
16:30	<i>Session: Comparing approaches - 2</i>	21:00	WORKSHOP DINNER	15:50	CONCLUDING REMARKS MIKEL LATASA
16:55	<i>Buchaca et al.</i>			16:20	Coffee break
17:20	<i>Rodrigues & Marinho</i>				END OF WORKSHOP
17:45	<i>Schmitt et al.</i>				

Session Information

Sunday morning

- 09:15 09:30 Check in.
09:30 10:00 Mikel Latasa.
Welcome and Opening remarks

Pigment Methodology (1) Chairperson: Kees van Lenning

- 10:00 11:00 Manuel Zapata.
*Looking at phytoplankton through HPLC pigment analysis:
When taxonomy is called chemotaxonomy*



- 11:00 11:30 **Coffee Break**

Pigment Methodology (2) Chairperson: Manuel Zapata

- 11:30 11:55 Kees van Lenning, Mikel Latasa, José Luis Garrido, Renate Scharek, Marta Estrada, Francisco Rodríguez, Manuel Zapata.
Losses of pigments in aqueous acetone and methanol extracts prepared for RP-HPLC analysis of pigments

- 11:55 12:20 Jacqueline Ruecker, A. Liepelt and H. Krumbeck.
Problems in estimating algal biomass by HPLC pigment analyses in acidic mining lakes

- 12:20 12:45 Michael Schaeferl, Martin Dokulil, Karl Donabaum, Wilfried Kabas, and Katrin Teubner.
Comparison of different phytoplankton quantification techniques in freshwater systems



- 12:45 14:45 **Lunch Break**

Sunday afternoon

Comparing Approaches (1) Chairperson: Carole Llewellyn

- 14:45 15:10 Ken Furuya and Neelam Ramaiah.
Application of CHEMTAX for estimating class-specific bio - mass of phytoplankton in a eutrophied coastal environment

- 15:10 15:35 Senka Terzic, Marijan Ahel, Sasa Vukelic, and Damir Vilicic.
Characterization of phytoplankton in a karstic estuary by biomark pigments and microscopy

- 15:35 16:00 Beatriz Díez, Mikel Latasa, Carlos Pedrós-Alió and Ramon Massana.
A spatio-temporal comparison of picoeukaryotes in the Alborán Sea (SW Mediterranean) by Denaturing Gradient Gel Electrophoresis and HPLC pigment analysis



- 16:00 16:30 **Coffee Break**

Comparing Approaches (2) Chairperson: Karen Wiltshire

- 16:30 16:55 Teresa Buchaca, Marisol Felip and Jordi Catalán.
Estimating algal class abundances in a stratified oligotrophic lake: HPLC measurements vs. direct cell counts
- 16:55 17:20 Silvana Rodrigues and Marcelon Marinho.
Phytoplankton of an eutrophic tropical reservoir: comparison of microscopy data with estimation from HPLC measurements of photosynthetic pigments using CHEMTAX
- 17:20 17:45 Mechthild Schmitt, Jacqueline Rücker, and Brigitte Nixdorf.
A comparison of phytoplankton dynamics in a lake outlet stream by microscopical and HPLC - pigment analysis

Monday morning

Chemtax (1) Chairperson: Louise Schlüter

- 9:00 10:00 Simon Wright.
Application of pigment analysis and CHEMTAX to field studies of phytoplankton communities
- 10:00 10:25 Carole Llewellyn and Denise Cummings.
Phytoplankton biomass and composition: CHEMTAX and microscopy compared



10:25 10:55 **Coffee Break**

Chemtax (2) Chairperson: Denis Mackey



- 10:55 11:20 Louise Schlüter and Flemming Møhlenberg.
Determining phytoplankton group composition by pigment ratios and size fractionations
- 11:20 11:45 Xabier Irigoien, Bettina Meyer-Harms, Roger Harris, Derek Harbour and Stuart Gibb
Using HPLC and CHEMTAX to investigate phytoplankton taxonomy: the importance of knowing your species
- 11:45 12:10 Sonja Eser and Wilfried Huber.
Monitoring of periphyton community shifts after application of herbicides using HPLC pigment analysis and microscopy
- 12:10 12:35 Emmanuelle Lemaire, Rutger De Wit, G. Abril and H. Etcheber.
Liquid Chromatography studies of photosynthetic pigments in estuaries need to consider continental inputs and degradation processes



12:35 14:35 **Lunch Break**


Monday afternoon

Practical Chemtax

- 14:35 15:35 Denis Mackey.
CHEMTAX - Not just a “Black Box”
- 15:35 Harry Higgins.
LAB SESSION
- Francisco Rodríguez.
LAB SESSION
-  **Coffee Break**
-  21:00 **Workshop dinner**

Tuesday morning

Pigment Results (1) Chairperson: Simon Wright

- 9:00 9:25 Vivienne Lochhead, M.W. Lomas, P.J. Lethaby, R.J. Johnson, N.R. Bates and A. H. Knap.
Long-term variability of phytoplankton community structure at the Bermuda Atlantic Time-series Study (BATS) site based on pigment analyses using the “CHEMTAX” matrix
- 9:25 9:50 Roberto Millán-Núñez. Charles C. Trees and Jim Aiken.
Pigment specific distribution and community structure of phytoplankton during Atlantic meridional Transect (AMT3)
- 9:50 10:15 Yumiko Obayashi and Koji Suzuki.
Correspondence of phytoplankton community structures estimated by pigment and CHEMTAX to hydrographic water masses, a case study on a Kuroshio warm-core ring
-  10:15 10:45 **Coffee Break**

Pigment Results (2) Chairperson: Millán-Núñez

- 10:45 11:10 Ilka Peeken.
Changes in phytoplankton community during the Southern Ocean Iron Fertilisation Experiment “EisenEx 1” based on marker pigments
- 11:10 11:35 Angela Wulff and Wängberg Sten-Åke.
Spatial and vertical distribution of phytoplankton pigments in the eastern atlantic sector of the southern ocean
- 11:35 12:00 Yosef Yacobi, Y. Suari and B. Herut.
Phytoplankton pigment markers along the Mediterranean coast of Israel

12:00 12:25 Christophe Brunet.
Sources of variability in cell and population pigment composition: Temporal, spatial and physiological scales



12:25 14:25 **Lunch Break**

Tuesday afternoon

Others

Chairperson: Mikel Latasa

14:25 14:50 Loïc Charpy.
Use of flow cytometry and extracted chlorophyll data to assess picoplankton groups contribution to phytoplankton biomass

14:50 15:15 Karen Wiltshire and M. Beutler.
Algal population composition and biomass determined in situ with accessory pigment induced fluorescence methods

15:15 15:40 John Wieland

15:40 15:50 Mikel Latasa.
CLOSING REMARKS



15:50 **Coffee**



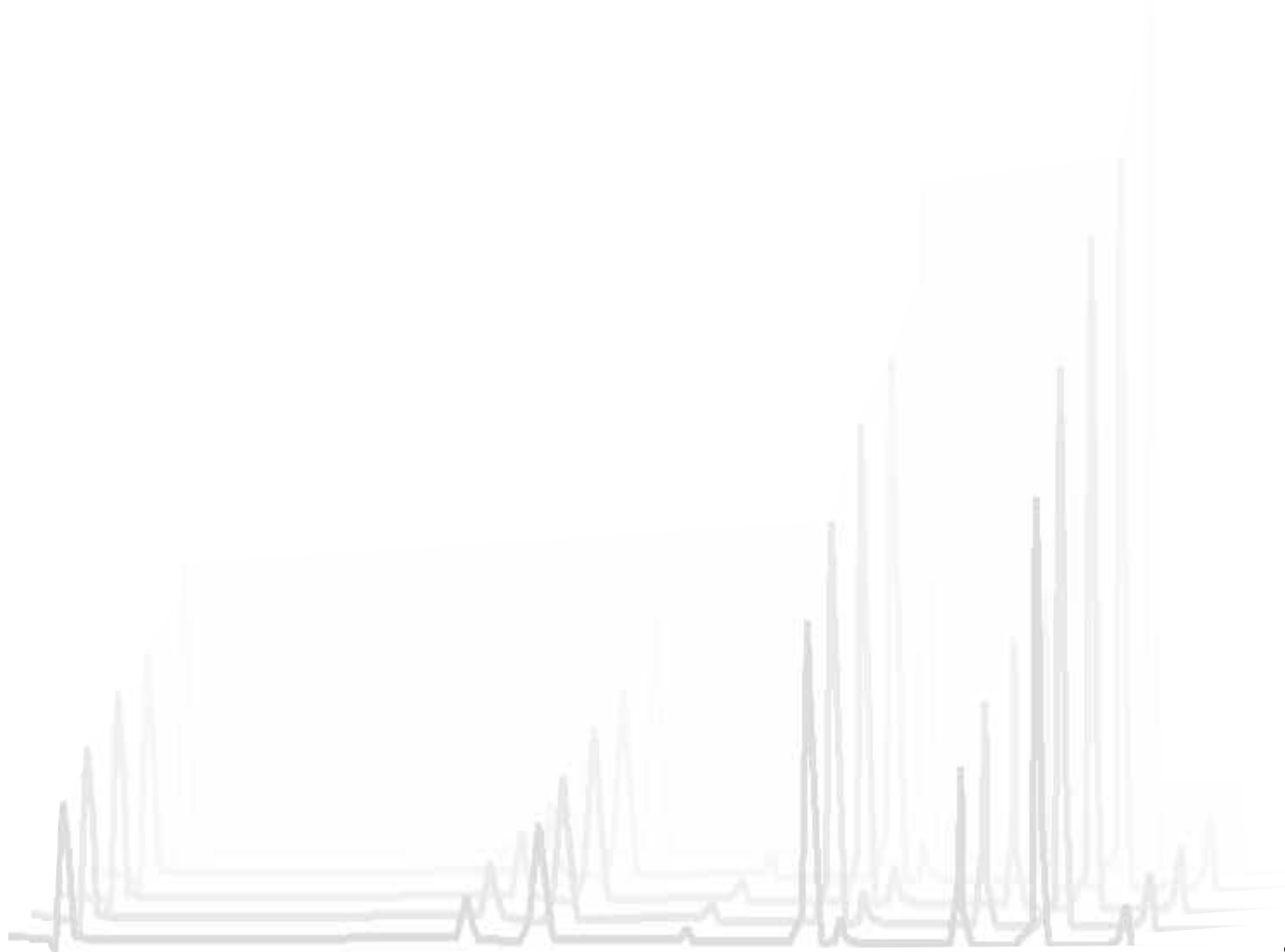
Sunday 29, 10:00

Looking at phytoplankton through HPLC pigment analysis. When taxonomy is called Chemotaxonomy

Manuel Zapata

CIMA, Conselleria de Pesca, Xunta de Galicia, Apdo. 13, 36620-Vilanova de Arousa, Spain.

New developments in chromatographic techniques have enlarged our knowledge on pigment composition of marine phytoplankton. Improvements in HPLC methods have allowed the separation of a wide array of pigment including both polar chlorophyll (chl) *c*-type pigments (chl *c*₁, chl *c*₂, chl *c*₃, MgDVP) and non-polar (i.e., esterified) chls (chl *a* and *b* plus its DV forms, non-polar chl *c*), as well carotenoids in a single run. These methods incorporate pyridine-containing mobile phases in combination with polymeric C18, or monomeric C8 columns. Paralleling HPLC improvements, novel pigments such as chl *c*₂-MGDG (a pigment consisting in a chl *c*₂ esterified to monogalactosyldiacylglycerol), monovinyl chl *c*₃, and 4-keto-19'-hexanoyloxyfucoxanthin have been detected in several bloom-forming haptophytes such as *Emiliana huxleyi* and *Chrysochromulina polylepis*. The combination of new HPLC methods, able to separate new marker pigments (and pigment signatures) and the new generation of mathematical tools (e.g. CHEMTAX) for interpreting the acquired data set, will provide invaluable information about the variability of phytoplankton populations associated to hydrographic structures and different oceanographic regions.



Sunday 29, 11:30

Losses of pigments in aqueous acetone and methanol extracts prepared for RP-HPLC analysis of pigments

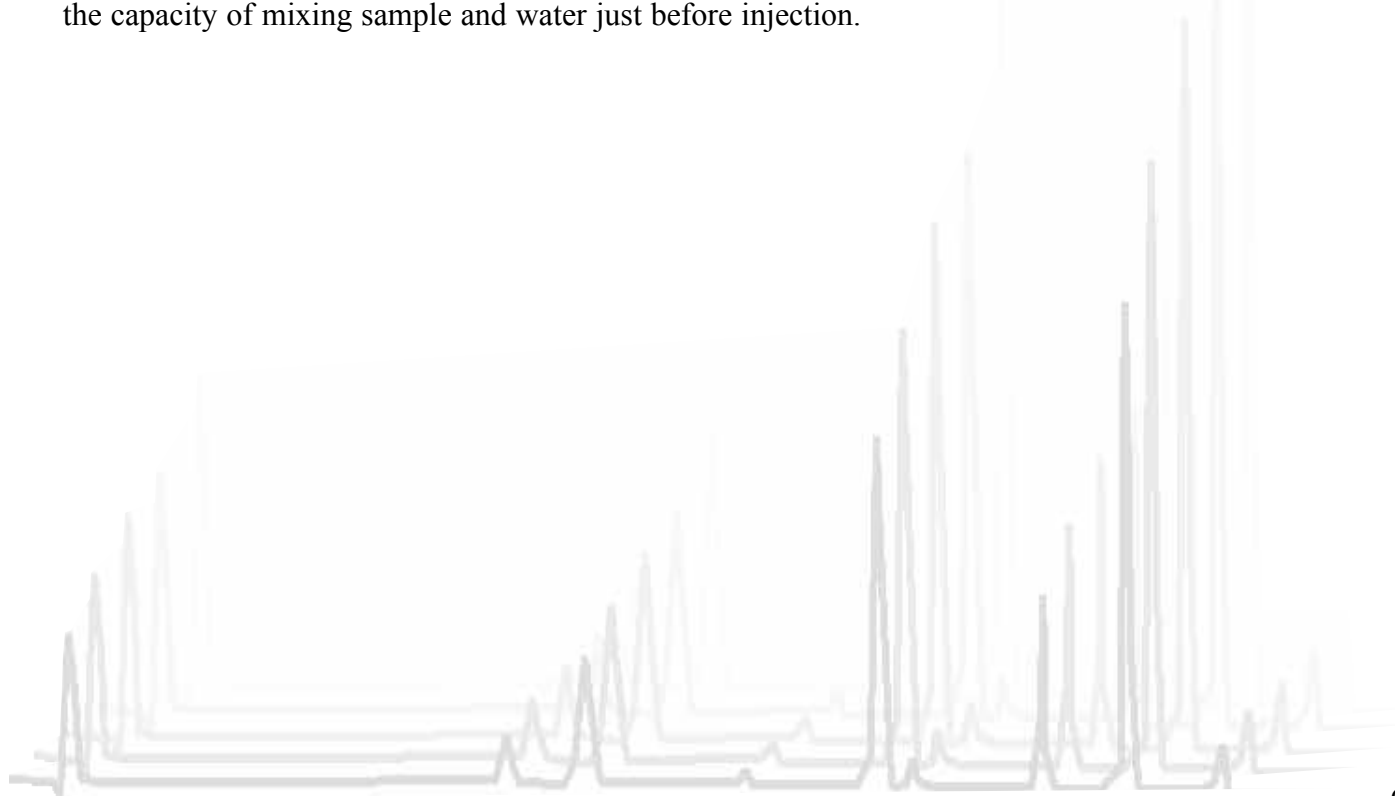
*Kornelis van Lenning*¹, *Mikel Latasa*¹, *José-Luis Garrido*², *Renate Scharek*¹, *Marta Estrada*¹, *Francisco Rodríguez*³ and *Manuel Zapata*³

¹ Institut de Ciències del Mar, C.S.I.C., Barcelona, Spain;

² Instituto de Investigaciones Mariñas, C.S.I.C., Vigo, Spain

³ Centro de Investigaciones Mariñas, Vilanova de Arousa, Spain

RP-HPLC protocols for analysis of photosynthetic pigments usually require water additions to methanol or acetone extracts to avoid peak distortion effect. We have investigated the short- (< 2 minutes) and long-term (up to 48 hours) effect of water addition to acetone and methanol extracts from two marine phytoplankton species, *Emiliania huxleyi* and *Dunaliella tertiolecta*. Solvent extracts were prepared and separated into fractions that were subsequently diluted with water to 90%, 80%, 70%, 60%, 50% and 40% for methanol, and extended to 30% and 20% for acetone. Changes in pigment concentration with time were followed using both spectrophotometric and chromatographic procedures. There was a clear loss of pigments due to precipitation immediately after dilution of extracts to acetone 60% or less and to methanol 80% or less. For chlorophyll *a* the most important losses were recorded in 50% acetone (up to 27% decrease) and in 70% methanol (31% decrease). This effect increased considerably with time. Only 90% and 80% acetone kept intact the initial concentration of all pigments after 24 hours, and even up to 48 h. On the contrary, more than 60% and 57% of the initial chlorophyll *a* concentrations were lost after 24 hours in 50% acetone and 70% methanol extracts, respectively. These losses increased to 83% and 60% after 48 hours. There was a clear correlation between the polarity of a certain pigment and the polarity of the solvent at which its maximum precipitation occurred. Pigment losses were also observed in pure acetone and methanol extracts with time, although we attribute them to pigment degradation rather than precipitation. Some of the losses occurring with time can be avoided using autosamplers with the capacity of mixing sample and water just before injection.



Sunday 29, 11:55

Problems in estimating algal biomass by HPLC pigment analyses in acidic mining lakes

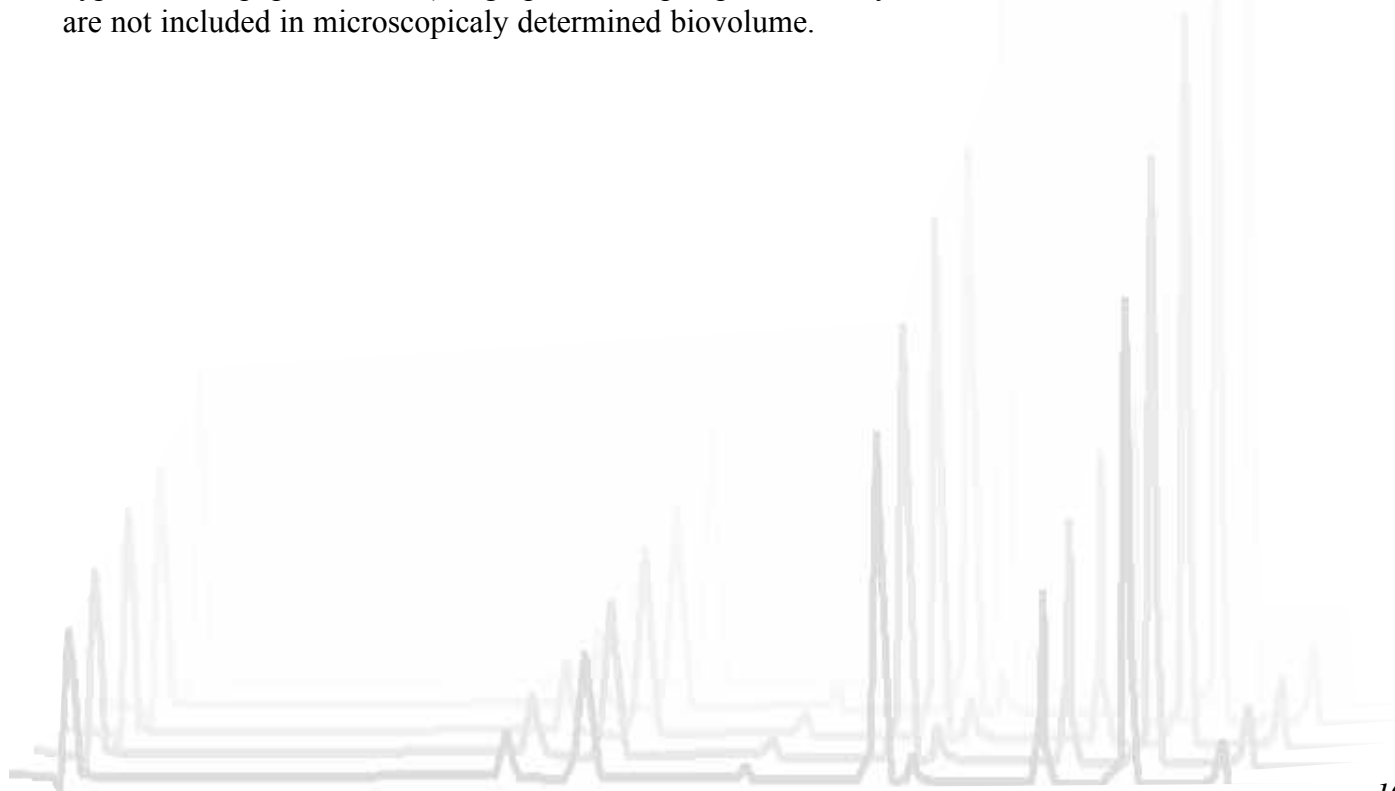
Jacqueline Ruecker, A. Liepelt and H. Krumbeck

Brandenburg Technical University of Cottbus, Bad Saarow, Germany

In the mining district of Lusatia (East Germany), more than four hundred lakes have been developed following closure of open-cast lignite pits. Most of them are characterised by high acidity, high concentrations of dissolved iron and sulfate but low carbon and phosphorous. Depending on the degree of acidity, the lakes are colonised by Chlorophyceae and Chrysophyceae (pH 2.5 to 3.5), and additionally by Dinophyceae and Cryptophyceae at moderate acidity (pH 3.5 to 4.0). The number of species usually do not exceed six. Cyanobacteria were found only in circumneutral lakes (pH > 6) which show a diverse mesotrophic phytoplankton dominated by Bacillariophyceae.

For monitoring algal composition and biomass, HPLC pigment analysis was tested and compared with microscopic counting. There are specific problems when analysing pigment composition of lakes in this landscape: i) Low biomass ($< 5 \mu\text{g Chl } a \text{ l}^{-1}$). ii) High contents of iron compounds which prevent filtration of water samples large enough to get a sufficient pigment yield in the extracts. iii) High acidity which accelerates enormously pigment degradation during extraction procedure and storage of samples.

To solve the problems, magnesium carbonate and ammonium acetate were tested as buffers for preventing pigment degradation. The best results were achieved with ammonium acetate. The correlation between algal biovolume obtained by microscopic counting and pigment content was not satisfying. Some reasons are: i) The very low biomass level involves high errors in cell countings as well as in pigment analyses. ii) Uncontrolled pigment degradation during pigment extraction and storage. iii) High variability of cellular pigment content between epi- and hypolimnetic populations. iv) High portion of picoplanktonic cyanobacteria in neutral lakes which are not included in microscopically determined biovolume.



Sunday 29, 12:20

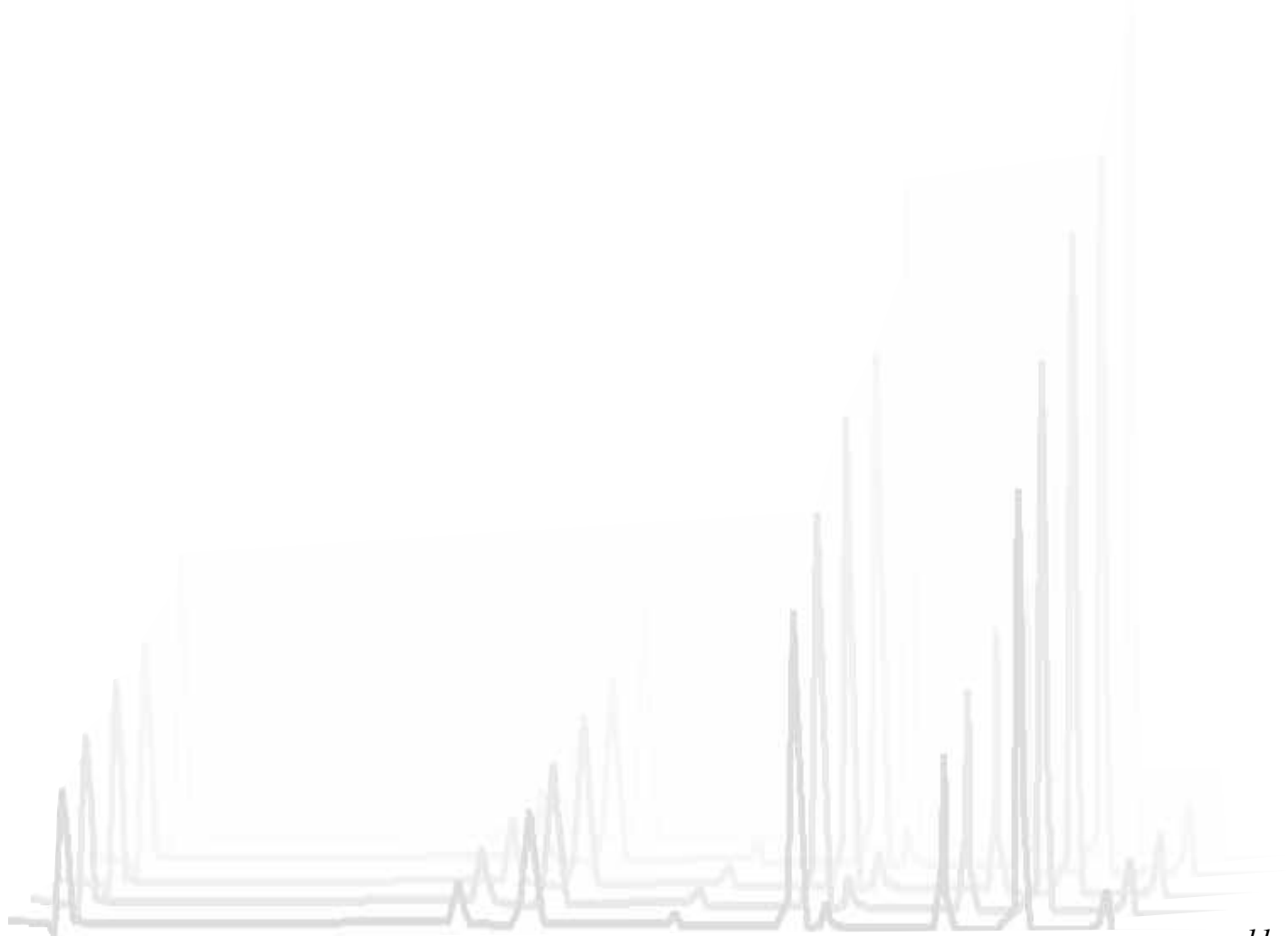
Comparison of different phytoplankton quantification techniques in freshwater systems

Michael Schagerl, Martin Dokulil, Karl Donabaum, Wilfried Kabas and Katrin Teubner

Institute of Ecology and Conservation Biology, University of Vienna, Vienna, Austria

From 1996 to 2000, phytoplankton samples were collected in 14 days intervals from the backwater “Alte Donau”, which is situated in the city of Vienna (Austria). Based on these data, phytoplankton quantification by means of the inverted microscope technique was compared to the results obtained by pigment markers. Calculations of pigment-based phytoplankton composition were done both with CHEMTAX and fixed ratios.

The comparison of the different methods showed high relation thus confirming other studies. Discrepancies could partly be traced back to the different approaches: (I) pigment based estimations are dependent on the physiological state of algae and (II) the inverted microscope technique is highly influenced by the person responsible for cell counting.



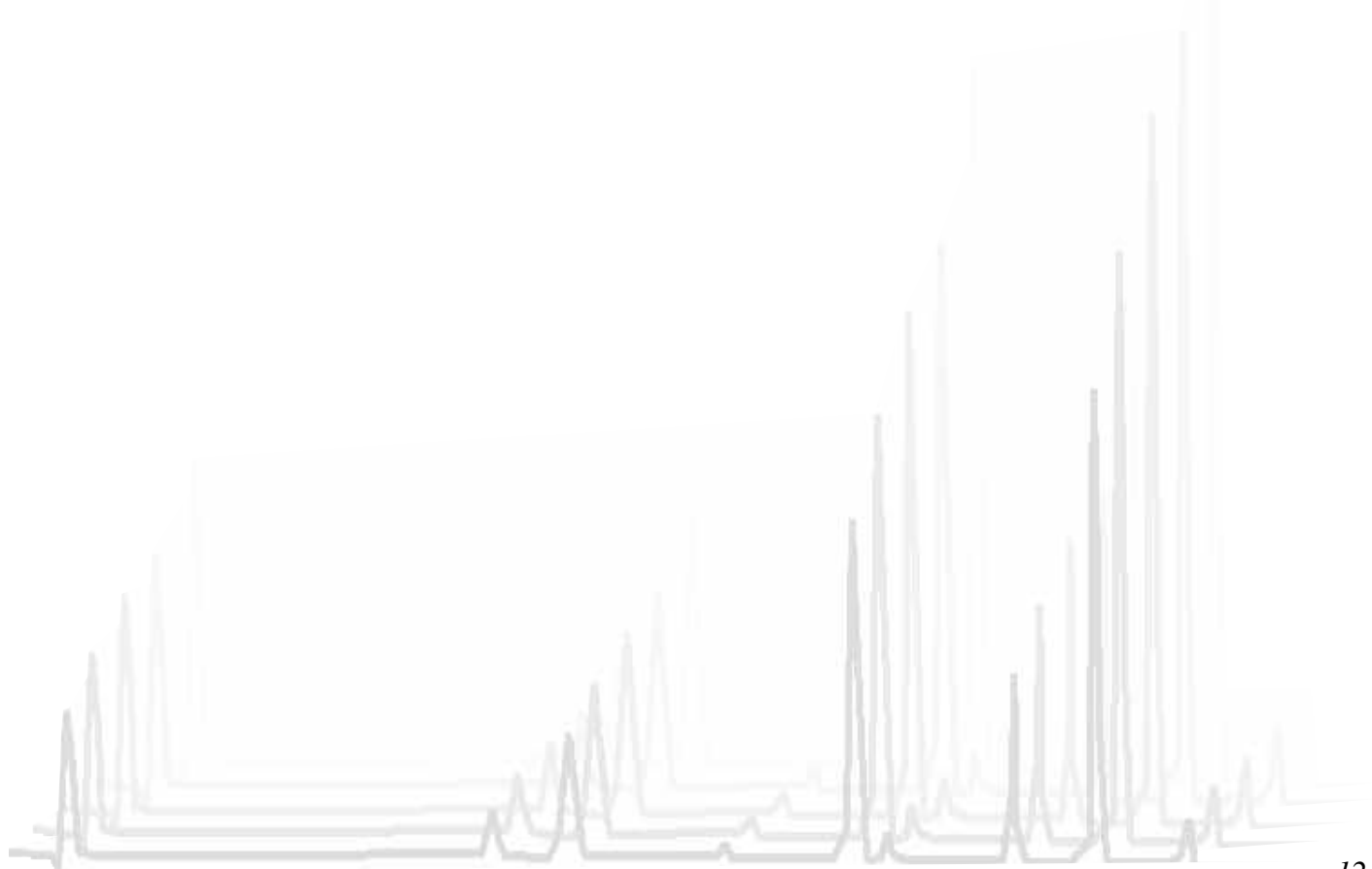
Sunday 29, 14:45

Application of CHEMTAX for estimating class-specific biomass of phytoplankton in a eutrophicated coastal environment

Ken Furuya and Neelam Ramaiah

Department of Aquatic Bioscience, Graduate School of Agricultural and Life Sciences, the University of Tokyo.

Annual variations in phytoplankton composition were investigated in Tokyo Bay. Blooms caused by red-tide organisms like diatoms, raphidophytes and dinoflagellates are an obvious impact of eutrophication in this bay. As an alternative approach to microscopy, particularly to quantify the fragile forms, we determined the group-specific abundance of phytoplankton using CHEMTAX. Nine groups were decided based on the pigment concentrations obtained by HPLC analysis. Initial pigment ratios used for groups other than the raphidophytes were modified from Mackey et al. (1996). Fucoxanthin and violaxanthin were considered as marker pigments for raphidophytes. In Tokyo Bay this group is represented by *Heterosigma akashiwo* which are not adequately quantified due to loss during preservation. We thus obtained the pigment ratios by exposing cultures of *H. akashiwo* to varying light intensity in order to account for the light adapted variations in the concentrations of pigments. Diatoms dominated throughout the year. Raphidophytes became abundant in late spring and summer and contributed substantially to total chlorophyll *a*. Other groups recorded were the dinoflagellates, prasinophytes, haptophytes, chlorophytes, chrysophytes, cryptophytes and cyanophytes. Temporal variations in raphidophytes estimated by CHEMTAX were in good agreement with that obtained by microscopic quantification. Total amount of chlorophyll *a* was primarily contributed by diatoms and raphidophytes. The chlorophyll *a* of prokaryotes and other eukaryotic flagellates did not exceed above a certain limit, indicating herbivorous control on the small-sized phytoplankton.



Sunday 29, 15:10

Characterization of phytoplankton in a karstic estuary by biomarker pigments and microscopy

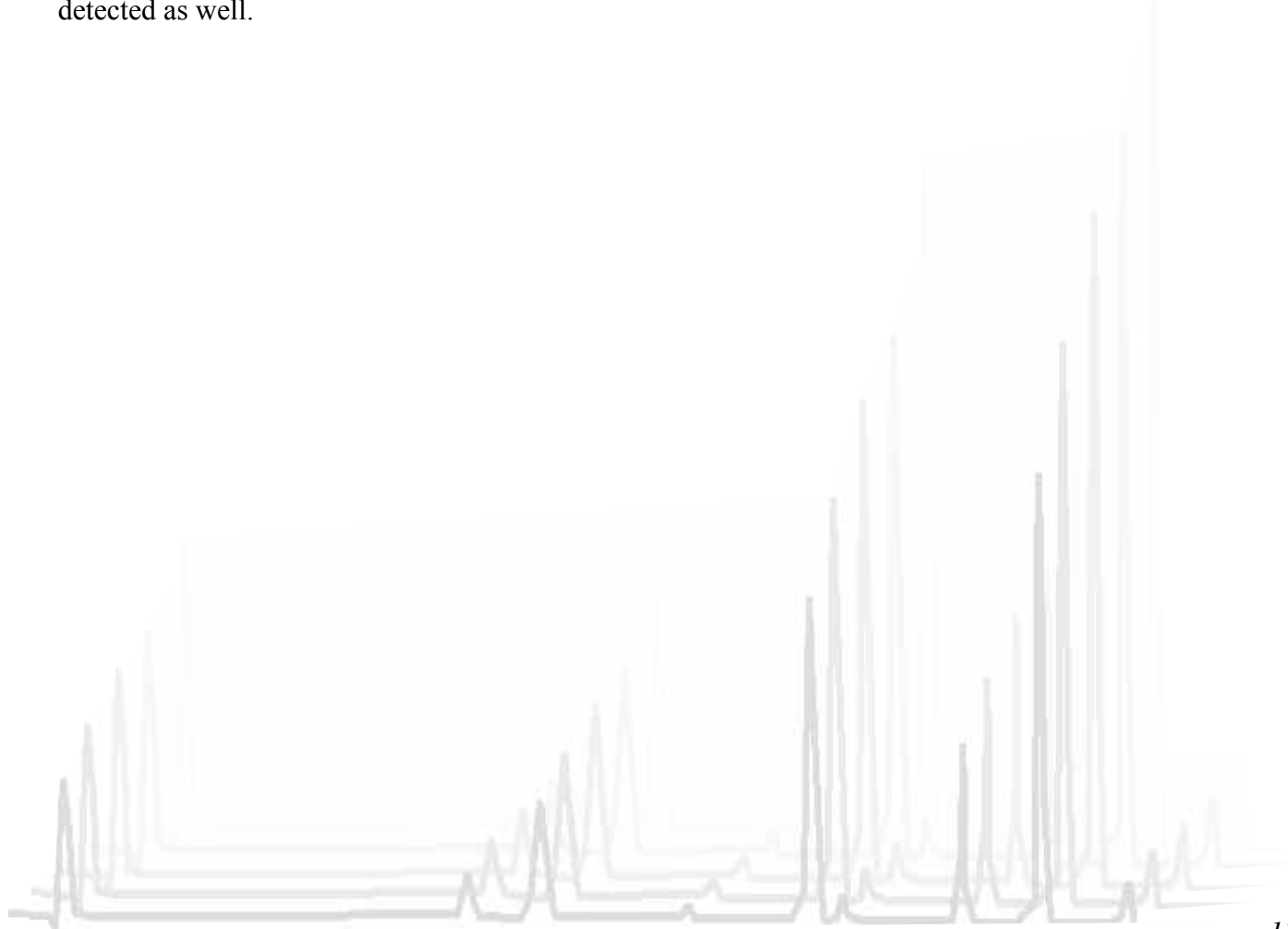
Senka Terzic¹, Marijan Ahel¹, Sasa Vukelic² and Damir Vilicic²

¹ Centre for Marine and Environmental Research, Rudjer Boskovic Institute, Zagreb, Croatia

² Department of Biology, Faculty of Natural Sciences and Mathematics, University of Zagreb, Zagreb, Croatia.

Recent investigations indicate that the salinity gradients in karstic estuaries belong to important factors, which govern the distribution of phytoplankton and suggest that small-sized phytoplankton could represent an important fraction of phytoplankton biomass. In this work, we present the first results of the characterisation of phytoplankton in the Zrmanja estuary (Croatia), as reflected by HPLC analysis of photosynthetic pigments and by classical microscopy. The chlorophyll and carotenoid pigments were determined using reversed-phase high-performance liquid chromatography (RP HPLC) equipped with serially-coupled spectrophotometric and spectrofluorimetric detectors.

The study indicated that the vertical and longitudinal distribution of the total phytoplankton biomass as reflected by chlorophyll *a* (chl *a*) strongly depended on salinity distribution, with higher concentrations being associated with salinity range of 15-25 PSU. According to biomarker pigment concentrations diatoms (fucoxanthin) and cryptophytes (alloxanthin) were the main phytoplankton groups during the investigated period. However, presence of dinoflagellates (peridinin), prymnesiophytes (19'-hexanoyloxyfucoxanthin) and prasinophytes (prasinoloxanthin) could be detected as well.



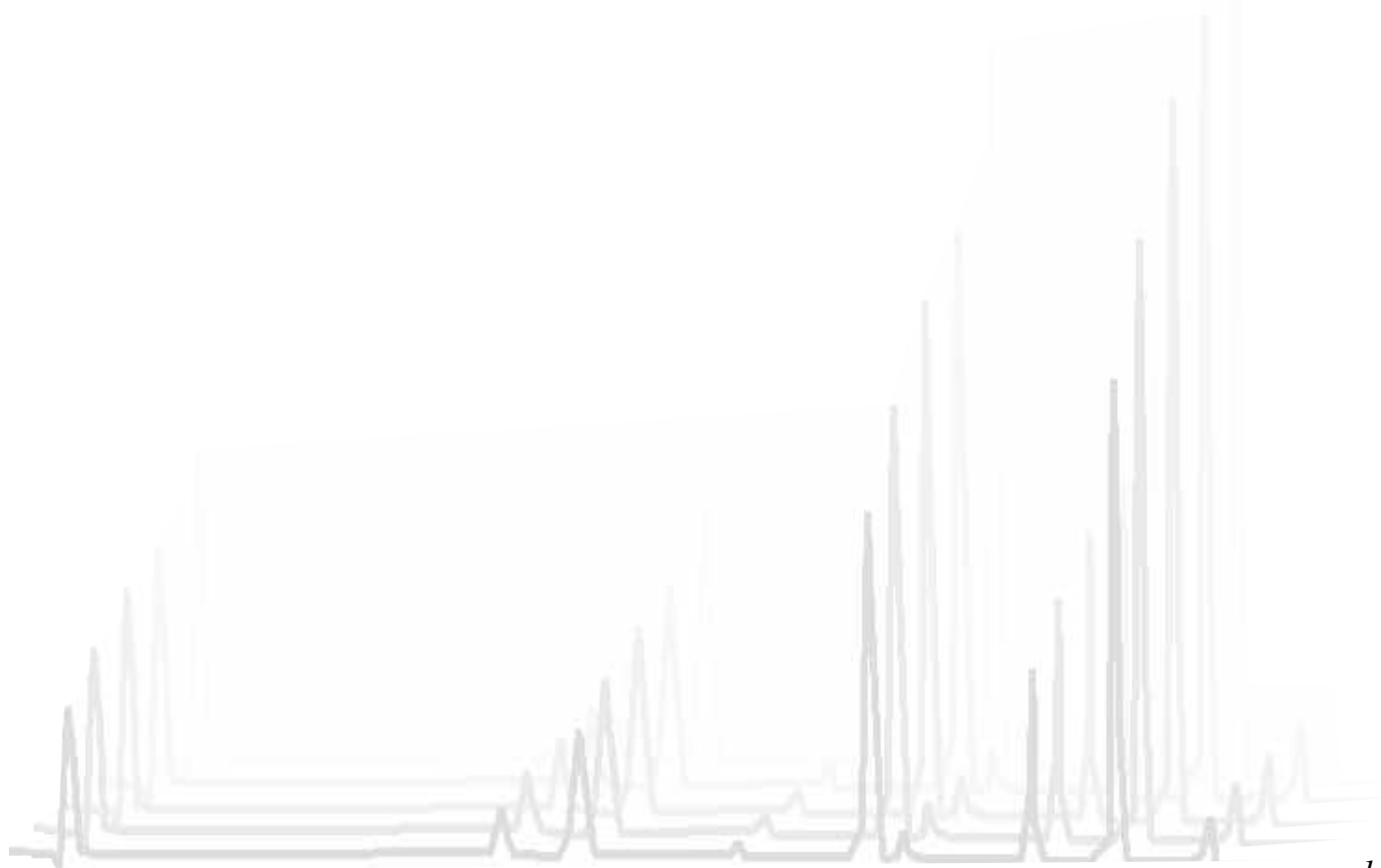
Sunday 29, 15:35

A spatio-temporal comparison of picoeukaryotes in the Alborán Sea (SW Mediterranean) by Denaturing Gradient Gel Electrophoresis and HPLC pigments analysis

Beatriz Díez, Mikel Latasa, Carles Pedrós-Alió and Ramon Massana

Institut de Ciències del Mar, CSIC, Passeig Joan de Borbó s/n, 08039 Barcelona. Spain.

Within the phytoplankton community, the picoplanktonic fraction is regarded as quasi-invariable in terms of biomass. In terms of taxonomical composition, however, its variability is very much unknown. This lack of knowledge is due, above all, to the technical difficulties to taxonomically classify the picoplanktonic organisms. Here, we used two marker techniques, HPLC-pigment analysis and rRNA-DGGE, to describe the spatial and temporal variability of the eukaryotic picoplankton community in the Alborán Sea (SW Mediterranean Sea) during Spring 1998 and Fall 1999. In this marine area there are different macroscale and mesoscale physical structures due to the input of surface Atlantic waters into the Mediterranean basin. While it is known that these structures cause a high spatial variability in the composition of large phytoplankton, their influence on the distribution of small picoplankton is unknown. Cluster analysis of the DGGE results from 1998 grouped the samples in a shore-offshore gradient. In 1999, however, the shore-offshore pattern was not revealed. DGGE and HPLC results showed that the strongest differences in picoplankton community occurred between seasons/years. The pigment matrix was converted to the relative contribution of the different picophytoplankton groups using CHEMTAX. The main DGGE bands were sequenced and compared to the genetic database. Both techniques agreed in presenting chlorophyll b-containing algae and prymnesiophytes as the main components of the picoplankton during our cruises in the Alborán Sea.



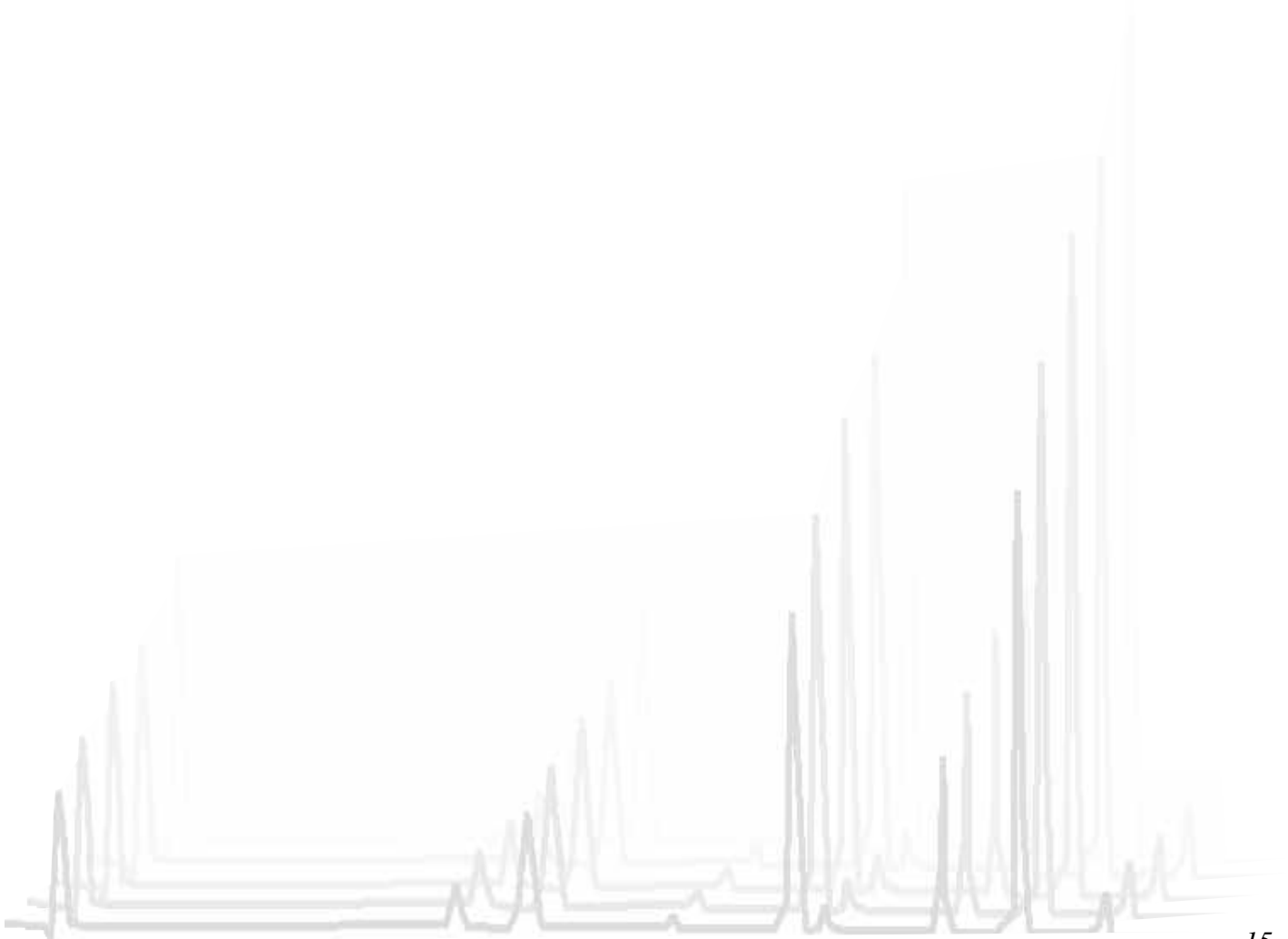
Sunday 29, 16:30

Estimating algal class abundances in a stratified oligotrophic lake: HPLC measurements vs. direct cell counts

Teresa Buchaca, Marisol Felip and Jordi Catalán

Department of Ecology, University of Barcelona, Avd. Diagonal 645, E-08028 Barcelona, Spain

We discuss the preliminary results obtained of applying the CHEMTAX program to a set of samples from a dimictic oligotrophic mountain lake. Samples were taken every 9 m depth along a 63 m profile and monthly from June-97 to December-97. The period sampled covers three main production episodes, the first one occurring during spring overturn, a second one in the upper hypolimnion during summer stratification and the last one under the ice at the beginning of the ice-covered period. Estimated algal class abundances were compared with direct cell counts. Flagellated chrysophytes and dinoflagellates dominated phytoplankton biomass; occasionally cryptophytes and chlorococcal chlorophytes were also significant.



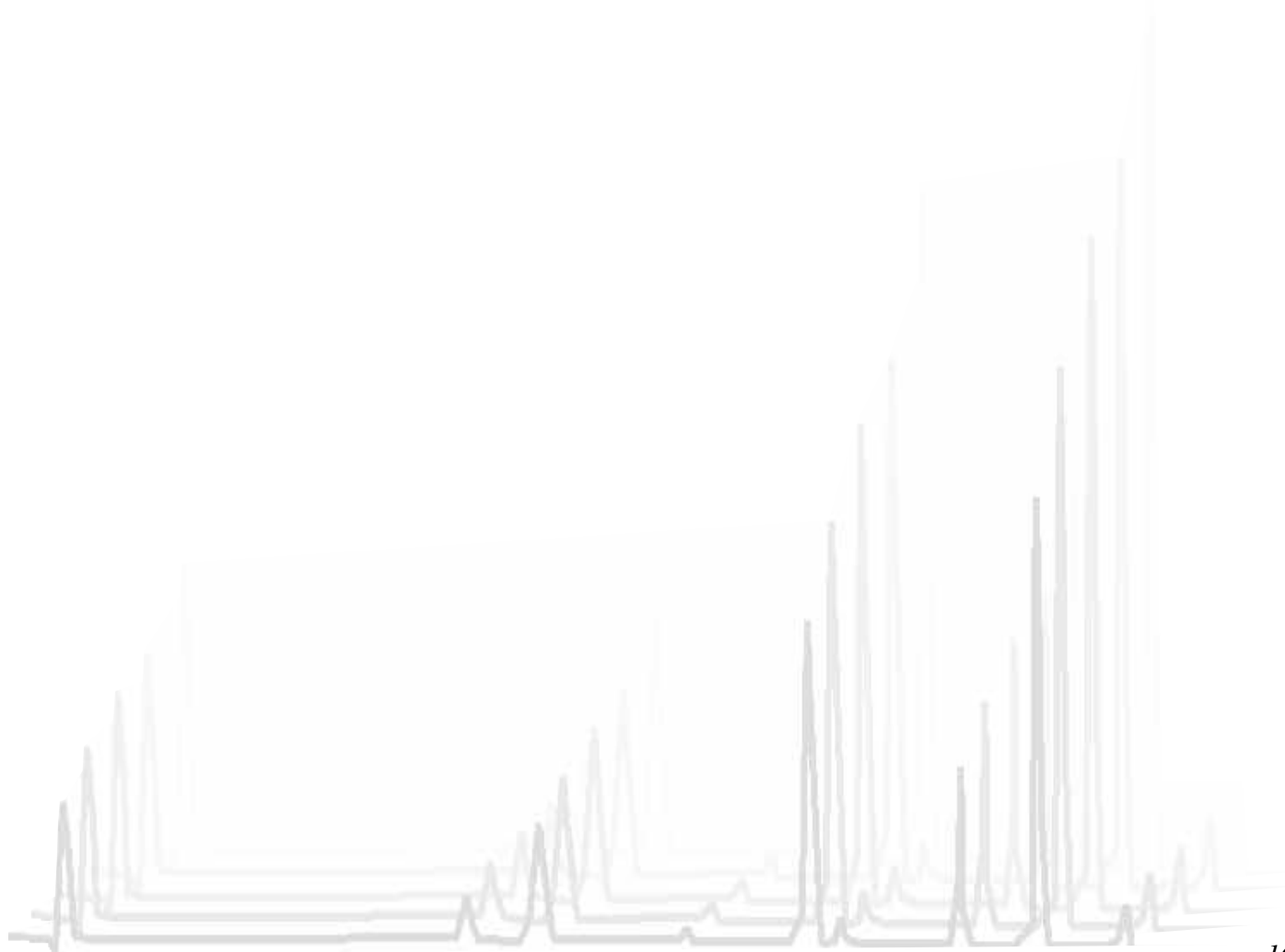
Sunday 29, 16:55

Phytoplankton of an eutrophic tropical reservoir: comparison of microscopy data with estimation from HPLC measurements of photosynthetic pigments using CHEMTAX

Silvana Rodrigues and Marcelo Marinho

Universidade Federal do Rio de Janeiro, Núcleo de Pesquisas de Produtos Naturais and Universidade Federal Fluminense, Departamento de Química Analítica, Niterói, Brasil

The seasonal variation of phytoplankton in an eutrophic reservoir was evaluated through photosynthetic pigments analyzed by HPLC. The contributions of algal classes to total chlorophyll *a* (TChl-*a*) were estimated, using CHEMTAX to analyze the pigment data. These results were compared with estimated biomass (biovolume) from microscope analysis. Although displaying some differences, the general pattern of phytoplankton community dynamics was described in a similar way by cell count and pigment data, and variations in phytoplankton biomass and composition were detected. The CHEMTAX software satisfactorily calculated the contributions of the algal groups to TChl-*a*, and provided better agreement with microscopy data than the calculations based on marker pigment/chlorophyll *a* ratios. These results demonstrated that, in spite of some inherent limitations, the HPLC method is a valuable tool for monitoring and for ecological studies of phytoplankton.



Sunday 29, 17:20

A comparison of phytoplankton dynamics in a lake outlet stream by microscopical and HPLC - pigment analysis

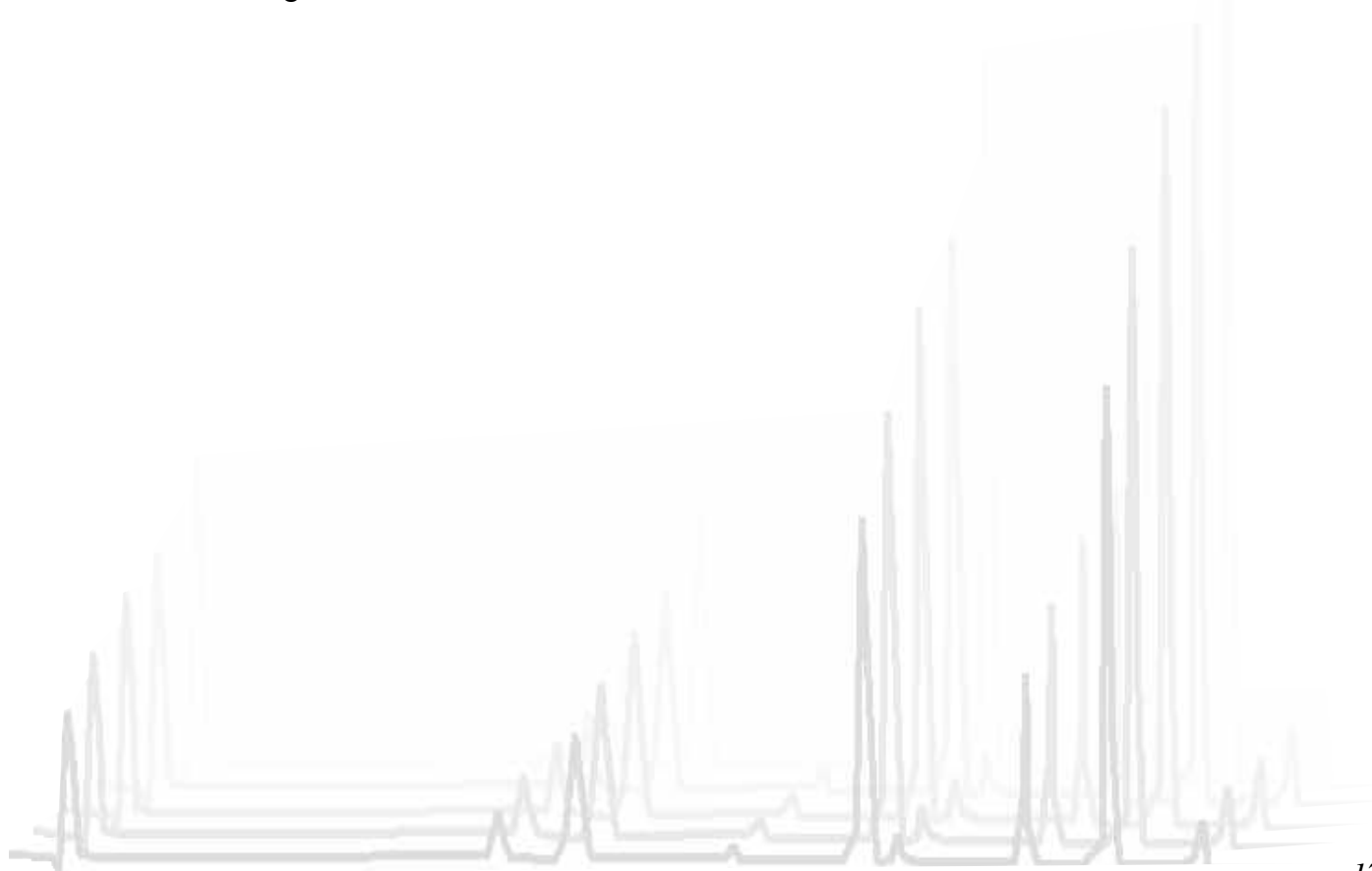
Mechthild Schmitt¹, Jacqueline Rücker² and Birgitte Nixdorf²

¹ Department of Chemical Ecotoxicology, UFZ, Leipzig, Germany

² Chair of Water conservation, Brandenburg Technical University of Cottbus, Bad Saarow, Germany

Landscape of North East Germany is characterised by river-lake systems. Shallow eutrophic lakes are connected by small streams, which are loaded with high amounts of lakeborn phytoplankton. In the stream, the lake-adapted plankton is faced with a changed abiotic and biotic environment. An increase in soluble nutrients was observed. Turbulence and the benthic-pelagic coupling intensified. The light climate diminished by a dense riparian vegetation.

Phytoplankton dynamics were investigated along a stream course by comparing upstream and downstream samples. Biomass and species composition were detected by microscopy (Utermöhl standard techniques) and by pigment analyses (HPLC). Lipid-soluble pigments were extracted in 90 % acetone and separated and determined by C18-reversed phase HPLC according to Voitke et al. (1994), modified by Lenhard (unpublished). The upstream lake was characterised by a plankton regime, dominated by cyanobacteria in summer and communities of chrysophytes and diatoms in winter. Both methods demonstrated an elimination of phytoplankton biomass along the stream course. Elimination rates varied seasonally and were highest in summer. Shifts in the relative portion of the marker pigments to chl *a* (carotenoid / chl *a* - ratio) between upstream and downstream samples were detected for fucoxanthin and zeaxanthin. This downstream changes of phytoplankton will be discussed in the context of adaptation processes to a changed abiotic environment along the stream course.



Monday 30, 9:00

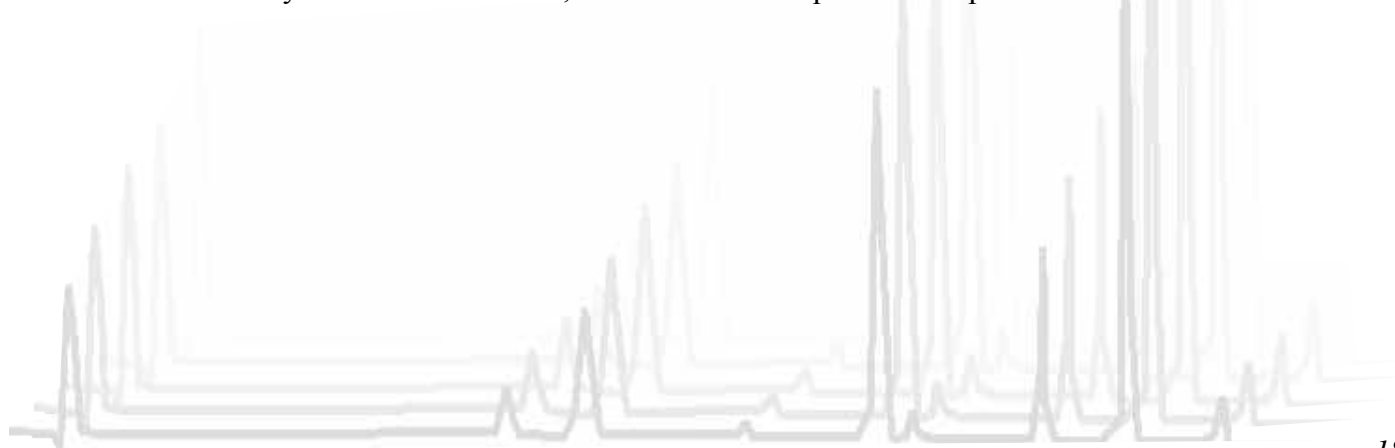
Application of pigment analysis and CHEMTAX to field studies of phytoplankton communities

Simon W. Wright

Australian Antarctic Division, Channel Hwy, Kingston, 7050, Tas., Australia

During the 1960's and 70's, pigments were recognized as providing a chemical means for estimating the composition and abundance of phytoplankton in the presence of zooplankton, bacteria and detritus. However the taxonomic distribution of pigments was poorly understood and thin-layer chromatographic techniques available at the time did not have the resolution nor the throughput of samples to make pigment analysis a practical tool for field studies. The development of HPLC methods during the 1980's provided increased resolution that led to the recognition of many new pigment markers, while automated equipment permitted analysis of large numbers of field samples. Interpretation of this pigment data was limited to assignment of single marker pigments to particular taxa. Increasingly though, analysis of cultured phytoplankton showed that the distribution of pigments was much more complex than originally envisaged. Few pigments were unambiguous markers – most were present in multiple taxa in various combinations with other pigments, and many of these pigment patterns crossed taxonomic boundaries. The emphasis thus changed from using individual markers to considering representative 'suites' of pigments, greatly complicating interpretation of the data. A number of computer methods were developed to aid interpretation, CHEMTAX being perhaps the most powerful and versatile. CHEMTAX proved valuable in interpreting field pigment data and in many cases its interpretations have been confirmed by direct techniques such as microscopy. Pigment analysis now provides a practical tool for analyzing phytoplankton populations, allowing detailed mapping that would not be feasible by other techniques.

However, pigment analysis and CHEMTAX are still limited, primarily by a lack of data on the pigment composition of phytoplankton. Only a tiny proportion of species has been analyzed by modern methods. Furthermore, laboratory studies have shown large changes in the ratios of pigments in response to light (and hence sample depth) and nutrient status. It is essential to allow for these changes during CHEMTAX analysis, but the software does not facilitate this as yet. Nor does the software identify or allow for changes of oceanic region and/or gross species composition within a sample set. This presentation will examine the role of pigments as markers in phytoplankton ecology and the current state of data interpretation by CHEMTAX using examples from the Southern Ocean as well as other regions. The necessity for determining species composition by microscopy is emphasized. Methods for optimizing pigment HPLC and subsequent CHEMTAX analysis will be examined, as well as current plans for improvements in the method.



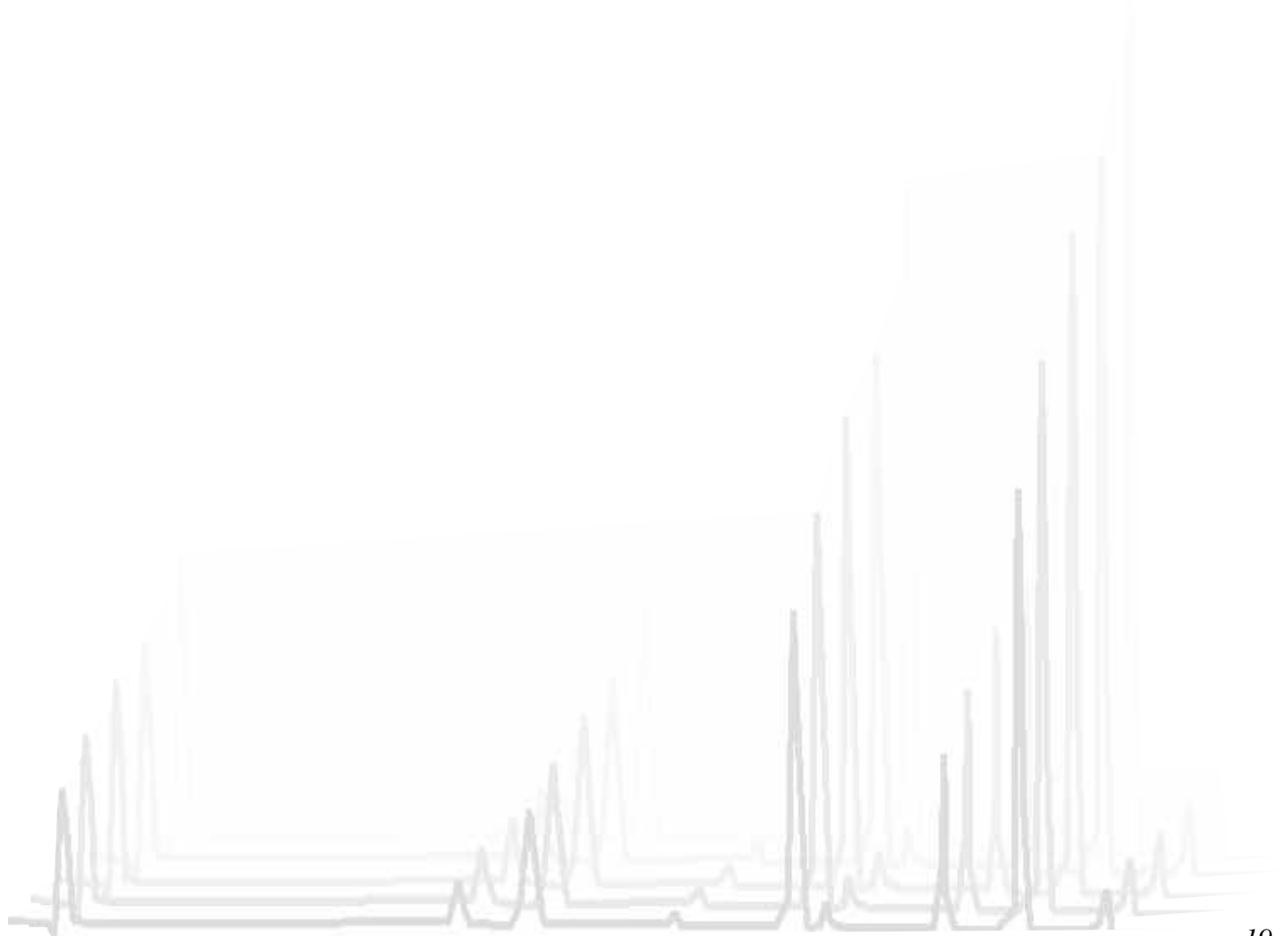
Monday 30, 10:00

Phytoplankton biomass and composition: CHEMTAX and microscopy compared

Carole Llewellyn and Denise Cummings

Plymouth Marine Laboratory, UK

CHEMTAX has enabled scientists to convert algal pigment concentrations determined using HPLC into estimates of phytoplankton class abundance. How do these CHEMTAX results compare to estimates determined using light and fluorescence microscopy? In this presentation pigment and CHEMTAX determinations are compared with microscopy counts for three data sets; the North Sea, the Iberian Sea and the English Channel. Comparisons are made for vertical profiles and a surface temporal data set. Are such comparisons valid? Errors associated with CHEMTAX and microscopy analysis are discussed.



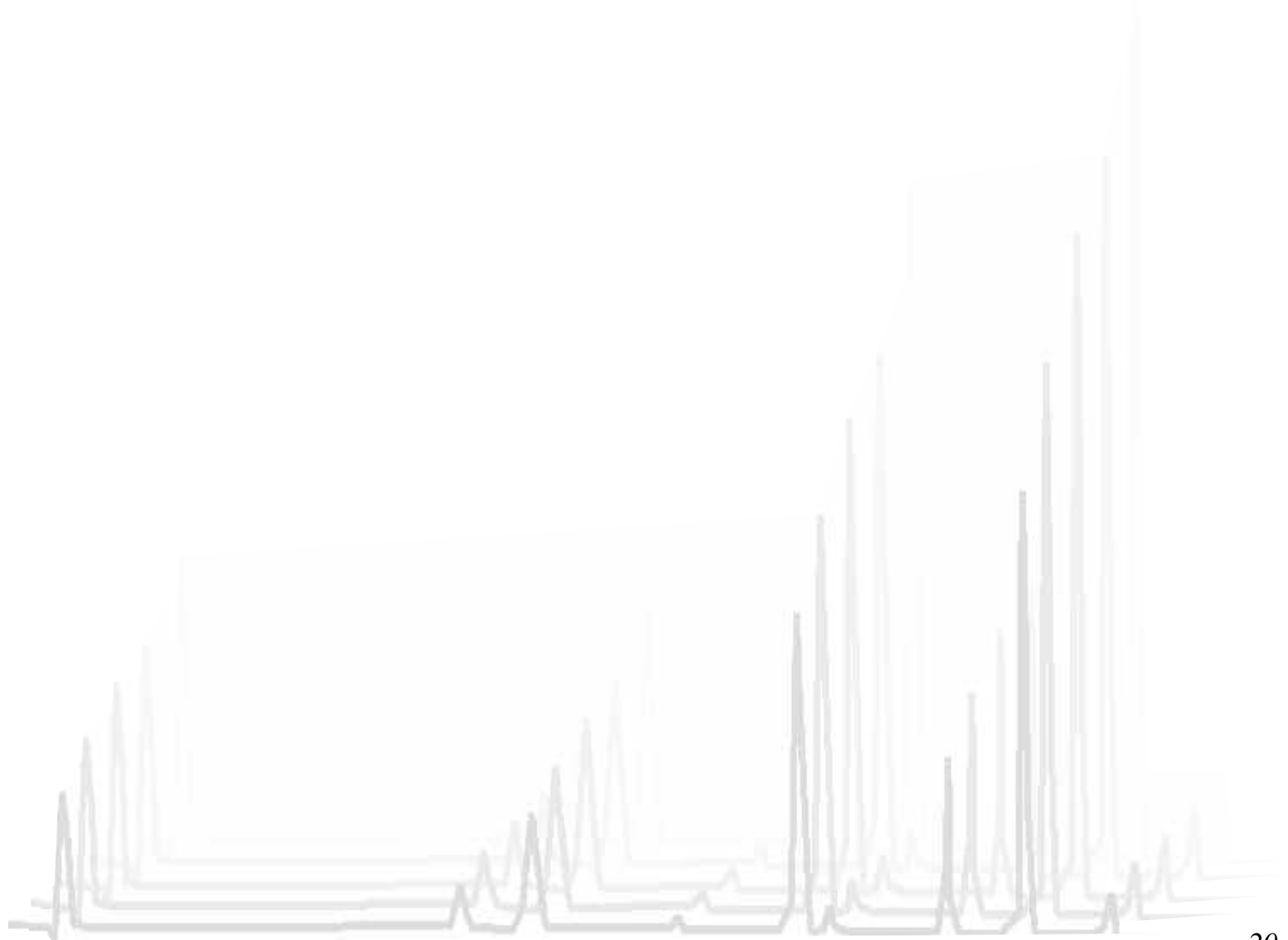
Monday 30, 10:55

Determining phytoplankton group composition by pigment ratios and size fractionations

Louise Schlüter and Flemming Møhlenberg

DHI – Water and Environment, Hørsholm, Denmark

The use of pigment ratios and size fractionation was investigated for the purpose of detecting the presence of phytoplankton groups without specific pigment signatures. These groups include prymnesiophytes, chlorophytes, some prasinophytes and cyanobacteria. Prasinophytes without the diagnostic pigment prasinoxanthin could be identified by calculating ratios of prasinoxanthin/chlorophyll *b* and lutein/chlorophyll *b* on data sets from Danish estuaries. Furthermore, these pigment ratios could be used to exclude the presence of chlorophytes, and ratios of zeaxanthin/chlorophyll *b* revealed that cyanobacteria were sporadically present. These informations are important when selecting the phytoplankton groups, which should be loaded in the CHEMTAX program for calculating the abundance of the individual phytoplankton groups as chlorophyll *a*. Subtypes of prymnesiophytes were detected by size fractionations on samples from a mesocosm experiment. The size fractionations, although time consuming and not always feasible, and the pigment ratio calculations proved to be able to provide the wanted information on which phytoplankton groups to load in CHEMTAX, when comparing the results of the CHEMTAX analyses to the biomass determinations of the phytoplankton groups made in the microscope.



Monday 30, 11:20

Using HPLC and CHEMTAX to investigate phytoplankton taxonomy: the importance of knowing your species

Xabier Irigoien¹, Bettina Meyer-Harms², Roger Harris³, Derek Harbour³

¹ Southampton Oceanography Centre, Southampton, UK

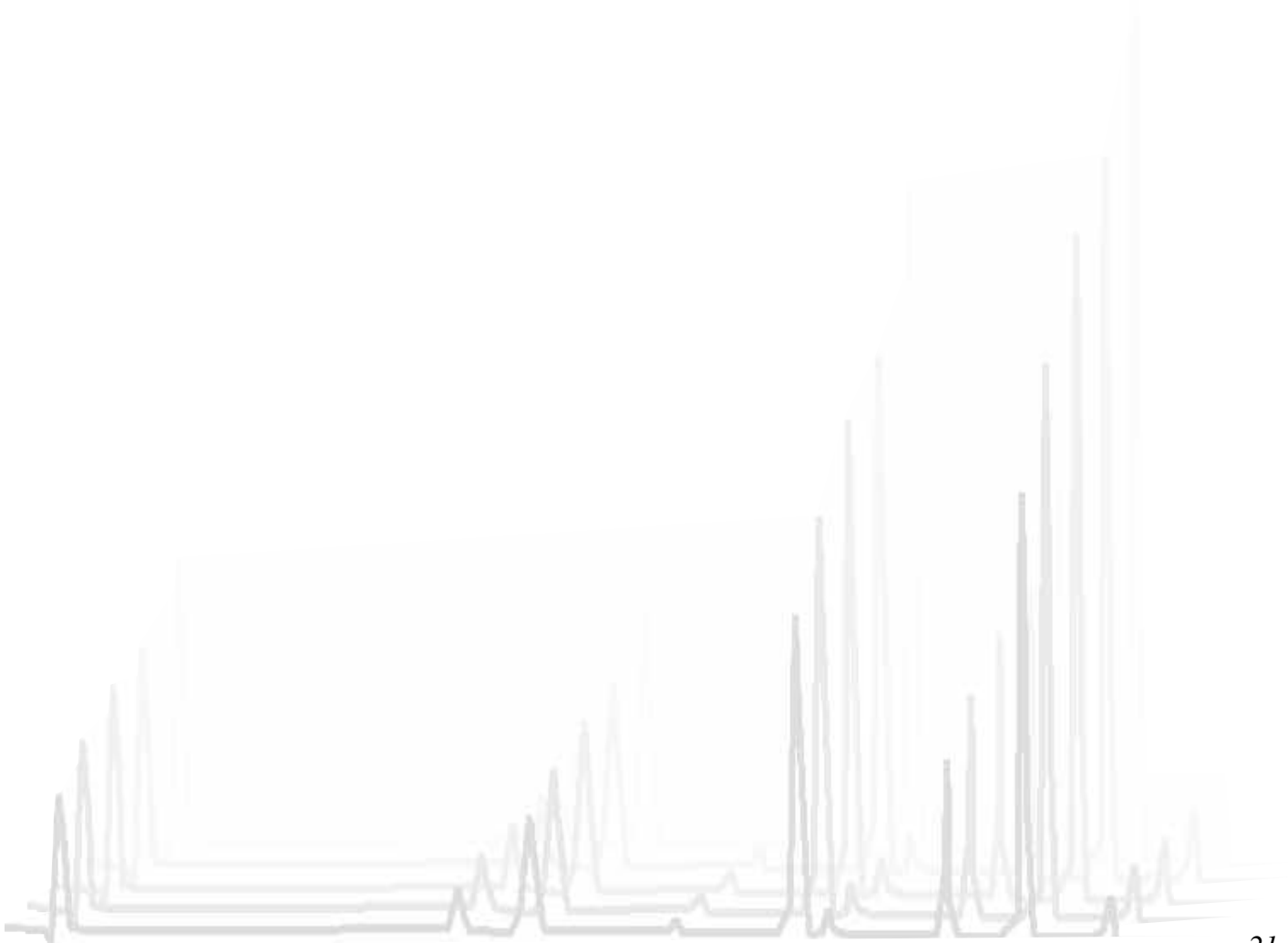
² Alfred-Wegener Institute, Bremerhaven, Germany

³ Plymouth Marine Laboratory, Plymouth, UK

HPLC and phytoplankton microscopic measurements were performed weekly for a complete year at coastal station in the English Channel. The phytoplankton taxonomy was analysed using the HPLC results and CHEMTAX in two different ways:

- Without using the species level taxonomic information obtained from the microscopic analyses (blind analyses)
- and including the information from the microscopic taxonomic analysis (directed analyses).

The results indicate that, due to the particular pigment composition of some species (*Gyrodinium aureolum* and *Phaeocystis pouchetii*), a blind HPLC and CHEMTAX analyses would produce extremely important errors in the taxonomic determination of the major blooms at this station.



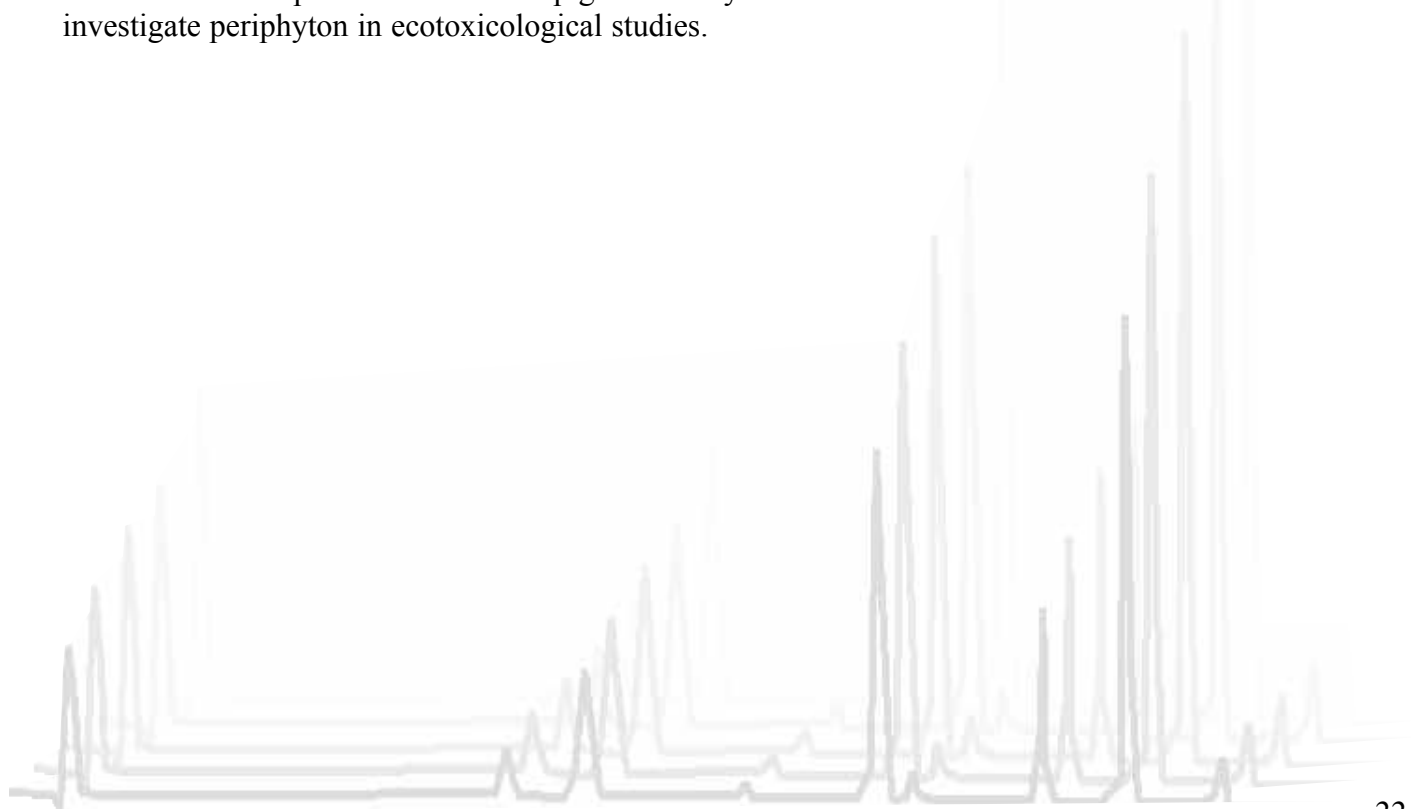
Monday 30, 11:45

Monitoring of periphyton community shifts after application of herbicides using HPLC pigment analysis and microscopy

Sonja Eser and Wilfried Huber

Technische Universität München, Fachgebiet Systematik und Ökophysiologie, Freising, Germany

HPLC pigment analysis was seldom applied to freshwater periphyton, although microscopy of the total periphyton community is often impossible due to their three-dimensional growth form. The algae stick to each other and their substrate and therefore often resist separation for microscopic counting. This is one reason why this community is normally not investigated in ecotoxicological studies. The presented study uses HPLC pigment analysis as a new method in pesticide studies to simplify periphyton investigations. In two outdoor studies the periphyton community was monitored for changes after the application of the PSII-blocking herbicides terbuthylazine and isoproturon. Periphyton samples were collected on glass slides and analysed under the microscope and with the HPLC analysis. Different HPLC methods and sampling preparations were tested for the use with periphyton. The pigment data were calculated with different methods to make them comparable to counting results. Cyanobacteria and diatoms were over- or underestimated with the program CHEMTAX, therefore multiple linear regression was chosen to calculate class composition. Dividing the samples into clusters helped to achieve better comparability. The calculated pigment ratios were nearly all in the range of phytoplankton literature data. Linear regressions between pigments and class biovolume were only middle to high correlated, but class compositions revealed the same pattern in community shifts. Additionally, new multivariate statistical analysis (PRC) showed a comparable reaction of the community to the herbicides as well as pigments and corresponding algal classes showed comparable sensitivity to the herbicides. Differences especially in diatom numbers compared to fucoxanthin contents were observed in the outdoor studies. Laboratory investigations showed that this was due to pigment changes in cells after herbicide exposure. The HPLC pigment analysis was shown to be a useful method also to investigate periphyton in ecotoxicological studies.



Monday 30, 12:10

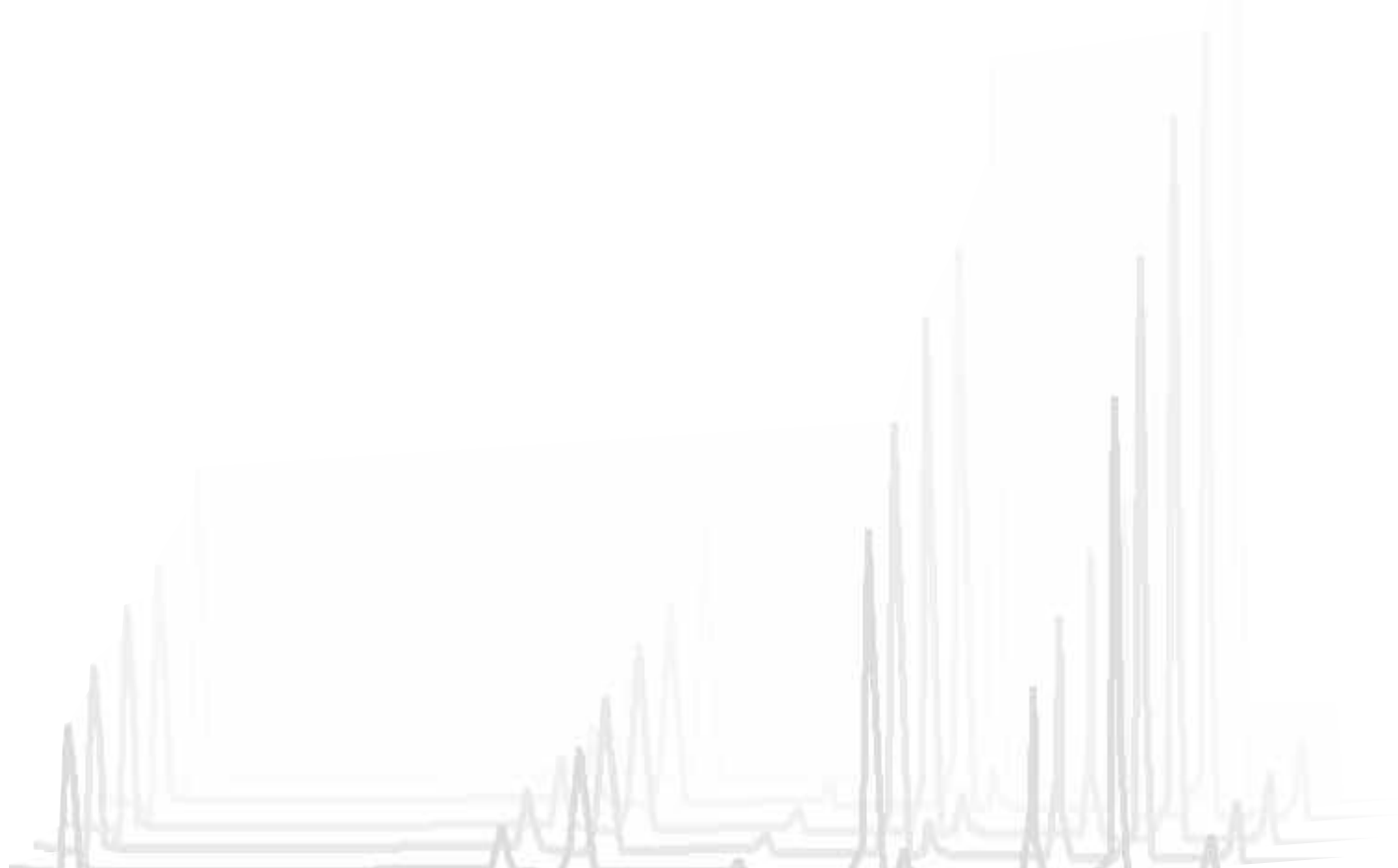
Liquid Chromatography studies of photosynthetic pigments in estuaries need to consider continental inputs and degradation processes

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² Département de Géologie et d'Océanographie - Université Bordeaux 1, Talence, France

Estuaries are characterised by highly variable environmental conditions such as salinity, oxygen and turbidity caused by mineral particles maintained in suspension. Photosynthetic pigments originate from different sources including terrestrial plants, freshwater algae from upstream rivers, marine algae brought by tidal movements inputs and autochthonous phytoplankton blooms. However, estuaries are most often net heterotrophic ecosystems. As a result, pigment degradation processes are very important, particularly in the maximum turbidity zones. Therefore, LC pigment studies are less straightforward in estuaries compared to the open ocean. The use of algorithms as developed in CHEMTAX is still difficult in estuaries, because terrestrial inputs and degradation processes need to be considered. Bianchi and Findlay have suggested in 1990 to use the chlorophyll *b*/lutein ratio as an index to distinguish between different higher plant sources and planktonic Chlorophytes. We combine this index with a quantification of pheophytin *b* to get an idea of the extent of the degradation process. Chlorophyll *b* is more labile than lutein; we have studied the first-order decay constants at different turbidity levels and different temperatures by following dark degradation of a chlorophyte in laboratory incubations. Using this information together with LC pigment analyses, we improve recognition of terrestrial inputs, quantify the extent of degradation and can thus provide a better description of the phytoplankton assemblage.



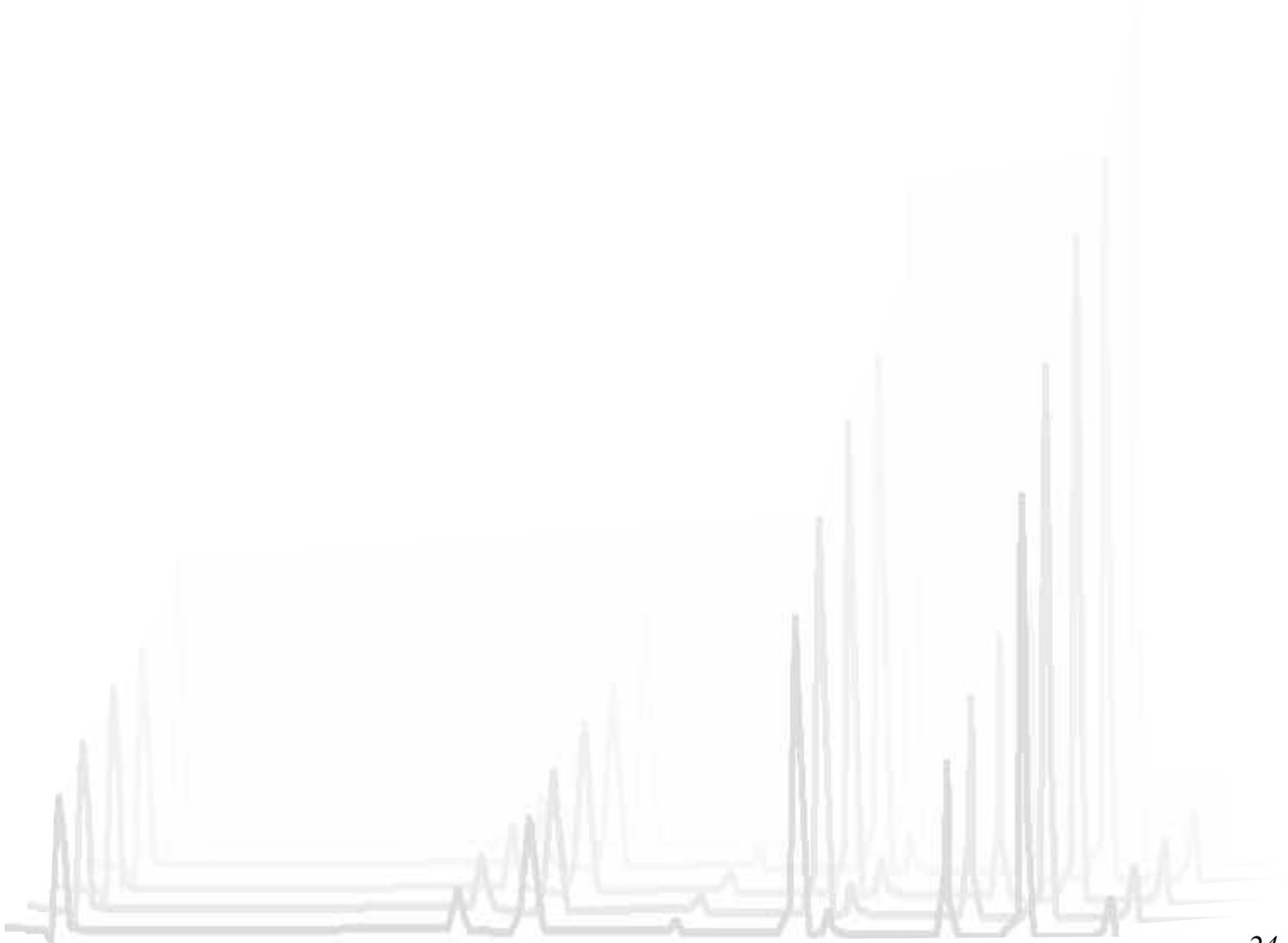
Monday 30, 14:35

CHEMTAX - not just a “Black Box”

Denis Mackey

CSIRO Marine Research, Hobart, Tasmania 7001, Australia.

Recent developments in the HPLC analysis of pigments have provided a wealth of information on the pigments produced by marine phytoplankton both in the field and in culture. In principle, these measurements should be able to provide information on the types of algal classes present in a given sample. In practice, this is not simple. The CHEMTAX program is one method that is currently being used in about 80 laboratories around the world to calculate the contribution that a given algal class makes to the total concentration of pigments or of a given pigment such as chlorophyll *a*. The assumptions inherent in the CHEMTAX approach will be discussed as well as the advantages and disadvantages over other techniques. The information gained from the analyses of carotenoid and chlorophyll pigments complements that obtained from other techniques, such as flow cytometry, conventional microscopy or molecular biology. As for many other software packages, the quality of the output depends critically on the quality of the input and considerable care is required in setting up the relevant input matrices - the program certainly should not be used as “black box” with a generic set of pigment ratios covering all the types of algal classes that may (or may not) be present in the samples. Some of the more common pitfalls in the use of the program will be presented as will the results from some recent cruises in the western equatorial Pacific.



Tuesday 1, 9:00

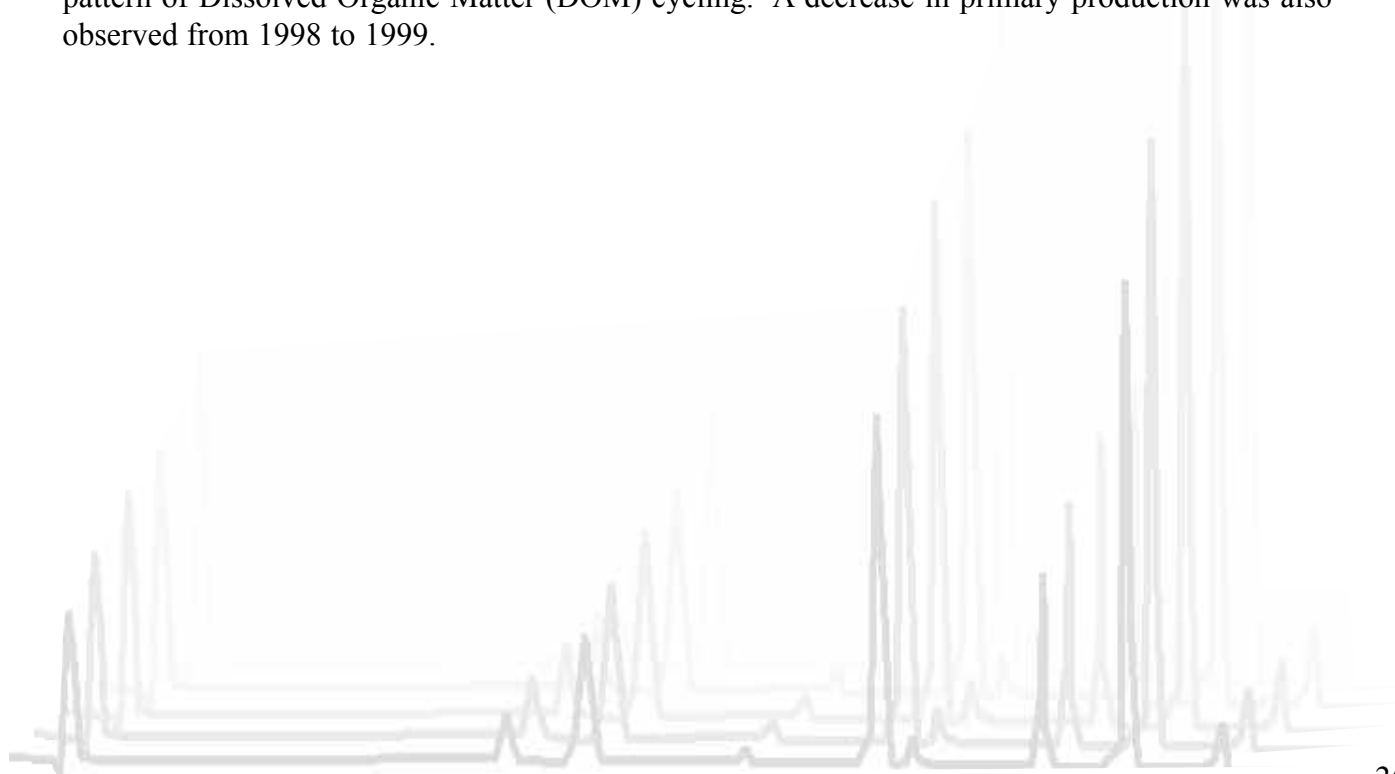
Long-term variability of phytoplankton community structure at the Bermuda Atlantic Time-series Study (BATS) site based on pigment analyses using the “CHEMTAX” matrix

Vivienne C. Lochhead, M.W. Lomas, P.J. Lethaby, R.J. Johnson, N.R. Bates and A.H. Knap

Bermuda Biological Station for Research, Ferry Reach, St. George, Bermuda

Upper ocean algal chlorophyll and carotenoid distributions have been measured monthly at the BATS site (31° 40.00' N, 64° 10.00' W) since December 1989 using high-performance liquid chromatography (HPLC) analysis. The “CHEMTAX” program was used to interpret the HPLC data from December 1989 to February 2000 and calculate the abundance of diatoms, dinoflagellates, haptophytes, prasinophytes, chlorophytes (+ prochlorophytes), and cryptophytes throughout the time series. The protocol we employ does not permit the resolution of Chl *b* and divinyl Chl *b* and therefore our chlorophyte group likely includes prochlorophytes. Results indicate the presence of a phytoplankton community structure seen throughout the upper 80 m that is dominated by chlorophytes (+ prochlorophytes) (> 50%) from 1991 to 1997 and 1999 to 2000 and a loss of the chlorophyte (+ prochlorophyte) (<20%) population from 1997 to 1999. From 1997 to 1999, the community structure was dominated by the cyanobacteria (> 40%) and the haptophytes resembling such species as *Emiliana huxleyi* (~30%). Data from 1989 to 1990 hint at a similar shift on community structure.

Macro-climate indices such as North Atlantic Oscillation (NAO) and Southern Oscillation Index (SOI) are being investigated as to their role in the biogeochemical change and indirectly the community structure. A negative NAO during the time period of 1997 to 1999 caused deeper mixing in the water column. In 1991, the NAO was positive but was close to zero and deeper mixing was observed in February. The change in species composition was coincident with a change in the pattern of Dissolved Organic Matter (DOM) cycling. A decrease in primary production was also observed from 1998 to 1999.



Tuesday 1, 9:25

Pigment specific distribution and community structure of phytoplankton during Atlantic meridional Transect (AMT3)

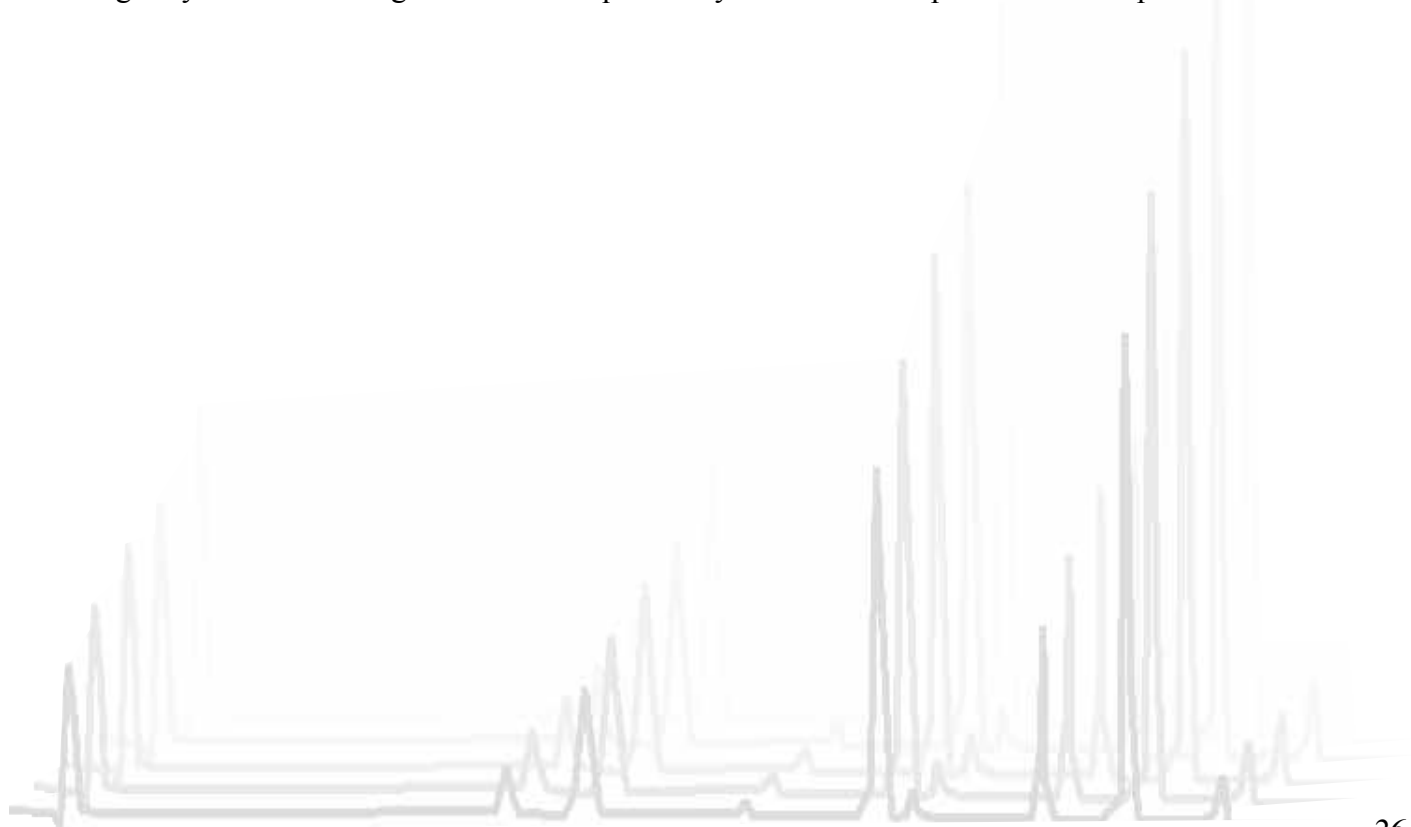
Roberto Millán-Núñez¹, Charles C. Trees² and Jim Aiken³

¹ Facultad de Ciencias Marinas, UABC, Ensenada B. C., México

² Center for Hydro-Optics and Remote Sensing, SDSU, San Diego, CA, USA

³ Plymouth Marine Laboratory, Plymouth, United Kingdom

Pigment samples were collected for HPLC analysis during the Atlantic Meridional Transect (AMT3) cruise from the United Kingdom to the Falkland Islands (50°N to 50°S). HPLC pigment samples were collected every two from a continuous underway system and from the daily CTD stations. Pigment compounds were separated and quantified using Wright et al. (1991) method. The distribution of divinyl chlorophyll *a*, a specific biomarker for prochlorophytes, was maximum in the tropical and subtropical areas, indicating that prochlorophytes contributed up to 50 % of the total chlorophyll *a* biomass. The ubiquitous nature of cyanobacteria during the cruise was also demonstrated using the the ratio of zeaxanthin to total carotenoid concentrations. Zeaxanthin is found in prochlorophytes, cyanobacteria, chlorophytes and prasinophytes, and, based on the relatively low to undetectable concentration of monovinyl chlorophyll *b* (chlorophyte biomarker) and prasinoxanthin, the latter two groups did not contribute significantly to the zeaxanthin signal. Using published zeaxanthin to divinyl chlorophyll *a* and monovinyl chlorophyll *a* to zeaxanthin ratios, it was estimated that cyanobacteria made up about 30-40 % of the chlorophyll *a* in these tropical waters. The latitudinal and vertical distribution of photoprotectant (PPC) and photosynthetic (PSC) carotenoids were quiet dramatic. High PPC to total carotenoid ratios were found in the tropical and subtropical surface waters, but decrease with depth. The ratio of PPC to total carotenoids was a function on the photoadative state of the phytoplankton. The change in this ratio for both near surface and vertical samples as function of radiant flux will be discussed. Obviously, the absorption of light by PPC will change estimates of quantum yield in these tropical and subtropical areas.



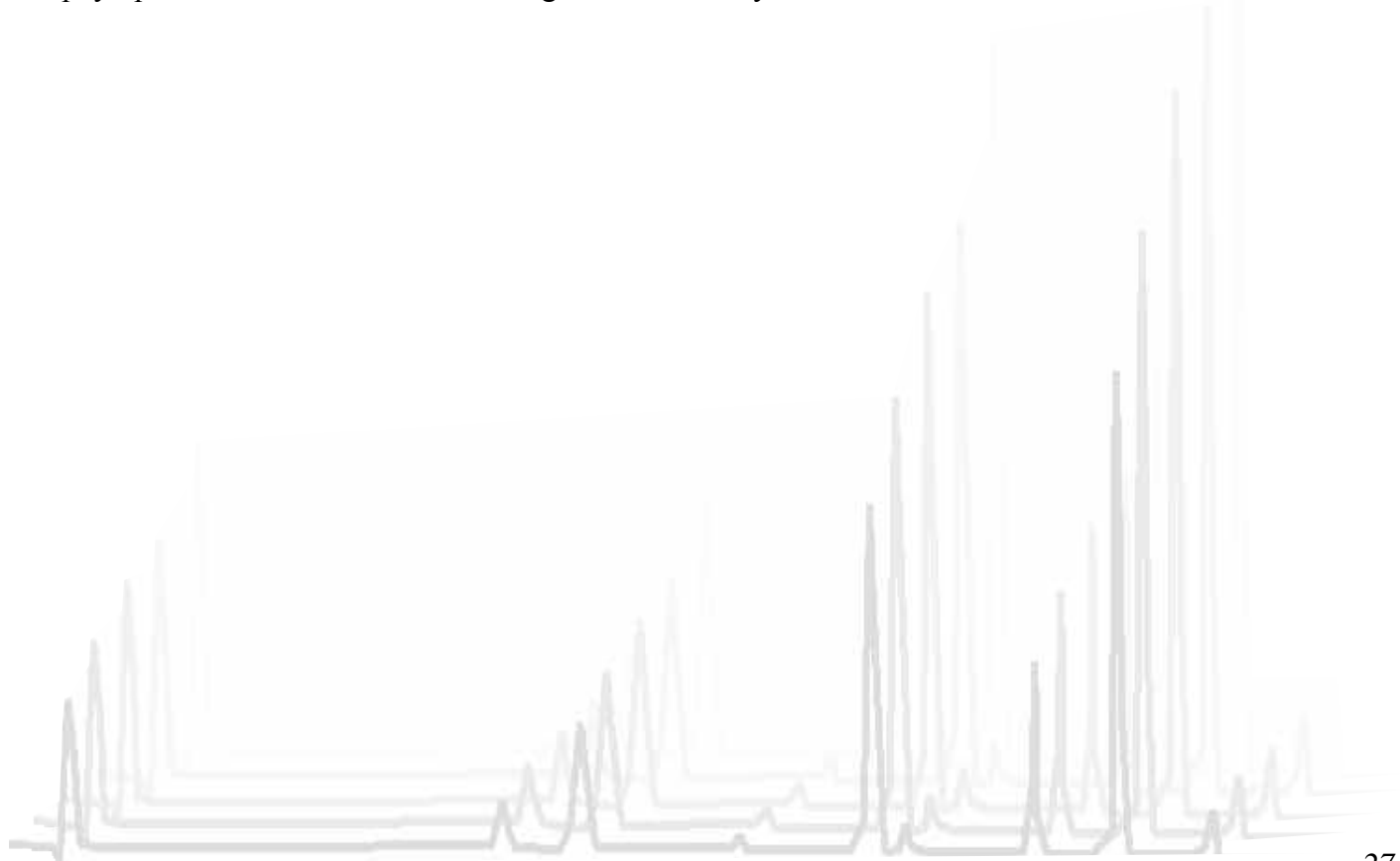
Tuesday 1, 9:50

Correspondence of phytoplankton community structures estimated by pigment and CHEMTAX to hydrographic water masses, a case study on a Kuroshio warm-core ring

Yumiko Obayashi and Koji Suzuki

Institute for Hydrospheric-Atmospheric Sciences, Nagoya Univ., Japan

Warm-core rings are mesoscale eddies which generally detach from western boundary currents in the world's oceans. These eddies are important not only for heat transfer, but also for biological productivity of the region. The Kuroshio warm-core rings in the sea east of Japan represent such a class of eddies. They evolve from a meandered Kuroshio Extension, a warm and high saline water current. Upon their formation, these warm core rings advect northward into the Oysahio region where they can remain entrapped for periods ranging from several months to several years. During this period, these high saline warm-core rings undergo several structural changes mainly in winter, as they entrain the cold, low saline, high nutrient waters of the Oyashio along their margins. In this study, we present results from an investigation into phytoplankton pigments within and around the Kuroshio Warm-Core Ring 93A (KWCR 93A). At the time of our sampling, the KWCR 93A had passed its fourth winter. Although it was still distinguishable from the waters surrounding it by NOAA/AVHRR imagery, the hydrographic features and chemical characteristics of KWCR 93A were substantially different from that of its source waters, the Kuroshio Extension. Pigment measurements in conjunction with CHEMTAX analysis (Mackey et al., 1996) at 7 stations in and around KWCR 93A enabled us to classify the resident phytoplankton community into four distinct groups. This classification corresponded well with the hydrographic features at each station, indicating that chemotaxonomic pigments could be a valuable tool for elucidating the structure of phytoplankton communities within regional non-steady water masses.



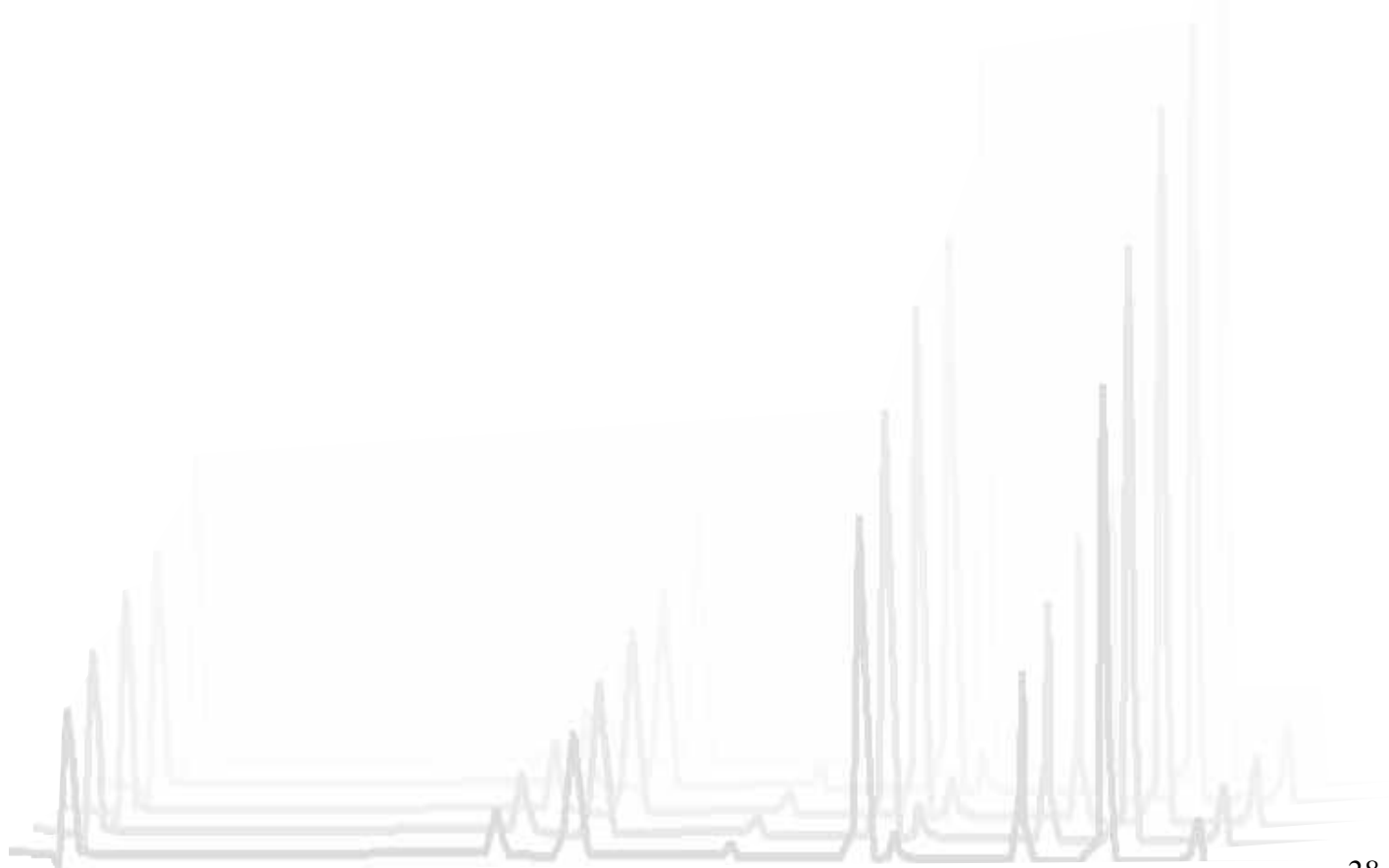
Tuesday 1, 10:45

Changes in phytoplankton community during the Southern Ocean Iron Fertilisation Experiment “EisenEx 1” Based on Marker Pigments

Ilka Peeken

Institute of Marine Research, Kiel, Germany

The first iron fertilization experiment in the Atlantic sector of the Southern Ocean (EisenEx 1) was carried out in early austral spring 2000 (October/November). Approximately 500 square kilometers were fertilized with iron to test the hypothesis concerning the role of iron as growth-limiting factor for marine phytoplankton in this region. During the course of the 3 weeks of the experiment water samples were taken inside and outside the iron-fertilized patch. The samples were analyzed by high performance liquid chromatography to determine changes in phytoplankton biomass and group composition inside and outside the patch, using chlorophyll *a* and a variety of marker pigments. Results indicate a fourfold increase in phytoplankton biomass inside the patch at the end of the experiment. Fucoxanthin, a marker for diatoms, showed a clear increase inside the iron patch, particularly during the last week of the experiment, suggesting a preferential growth of diatoms due to the iron fertilization. A slight increase of the diatom marker was also found outside the patch, indicating a succession towards a diatom-dominated phytoplankton community outside the iron-patch. In contrast, the marker for prymnesiophytes, 19'-hexanoyloxyfucoxanthin, decreased inside and, less pronounced, outside the patch during the experiment. In addition, markers of autotroph dinoflagellates, chlorophytes, pelagophytes, cryptophytes, and cyanophytes were present during the investigation, but these species seemed to be less affected by the iron fertilization than diatoms. Preliminary analyses using the CHEMTAX program (Mackey et al., 1996) will be presented.



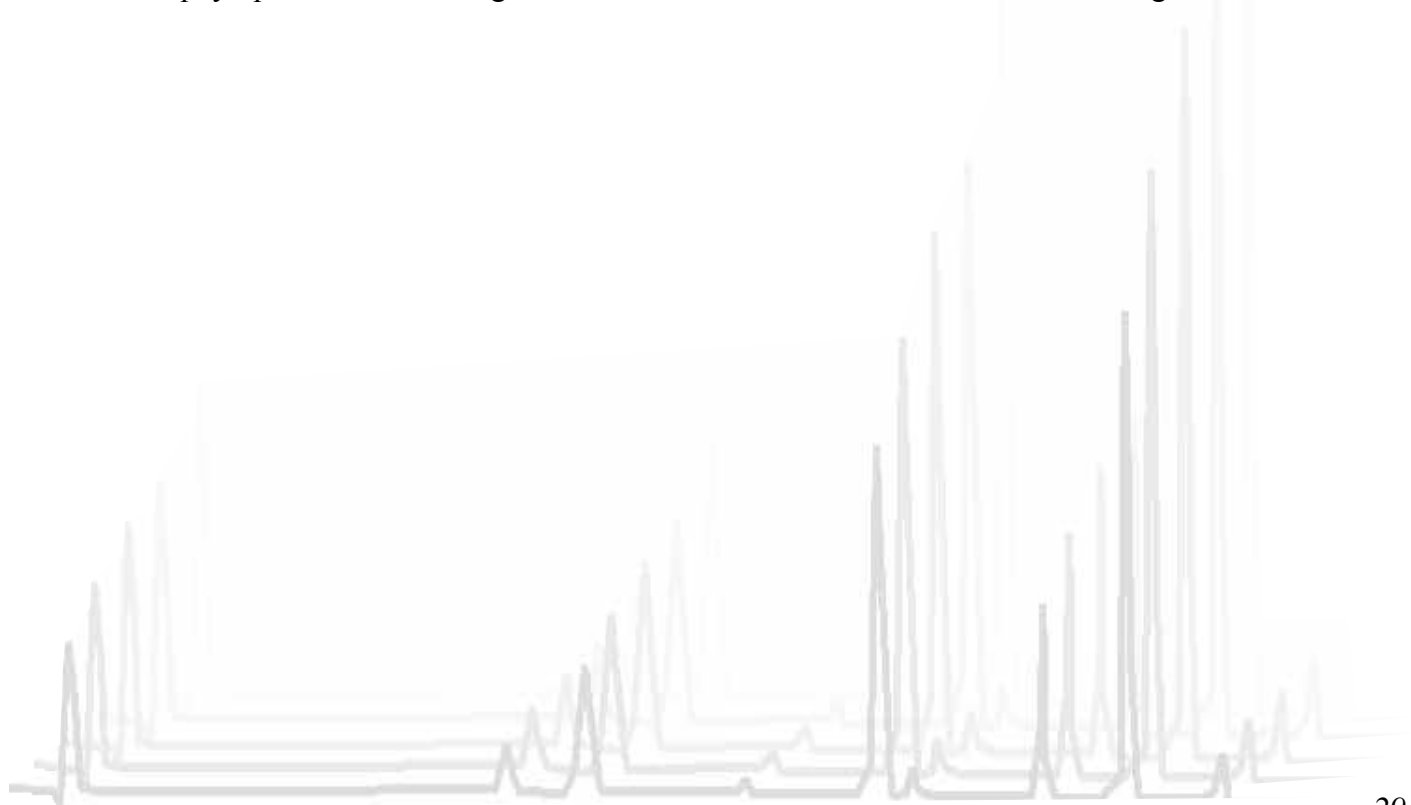
Tuesday 1, 11:10

Spatial and vertical distribution of phytoplankton pigments in the Eastern Atlantic section of the Southern Ocean

Angela Wulff and Wängberg Sten-Åke

Marine Botany, Göteborg Univ., Göteborg, Sweden

The spatial and vertical distribution of phytoplankton pigments was analysed during the SWEDARP 97/98 cruise of the S.A. Agulhas from December 31, 1997, to January 26, 1998. In total, seawater from 12 different stations along a transect at 6°E from 60.38 to 49.82°S were sampled at water depths of approximately 2, 10, 20, 30, 50, 75 and 100 m. Pigment concentrations were analysed using high performance liquid chromatography (HPLC). The highest chlorophyll *a* (chl *a*) concentrations were found within the Marginal Ice Zone (MIZ) at water depths of 2 to 30 m (1.5-2.0 $\mu\text{g l}^{-1}$). The lowest phytoplankton biomass (chl *a*) was found within the Inter Frontal Region (IFR) with typical values around 0.3 $\mu\text{g l}^{-1}$. While the chl *a* concentrations decreased with depth within the MIZ, no apparent difference between depths was found within IFR. The same trend with similar chl *a* concentrations (0.8-1.2 $\mu\text{g l}^{-1}$) at all depths was found for the northernmost part of the Antarctic Polar Front (APF) except for samples from 100 m. Principal Component Analysis (PCA) and the matrix factorisation program, CHEMTAX, were used to interpret the pigment data. The PCA indicated small differences between the samples in IFR and APF while the MIZ samples were separated into three groups. At the southernmost stations a clear separation was found between surface samples and deeper samples, this was not seen at the two northernmost stations within MIZ. Along the whole transect, CHEMTAX suggested a dominance of diatoms and haptophytes. In general, the haptophytes contributed more to total chl *a* further south and the diatom contribution increased from south to north. Within IFR, CHEMTAX indicated that cyanobacteria were contributing to the total autotrophic biomass. In APF, a more diverse phytoplankton community was found compared with IFR and MIZ. The pigment patterns interpreted by CHEMTAX showed that different phytoplankton assemblages were associated with distinct water masses along the transect.



Tuesday 1, 11:35

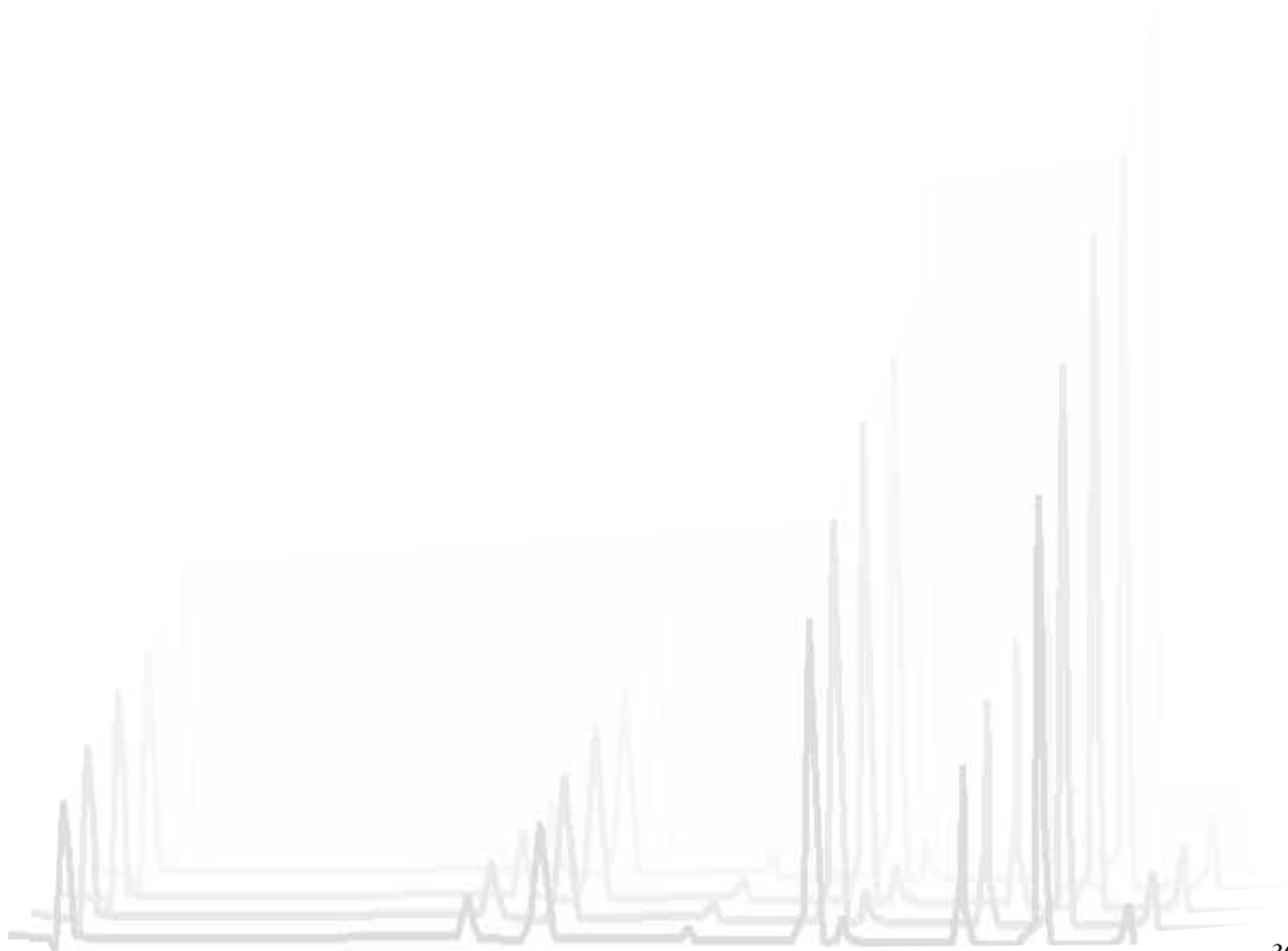
Phytoplankton pigment markers along the Mediterranean coast of Israel

Y. Z. Yacobi¹, Y. Suari² and B. Herut²

¹ National Institute of Oceanography, Israel Oceanographic & Limnological Research, Tiberias, Israel

² Kinneret Limnological Laboratory, Israel Oceanographic & Limnological Research, Tiberias, Israel

Phytoplankton densities along the Mediterranean coast of Israel are extremely low. Chlorophyll *a* (chl *a*) concentrations are seldom higher than 1 mg l⁻¹, and in most sites much lower, all round the year. Exceptional is the Haifa Bay area, at the northern part of the Israeli Mediterranean coast, where chl *a* concentrations reach values of tens of mg l⁻¹. HPLC analysis of the particulate matter showed about 15 pigments in all samples that were studied. Pigment-based calculation of the contribution of different groups showed the domination of diatoms in Haifa Bay close to the coastline and decreasing gradient of their contribution with the shift towards the open sea. The decline in the relative concentration of diatoms coincided with the decreasing proportion of chl *a* harbored by particles >2 and >10 μm, indicating the increasing importance of small phytoplankton forms, with the increase of the distance from the shoreline. The taxonomical identity of the former changed seasonally, but in 5 out of 6 cruises cyanophytes were the most abundant group. Cyanophytes and prymnesiophytes were the dominant groups out of the boundaries of Haifa Bay. Prochlorophytes did not make up more than 20% of the phytoplankton biomass along the open shoreline, and mostly much less within the Haifa Bay.



Tuesday 1, 12:00

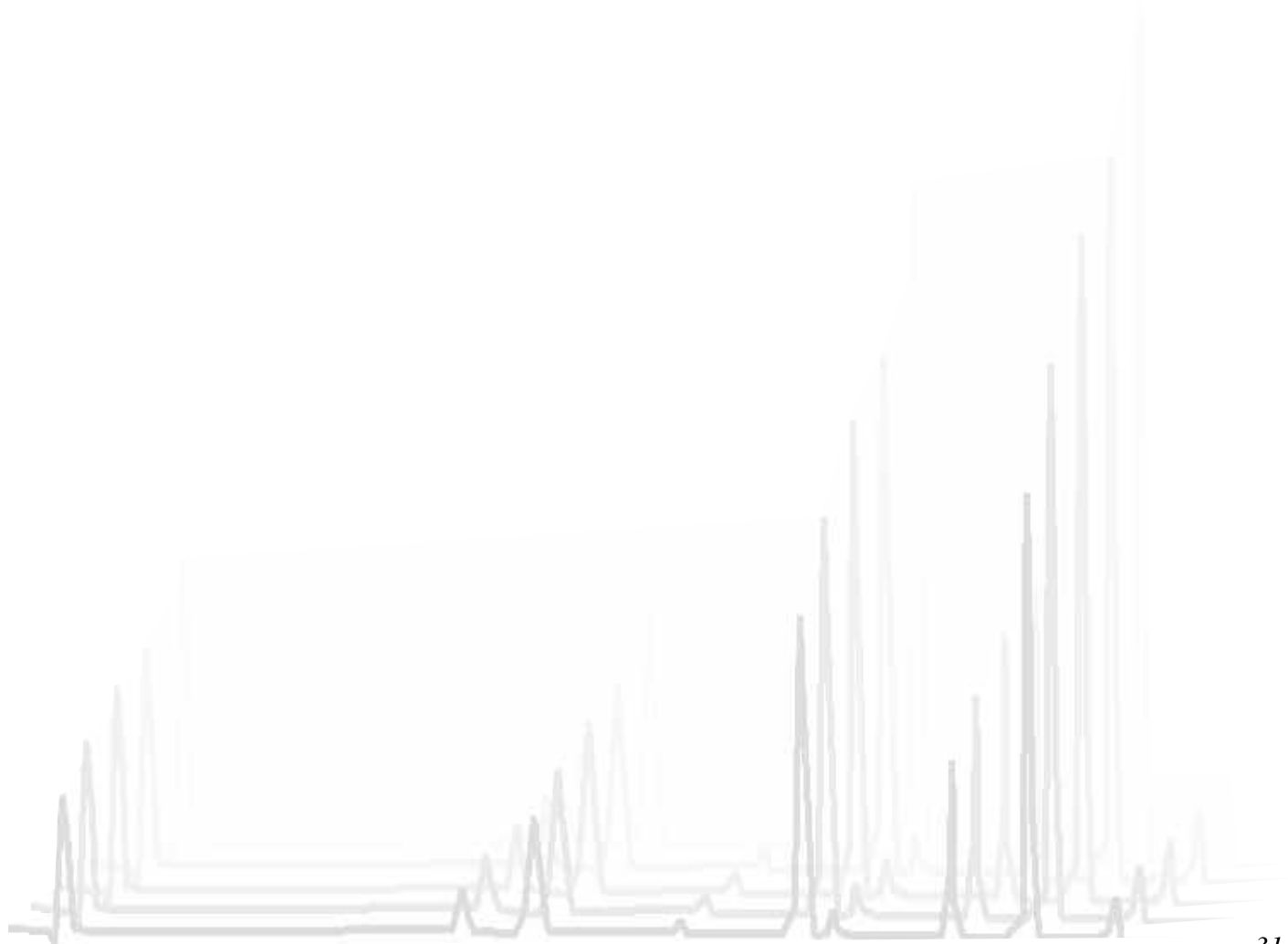
Sources of variability in cell and population pigment composition: Temporal, spatial and physiological scales

Christophe Brunet

Stazione Zoologica di Napoli, Villa Comunale, Napoli, Italy

Plant pigment analysis by HPLC is a very powerful tool to assess the composition and physiology of phytoplankton and it is now commonly used in oceanography both for descriptive and functional studies of marine ecosystems. Pigments can be used to give information on phytoplankton physiological and ecological processes, such as photoacclimation, absorption coefficients (from reconstructed spectra), stress, senescence and grazing. We present pigment data both from culture and from experiments in situ, emphasizing pigment ratio variations in response to environmental changes as a function of different spatial and temporal scales.

Culture experiments on different Chromophyte algae helped in characterizing photoacclimation processes, and results show that pigment composition (carotenoids, xanthophylls and chlorophylls) significantly varies with light quantity and quality. Data from in situ studies show significant pigment variations in response to environmental constraints at different temporal and spatial scales. All these results are discussed with the aim of showing the kind of information that have been obtained at the different levels of ecosystem organization and its variability.



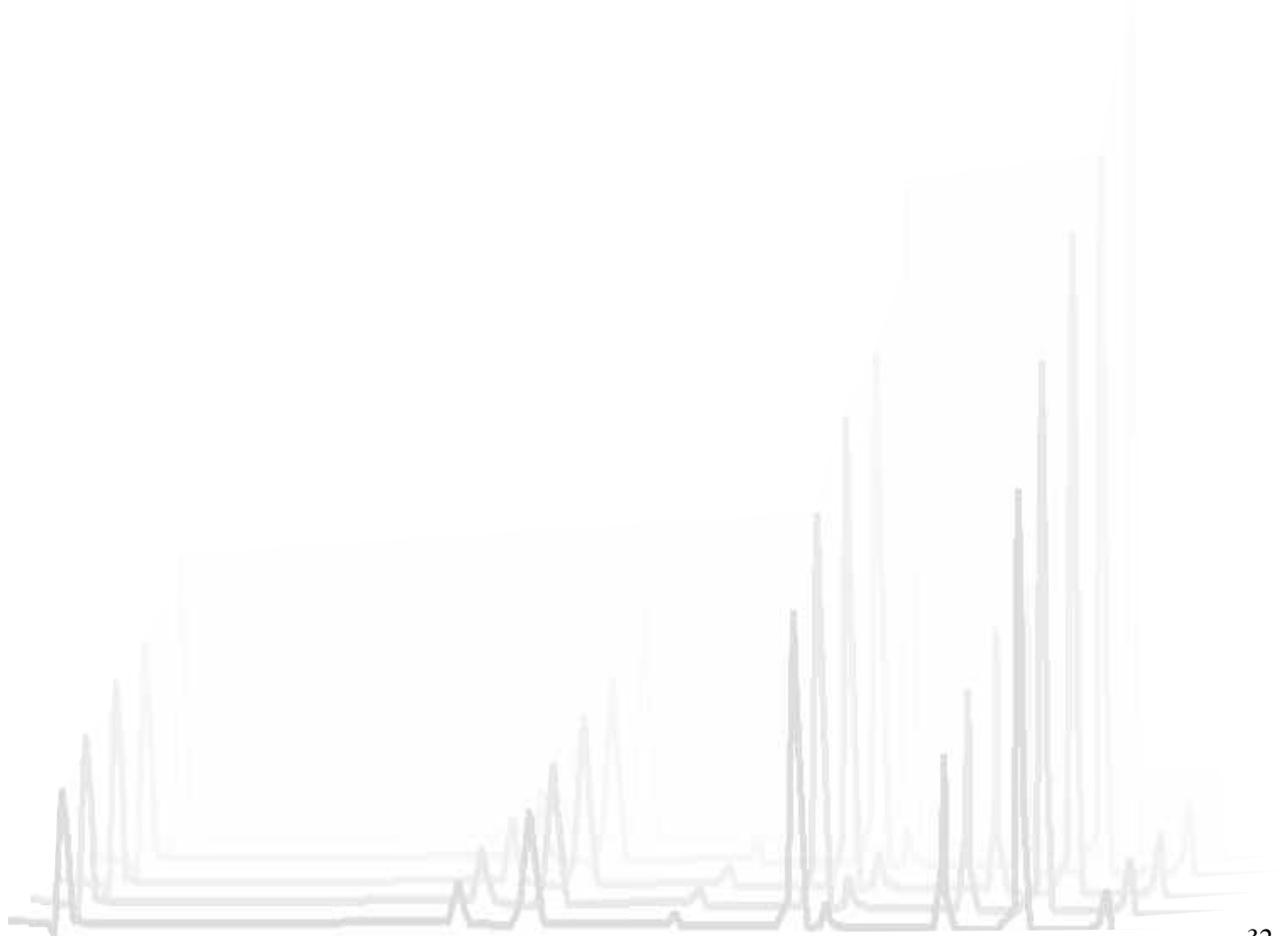
Tuesday 1, 14:25

Use of flow cytometry and extracted chlorophyll data to assess picoplankton groups contribution to phytoplankton biomass

Loïc Charpy

IRD, COM, Marseille, France

Flow cytometry data allow the direct calculation of picoplankton cell abundance and relative fluorescence, and an indirect estimation of their cell size. The measure of extracted chlorophyll in the $<3 \mu\text{m}$ size fraction give a good estimation of the picoplankton biomass and its contribution to total phytoplankton biomass. The red fluorescence (RF) measured by flow cytometry is a proxy for chlorophyll as evidenced by a simple correlation between chlorophyll $<3 \mu\text{m}$ and total RF. Therefore, the contributions of the 3 picoplankton groups (*Prochlorococcus*, *Synechococcus* and picoeukaryotes) to the phytoplankton biomass can be assessed from their RF and their specific chlorophyll content per unit of red fluorescence (F). If the number of replicates is large enough (>4), F values can be calculated by regression using measured values of chlorophyll and picoplankton groups RF. An application of this method was made on atoll lagoon data and its validity is discussed.



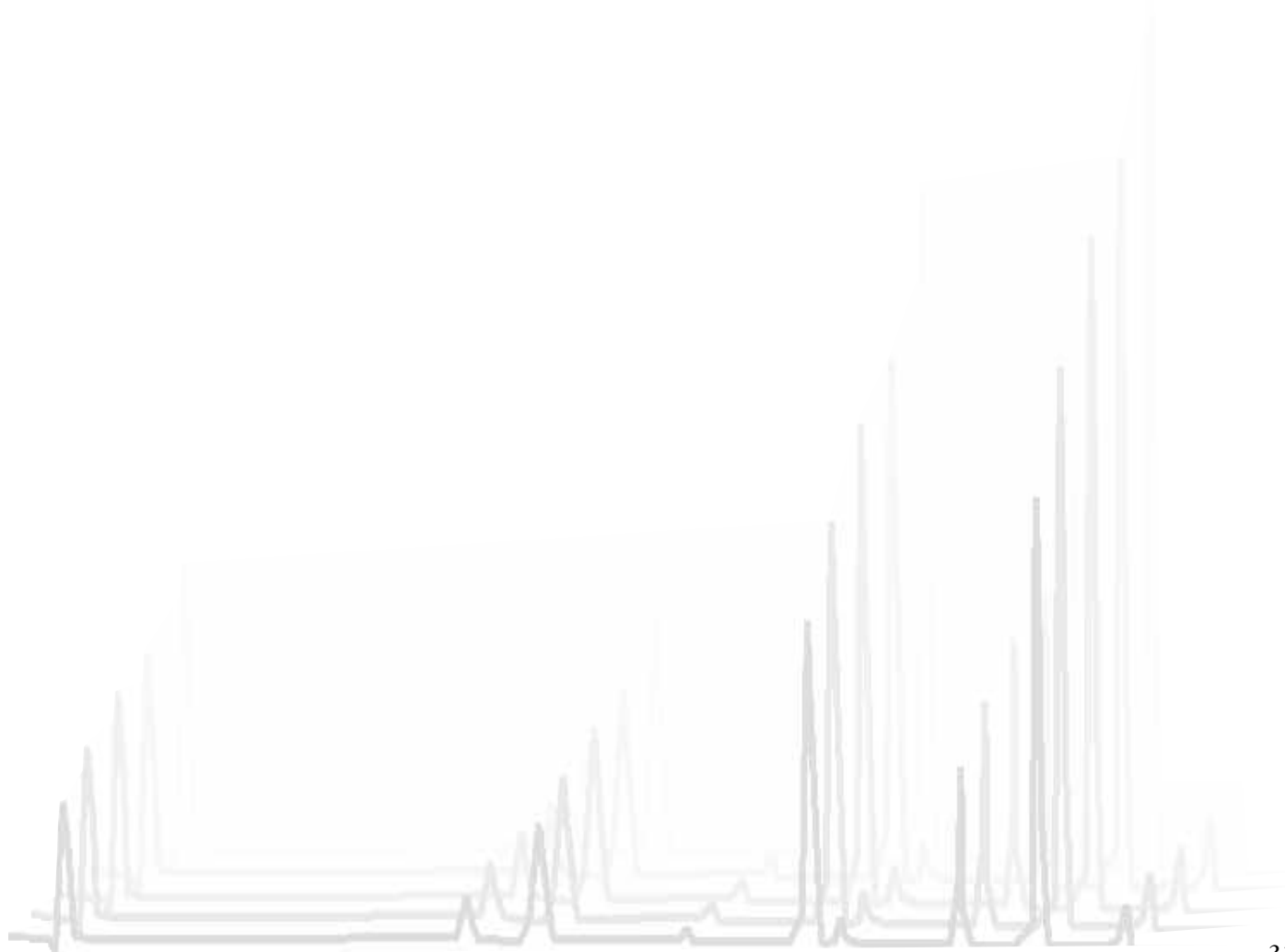
Tuesday 1, 14:50

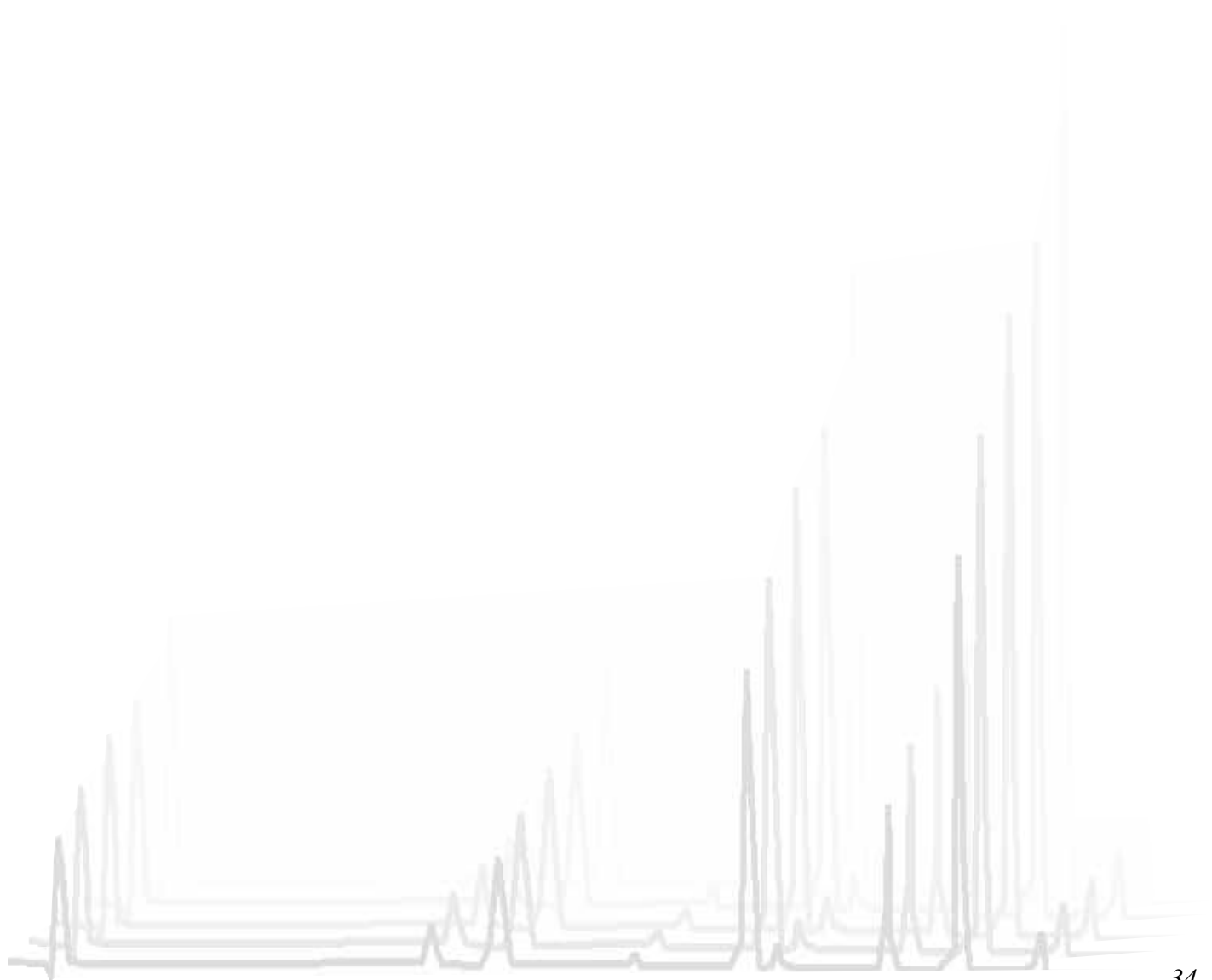
Algal population composition and biomass determined in situ with accessory pigment induced fluorescence methods

Karen H. Wiltshire and M. Beutler

Biophysics Group, Centre for Biochemistry and Molecular Biology, University of Kiel, Kiel, Germany

Algal fluorescence is influenced by excitation wavelengths due to light absorption by antennae pigments. Based on this a submersible instrument was developed which utilizes this specific absorption to induce fluorescence. This was used to differentiate algal classes in algal populations in situ. Fluorescence profiles in aquatic systems in which algal groups, associated pigment relationships and biomass as automatically detected from fluorescence are shown. This is compared with pigment HPLC data. Yellow substances (coloured dissolved organic matter) may interfere with the measurement because of overlap in the excitation spectra with phytoplankton. In a new instrumental approach we correct for the influence of yellow substances on the chlorophyll fluorescence. This method is seen as a substantial step forward for in situ estimation and determination of algal biomass in aquatic systems.

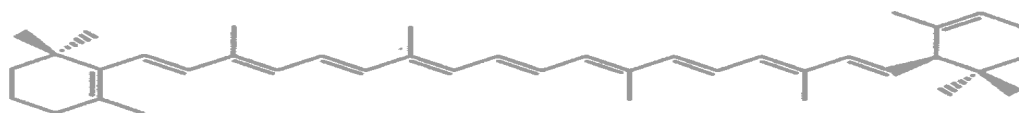




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