ISTITUTO SUPERIORE DI SANITÀ

Algal bloom detection, monitoring and prediction. 3rd Workshop: "Public health"

Istituto Superiore di Sanità
Rome, April 21-22, 1998

Proceedings edited by
G. Catena and E. Funari

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The Workshop focused on the problem of the harmful algae in the Mediterranean basin with the aim of verifying the possibilities of using remote sensing techniques together with conventional ones. These proceedings report the main aspects of the problem, as the ecological one, the conventional monitoring techniques, the activities of international organizations and finally some of the current research activities in Italy aimed at using remote sensing techniques.
Key words: Algal blooms, Mediterraneum, Pigment optical properties, Remote sensing

Istituto Superiore di Sanità
Determinazione, monitoraggio e previsione delle fioriture algali. 3°Seminario: "Salute pubblica". Istituto Superiore di Sanità. Roma, 21-22 aprile 1998.
A cura di Giorgio Catena e Enzo Funari
1999, 95 p. Rapporti ISTISAN 99/8 (in inglese)

Il Workshop è incentrato sul problema delle alghe tossiche marine all'interno del bacino del Mediterraneo con lo scopo di verificare le possibilità di utilizzazione delle tecniche di telerilevamento in modo complementare rispetto a quelle convenzionali. Si affrontano alcuni degli aspetti principali di questa problematica quali l'ecologia, le tecniche convenzionali di monitoraggio, le attività di organismi internazionali e infine le attività in corso in Italia finalizzate all'utilizzazione delle tecniche di telerilevamento.
Parole chiave: Fioriture algali, Mediterraneo, Proprietà ottiche pigmentarie, Telerilevamento

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INTRODUCTION

The European Concerted Action on Algal Bloom Detection, Monitoring and Prediction (ABDMAP) aims at bringing together leading European academics and organizations interested, or playing statutory role in facing the problems of marine and coastal algal blooms. In particular, the ABDMAP is pointing out the role of Earth Observation data in the detection, monitoring and prediction of algal blooms in the marine environment.

The Concerted Action is co-ordinated by the University of Dundee, Scotland, and has partners belonging to five European countries. Over the two-year programme, 7 workshops has been envisaged focusing on different main topics. The Rome Workshop, held on 21th and 22nd April 1998, focused on the problem of the Mediterranean basin and in particular on harmful algae. This Workshop was organized in sessions of presentations by qualified experts, followed by sessions structured in small discussion groups involving end-users. In the discussion sessions, end-users were invited to consider the way remote sensing techniques can be used for their research and/or monitoring activities.

The workshop was particularly successful. It was attended by a high number of end-users, belonging to many countries in the Mediterranean area.

This volume presents the proceedings of the Rome Workshop.

POTENTIAL USE OF REMOTE SENSING IN THE STUDY AND PREVENTION OF WATERBORNE HUMAN DISEASES.

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Introduction

The main aim of the European Concerted Action on Algal Bloom Detection, Monitoring and Prediction (ABDMAP) is to consider the current use and future possibilities of the use of Earth Observation (EO) data in this field.

The Italian National Institute of Health (ISS) is actively involved in the activity of the Concerted Action and fully shares its objectives.

Earth Observation (EO) data in the prediction, detection and monitoring of algal blooms in the marine environment is of particular interest for ISS, especially because some of these algal blooms can be produced by toxic algae, which can represent a threat to human health especially because of the occurrence of algal toxins in seafoods.

EO data can be conveniently used also for studying, monitoring and preventing the microbiological and chemical risks associated with the quality of waterbodies.

ISS has the scientific expertise in this fields (Catena e Palla, 1980; Catena e Palla, 1988; Catena e Palla, 1989a; Catena e Palla, 1989b; Funari, 1999; Draisci et al., 1998; Funari and Silano, 1997) and consolidated relationships with national and local possible end-users of EO data.

EO data and toxic algal blooms

Even though at present many problems must be solved before using EO data for toxic algae blooms in the Italian (as well as other) Seas, these techniques could offer favourable conditions for these activities in the near future.

Traditional local, regional and national activities of detection and monitoring of toxic algal blooms are carried out through *in situ* programmes, with increasing frequency in the main algal bloom season. The presence and extention of the phenomenon are detected through the determination of algal species in water samples coming from a necessarily limited number of stations: the research is time-consuming and requires a demanding organization and the use of boats.

Compared to traditional research methods, remote sensing has the potential to provide greater spatial and temporal coverage, and additional environmental information.

The use of EO data would allow to better describe the presence, extension and evolution of the phenomenon of a toxic algal bloom in a certain waterbody and represents a predictive tool for a whole basin. Moreover, the availability of EO data, which show the course of surface currents, wind speed and direction, would permit shifts of algal masses to be forecast and possible immediate measures to be taken.

The Northern Adriatic Sea is particularly affected by the eutrophication phenomenon. This is due to the huge number of nutrients discharged through the rivers, to the conformation of the coastline and its shallow waters.

The first algal bloom in this area was observed in 1969. Then, after some years of absence, the phenomenon reappeared in 1975 and rather regularly in the following years. These blooms caused in particular a diffused anoxia of deep waters, and consequently death of benthic organisms. This has led to significant changes of the benthic ecosystem and a general reduction of the original populations. Algal blooms have given rise to negative implications also on tourists activities, fishery and mollusc cultivation.

Algal blooms in the northern Adriatic Sea are due to diatoms and dinoflagellates. Diatoms produce blooms in the winter-spring period. Dinoflagellates produce algal blooms in the autumn-winter period. The occurrence of *Dynophisis* spp. producing DSP toxins has led in many occasions to the impossibility of gathering molluscs for commercial purposes.

A number of phytoplankton-related phenomena due to toxic dinoflagellates have been repeatedly recorded in Italy in the last decades. Yet, the first case of diarrhoeic shellfish poisoning (DSP)-contaminated mussels collected from the northern Adriatic Sea (Emilia Romagna region) has only been recently reported (Boni et al., 1992). The episode was associated with the simultaneous presence of some toxic algae of the *Dinophysis* genus, including *D. fortii*, both in sea-water and in shellfish hepatopancreas.
In order to prevent the risk of DSP-contamined seafood consumption, the Italian Health Authority has established measures involving the monitoring of shellfish growing areas to examine phytoplankton in sea-water and to determine DSP toxicity in molluscs by mouse bioassay (G.U., 1990).

Particularly, in the monitoring of phytoplankton(1989-1994) along Emilia Romagna coast (northern Adriatic Sea) significant levels of *D. sacculus* and *D. sp.* similar to *D. acuminata* were usually observed at the beginning of summer, whereas *D. fortii* and *D. caudata* generally predominated at the beginning of autumn, which corresponded to the highest levels of toxic phytoplankton.

To date, DSP phenomena in Italy have been related to the presence of okadaic acid (OA) (Fattorusso et al., 1992; Draisci et al., 1994, 1995, 1996) and dinophysistoxin-1 (DTX-1) (Draisci et al., 1995) in Adriatic mussels, although the presence of other toxins has been repeatedly suggested (Zhao et al., 1993, 1994; Draisci et al., 1994, 1995).

As the *Dinophysis* species have successfully been brought into culture, analyses of DSP in these organisms are limited on field collected material. Although a direct correlation between the presence of *D. fortii* in sea water and the OA concentration in shellfish suggested that this species was involved in the Adriatic OA mussel contamination (Della Loggia et al., 1993), *D. fortii* has only recently been unambiguously shown to transmit OA to shellfish (Draisci *et al.*, 1996).

In 1994, the first case of PSP mussel contamination at levels above those admitted by the national regulations was recorded in Italy in an area offshore Cesenatico (Poletti *et al.*, 1998).

EO data and microbial and chemical risks

Remote sensing data can represent a significant advantage also in the study and management of the risk posed to human health by chemical and microbiological agents.

Monitoring activities to control microbial and chemical agents are conventionally carried out through the direct determination of these agents or of some of their indices. These activities are often planned without adequately considering the nature and the origin of the inputs and the conditions which can be responsible for their transport from the area of the input to that of concern (where drinking waters are collected, recreational activities are performed or waterbodies are devoted to mussel and fish farming). So, without an adequate knowledge of the sources and processes of these pollutions, the traditional monitoring systems hardly permit any measure to be taken.

The use of EO data in monitoring microbial and chemical agents in waterbodies can be complementary to the traditional activities. Through these EO data it would be possible to obtain information on a large scale, to identify the sources of contamination and follow their transport processes.

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THE ROLE OF METADATA IN ALGAL BLOOM DETECTION MONITORING AND PREDICTION (ABDMAP)

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Introduction

The primary aim of ABDMAP is in advancing and encouraging the role of Earth Observation data to detect, identify and predict algal and cyanobacterial blooms in European waters. A number of organisations and research institutes conduct regular measurement campaigns or partake in valuable research of relevance to the study of algal blooms, yet much of this activity takes place in isolation. The dissemination of the results is limited, and those research data that *are* made available are not always applicable to the problems of interest to the user community. ABDMAP was developed in response to this problem of bridging the knowledge gap between EO researchers and current and potential users. As part of the process of exchange and co-operation among research groups, and amongst those organisations involved in operational water quality studies, the project requires the consolidation of a data archive comprising descriptions of available EO data and complementary ancillary/auxiliary datasets. The archive is necessary to assess the relative importance of remote sensing (EO) data in providing relevant information for predicting the onset of blooms. Such an archive is best achieved by the development of a metadatabase, which does not require the extensive organisation of creating a complete data archive, but rather is a database of descriptive information, so does not include the raw data itself.

This paper describes the value and importance of metadata and summarises how metadata will be used to develop an ABDMAP metadatabase. To remain consistent with current trends in European metadata standards, the CEO recommendations on metadata have been followed. These are given in more detail in the CEO, "Recommendations on Metadata: Describing the data, services and information you have available" (Published by CEO, Version 1.1, May 1997).

What are metadata?

Metadata are 'data about the content, quality, condition and other characteristics' of the data. There are many specific definitions of metadata, but it can be broadly considered as 'information about information'. A metadata base is therefore a database of information that describes properties of other data sets (and therefore need not include the raw data itself). For a data-holder, metadata addresses such issues as:

- Do you know what data you have?
- Can you describe what you have to someone else who might want to use it?
- Can they figure out if the data will be useful to them?
- In 20 years time, can they (or you) figure out if the data is still useful?

Within the ABDMAP project, the metadata will describe relevant information about data (and other resources) that exists within various organisations and locations, and are of potential value to those people or organisations interested in the study or monitoring of algal blooms in European waters.

A further advantage of a metadatabase is that once it is open to use outwith the ABDMAP project partners, researchers and organisations are able advertise their resource, and provide relevant descriptive information while maintaining control over the use and distribution of the raw data. This is particularly important to those organisations that consider their data sets to be of potential commercial value.

The Purpose of Metadata. - In general metadata can be considered to have three principal purposes:
- To catalogue a resource for efficient storage and retrieval within an organisation and ensure consistency and integrity of information, through efficient housekeeping.
- To externally advertise a resource to other potential users, and provide easy access to the information concerning the nature, purpose and current status of the resource.
- To describe the nature of a resource, the processes by which the resource was generated and the way in which the resource should be used, including descriptions of the available formats and other supply details for the resource.

From the perspective of the potential user, the metadata should (from CEO, 1997):
- Allow efficient location of relevant resources
- Allow the assessment of the suitability of a resource with respect to the intended application.
- Allow the assessment of the cost and relative benefit of the resource, and any restrictions or access limitations to the data
- Stimulate initial confidence in the quality and validity of the resource.

The CEO and Metadata. - In general, information systems use different methods to describe data and knowledge resources. Often different terms are used for the same concept, or sometimes the same term for different concepts. A number of metadata standards have evolved to answer the growing user requirement for ease in locating data which meet specific criteria, ease in determining the quality and value of these data and ease in accessing these data across different information systems.

The Centre for Earth Observation (CEO) of the European Commission started activities on metadata as a response to the strong requirement of European EO users for a consistent and integrated information system and their wish that CEO provides recommendations about metadata description in harmony with the existing programmes. As a consequence of the CEO activities a number of metadata standards have evolved. These standards define terms that either use free text or keywords from well-defined lists -called 'valids'. The proliferation of parallel developments of these 'metadata standards' and 'valids' led to the need for guidelines for usage by small and medium size information holders.

The CEO recommendations are fully aligned with the major European standard CEN TC287, other international initiatives with a major European interest (CIP and GELOS) and US major standards (GILS, GEO). Furthermore, the 'CEO Metadata' is meant to act in a complementary manner to the CEO's future information system, INFEO.

Metadata within ABDMAP

The aim is not to introduce any new metadata standards or specifications, but rather conform to the recommendations of the CEO and therefore remain consistent with the European Earth Observation community, and with the existing international and European metadata standards.

It is hoped that the adoption of a consistent metadatabase within the ABDMAP project will :

- Help the partner organisations in their own internal data management and house-keeping tasks.
- Help partners review, assess and acquire relevant data from other partners within ABDMAP.
- Encourage other organisations and research groups to contribute metadata descriptions to the database.

The final aim would be to make the database widely available, allowing a range of researchers, statutory bodies, and organisations to review, assess and subsequently acquire data that is specifically relevant to their individual requirements.

Resource types. - The CEO has identified 10 simple resource types, and each one is described with a well-defined number of pieces of information ('elements'). These elements may be Descriptive (describing qualities of the resource itself) or Administrative (describing the record that contains the information, rather than the information itself). A metadata record therefore consists of a collection of elements, most of which can be edited as free text, but with some elements getting their value from pre-defined lists of controlled keywords, known as a 'controlled list'. The use of controlled keywords allows for better keyword searching, and avoids difficulties arising from spelling mistakes or dubious semantics.

Of the ten CEO resource types, the one of most interest (and therefore highest priority to complete) within the ABDMAP project is the 'Dataset', which will make available information on measured data related to algal blooms. This resource type may describe other products as well as datasets, and may include EO datasets at any processing level, cartographic datasets, maps, statistics, in situ measurements, etc.

Of course, interest is not exclusively in the datasets themselves, but the associated organisations that collect the data, the project within which the measurements were undertaken, models used for prediction, etc. The ABDMAP metadatabase will therefore aim to include the following resource types as a secondary priority:

- Organisation. General information on an organisation such as services offered and contact details.
- Person. Outlining the experience or expertise of an individual.
- Document. Describing textbooks, journal papers, technical reports, users manuals, on-line documents, etc.
- Project. Summarising a research project, or a demonstration case that describe successful applications of EO data.
- Software/model. Describing a software package or model.

Because of the specific nature of the ABDMAP project, in each of the above cases, the metadata resources will explicitly cover projects of relevance to algal bloom research.

Finally, consideration will also be given to the value of adding the following resource types:
Service (value added, consultancy and other services), Event (workshops, seminars, conferences, training courses, etc), Discussion forum (electronic or other discussion fora), Promotion (special offers, job opportunities, etc).

Format and populating the database

One of the important qualities of standardised metadatabases must be in its portability and its accessibility. Since no raw data is kept within the database it is easy to maintain standard field formats and entry types. The basic (Level 0) metadatabase will therefore consist of ASCII text files (with a suitable naming convention). This will allow for easy access and simple text searches to be carried out when no further interactivity is available.

A further level of the database (Level 1) will be fully interactive within on-line web pages, using the Common Gateway Interface protocol. The user will then be able to search a number of fields and keywords simultaneously, and will allow for hyperlinks within the metadata fields (to direct the user straight to sites of further interest or other databases).

For users adding information to the database, there will be two options. The first is to supply a text file (based on a standard ASCII format that will be made available from the University of Dundee) in which the relevant elements will be typed in, according to a list of possible keywords. The file can then be sent or emailed to the database manager.

The second method will be to complete an on-line HTML form that will contain the list of keywords for each element (an example is shown in Figure 1). The form will then be added to the database (after suitable review of the contents). This will provide a simple and convenient method of populating the database.

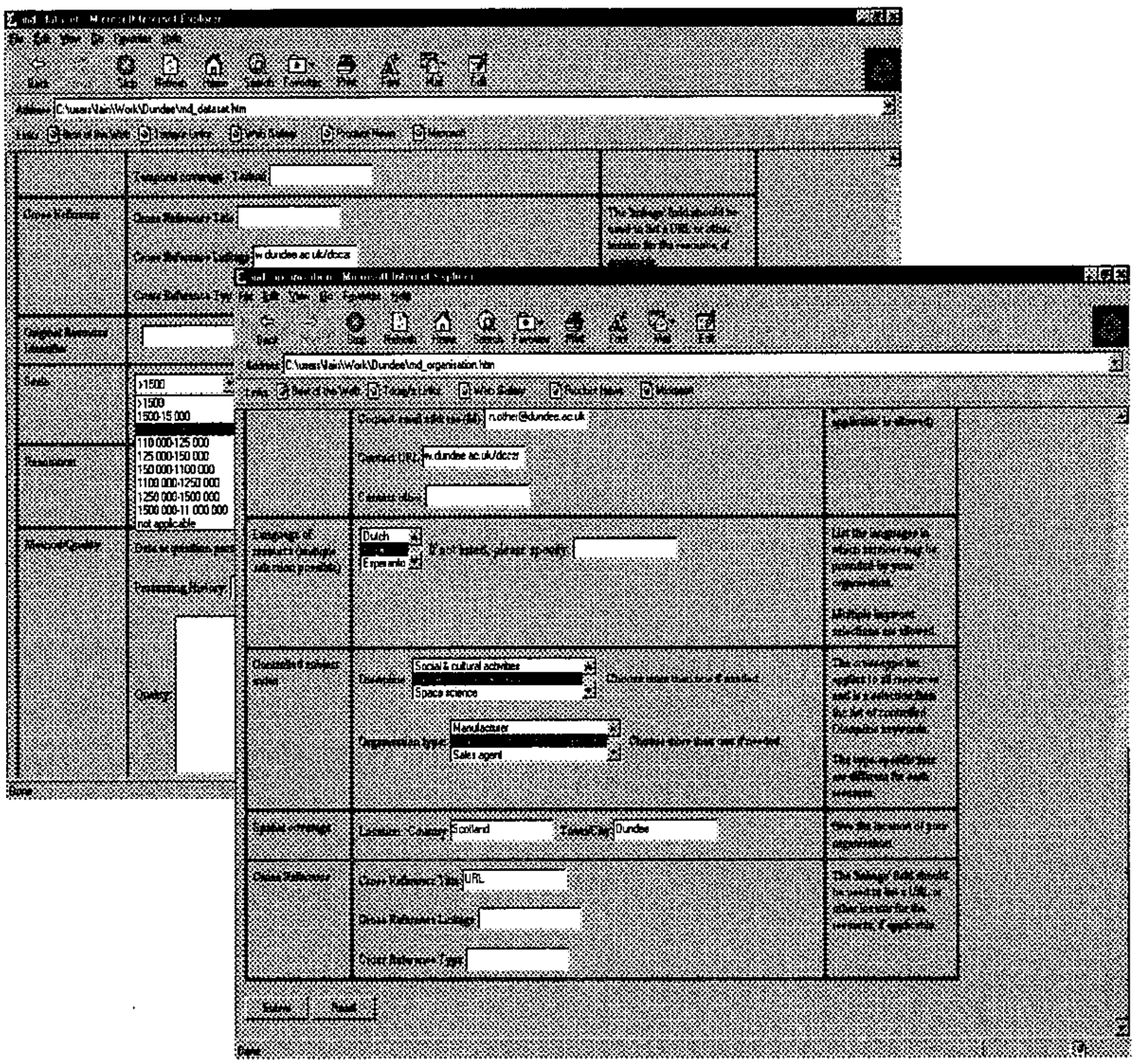

Figure 1. - *Screen dumps showing the format of the on-line submission forms*

Conclusions

The ABDMAP database will provide a convenient focus for people with an interest in resources on algal blooms. It will provide an opportunity for the efficient dissemination of information and data that may be of value to a wide range of organisations and researchers interested in the application of Earth Observation data to algal bloom detection, monitoring and prediction. As a starting point, the database will allow easy access to information on the availability of datasets around Europe and allow an assessment of the suitability of such data. In addition, it will allow contact to relevant individuals and organisations, and by maintaining standardised metadata elements it will provide convenient cross-referencing between other database systems (such as the CEO-INFEO) that may contain additional relevant information.

THE IOC HARMFUL ALGAL BLOOM PROGRAMME

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A rather wide array of different phenomena are grouped under the generic expression 'Harmful Algal Blooms' (HABs), which are caused by different marine microalgae and affect human health and economic activities in coastal waters all around the world (Table 1).

Of about 4000 marine phytoplankton species, less than 300 are potentially harmful and less than 80 are toxic (Sournia 1995). However, these belong to several different algal groups (Table 1) and consequently are notably different in terms of toxicity or damage characteristics, ecophysiological requirements and bloom dynamics.

Table 1. - *Overview of problems caused by harmful algae and harmful algal blooms*

PROBLEMS	CAUSATIVE ORGANISMS
Human health	
Paralytic Shellfish Poisoning (PSP)	dinoflagellates (e.g. *Alexandrium* spp.)
Diarrhoeic Shellfish Poisoning (DSP)	dinoflagellates (e.g. *Dinophysis* spp.)
Neurotoxic shellfish poisoning (NSP)	dinoflagellates (*G. breve*)
Amnesic Shellfish Poisoning (ASP)	diatoms
ciguatera	benthic dinoflagellates
Natural and cultured marine resources	
haemolytic substances	dinoflagellates, raphidophytes
other toxins	dinoflagellates, prymnesiophytes
mechanical damages	diatoms
mucilages	*Phaeocystis* spp., diatoms
Touristic and recreational activities	
foams, mucilages, discolouration, repellent odours, allergic reactions	dinoflagellates, *Phaeocystis* spp., diatoms, *Aureococcus anophagefferens* (Chrysophytes)
Ecosystem health	
Hypoxia, anoxia	dinoflagellates, diatoms
Negative effects on feeding behaviour	*Aureococcus anophagefferens*
Toxicity to marine invertebrates, mammals, birds	Dinoflagellates, diatoms

The main problem is constituted by risks for human health: several kinds of syndromes are known that are caused by algal toxins which are accumulated by molluscs and, in one case, by wild fish. These problems in turn restrict the possibility to exploit food of marine origin and affect economic activities related to aquaculture and fish trading. Some other microalgae can produce ichtyotoxic substances which directly affect food resources, both natural and cultured, or can cause mechanical damages to fish gills, with consequent huge economic loss when occurring in fish farming areas. Mucilage-forming algal species can limit fishing activities by damaging fishing nets, in some cases, algal blooms may spoil the aesthetic quality of seawater or provoke allergic reactions through respiration, thus hampering touristic and recreational activities. Finally, harmful algae can cause problems to selected marine organisms, or even to the whole trophic chain, with negative consequences on ecosystem health, which also implies serious economic losses (Costanza *et al.*, 1997).

The geographic distribution of HABs is almost worldwide: temperate and subtropical coastal areas mainly face the problem of PSP, DSP, ASP and NSP, whereas tropical areas show the negative effects of PSP and ciguatera. Apparently, the world areas less affected, besides the Polar regions, are those for which no information is available, which raises the suspicion that the extent of these problems may be even wider.

The complexity of HABs in terms of species involved, mechanisms of toxin release and practical consequences, coupled with their wide geographic distribution, have urged the Intergovernmental Oceanographic Committee (IOC) to establish an international programme, the Harmful Algal Bloom programme, with the following general aim:

'to foster the effective management of and scientific research on harmful algal blooms, in order to understand their causes, predict their occurrence, and mitigate their effects'.

An intergovernmental Panel on harmful Algal Blooms (IPHAB) was established at the 16th IOC Assembly (Paris 7-21 March 1991), through Resolution XVI-4. Since 1992 four IPHABs have been held, the first three in Paris (1992, 1993 and 1995) and the last one (1997) in Vigo (Spain). Participation in these meeting has increased continuously over the years, from 28 participants from 15 countries in 1992 to 40 participants from 27 countries in 1997, thus demonstrating a growing awareness of the problem. Attendance covers a consistent part of world coastal areas, although whole regions, such as the Caribbean, the southern Asia coast and almost all the African coasts have been scarcely represented so far. Besides delegates from Member States, the panel has included representatives of several agencies which have HABs among their main interests, such as SCOR, ICES, FAO, IMO, IUPAC, WHO-IPCS, CEC, and related IOC programmes, regional bodies and Group of Experts (WESTPAC, IOCARIBE, GEEP, GEMSI, TEMA, etc.).

The IOC-HAB activities are based on a comprehensive and detailed programme consisting of educational, scientific and operational elements, which are listed in Table 2, along with their respective specific aims. This programme was outlined by a broad spectrum of experts at an IOC-SCOR Workshop, Newport, USA, in November 1991, and approved at the first IPHAB (Paris, June 1992).

The IOC-HAB Programme includes an intense and continuous training activity on taxonomy, toxin chemistry and monitoring of HABs. More than 300 students have been trained in about 15 courses organized in the last 6 years, often in co-operation with other agencies. Several relevant publications have been prepared, including the multiauthored Manual on Harmful Marine Microalgae (Hallegraeff et al., 1995), a directory of experts also searchable on the net, several manuals and guides. A newsletter, Harmful Algal News, edited by Timothy Wyatt, is distributed free to about 2000 subscribers and is available on the net.

Table 2. - *Outline of the IOC-Programme on Harmful Algal Blooms*

EDUCATIONAL PROGRAMME ELEMENTS
Information Network: To develop, encourage and maintain the flow of information, technology and expertise to scientists, administrators and the general public
Training: To promote and facilitate the development and implementation of appropriate training programmes in order to distribute the necessary knowledge and expertise on a global basis.
SCIENTIFIC PROGRAMME ELEMENTS
Ecology and Oceanography : To understand the population dynamics of harmful algae.
Taxonomy and Genetics : To establish the taxonomy and genetics of the causative organisms at the appropriate levels.
Toxicology and Toxin Chemistry : To determine the physiological and biochemical mechanisms responsible for toxin production and accumulation, and to evaluate the effect of phycotoxins on living organisms.
OPERATIONAL PROGRAMME ELEMENTS
Resource Protection: To develop and improve methods to minimize the environmental and economic consequences of harmful algae.
Monitoring: To promote and facilitate the development and implementation of appropriate monitoring programmes.
Public Health and Seafood Safety : To protect public health and ensure seafood quality.

To stimulate scientific research on HABs, workshops and study-groups have been organized focusing on several scientific aspects of the problem, in co-operation with SCOR, ICES and IMO.

The IOC-HAB activities are co-ordinated by a Programme Office, established at IOC in Paris since 1992, and two HAB Science and Communication Centres opened in Copenhagen (DK) and Vigo (Spain) in 1994 and 1996, respectively. The staff presently includes Henrik Enevoldsen, IOC project coordinator, Jane Grooss at the Programme Office, Jacob Larsen and Gert Hansen, operating at the Copenhagen Centre and Jorge

Diogene, coordinating the Vigo Centre. A number of task-teams, constituted by panel members and experts, have been established to address specific issues, i.e., the Aquatic Biotoxins, Algal Taxonomy and Monitoring and Management. The latter task-team has co-operated with ICES in the preparation of a Manual on the Design and Implementation of some Harmful Algal Monitoring System (UNESCO 1996).

An updated database on monitoring systems and an on-line questionnaire on the dynamics of harmful algal blooms, are available at the IOC Web site (http:\\www.unesco.org\ioc\oslr\hab.html).

Ongoing activities of the HAB programme include the preparation of a global scientific agenda on HABs (Global Ecology and Oceanography of Harmful Algal Blooms, GEOHAB) in co-operation with SCOR, and the organization of a Workshop on Harmful Algae Monitoring and Management. Other initiatives are planned aimed at improving scientific and managerial co-ordination in different areas of the world, including Europe, the Caribbean and the circum-Pacific area.

One of the most frequently asked questions on HABs is whether these phenomena have increased in frequency and intensity in recent decades. This is almost impossible to answer, due to the lack of time-series at the proper scale to detect trends in algal blooms (Wyatt 1995). However, there is no doubt that an effective exploitation of marine resources is increasingly important, due to the growth of human population and of demographic pressure on coastal areas, and that HABs constitute a serious restriction for the sustainable development of coastal areas. A co-ordinated HAB management effort, backed by scientific knowledge, is necessary to protect human health, food resources, touristic and recreational activities and the ecosystem health. In this context, present applications of remote sensing can constitute a valuable tool to complement hydrographic information and significantly contribute to knowledge of the dynamics of algal blooms. Future developments of the technique are warranted aimed at extending its application to the detection of harmful blooms and to an effective contribution to the coastal zone management.

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NATIONAL INVOLVEMENT IN OCEAN PROJECTS ON REMOTE SENSING

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Introduction

A number of projects being carried out involve Italian research institutions in which Remote Sensing activity is recognized as the primary means of exploring the environment. We must acknowledge that much of the activity is directed at the experimental research required to support and promote the operational system which concerns some aspect of marine applications not yet consolidated. To attempt an environmental study and more specifically in the hydrosphere requires in fact the use of technologies and methodologies operating on the basis of the time-space characteristics of the problems. Actually, a very clear picture emerges of the overall activity especially with respect to active and passive remote sensing that operate in the visible and infrared bands, where there is some commitment to develop procedures to obtain as much as is possible quantitative type data. It therefore is rather evident the existence of programmes oriented to applications or methodologies in which there was an attempt to keep the interests complementary. Not only the Italian, but the whole scientific community is involved, as revealed from the projects financed by the EC, to develop the use of remote data to study oceans with respect to their being the primary source of resources.

The programmes that are presented here are financed solely by different funds from the Italian ministries and the EC-DGXII. The main ones are cited because other existing programmes with specific objectives are backed financially by other institutions for example ASI (Italian Space Agency) and ESA (European Space Agency).

Experimental Research Programme for the protection of the Adriatic Sea (PRISMA-2)

In the framework of big Italian programmes, mention must be made of the **PRISMA-2** (coordinated by Prof. Roberto Passino) financed by MURST (Ministry of Scientific Research and Technology) under agreement with the Consiglio Nazionale delle Ricerche (Italian National Research Council), begun in 1996 for a 3-year duration in which the use of remote sensing technology is required to better develop a model to monitor the Adriatic Sea.

The main objectives of the project are:

- develop an observation system to scientifically manage environmental problems of the Adriatic ecosystem by:
  - a permanent observation system used in controlling coastal and pelagic areas
  - identification and quantification of pollution loading and the means for their dispersion
- specific investigations, laboratory analyses, development of methods and protocols
- development of research support structures.

Subprojects.- To reach the objectives of PRISMA-2, activities are carried out in the framework of seven subprojects that are essential to be able to fully comprehend and understand the different related issues. The subprojects and their respective scientific leaders are:

1. Physical Oceanography, Chemistry and Biology, Prof. G. Spezie
2. Bio-geo-chemical Cycles, Dr. G. Catalano
3. Changes in Benthic Communities, Prof. E. Fresi
4. Boundary Conditions, Dr. A. Brondi
5. Monitoring and Remote Sensing, Dr. L. Alberotanza
6. Human Health, Dr. E. Funari
7. Data Bank, Dr. L. Rossi.

Specific objectives of Subproject 5 "Monitoring and Remote Sensing" are:

- define a global model to monitor the Adriatic Sea on the basis of the ecological, methodological, and institutional aspects;
- support the experimental activity of the other subprojects (especially that of Subprojects 1 and 2).

These subprojects required the development of activity so as to:

- define a monitoring system based on specific needs that can satisfy these requisites:
  - to be a tool for management,
  - to represent a continuous acquisition system of coherent data to use in information on the state and operating of the system,
  - to build a database sufficient to describe the set of processes and conditions on running the system.
- support at various levels some of the experimental activity of the Subprojects by:
  - historical data analysis of CZCS images (from '79 to '85, pigment maps), AVHRR/NOAA and ATSR/ERS1-2 (SST maps, different periods) and SAR (ocean currents),
  - quasi-real time maps of SST from AVHRR/NOAA images and surface currents from the coastal radar CODAR (Figure 1),

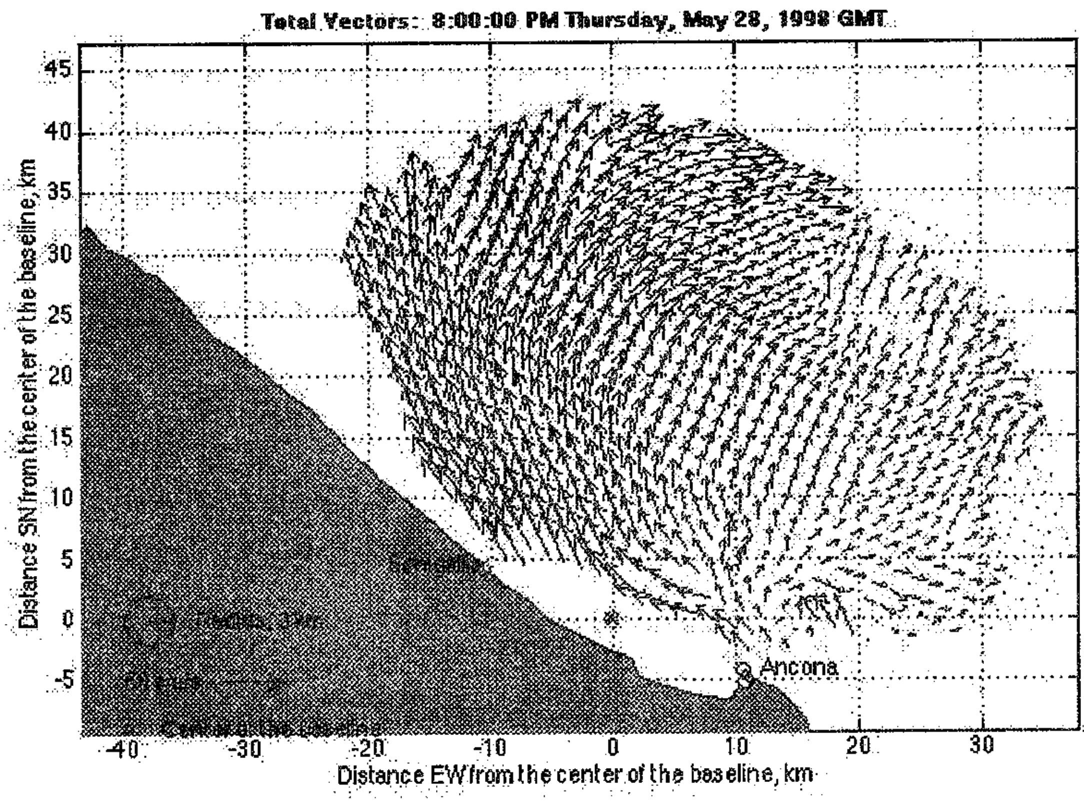

Figure 1. - *An example of current-mapping with CODAR-type HF radar*

- pre-exploration measurements from ship with the FLIDAR-3 (laser-optical pigment profiles, DOM and TSM) and SARAGO (underwater profiles of T, S, primary production, O, etc.).

– develop new or more opportune methods and technologies considering:
  - the integration of TLR data in numerical models,
  - the analysis of data in terms of time and space,
  - developing measurement systems: PRISMA's spectral radiometric and multi-parametric profilers and PTF (Oceanographic Platform "Acqua Alta", Fig. 2) measurement systems.

– improve the understanding of certain phenomenologies on the basis of new, scientific instruments among which:
  - data acquisition from satellite with TM, AVHRR, ERS1-2 and SeaWiFS relative to the experiment periods of the project;
  - observed data from aircraft with MIVIS and FLIDAR-3;
  - sea-truth activity from both ship and the oceanographic platform, "Acqua Alta".

The afore-mentioned research activities are carried out under nine Operational Units plus two Associated Units with acting heads at different CNR Institutes, the University of Tor Vergata, Rome, and JRC (Joint Research Centre, Ispra).

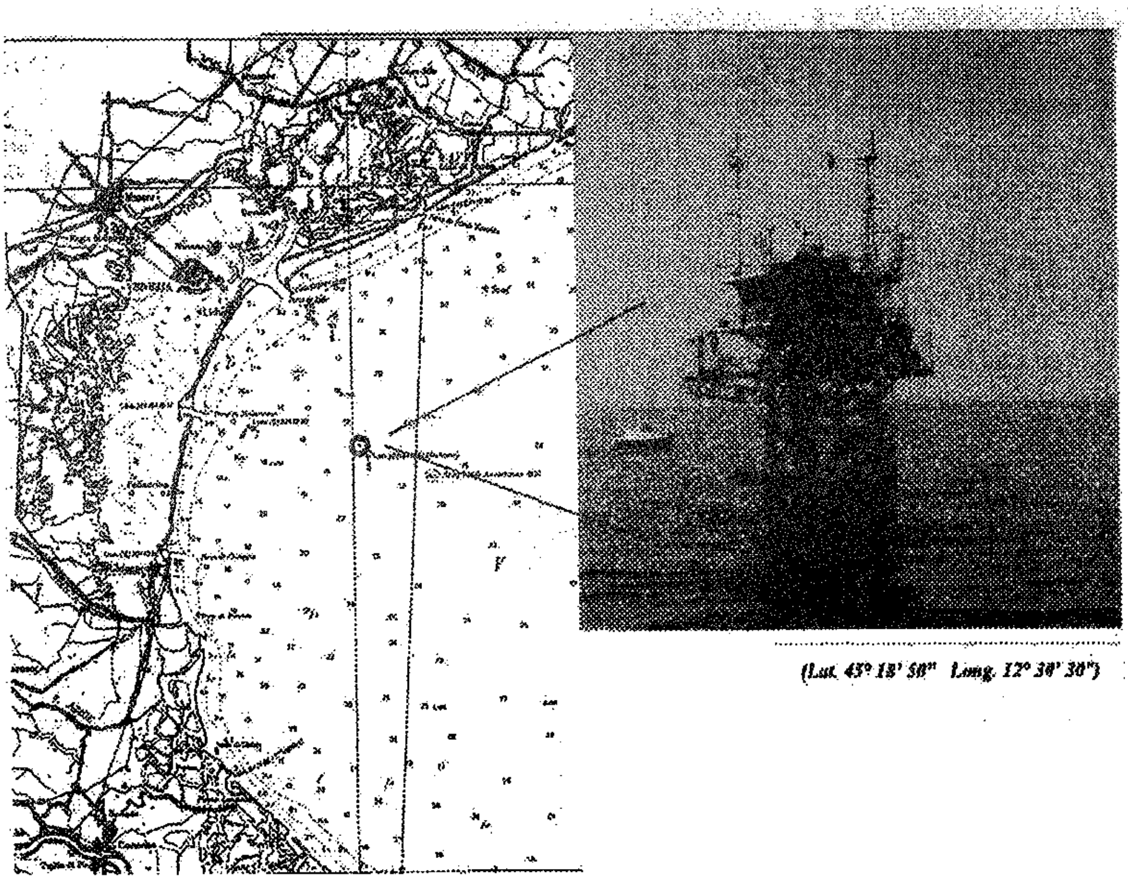

Figure 2. - *Oceanographic platform "Acqua Alta". Its location is indicated on the map of the North Adriatic Sea.*

Colors

With the SeaWiFS sensors, NASA has fixed to be able to define the leaving water radiance from the sea with an error of 5% and the measurements of chlorophyll concentrations in oceans with 35%. With these goals it was necessary to guarantee an accurate programme of calibration and validation (Cal/Val). The programme **COLORS**, Coastal Region Long-term Measurements for Color Remote Sensing Development and Validation, finanzed by the EC-DG XII for Marine Science and Technology III (MAST III) with a three year duration starting at the end of 1997. The project, coordinated by Dr. G. Zibordi of the JRC, and carried out by Italian, English, German, French, Dutch and Irish institutes, for their respective specific tasks in the three test sites (Adriatic, English Channel, North Sea) proposes:

- to establish the basis for a European network of three differing representative sites on which a systematic program of long-term relevant measurements in water and in the atmosphere is to be carried out.
- all three sites will be equipped with identical instrumental packages and special attention will be given to their inter-calibration.

COLORS encompasses the following main activities:

- long-term, seasonal, in-situ, atmospheric and sea water measurements, data calibration and data archiving;
- analysis and interpretation of the acquired data in terms of water quality and bio-geo-optical parameters including their use in models/algorithms development and assessment;
- a first level application of the developed algorithms/models to validate the satellite and aircraft data products and estimate their accuracy in the European coastal and shelf water.

Measurements in the Adriatic are made from the oceanographic platform, "Acqua Alta" [1] offshore the littoral of Venice in the North Adriatic since the site, well representative of the marine features of the entire Adriatic Sea, is located in a transition region characterised by oceanic or coastal aerosol and Case-1 or Case-2 water [2], depending on the prevailing winds and currents. Both marine and atmospheric measurements are taken. Atmospheric measurements are continuously acquired while those along the water column are taken during field measurements made one week every month. Procedures followed for the acquisition of bio-optical and oceanographic parameters and for laboratory determination follow the indications supplied by NASA [3] and the Joint Global Flux Study [4]. Obviously some procedures have been modified with respect to the mean characteristics of the Adriatic sea water being that they were validated for typical ocean environments.

A very demanding experimental programme was made and instruments set up (atmospheric and underwater radiometers) even with the estimation of the significance of optical data with respect to the effects of diffusion of the light induced by the platform structure in the body of water surrounding it. Surely the results of this project will represent a valid contribution in making the use of satellite images more operational to study bio-physical characteristics of the marine environment. The use in the activation of calibration/validation of the SeaWiFS images has obviously an extended use because space programmes are ready to put in orbit the sensors MODIS (NASA), MERIS (ESA) and ROSIS (ESA-DLR) and the experience derived will be very useful.

Inland waters

Also another two programmes presently being carried out concern more the inland waters: coastal lagoons and lakes. For these there is an interest in developing specific methods for application related to algal biomass recognition and on the quality of fresh water in the larger Alpine and northern European water basins.

Orbetello lagoons.- The lagoons of Orbetello, located along the southern coast of Tuscany are of great ecological interest for their peculiar characteristics, among the brackish wetland still preserved in Italy. The natural, environmental equilibrium is very fragile due to the large seasonal fluctuations of several parameters, such as water temperature and salinity. Anthropogenic influence on this region has induced a further decrease in the stability of this environment. In fact, anoxic crises have been observed with increasing frequency over the past few years due to eutrophication. Primary productivity in the lagoons is mostly sustained by macrophytes, while phytoplankton is relatively unimportant. The abundance of nutrients favours the proliferation of algal biomass, and the oxygen demand related to the respiratory activity of large masses of macroalgae can cause an anoxic crisis dangerous for aquatic life. Macrophytes are an evident manifestation of the instability of those lagoons. The more abundant macrophytes are *Chaetomorpha* sp., *Gracilaria* sp., *Ulva* sp. and *Cladophora* sp. which are taxa typical of eutrophic and hypertrophic waters.

To follow the seasonal evolution of algae masses, eight aerial surveys were repeatedly taken at regular intervals over a two-year span using the MIVIS hyperspectral, Daedalus scanner. It operates with four spectrometers covering the Visible, Near-IR, Mid-IR and Thermal-IR spectral regions with 102 channels (Table 1). A series of *in situ* spectral radiance were collected during some

of the MIVIS over flights on the different algal species from a small boat with a portable, multispectral, field radiometer operating between 380 and 780 nm with 4 nm resolution.

Images were radiometrically and geometrically corrected for atmospheric effects to make a mosaic composition of the different flight paths. A selection, therefore, was validated of the reference spectra of submerged vegetation after a qualitative comparison of spectral signature collected *in situ* and of the upwelling radiances extracted from the MIVIS imagery [5]. The *training* sites were selected on a quasi-true color image and on the indication derived by a continuous algal survey. Then the Spectral Angle Mapper (SAM), supervised classification techniques, has been adopted as an automated method that permits rapid mapping of spectral similarity of image spectra to field spectra. Thus distribution maps of the different species of algae were reproduced (Figure 3).

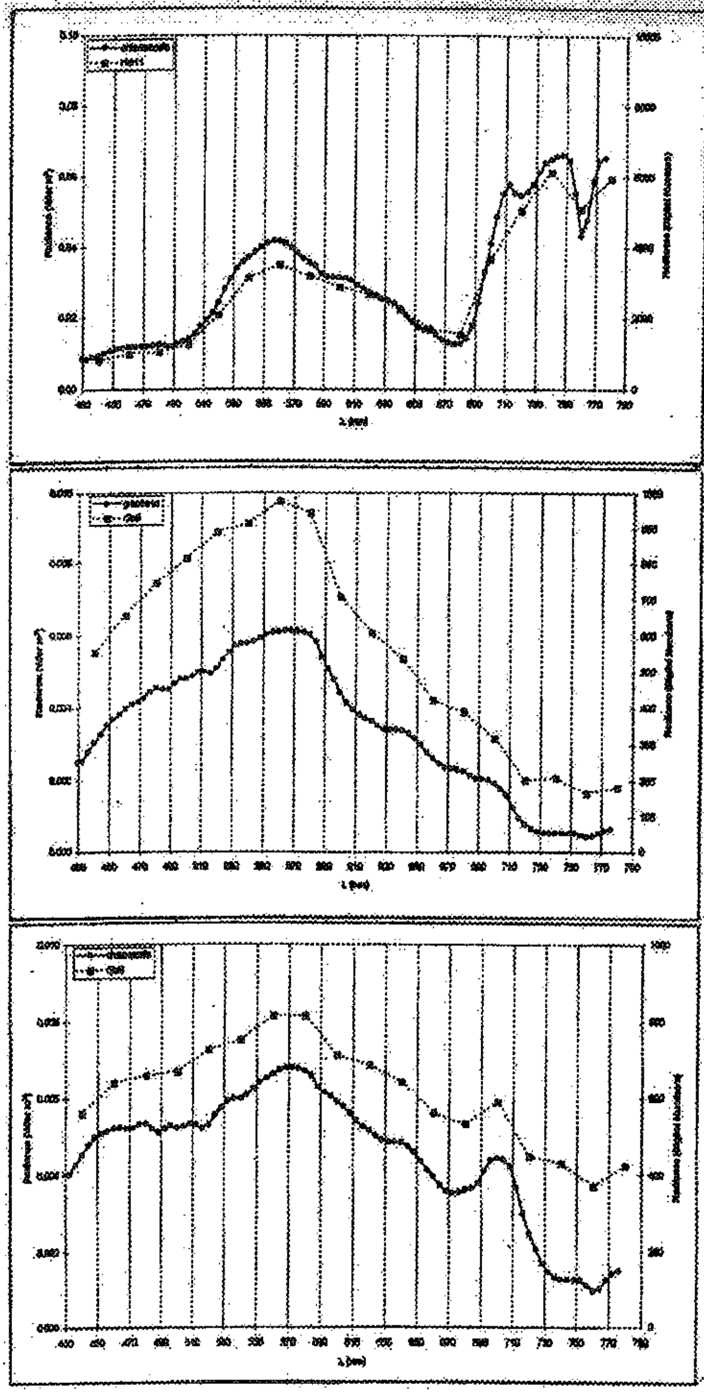

Figure 3. - *Spectral radiances of the different algae: comparison between in situ data (dark line and circles) and MIVIS observations from aircraft (dotted line and black squares).*

Quantitative evaluation of the seasonal variability (correlated to the different environmental parameters) is being made to verify the growth rate of the biomass with respect to restoration works made in the lagoons (harvesting, waste treatment and increased exchange with the sea).*Salmon.* - Even large fresh water reservoirs (lakes) undergo actions of degradation very similar to those of oceans and seas, but moreover represent the freshwater reservoir necessary to human, agriculture, and energy demands. The project SaLMon (**S**atellite Remote Sensing for **L**ake **Mon**itoring), coordinated by Dr. E. Zilioli of the CNR-IRSS, Milan, financed by the EC-DG XII *Climate & Environment* begun in 1996 for a duration of three years..

The principal objective of the project is the definition of data needs and specifications for environmental monitoring of European lakes with the primary concern being water quality.

The goal will be reached through:

- interaction with end-users to define their needs for the monitoring of lake water quality (parameters, accuracy, frequency);
- evaluation of the capabilities of current instruments and methods to fill these needs;
- acquisition and comparison of aerial satellite and in-situ data during field measurements involving instruments similar to the next generation of space borne instruments (e.g. hyperspectral passive observation, fluorescence lidar);
- improvements of the processing methods for remote sensing data including calibration, atmospheric correction for solar zenith angle;
- definition of an optimal space borne sensor for the purpose of lake water quality monitoring.

The expected results of the research and an evaluation of the remote sensing capabilities will be presented to potential end-users. Main outcomes are:

- guide-lines and protocols for the definition of a suitable tool in the water quality monitoring and management;
- proposed set up of wave-lengths, wave-widths and other requirements for future more effective use of satellite imagery for detecting specific limnological variables;
- an end-user evaluation of remote sensing usefulness for specific purposes, together with a cost effectiveness analysis;
- a package of "dissemination products".

In the experimental measurements of this project we have used the procedures and execution of optical measurements along the water column based on the experience consolidated by the programmes (COASTS and CEVEX) that preceded COLORS.

To conclude, the briefly illustrated national programmes are a need to scientific research to improve the methods of extracting environmental parameters from remote sensed images and therefore are an undertaking in the applications that could contribute to the monitoring and the management of marine environments.

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IN VIVO OPTICAL PROPERTIES AND CHARACTERIZATION OF PHYTOPLANKTON PIGMENTS FOR ALGAL BLOOM DETECTION AND MONITORING

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Introduction

Detection and characterization of algal blooms have been, since a long time, a major objective in basic oceanography and limnology, as well as in applied aquatic sciences concerning coastal management. In the last decade, the use of optical data, remotely sensed or *in situ* measured, has largely improved our knowledge of blooms distribution and dynamics, covering spatial and temporal scales previously unexplored and allowing estimates of the global ocean carbon production (Longhurst et al., 1995; Antoine et al., 1996; Beherenfeld and Falkowski, 1997). The passive remote sensing of phytoplankton biomass and activity, by satellite or by aircraft, is based on the *in vivo* optical properties of pigments, which strongly affect those of the entire water body.

These optically-based methods can show significant advantages, since they give access to spatially integrated and/or time resolved information, circumventing problems derived from under-sampling and allowing correlations with environmental factors. A variety of methods make use of microalgal cells optical properties like absorption, backscattering and attenuation coefficients: i) remote sensing of backscattered light from the upper sea layers (Gordon and Morel, 1983); ii) spectrophotometry of natural phytoplankton (Kishino et al., 1985; Bricaud and Stramski, 1990); iii) multi-wavelength optical sensors deployed on buoys or vertical profilers to investigate the underwater light field (Cullen et al., 1997 and references therein). Fluorescence emitted by the photosynthetic pigments of algae *in vivo,* is also an optical property which has been extensively used (Prezelin and Boczar, 1986; Geider and Osborne, 1992) to study both the phytoplankton biomass distribution and its physiological condition, by means of: i) classical *in situ* fluorometers with blue excitation and red emission detection (Lorenzen, 1966); ii) sun-induced passive fluorescence, through measurements of upwelling radiance at 683 nm (Gordon, 1979; Chamberlin et al., 1990); iii) measurements of the spectral excitation and emission characteristics of natural phytoplankton (Yentsch and Yentsch 1979; Sohoo et al., 1986; Hilton et al., 1989; Poryvkina et al., 1994); iv) LIDAR fluorescence (Hoge and Swift, 1983); iv) flow cytometry (Li, 1989); v) variable fluorescence: DCMU-induced (Cullen and Renger, 1979), with PAM technique (Schreiber et al., 1986), with pump & probe or FRRF (Kolber and Falkowski, 1993; Falkowski and Kolber, 1997) and laser-induced pump & probe (Barbini et al., 1997).

The link between phytoplankton distribution and remote sensed data (Figure 1) is made of a rather long chain of relationships which in turn relate the optical properties of the aquatic medium to the abundance and activity of phytoplankton.

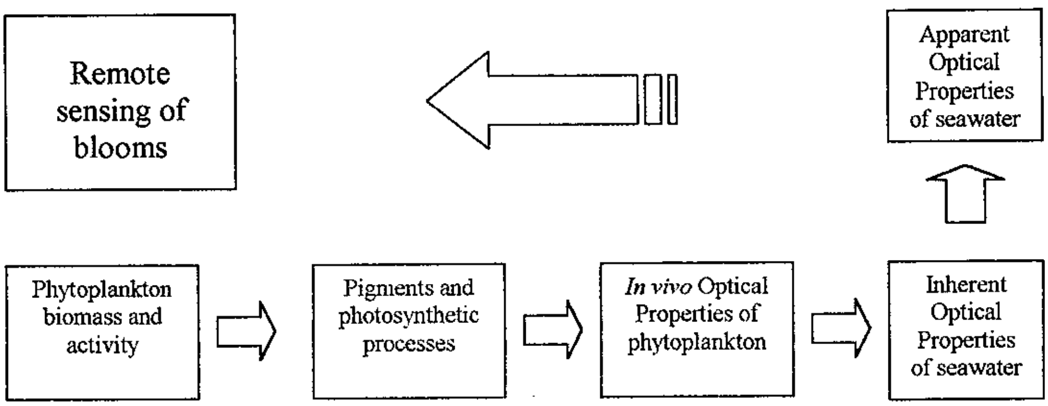

Figure 1. - *Chain of links between microalgal blooms and its remote detection*

The optical properties of seawater must be distinguished into « inherent », which are independent of the spatial distribution of the radiance field, like attenuation, scattering and absorption coefficients, and « apparent » which depend on the formers as well as on the radiance field (Kirk, 1994). These latter, like reflectance, are those practically measured by the optical remote sensors. On the other hand, only the inherent optical properties, namely the absorption coefficient, are directly linked to the concentrations of the optically active substances in the water. However some approximate relationships have been established between these properties. For instance, the radiance reflectance (R_r), the ratio of upwelling radiance to downwelling irradiance (L_u/E_d), that is what optical sensors measure above the sea surface, is proportional to the ratio of the backscattering coefficient (b_b) to the absorption coefficient (a) :

$$R_r \propto b_b / (a + b_b)$$

Dissolved organic matter, phytoplankton, other suspended particles and water itself are optically active substances and all of them contribute to absorption and backscattering in different ways, but fortunately the spectral absorption of phytoplankton is strongly featured, with two or three main bands (see below Figure 2). Then what finally makes possible the *in vivo* optical detection of phytoplankton in the sea, is that absorption of light by water, DOM and detritus lacks strong spectral features, right in the spectral domains where phytoplankton has the main and characteristic absorption bands (Kirk, 1994).

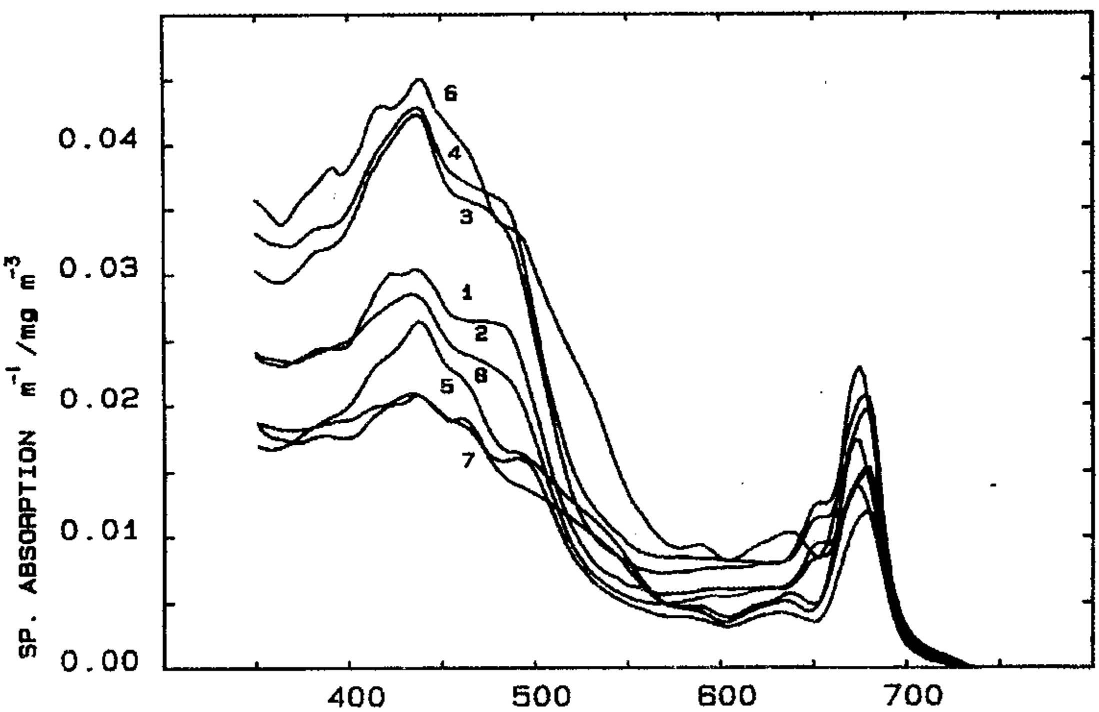

Figure 2. - Spectral absorption coefficients, per unit of chlorophyll *a*, for eight phytoplankton species of three diffent classes: clorophytes (1 - *Platymonas suecica*, 2 - *Dunaliella marina*, 3 - *Tetraselmis maculata*, 4 - *Platymonas* sp.), coccolitophorids (5 - *Hymenomonas elongata*) and diatoms (6 - *Chaetoceros didymum*, 7 - *Chaetoceros protuberans*, 8 - *Skeletonema costatum*). Redrawn from Sathyendranath et al. (1987).

We will examine hereafter, with some detail, the main spectral features of phytoplankton absorption and fluorescence *in vivo*, as they can be measured in natural populations, in order to relate them to water color remote sensing and its interpretation for blooms detection.

Phytoplankton *in vivo* absorption spectra

First of all, it is to be stressed that optical absorption per unit of pigment, for a suspension of microalgal cells is reduced, when compared to absorption of the same amount of absorbing material dissolved in solution. This is due to the so-called « package effect » (Duysens, 1956; Kirk 1975; Morel and Bricaud, 1981) which lowers the absorption maxima more than the minima (a flattening effect) depending on the size and the intracellular concentration of the absorbing particles (the cells, as first approximation). It is possible to completely account for this effect knowing the size, shape and concentration of cells and the concentration of differentpigments, for different species in colture (Sathyendranath et al., 1987; Hoepffner and Sathyendranath, 1991).

Typical absorption spectra per unit pigment of phytoplankton belonging to three different classes (diatoms, clorophytes and coccolitophorids) are shown in figure 2, where the presence of the main pigments can be detected: chlorophyll a (at 440 nm, all the lines), chlorophyll b (at 480 and at 655 nm, lines 1,2,3 and 4), chlorophyll c (at 460 and at 640 nm, lines 5,6,7 and 8) together with that of carotenes (between 460 and 490 nm, all the lines) and xanthophylls like fucoxanthin (between 490 and 540 nm, lines 5,6,7 and 8). It can be noted also, that peaks which characterize pigments are red-shifted of 5 to 10 nm, compared to their typical wavelengths in organic solvents.

Spectral absorption of natural phytoplankton has been extensively studied (Bricaud et al., 1995, Stuart et al., 1998 and references therein) through measurements of particulated matter concentrated on filters and bleached with organic solvents. The artifact of amplifying the optical path-length has to be corrected through an empirical algorythm (β factor) which can be somewhat critical and species-dependent (Mitchell and Kiefer, 1988; Bricaud and Stramski, 1990; Hoepffner and Sathyendranath, 1992; Cleveland and Weidman, 1993; Moore et al., 1995). Notwithstanding this variability, the pigment composition of natural populations can be widely analyzed, as well as quantified the variance linked to the package effect (Stuart et al., 1998). Unfortunately, with these methods, the presence of hydrosoluble pigments like phycobilins (mainly phycoerythrin, phycocyanin and allophycocyanin) remains underestimated or neglected.

Phytoplankton *in vivo* fluorescence spectra

An increased sensitivity in phytoplankton detection is reached by the use of fluorescence techniques as they directly measure a flux of photons, not ratios of fluxes as absorption does. Chlorophyll fluorescence *in vivo* is emitted with low (up to 5%) and variable efficiency (Loftus and Seliger, 1979), which depends on several distinct processes such as photochemistry of the electron transport chain, energy transfert to PSI and radiationless dissipation. This efficiency is strictly dependent on light intensity, as revealed by strong day to night variations with constant chlorophyll concentrations or by variation induced by different cloud coverage (Owens et al., 1980, Abbott et al, 1982).

Spectral fluorometry of phytoplankton give information about its excitation as well as emission properties, and therefore they reveal the presence of the emitting pigment and also signal the event of energy transfer from the accessory pigments to the PSII reaction centers. The presence of the accessory pigments makes possible to rapidly *in vivo* discern between four « pigmentary groups » in natural phytoplankton; with organisms spectrally characterized by: i) chlorophyll *b*, ii) chlorophyll *c* and xanthophylls, iii) phycobilins 1986, Lazzara et al., 1992, Cowles et al., 1993, Lazzara *et al.*, 1996).

The spectral fluorescence properties of phytoplankton populations of the tropical Atlantic Ocean, have been analysed in two distinct trophic conditions with direct measurements on suspensions after gentle concentration (Lazzara *et al.*, 1996). The oligotrophic site (Figure 3a) was characterized by the presence of a deep chlorophyll maximum, below the euphotic zone, dominated by the widespread prokaryote *Prochlorococcus marinus*, as revealed, in the fluorescence excitation spectra, by the peaks due to the specific pigment divynil-chlorophyll b, at 480 and 600 nm. At the mesotrophic site (Figure 3b) the more abundant surface phytoplankton was dominated by phycoerythrin (PE)-containing organisms : both cryptophyceans and cyanobacteria of the genus *Synechococcus*, characterized by a peak in the excitation spectrum at 546 nm. A direct emission of fluorescence by the phycoerythrin could also be measured at 585 nm and this allowed to distinguish between oligotrophic and mesotrophic PE-organisms, with respectively high and low PUB/PEB cromophores ratios (with peaks at 490 and 546 nm).

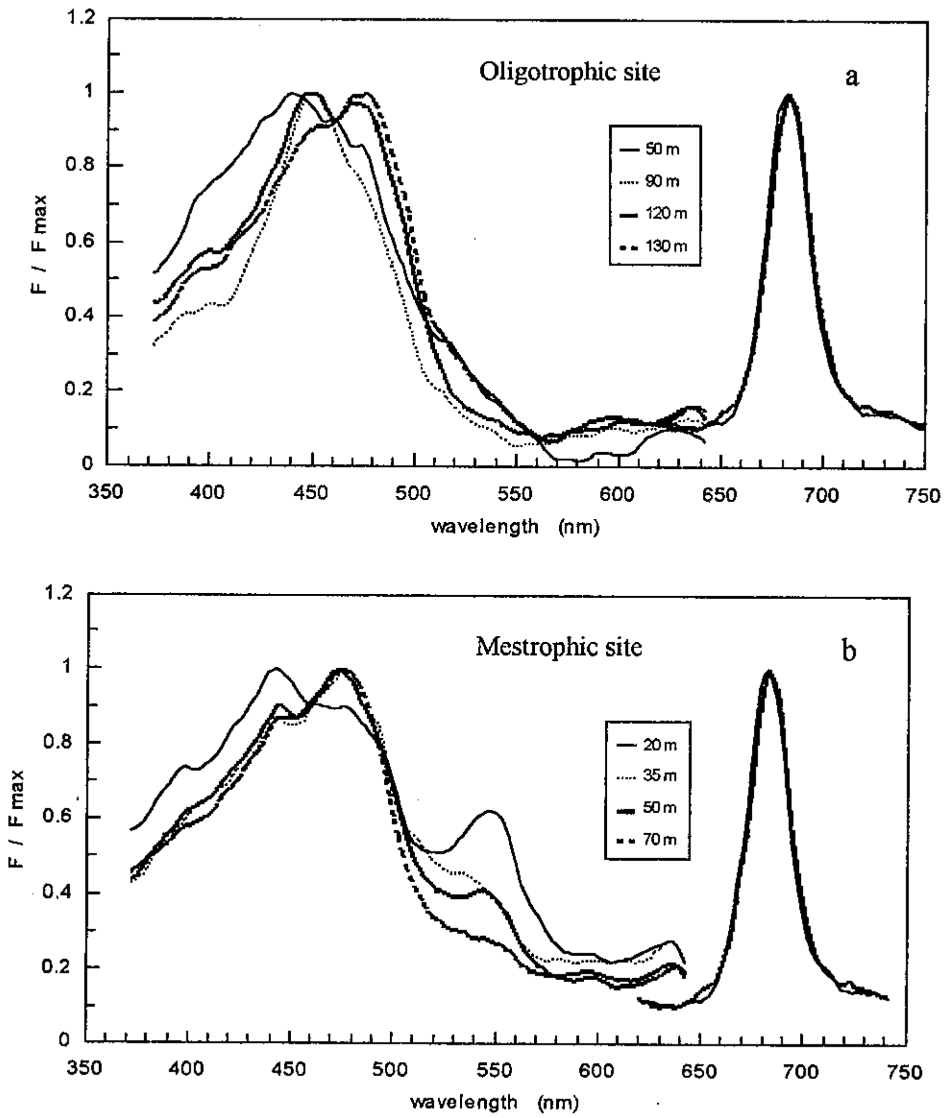

Figure 3. - *Spectral fluorescence of natural phytoplankton in the tropical Atlantic Ocean (excitation at 432 nm and emission at 682 nm), from the surface to below the euphotic zone. 3a) At an oligotrophic site; 3b) At a mesotrophic site.*

Final considerations

A qualitative assessment of phytoplankton assemblages through remote sensing, in addition to what above mentioned, is still limited at present by a series of constraints such as: i) overlapping of pigments spectral features; ii) scarce resolution of remote sensing spectral analysis, which is made through bands; iii) absorption and fluorescence interaction leading to modification of spectral properties.

As a matter of fact, Garver et al. (1994) did not observe significant variations in the shape of a large set of absorption spectra from particulate matter and concluded that it will be extremely difficult to retrieve phytoplankton composition from satellite ocean-color imagery. However, specific optical features such as unusually high backscattering coefficients of coccolitophorids, has allowed the specific detection of blooms for that algal group (Balch et al., 1991).

Millie et al. (1997) recently explored the possibility to detect the red tide dinoflagellate *Gymnodinium breve*, from absorption signatures of a specific pigment, the carotenoid gyroxanthin-diester, in mixed cultures. They concluded that it is possible to discriminate and quantify the presence of the species through a stepwise discriminant analysis applied on the 4th derivative of the absorption spectra, but the same task should not be achieved in natural assemblages, due to the variable package effect and to the mixed composition of natural samples.

At present it appears that only organisms with phycobilins and/or DV-chlorophylls (pigmentary groups iii) and iv), see above), wether dominant, can be remotely discerned in natural populations from the most common group ii) (diatoms and coccolitophorids), thanks to their pigments properties. On the other side, joined *in situ* and remote sensed optical measurements represent an extremely powerfull tool for the quantitative assessment and monitoring of phytoplankton blooms and phytobiomass distribution.

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REMOTE AND LOCAL FLUORESCENCE DETERMINATION OF ALGAE PIGMENTS AND PHOTOSYNTHETIC EFFICIENCY

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1. Introduction

Spectrally resolved local and remote Laser Induced Fluorescence (LIF) measurements are suitable to investigate water dispersed and dissolved chromophores [1]. In fact the signatures of dispersed impurities, such as organic pollutants, oils, DOM and phytoplankton, can be extracted from the detected LIF spectra [2, 3]. Nowadays the importance of lamp or laser excited fluorescence methods for in-situ determination of chlorophyll in terrestrial and marine vegetation samples has been well established [4].

Reliable information on the photosynthetic efficiency of a living vegetation target can be obtained from fluorescence measurements performed by externally perturbing the system, plant or alga, in its actual conditions [5]. In this way the vegetation health can be monitored and, in the case of phytoplankton, the growth rate of specific algal species responsible for hazardous blooms can be timely detected.

Techniques based on active Chlorophyll fluorescence detection [6] were developed in alternative to different *in-situ* photosynthesis measuring methods, including the determination of dissolved oxygen evolution from phytoplankton [7]. Differential fluorescence instruments, like the Pulse Amplitude Modulated (PAM) fluorometer, have been certified for application to vegetation living tissues being currently used to locally measure the fluorescence yield at the onset of an actinic lamp [8].

The techniques of inducing fluorescence in living tissues is also suitable to remote applications in a lidar fluorosensor. In fact, intense laser pulses can be used to give rise to differential LIF signals according to the *Pump-and-Probe* (PP) scheme [9], which requires detecting the chlorophyll red signal induced by a probe laser pulse both before and after the action of a pump saturating laser pulse. From Pump-and-Probe measurements, performed on phytoplankton cultures illuminated at different light levels, the Electron Transfer Rate (ETR) characteristic of each family can be obtained, which supply information on the relevant photosynthesis efficiency.

A mobile lidar fluorosensor system was developed at the ENEA remote sensing laboratory and installed inside a standard ISO 20" container. The instrument, designed to measure time integrated LIF spectra and wavelength selected time profiles, was extensively used in marine campaigns [10-12]. The lidar has recently been upgraded by adding a newly designed laser source suitable to remotely perform differential measurements based on the PP laser excitation scheme in order to monitor the photosynthetic activity variations directly in the sea water [9]. A lamp spectrofluorometer, a pulsed amplitude fluorometer (PAM), a solar radiance detector and a GPS complete the set of sensors on board, used both to calibrate lidar data and to extend the monitoring capabilities of the system.

Laboratory experiments, utilizing the available laser and lamp baser fluorometers are currently carried on, prior to marine campaigns in order to form a database for characterization of natural phytoplankton communities. As an example, laboratory results obtained on Dinoflagellates are presented in the following. Field experiments aimed to monitor the photosynthetic activity and the growth rate of some species belonging to this algal class have been performed during the ICES/IOC marine campaign (Kristineberg, Sweden, Sept. 1996), the results obtained are here discussed in comparison to concurrent PAM data and to Oxygen evolution determination.

2. Local and remote LIF characterization of phytoplankton

2.1 *Spectrofluorimetric* - pigment analysis. - Visible and near-UV light is suitable to excite chromophores in algae, thus the LIF technique is commonly used to investigate Chl-a and related pigments concentration both locally and remotely [13]. As an example, the complete near UV visible emission spectrum measured upon laser excitation at 355 nm of a concentrated solution of *Synecochoccus 625* is shown in Figure 1, together with a deconvolution in bands relevant to the main features. In this figure, apart from the backscattered radiation at the laser wavelength, we observe the water Raman peak red shifted at 402 nm, which is currently used for normalising remotely sensed data [14, 15], the visible emission mostly due to DOM (Dissolved Organic Matter), which is present in natural waters and in some culture media, and different phytoplankton pigments. In fact, the phytoplankton fluorescence spectrum shows structures, extending from the blue to the red region, which occur in spectral windows suitable to remote determination [16]. The most important red fluorescing pigments [17] include phycoerythrin (580 nm), phycocyanin (660 nm), and chlorophyll-a (peaked around 680 nm for molecules contained in PSII and around 730 nm in PSI). By using an UV excitation source it might be possible to detect also the blue pigments emission overlapping the DOM broad band. In fact, in this spectral region contributions due to absorption and re-emissions of xanthophyll, NaPDH, fucoxantin [18] and minor light harvesting carotenoids [19] occur. In particular, in the blue absorption band some structures have been recently related to the Peridinin-Chl a-Protein (PCP) characteristics of dinoflagellates [20].

In order to link LIF intensities measured on different spectral channels to corresponding pigment contents, the spectra collected upon UV excitation are successively processed by integrating with 10 nm bandwidth both the water Raman peak and the different pigments bands shown in Figure 1 after a multiple gaussian deconvolution taking into account the contribution of nearby spectroscopic lines to each integral.

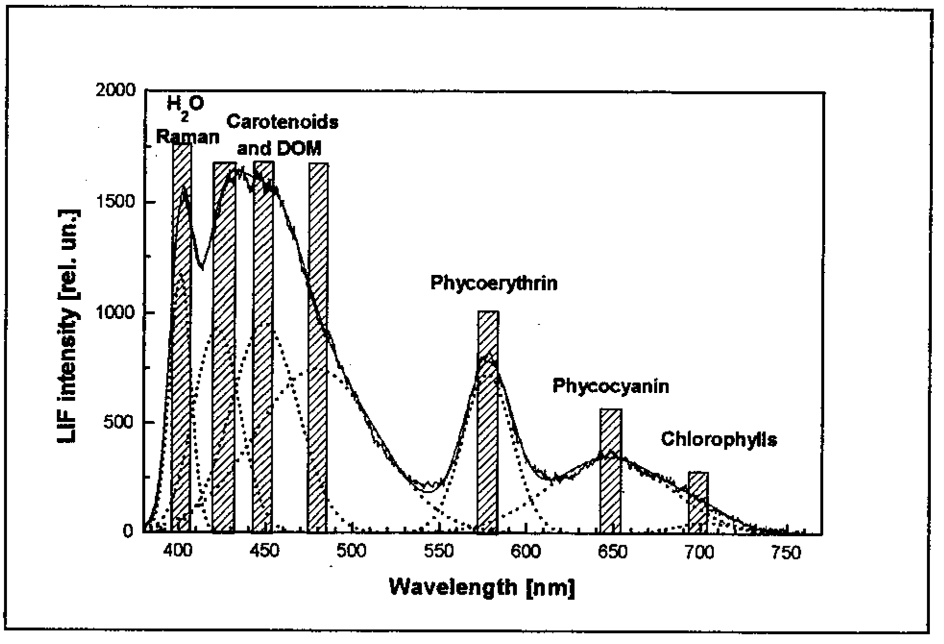

Figure 1. - *Gaussian deconvolution of a laboratory LIF spectrum obtained from a concentrated solution of Synecochoccus 625. Laser excitation at 355 nm. The assignment of the pigments is indicated and different integration bands are marked*

In linear regime it is possible to relate each remotely measured LIF signal to the cross section efficiency [21]:

$$F_{\lambda_{em}} = \frac{A(R_0)}{n^2} E_{ex} \frac{\sigma(\lambda_{em}, \lambda_{ex})}{k_T} \qquad (1)$$

where F is the time integrated signal emitted at the wavelength λ_{em}, n is the refraction index of water, $A(R_0)$ is a system depending constant at the distance R_0 from the water surface, E_{ex} is the excitation laser energy, σ is the fluorescence efficiency of the process, k_T is the total extinction coefficient given by the sum of the extinction terms at the excitation and emission wavelength $k_T = k_{ex} + k_{em}$.

In order to evaluate the concentration of different chromophores including the fluorescing phytoplankton pigments, LIF signals are calibrated through a normalization using the water Raman signal as internal standard reference and released in Raman units [1]. The ratio of the chromophore fluorescence signals on the water Raman scattering intensity can be expressed as:

$$\frac{F}{R} = \frac{\sigma_F}{\sigma_R} \frac{k_{ex} + k_R}{k_{ex} + k_F} \qquad (2)$$

where the σ and the k's refer to the respective wavelengths (*ex* to laser excitation, R to Raman scattering and F to fluorescence emission). The ratio of the extinction coefficients can be set approximately to be a constant and neglected, provided a careful choice of excitation and emission wavelengths. This normalization to the Raman signal allows for eliminate the dependence of the LIF signal intensity on the system parameters, on the laser energy and on the water optical properties. Remotely measured LIF intensities expressed in Raman units can be successively calibrated towards different pigments contents determined by conventional *in situ* methods (e.g. fluorometric, HPLC, etc,).

From the integrated LIF intensities, spectral ratio's can be also obtained for each species in order to allow for the recognition of dominant species in natural ecosystems. To this aim a large number of algae cultures are currently analyzed in laboratory by means of a laser fluorometer and a spectrofluorometer, the results are collected in a reference database which finds its natural application in remote recognition of the different algae taxa over large marine areas.

2.2 *The laser Pump-and-Probe remote technique.* - Laser pump-and-probe experimental tests carried upon living vegetation tissues with excitation at 355 nm or 532 nm already allowed to detect the effect of chemical toxicants [22], of exposure to thermal stresses [23] and to low levels of gaseous pollutants [24]. Remotely measured fluorescence yields have been compared to those obtained by a local PAM fluorometer and seen to approach the theoretical value expected for a healthy dark-adapted target, provided the proper choices of experimental parameters (pump and probe laser pulse intensities) are adopted [23].

Within the laser PP technique, chlorophyll LIF signals in the 690 nm band (labeled F_i) provide information upon the investigated vegetation photosynthetic efficiency. An isolated probe laser pulse in the dark or in the presence of background illumination triggers a fluorescence signal (F_0 or F_s) corresponding to the actual state of closure of the Reaction Centers (RC's). An intervening intense laser pulse (pump) switches all the RCs to the closed state, whose fluorescence response (F_m or F'_m) can be tested by a second probe pulse, provided the pump-to-probe delay is kept within the quenchers' lifetime, which is of the order of 100 μs. The conditions necessary to fully close the RC's by the pump pulse and to leave them unperturbed when probed have been studied in detail in [25].

The laser fluorescence quantum yield Y_{pp} can be computed as:

$$Y_{PP} = \frac{\Delta F}{F_m^{'}} = \frac{F_m^{'} - F_s}{F_m^{'}} \qquad (3a)$$

which at dark corresponds to:

$$Y_{PP} = \frac{\Delta F}{F_m} = \frac{F_m - F_0}{F_m} \qquad (3b)$$

Similar quantities, fluorescence ratios in the light ($\Delta F/F'_m$) or at dark ($\Delta F/F_m$) are currently measured by local PAM fluorometers and labeled as Φ_{p0} and F_v/F_m respectively [26].

Pulses are emitted by our laser in bursts whose time microstructure is sketched in Figure 2, where the optimum P2-to-Pump time delay has been experimentally determined to be 50 μsec [23]. Correspondingly, raw induced LIF signals F(P1) and F(P2) are collected and normalized to the relevant exciting pulse energy E_{ex} to obtain:

$$F_0, F_s = \left.\frac{F_{P1}}{E_{ex1}}\right| = \frac{A}{n^2}\frac{\sigma_{P1}}{k_T} \qquad (4a)$$

$$F_m, F'_m = \left.\frac{F_{P2}}{E_{ex2}}\right| = \frac{A}{n^2}\frac{\sigma_{P2}}{k_T} \qquad (4b)$$

where the other symbols have already been defined in eq. (1)

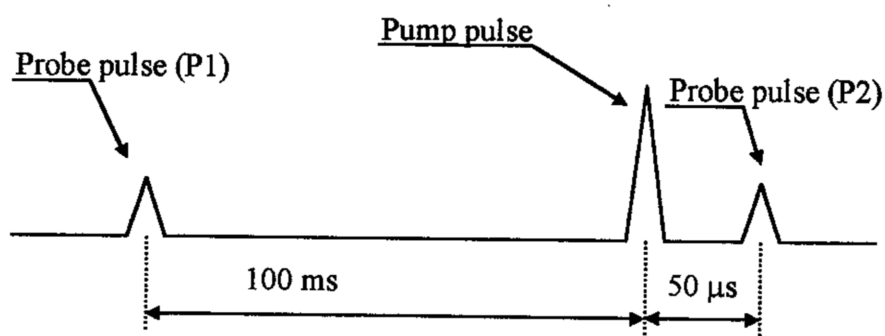

Figure 2. - *Time sequence of a laser pulses burst used in the PP technique: probe pulse P1 is used to test the actual fluorescence status; pulse P2 is sent 50μs after the pump pulse, to stimulate the maximal fluorescence emission.*

By introducing eqn.s (4) into eqn.s (3) we obtain:

$$Y_{PP} = \frac{\sigma'_m - \sigma_s}{\sigma'_m} \qquad (5a)$$

and

$$Y_{PP} = \frac{\sigma_m - \sigma_0}{\sigma_m} \qquad (5b)$$

for a vegetation target exposed to light and dark-adapted, respectively. Due to this normalization all the instrumental factors are removed, thus resulting in an absolute value of the fluorescence quantum yield.

By simultaneously measuring the PAR (Photosynthetically Active Radiation [μmoles quanta m^{-2} sec^{-1}]) and the laser fluorescence yield it is possible to calculate the ETR representing the apparent photosynthetic electron transport rate in μmoles electrons m^{-2} sec^{-1}:

$$ETR = 0.5 \cdot k \cdot Y_{PP} \cdot PAR \qquad (6)$$

where the factor 0.5 considers the absorption of two quanta for the transport of one electron. The constant k includes the collecting optical efficiency of the harvesting antennas and the water extinction coefficient at the used excitation wavelength in the case of marine measurements. For phytoplankton the collecting efficiency is generally assumed to be close to 1 and a 10% of absorption for the extinction coefficient of water at 355 nm in the first few layers, gives rise to $k = 0.9$, used in the present data analysis.

3. Experimental set-up

3.1 *The lidar system.* - The present version of the lidar apparatus [27] has been especially designed for field applications from ship-borne platforms, either installed in the mobile laboratory as single components. The easiness of use and flexibility are the main characteristics of this mobile system.

A coaxial arrangement has been adopted between transmitter and receiver, which are assembled on a rugged common chassis in order to minimize optical mismatches induced by mechanical vibrations (Figure 3). The UV laser radiation (@ 355nm) excites fluorescence directly in the sea water (Figure 4a) and the backscattered overall radiation is collected by a Cassegrain telescope and focused on a special type fiber optic bundle driving it to photomultipliers for detection (Figure 4b). by placing suitable interference filters in front of the gated detectors, different spectral channels (from 4 to 12) are selected in wavelength in order to monitor main algal fluorescence peaks with respect to the water Raman signal (peaked at 402 nm). The laser transmitted pulse energy, which is required as internal reference for normalization, is monitored by an external photodiode.

Detailed characteristics of the apparatus, which can perform remote differential measurements of chlorophyll fluorescence yield Y_{PP} according to the above described Pump-and-Probe scheme, are listed in

Table 1.

A personal computer controls the experimental settings, i.e. the laser operation mode (either single or dual pulses emitted at each trigger signal), high voltage and gating of the photomultipliers, digital conversions of signals and the storing of data. An adjustable gated HV power supply has been home-developed in order to minimize the background radiation contribution to collected data. Finally, a dedicated software performs both data acquisition and preliminary analysis in real time, allowing for continuos automatic operation during the marine campaigns. The same acquisition electronic also stores GPS georeferentiation coordinates and the overall background light intensity at the sea surface as measured by a radiometer and can collect also the oxygen electrode signals. During shipborn experiments the average speed cruise is in general maintained within 5 to 10 marine knots.

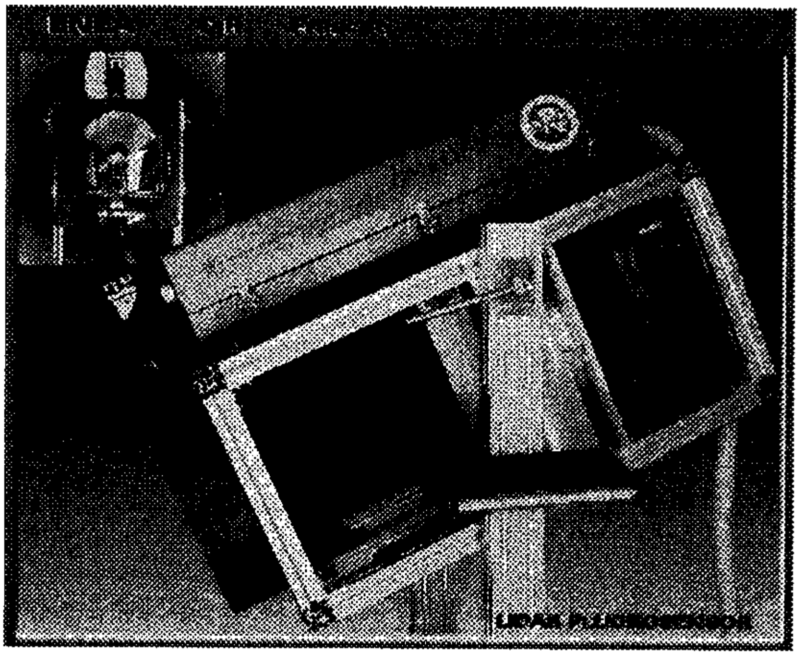

Figure 3. - *The ENEA lidar fluorosensor, transmitter receiver unit.*

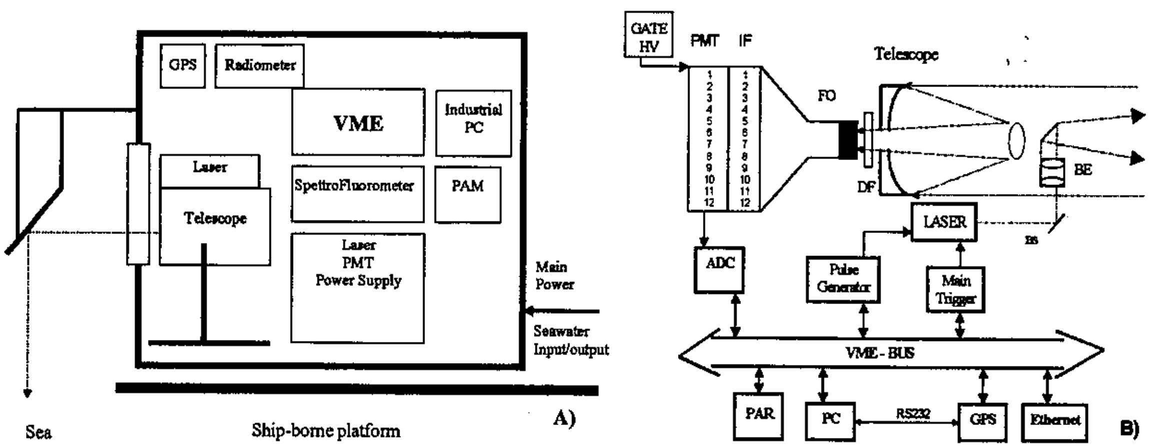

Figure 4. - *Lidar fluorosensor for marine application: a) sketch of the on board installation, b) optoelectronic schematics*

Table 1. - *Main characteristics of the lidar apparatus*

Transmitter	Nd-YAG laser	@ 355 nm
Pump	Energy	30 mJ
Probe	Energy	3 mJ
	Pulse length	10 ns
	Ppr	10 Hz
Expander	Variable	1x - 20x
Detectors	Hamamatsu PMT	R 3896 (2), R1477 (2), R928 (8)
	Gated HV	100 - 200 ns
Filers	Dichroic	T> 90% (@ 400 nm)
	Interferential FWHM 5 nm	355, 402, 435, 450, 475, 550, 580, 650, 660, 680, 730, 800 nm
Telescope	Cassegrain	40 cm dia. F# 1.5
	Focal length	165 cm
Fiber Optic	Plastic Multifiber	Twelve branches
	Diam.	input 25.4 mm, output 7 mm
	Length	1 m
Electronic	VME	ISA-VME-mixed bus
	ADC	V265 CAEN 15 Bit
Computer	VME-CPU embebbed	486-100 MHz

Spectrofluorometer. - In order to complete the sea water characterization, lidar measurements on single channels are accompanied by multispectral analysis at different UV excitation wavelengths either through a laser fluorometer [28] or through a PTI Quantamaster spectrofluorometer.

The latter instrument allows for acquisition of emission, excitation and synchronous spectra of liquid samples, whose comparison is useful in identification of different chromophores group for the recognition of natural components or pollutants and algal pigments. Fluorescence excitation/emission maps are also produced for particular samples to be analyzed. The water Raman signal, detected as well, is useful to release data in units consistent with lidar remote measurements.

Pulsed Amplitude Fluorometer. - Time resolved Chlorophyll fluorescence of various natural sea algal communities or cultures is measured also by using a Walz PAM-101 fluorometer and a PAM-103 accessory module for saturation pulse control. This instrument, by measuring the fluorescence yield, allows to investigate the photochemical and non-photochemical quenching processes at varying an external actinic light intensity. The growth rate and the relative electron transport of the investigated sample are determined, which can be useful for an estimation of the primary productivity of the sea area after taking into account the analytical properties of the phytoplankton dominant population (Chl-a content, absorption coefficient) and the natural environmental parameters (temperature, spectral radiance and intensity).

Clark-type polarographic electrode. - A Clark-type polarographic electrode can be coupled to the lidar fluorosensor data acquisition for contemporary measurements of oxygen evolution related to photosynthesis [7]. The electrode was used during the ICES/IOC marine campaign to determine the photo-synthetic performance of an enriched mesocosm (300 L polyethylene barrel), which was placed in the shade of the quay and filled with water from 3 m depth by a low volume diaphragm pump. Phosphate and ammonium were added to achieve a starting concentration of about 0.5 and 5 μM, respectively in the enriched mesocosm barrel. The reaction chamber was made up of a glass bottle 300 ml in volume screwed around a steel container which was kept in its upper position, just below the surface of the water, by a vertical steel float.

Photorespiration was monitored continuously, and automatically stored in the lidar acquisition system together with the relevant environmental parameters. Photosynthetic Active Radiation (PAR) is contemporarily measured in μmoles quanta m^{-2} sec^{-1} by using a quantometer (LI-COR 189). In order to obtain the value of the oxygen concentration the glass bottles connected to the electrodes were changed every two hours, in relation to PAR major variations. Three electrodes and the quantometer were immersed in the enriched mesocosm under control.

Results

Phytoplankton characterization. - Different algal cultures were analysed in laboratory in order to check the feasibility of their remote field recognition. Band integrated spectral contents F_λ at four different wavelengths, normalised to the total visible intensity F_{tot}, are reported in Figure 5 for the algal groups listed in Table 2. Due to the wide overlap between carotenoids and DOM emissions, the blue channel intensity F460 is seen not to provide any unambiguous discrimination among different algal classes, while the red channels (F580, F660 and F680) appear more useful for this purpose. Actually, the chlorophyll fluorescence intensity F680 turns out to be high for all the Dinophyceae species, lower but still detectable for the reported Chlorophycea and Bacillarophycea. The Cyanophycea shows also a relatively low Chl-a content, although it can unambiguously be identified by its intense phycoerythrin and phycocyanin emissions (F580 and F660, respectively), which do not occur in any of the other classes. It has to be mentioned that red chlorophyll fluorescence intensity is related to the chl-a content in each species (also reported in Table 2), but the relationship depends on the species morphology and on the accessory pigment contents, since cellular membranes and accessory pigments can be responsible both for losses of excitation energy and for re-absorption processes.

Table 2. - *List of the examined algal cultures with typical concentration*

Class	Alga	Cells conc. [cell/ml]	Chl-a [μg/l]	Chl-a/cell *10^{-6}
Dinophyceae	*Prorocentrum minimum*	3.5 10^4	173	4.9
"	*Prorocentrum micans*	1 10^4	146	14.6
"	*Alexandrium lusitanicum*	10 10^4	720	7.2
"	*Alexandrium tamarense*	5 10^4	366	7.3
Chlorophyceae	*Clamydomonas sp.*	3.5 10^4	343	9.9
Bacillarophyceae	*Phaeodactylum tricornutum*	7 10^4	692	9.9

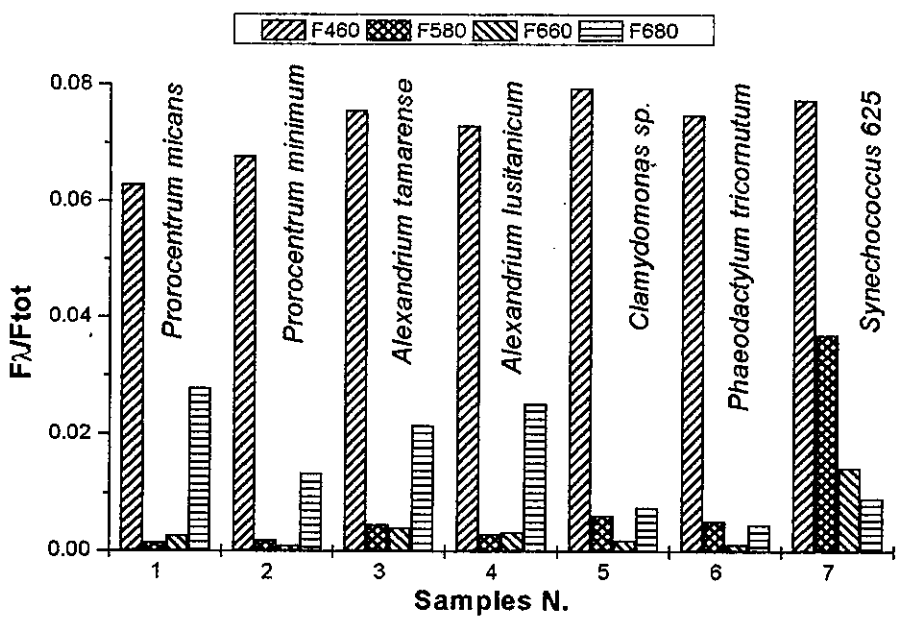

Figure 5. - *Spectral ratio's at different emission wavelengths for 7 different algae species: 1-4 Dinophyceae, 5 Chlorophycea, 6 Bacillarophycea, 7 Cyanobacteria.*

Laboratory spectrofluorometric experiments on concentrated samples allow also for identification on non fluorescent pigments directly coupled to the chlorophyll-a emitter. As an example, the excitation spectra of some of the algae, reported in Table 2 at the given concentration, are shown in Figure 6, as measured on the chl-a fluorescence channel at 680 nm. In this figure peaks relevant to chlorophyll c and b can easily be identified near 460 and 480 nm, together with the large carotenoids band (between 490 and 530 nm). In particular the chl-c appears to be the absorption pigment dominant in all dynophyceae, while chl-b is characteristic of *Clamydomonas sp.* (clorophyceae); the large contribution of carotenoids and fucoxantine [18] is evident in *Pheodactylum tricornutum* (bacillarophyceae).

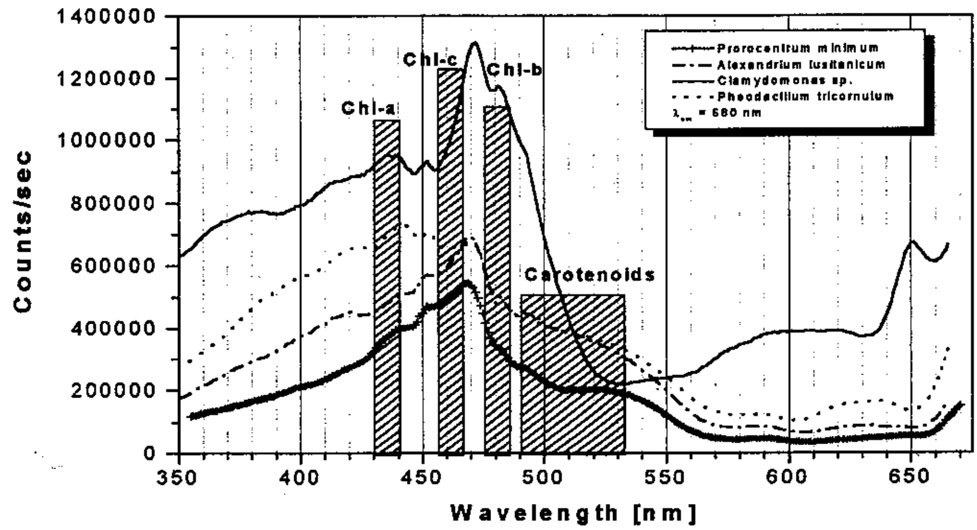

Figure 6. – *Excitation spectra of some of the algal cultures listed in Table 2 at given concentration*

As and additional example of phytoplankton spectrofluorometric characterization, the excitation spectrum of an Antarctic Chlorophycea species (found in proximity to the Italian base BTN and thus labeled as "Base") is compared in Figure 7 with the spectrum of a *Clamydomonas sp.* The identification of this Base sample as a Chlorophycea, independently performed by Prof. Andreoli's group at Padua University by using different methods [29], is remarkably well supported by the spectra in Figure 7, where a relatively higher content of chl-a in the Antarctic species also appears evident.

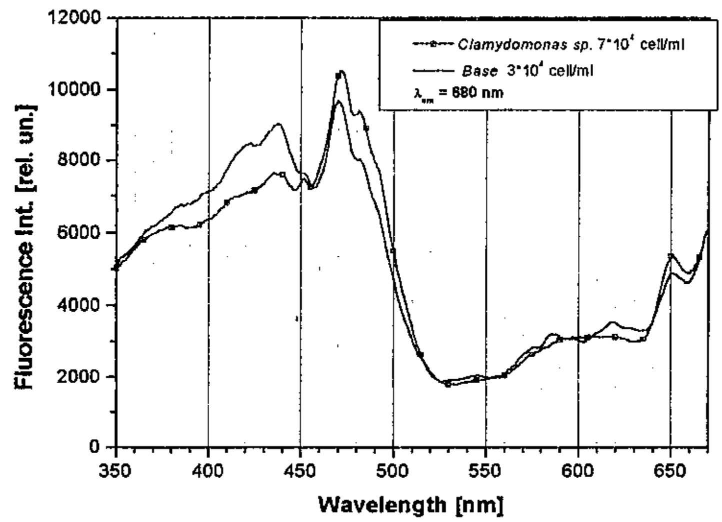

Figure 7. - *Excitation spectra of an Antarctic species labeled as Base compared with a concentrated solution of Clamydomonas sp.*

Results of laboratory phytoplankton characterization are currently used during marine campaigns: for instance the investigation on dinophyceae was introductory to the ICES/IOC marine campaign at Kristineberg where instrumental tests were performed, which included monitoring the natural community by a laser fluorometer. The collected spectra were typical of dinophyceae [28], which were actually the dominant population, although some other species were also present, as observed under the microscope by the other participant groups.

PAM and PP local fluorescence yield measurements. - In order to obtain information of photosynthesis process in dinoflagellates, laboratory experiments have been performed on these species by differential fluorescence instruments.

Some results of PAM measurements conducted on concentrated cultures of *Alexandrium tamarense* and *Prorocentrum micans* illuminated at different actinic light intensities are shown in Figure 8 and Figure 9, where ETR data elaborated according to eq. (6) are also reported. In Figure 8b and Figure 9b the I_K value and the light saturation intensity (PAR_{sat}) are graphically extrapolated from the initial slope of the ETR vs. PAR and from the final plateau, respectively. These parameters measured for different dinoflagellates are summarized in Table 3. Results obtained for *Prorocentrum* are in satisfactory agreement with a former PAM determination [30].

Table 3. - *PAM laboratory measurements*

Species	Cell conc. [cell/ml]	PAR_{sat} [μmoles quanta m^{-2} sec^{-1}]	I_K [μmoles electrons m^{-2} sec^{-1}]
Prorocentrum minimum	$3.5 \cdot 10^4$	300	194
Prorocentrum micans	$6.0 \cdot 10^4$	400	260
Alexandrium lusitanicum	$10 \cdot 10^4$	180	140
Alexandrium tamarense	$5.0 \cdot 10^4$	390	220

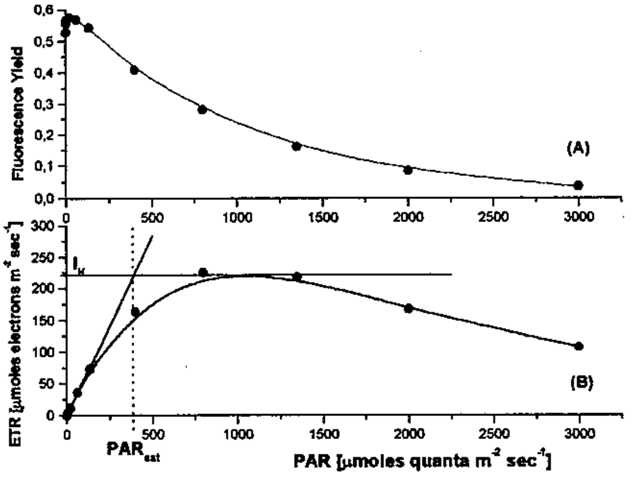

Figure 8. - *PAM measurement of light saturation effects on Alexandrium tamarense culture[$5 \cdot 10^4$ cell/ml]: A) fluorescence yield, B) relative electron transport rate; I_K=220 [μmoles electrons m^{-2} sec^{-1}]; PAR_{sat}=390 [μmoles quanta m^{-2} sec^{-1}]*

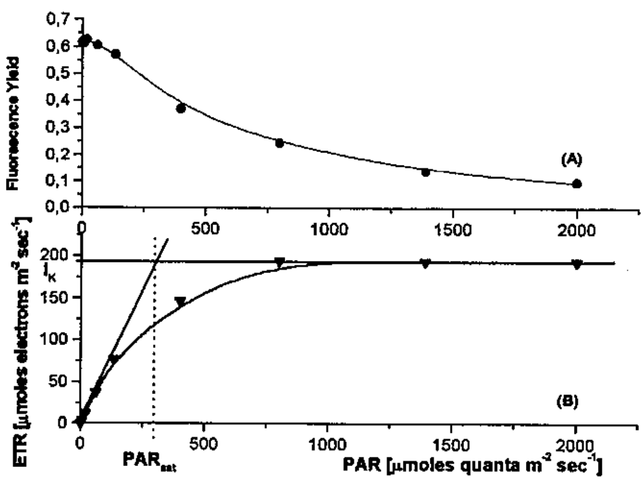

Figure 9. - *PAM determinations of light saturation effect on Prorocentrum minimum culture [45·10⁴ cell/ml]: A) fluorescence yield, B) relative electron transport rate, I_K = 194 [µmoles electrons m^{-2} sec^{-1}]; PAR_{sat} = 300 [µmoles quanta m^{-2} sec^{-1}]*

Local laser PP measurements have been performed on diluted cultures during the preparation of the ICES/IOC campaign. In this experiment, the samples were contained in a cell equipped with optical windows for laser access and signal collection, and for external illumination at right angle. The averaged actinic light reaching the sample was measured by means a radiometer at the center of the cell. A field like geometry has been adopted by looking at the cell through the complete lidar transmitter/receiver optics and placing the sample cell at about 10m from the laser.

Results are shown in Figure 10 and Figure 11 for *A. tamarense* and *P. micans*, respectively, where the average intensities and duration of the actinic light reaching the sample are also indicated. In both cases, a high Ypp value is observed in the dark (Y_{PP} = 0.73 for *A. tamarense* and Y_{PP} = 0.55 for *P. micans*, respectively) indicating the cultures examined to be in their optimal condition for photosynthesis; the values at dark approaching 0.6 are in good agreement with PAM measurements performed on all our dinoflagellate cultures. As the light increases, Ypp decreases due to the onset of the photosynthesis accompanied by the photo-adaptation. Due to the short time intervals at different exposure intensities (about 100 s), the minimum value reached at saturation is close to 0.2 and does not reach zero. When the light is switched off, a partial recovery starts for both the samples darkened again.

As in the case of PAM data, PP fluorescence yield have been used to obtain averaged ETR values, shown in Figure 10b and Figure 11b. In this case I_K cannot be determined with accuracy since the few points measured do not allow to establish whether saturation has been reached. However, assuming saturation above 1500 μmoles quanta m^{-2} sec^{-1}, the I_K values for *A. Tamarense* and for *P. micans* are found in overall agreement with PAM determination (Table 3). Discrepancies in PAR_{sat} values between PAM and PP results must be ascribed to the uncertainty in PAR measurements relevant to the latter experiment. In fact, the field-like arrangement used in the present PP case made difficult both to homogeneously illuminate the whole cell and to carefully determine the average actinic light intensity.

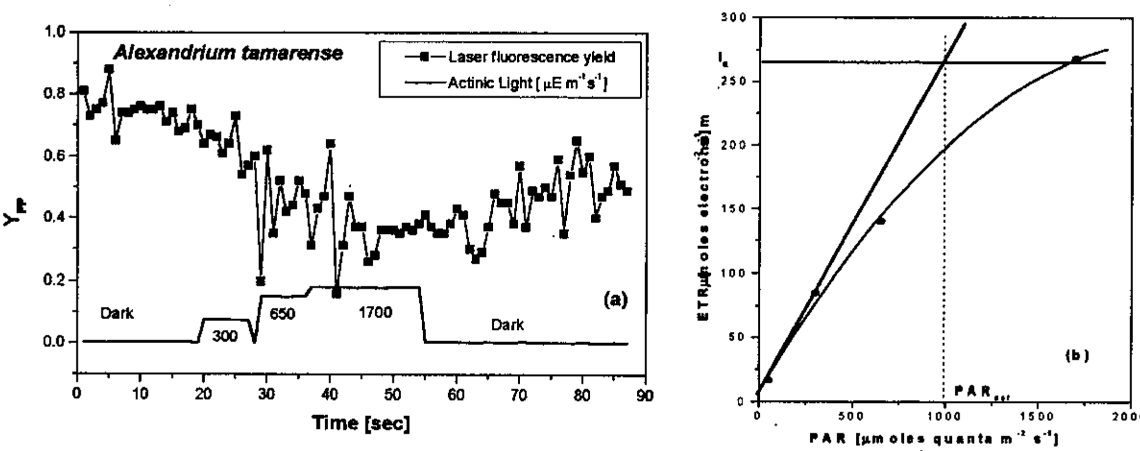

Figure 10. - *Laser fluorescence ratios measured on Alexandrium tamarense culture: a) Y_{pp} ; b) ETR at different light intensity exposure $I_K = 265$ [μmoles electrons m^{-2} sec^{-1}];*
$PAR_{sat} = 990$ [μmoles quanta m^{-2} sec^{-1}]. Chl-a = 5.8 μg/l. Date 10 Sep. 96.

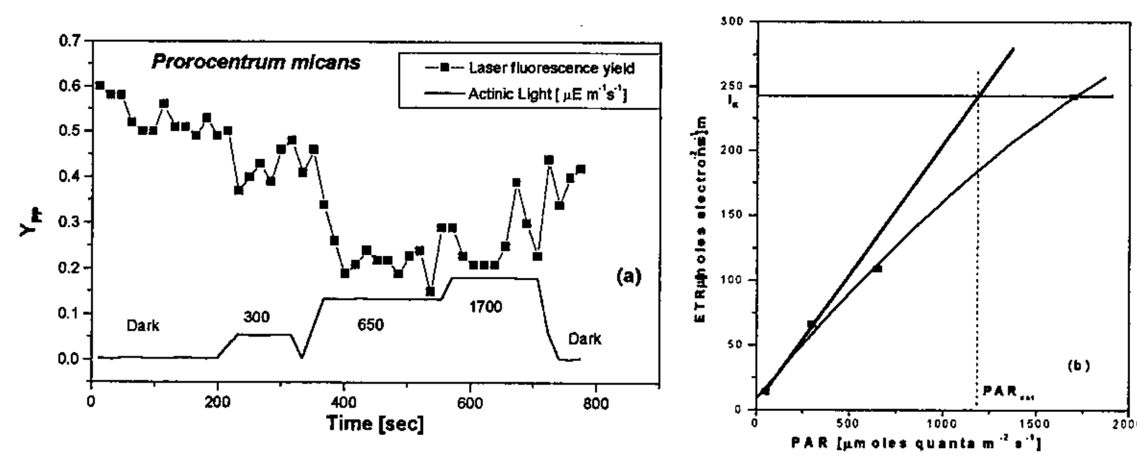

Figure 11. - *Laser fluorescence ratios measured on Prorocentrum micans culture: a) Y_{pp} ; b) ETR at different light intensity exposure $I_K = 244$ [μmoles electrons m^{-2} sec^{-1}];*
$PAR_{sat} = 1180$ [μmoles quanta m^{-2} sec^{-1}]. Chl-a = 4.2 μg/l. Date 10 Sep. 96.

Lidar PP measurements. - Laser Pump-and-Probe yields from phytoplankton have been measured on field by the lidar fluorosensor during the ICES/IOC workshop held at the Kristineberg Marine Station (Sweden) in 1996 both from the quay and from a ship along a sea transect.

Lidar data relevant to the joint monitoring programme of an enriched mesocosm are shown in Figure 12, as measured from the quay in combination with the contemporary oxygen evolution measured by the polarographyc electrode. As expected, a general anticorrelation has been found between PAR and Y_{PP} values, which indicates an efficient photoadaptation of the algae present (mostly dinoflagellates). A similar trend is observed in comparing PAR with O_2 evolution data.

The photoadaptation gives rise to a large increase of the Y_{PP} fluorescence yield at dark. Note that this effect might lead to a wrong determination of chlorophyll concentration during day-night transition, if the overall photochemical efficiency is not taken into account by differential measurements coupled to PAR determination.

A relative decrease at dark of the O_2 evolution has also been detected, contemporary to the ETR zero values reached after 19:00.

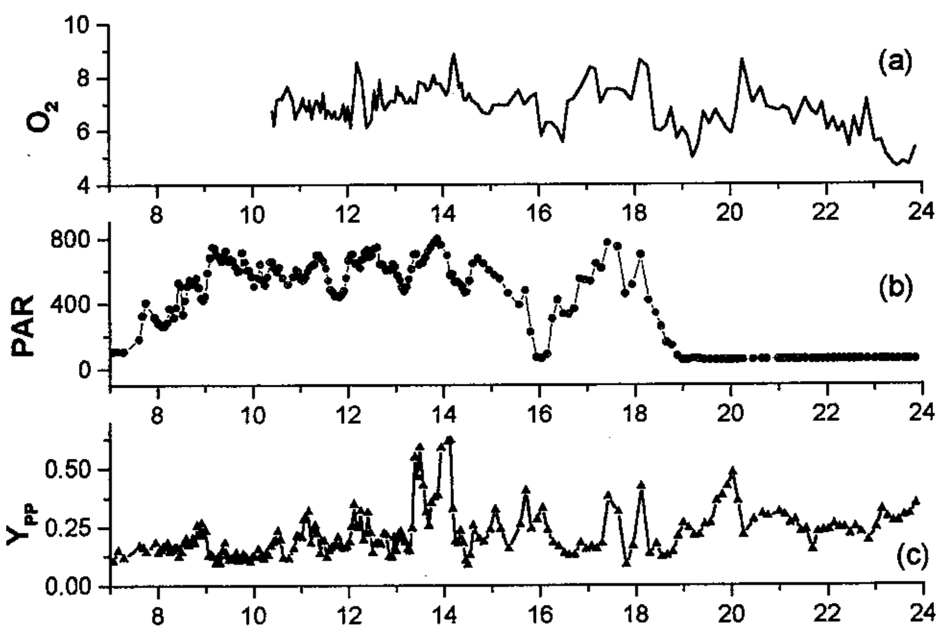

Figure 12. - *Mesocosm photosynthetic activity monitored from the quay: a) O_2 evolution [mg/l]; b) PAR [μmoles quanta m^{-2} sec^{-1}]; c) Differential chlorophyll fluorescence yield Y_{PP}. Date 12 Sep. 96.*

The cruise on board of the R/V Arne Tiselius, of September 12th afternoon, started at the Kristineberg Marine Research Station (KMF), crossed the Gullmar fjord and reached the Ellöse fjord after passing near the open sea at an average speed of 5 knots.

During the navigation, the lidar fluorosensor was continuously collecting data on water turbidity (at 402 nm), DOM (at 450 nm), Chl-a concentrations (at 690 nm) and Chl-a fluorescence yield Y_{PP}, the latter by taking advantage of the lidar pump-and-probe operation mode.

Chl-a concentration and remotely sensed ETR are reported in Figure 13a and Figureb, respectively, vs the cruise running time. We may notice changes in Chl-a concentration from the different fjords to the open sea. The evening increase on chlorophyll channel, recorded on the way back, has been preferable ascribed to phenomena of nocturnal bioluminescence caused by *Ceratium* (dinoflagellates) whose presence was detected independently. However it could be also partially due to relaxation at dark of quenched fluorescence.

Both because of afternoon irradiation conditions and a cloudy weather, rather small radiance changes were detected at the sea surface level, ranging from 500 to 50μmol m^{-2} sec^{-1}, which originating the rather low ETR value measured during the investigation time interval (ETR_{max} = 120 μmoles electrons m^{-2} sec^{-1}, $ETR_{average}$= 25 μmoles electrons m^{-2} sec^{-1}).

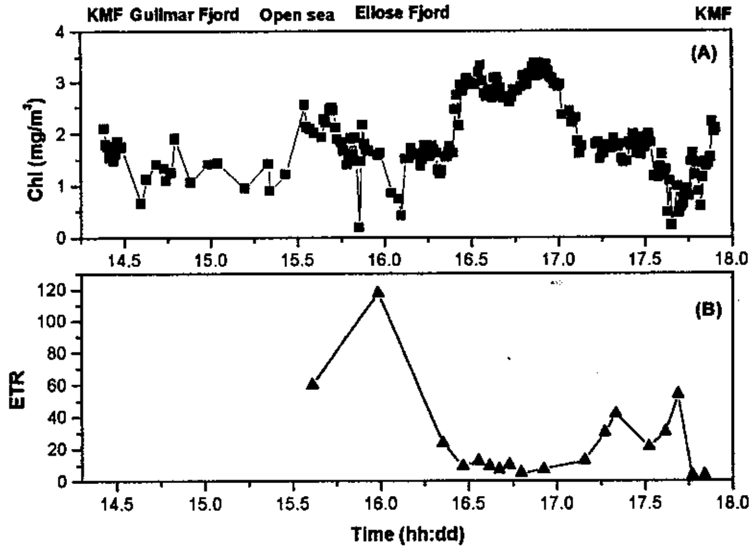

Figure 13. - *Lidar data measured during the cruise: (a) Chl-a [mg/m³], (b) ETR [μmoles electrons m⁻² sec⁻¹]. Kristineberg (S) 12 Sep. 96*

Conclusions

Results reported in the present work show that the local and remote methods based on red chlorophyll fluorescence detection are suitable to investigate in detail marine phytoplankton, both in laboratory controlled conditions and during field experiments. Intercalibration of data with results of various point determinations, based on either fluorescence and biochemical methods, was performed thus allowing to release absolute values for the measured parameters (chlorophyll concentration, photosynthesis yield).

Differential laser yield measurements have been performed on phytoplankton cultures in order to test its sensitivity to the occurring of photo-inhibition induced by proper light sources. These measurements, performed in combination with PAR determinations, have allowed us to obtain the ETR during laboratory and field experiments.

On the occasion of Kristineberg Workshop the complete LIF system, including its ancillary sensors (GPS, radiometer, O_2 electrodes), designed for phytoplankton monitoring in the Ross Sea during the XIII Italian Antarctica mission, was successfully tested, running in automatic operation from the quay and from the boat in spite of adverse meteorological conditions (strong wind and rain).

Acknowledgments

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Thank are due to Prof. Andreoli for supplying some Antarctica species collected in a former campaign.

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BLOOMS OF TOXIC ALGAE IN THE MEDITERRANEAN SEA

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Introduction

Until 1970 toxic phytoplankton blooms were known only in temperate waters of North America, Europe and Japan (Hallegraeff, 1995). During the following two decades a global increase of intensity and frequency of these phenomena, spreading to new areas of both hemispheres, has been observed (Anderson, 1989; Smayda, 1990).
The first toxic blooms with shellfish contamination in the Mediterranean Sea date back to 1987, although the presence of potentially toxic species had been previously recorded (Honsell et al., 1995). Since that date toxic algal blooms have become a common event in the Mediterranean Sea, being frequently recorded from various parts of the basin.
The toxic algae responsible of blooms in the Mediterranean Sea are dinoflagellates producing two different types of toxins: diarrhogenic DSP (Diarrhetic Shellfish Poisoning) toxins, causing symptoms as nausea, vomiting and diarrhoea, and PSP (Paralytic Shellfish Poisoning) neurotoxins causing neurological symptoms, and, in extreme cases, muscular paralysis and death through respiratory paralysis. The toxins are concentrated by filter-feeding bivalve molluscs, and, by these vectors, they can produce human intoxication.

Until now toxic blooms have occurred only in some Mediterranean coastal areas: the Alboran Sea (PSP), the Catalan coast and Balearic Islands (PSP), the Gulf of Lions (DSP and PSP) and the Northern and Middle Adriatic Sea (DSP and PSP).

DSP

DSP outbreaks are known only in two coastal zones of the northern part of the basin: the Languedoc-Roussilon coast of France and the northern and middle Adriatic Sea. Both areas are characterised by shallow waters and great river outflows.

The first DSP events occurred on the French coast in 1987 (Belin, 1993) and in Italy (Emilia-Romagna coast of the northern Adriatic Sea) two years later in 1989 (Boni et al., 1993). In both cases, DSP contamination revealed to be recurring events repeating more or less regularly every year . In the Adriatic Sea DSP presents a very wide extension along the western Italian coastline (from Friuli-Venezia Giulia to Marche) and along the eastern coastline of Slovenia and Croatia. The organisms responsible of DSP outbreaks are dinoflagellates belonging to the genus *Dinophysis*: *D. fortii* in the Adriatic Sea (Della Loggia et al., 1993), with a possible minor role of other species (*D. caudata, D. rotundata, D.* cf. *sacculus*), and the *Dinophysis acuminata.* complex on the French coast (Belin, 1993)

Dinophysis species are generally found in seawater at relatively low concentrations, ranging from 10 to 10^3 cells/L: higher values are only rarely encountered (Maestrini, 1998). The highest value of *Dinophysis* detected along the NW coast of the Adriatic Sea from 1989 to 1994 is 36240 cell/L (Poletti et al.,1998). However, also very low numbers of cells of *Dinophysis* cells in seawater (50-200 cells/L) can determine DSP contamination of shellfish (ICES, 1992).

Potential DSP toxins producers, as *Dinophysis* species, are widely distributed in the entire Mediterranean Sea: however, it is not clear if the lack of DSP contamination in the other parts the basin is a real fact or it is only due to the lack of monitoring and to the misinterpretation of DSP intoxications, as gastro-intestinal disorders due to microbial causes.

PSP

Blooms of PSP dinoflagellates have been reported in the western Mediterranean and in the Adriatic Sea since 1989. The species responsible are *Alexandrium minutum* and *Gymnodinium catenatum*. Also other *Alexandrium* species, as *Alexandrium catenella, Alexandrium lusitanicum* and *Alexandrium tamarense* have been found in the Mediterranean. Although they are known to present toxic strains, and sometimes caused blooms, no toxicity, due to these species, was detected until now. A list of PSP dinoflagellate blooms and cases of shellfish contamination in the Mediterranean Sea is reported in Table 1.

Alexandrium minutum is a small dinoflagellate widely distributed in the Mediterranean basin. It was described as a new species by Halim (1960) when it formed a red tide in the harbour of Alexandria (Egypt). Its presence was then reported in other parts of the Mediterranean, along the Atlantic coast of Spain and Portugal and in temperate waters of North America, Australia, New Zealand and Japan (Honsell et al.,1996). Its ability to produce PSP toxins was not known until 1989 when it caused shellfish contamination in Australia (Oshima et al., 1989) and then, few years later, in Spain (Delgado et al., 1990), France (Belin, 1993), New Zealand (Chang et al., 1995) and Italy (Honsell et al., 1996). *Alexandrium minutum* blooms and toxicity episodes in the Mediterranean Sea are reported in Table 1.

After the bloom recorded by Halim (1960), the presence of this species has been only sporadically detected in phytoplankton until the end of Eighties (Montresor et al., 1990). The first *Alexandrium minutum* toxic bloom in the Mediterranean Sea dates back to May 1989 in the harbour of Sant Carles de la Ràpita at the Ebro delta (Spain) (Delgado et al., 1990). In 1990 an *Alexandrium* cf. *minutum* bloom (180×10^6 cells/L) occurred in the Toulon harbour (France) with PSP mussels contamination (Belin, 1993).

Table 1. - *PSP dinoflagellates in the Mediterranean Sea*

SPECIES	REPORTS	CELLS / L	TOXICITY
***Alexandrium catenella* (Whedon et Kofoid) Balech**	Catalan coast and Balearic Islands (Spain) 1983 (Margalef and Estrada, 1987)	few	-
***Alexandrium* sp.**	Emilia Romagna coast, Adriatic Sea (Italy) 1985 (Boni et al., 1986)	bloom	no toxicity
***Alexandrium lusitanicum* Balech**	Gulf of Trieste, Adriatic Sea (Italy) 1987 (Honsell et al., 1992)	-	toxic cultures
***Alexandrium minutum* Halim**	Alexandria harbour (Egypt) 1958, 1994 (Halim, 1960; Halim and Labib)	26×10^6 24×10^6	- fish kills (1994)
	Gulf of Naples (Italy) 1985-86 (Montresor et al., 1990)	-	-
	Ebro delta (Spain) 1989 (Delgado et al., 1990)	28×10^6	110 µg STX eq/100g tissue
	Toulon Bay (France) 1990 (Belin, 1993)	180×10^6	80 µg STX eq/100g tissue
	Ganzirri lagoon, Sicily (Italy) 1990-92 (Giacobbe and Maimone, 1994)	9×10^4	-
	Izmir Bay (Turkey) 1990-91 (Koray et al., 1992)	bloom	-
	Emilia Romagna coast, Adriatic Sea (Italy) 1994 (Honsell et al., 1996)	7.3×10^4	192 µg STX eq/100g tissue
	Kastela Bay, Adriatic Sea (Croatia) 1992, 1995 (Marasovic et al., 1995; 1997)	7.5×10^4	traces (HPLC)
	Balearic Islands (Spain) 1995 (Forteza et al., 1997)	45×10^6	1170 µg STX eq/100g tissue
***Alexandrium tamarense* (Lebour) Balech**	Catalan coast (Spain) (Margalef, 1969)	few	-
	Emilia Romagna coast (Adriatic Sea) 1982 (Boni et al., 1986)	11×10^6	no toxicity
***Gymnodinium catenatum* Graham**	coast of Malaga, Alboran Sea (Spain) 1989 (Bravo et al., 1990)	3×10^3	200 µg STX eq/100g tissue
	Tetouan, Alboran Sea (Morocco) 1994 (Tagmouti-Thala et al., 1996)	bloom	30,000-40,000 M.U./100g meat

In May 1994 a PSP bloom of *Alexandrium minutum*, reaching concentrations of 73000 cells/L was reported along the coast of Emilia Romagna near the Po river delta (NW Adriatic Sea) (Honsell et al., 1996). During April and May of 1995 a proliferation of *Alexandrium minutum* in the Port of Palma de Mallorca (Balearic islands, Spain) reached cell concentrations of up to 45 x 10^6 cells/L, causing PSP contamination of shellfish (Forteza et al, 1997). Blooms of this species, without toxicity, have occurred also in other Mediterranean areas (see Table 1).

The *Alexandrium minutum* blooms, so far reported in the Mediterranean Sea, generally occur in spring (April-May) in estuarine coastal areas or in restricted embayments such as harbours, enclosures or lagoons. Data on their dynamics are few: a study carried out in a Thyrrenian coastal lagoon indicates that the appearance of this species in spring coincided with increased rainfall and freshwater runoff and stabilisation of the water column (Giacobbe et al., 1996); the meteorological and hydrographycal conditions of the bloom in the harbour of Sant Carles de la Ràpita were characterised by mixing caused by strong winds followed by warm weather and stratification of the water column (Delgado et al., 1990). In general terms, the occurrence of blooms in estuarine areas or restricted embayments in spring or summer suggests that river runoff and water column stabilisation play an important role for the development of blooms. However, the understanding of bloom dynamics in each specific area needs more research, considering not only physical forcings but also their coupling with the biological behaviour of *Alexandrium*, such as swimming, vertical migration, or physiological adaptations (Anderson, 1998). In addition the role of cyst "seedbeds" in bottom sediments, as bloom inoculum, should be considered.

Gymnodinium catenatum is a chain forming gymnodinioid dinoflagellate producing PSP toxins. In the SW of Europe it has a reported distribution from Alboran Sea to Cape Finisterre (Fraga, 1996; Hallegraeff and Fraga, 1998). In the Mediterranean Sea it seems to be present only in the most western part of the basin (Alboran Sea): previous reports of this species in other parts of the Mediterranean are more likely to be related to the morphologically similar non toxic species *Gyrodinium impudicum* (Fraga et al., 1995). *Gymnodinium catenatum* was first recorded in the Mediterranean on the coast of Malaga (Spain) in 1989, after the detection of PSP toxins in shellfish (Bravo et al.,1990). In November 1994 a bloom of this species along the Atlantic and Mediterranean coast of Morocco caused a dramatic PSP seafood contamination with 64 victims (23 hospitalised and 4 dead) (Tagmouti et al.,1996). Blooms of *Gymnodinium catenatum* in areas of the west and south coasts of the Iberian peninsula, where they cause serious damages to aquaculture, seem to be local expressions of a wider phenomenon related with the general oceanic circulation (Fraga, 1996)

Conclusions

Although toxic phytoplankton blooms in the Mediterranean Sea represent a recent phenomenon , that started only twelve years ago, they became recurring events in certain areas with relevant economic damages to aquaculture, as DSP outbreaks in the Adriatic Sea, and danger for human health, as *Gymnodinium catenatum* blooms along the coasts of Morocco. Until now toxic blooms have been detected only in the western part of the basin and in the Adriatic Sea; however, the global increase and spreading of these phenomena, observed during the last years, make necessary a control of phytoplankton blooms in all coastal areas of the Mediterranean Sea. A special effort should be given to the understanding of the dynamics of toxic phytoplankton blooms by biological and hydrographic data collected by field programs and satellite images to get a synoptic view of larger scale processes.

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MONITORING ACTIVITIES ON MARINE TOXIC ALGAE IN THE ADRIATIC SEA

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Introduction

A serious episode of benthic fish kill happened in the Adriatic Sea along the Emilia Romagna coast in the area between Porto Garibaldi and Cesenatico in the summer of 1975. Dozens of tons of fish suddenly beached themselves, also causing troubles to tourists because of the unpleasant smell that this putrefying organic substance lying on the shoreline emitted, even though it had immediately been removed.

On the basis of the tests analyses conducted at the Centro Ricerche Marine di Cesenatico, it was possible to identify the lack of dissolved oxygen (anoxia) due to the presence of microalgal bloom of the *Ceratium* genus.

The episode hit nationally the headlines, conjuring up future danger to the Emilia-Romagna coast, Europe's biggest bathing complex, to the biological balance and consequently to fishing.

After this first serious eutrophication episode, the Region of Emilia-Romagna carried out research to spot the features of the phenomenon, its causes, and its health implications.

In the last 20 years, research carried out at the Centro Ricerche Marine di Cesenatico along Emilia-Romagna coast on qualitative and quantitative phytoplankton distribution, on the concentration of phytopigments in sea water and possible toxic effects of phytoplankton on marine fauna and man has seemed to spot two separate periods characterised by a different qualitative and quantitative evolution of microalgal blooms.

Monitoring activities from 1976 to 1985

The first years of research were used to develop a monitoring system particularly aimed at the problem of microalgal blooms.

Figure 1 (shows the sampling stations of the monitoring. Such monitoring activity is carried out by the oceanographic vessel Daphne, which is part of ARPA (Agenzia Regionale Prevenzione Ambiente - regional agency for environmental protection).

The monitoring, aimed at identifying the features of coastal waters within 20000m, is made through a network of 32 coastal stations between Bagni di Volano and Cattolica.

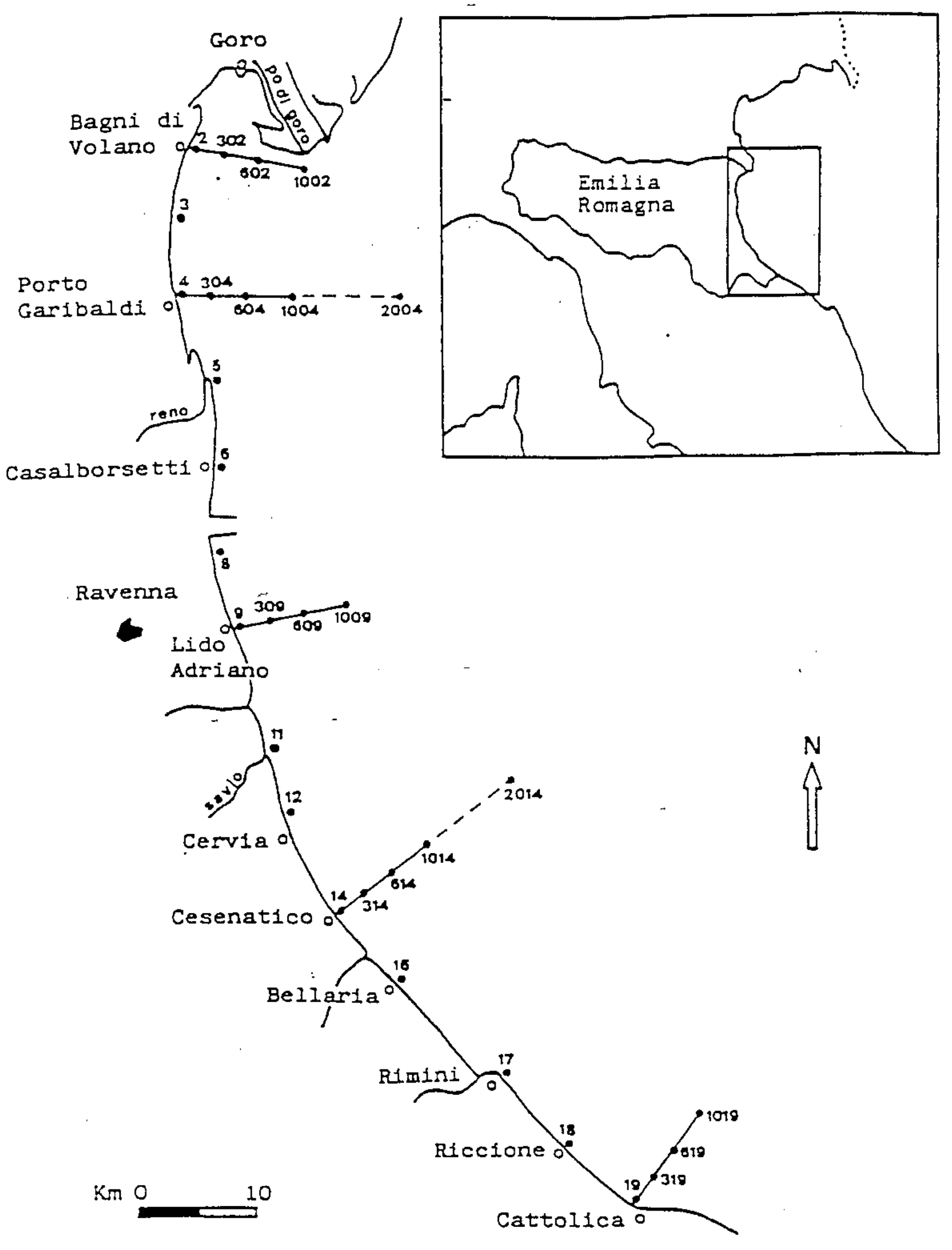


Figura 1. - *Programma di monitoraggio. Distribuzione delle stazioni di prelievo e di misura*

The stations located at 500 m (2, 3, 4, 5, 6, 8, 9, 11, 12, 14, 16, 17, 18, 19) and at 3000 m (302, 304, 309, 314, 319) from the coast are weekly monitored, while those along lines 4, 9, 14, 19, which go as far as 20000m from the coast, are monitored every two weeks.

Sea water is tested in situ with a probe which spots some biological , physical and chemical parameters (temperature, salinity, pH, dissolved oxygen and chlorophyll *a*) in the water column.

A sample of this water is taken to be tested ashore for nutrients (phosphate, nitrogenous, silicate) and phytoplankton quality and quantity.

In short, research conducted at sea through monitoring activities pointed out as follows:

1. chemical, physical and biological parameters show a great coastal variability;
2. phosphorus is considered as a limiting factor even if nitrogen has an important role along the coast in summertime;
3. diatoms outnumber dinoflagellates which mainly develop along the coast in the warmest months;
4. microalgal blooming is linked to nutrients and particularly intense blooming generally happens after a prolonged period in which the sea is calm;
5. the River Po affects the whole area in terms of nutrients; other nutrients from smaller rivers appear in the summertime and sometimes have an important role in coastal blooming.

All data pointed out that the monitored area can be divided into 3 sub areas (Figure 2):

1. sub area A between stations 2 (Bagni di Volano) and 6 (Casalborsetti) is mainly affected by the River Po; this area has a high productivity and level of chlorophyll "a";
2. sub area B between stations 6 (Marina di Ravenna) and 12 (Milano Marittima) in which production is affected by the Po but even by smaller rivers ;
3. sub area C between stations 14 (Cesenatico) and 19 (Cattolica) in which microalgal production is affected by nutrients coming from urban areas (water-treatment plants) and smaller watercourses.

Through the years monitoring activities also pointed out the seasonal repetitive emergence of blooms of microalgal species (Table 1) such as:

1. diatom *Skeletonema costatum* appears in the whole area in late winter, it makes surface and deep waters brown. More than 200 million algal cells per litre and chlorophyll higher than 100 µg /l are present;

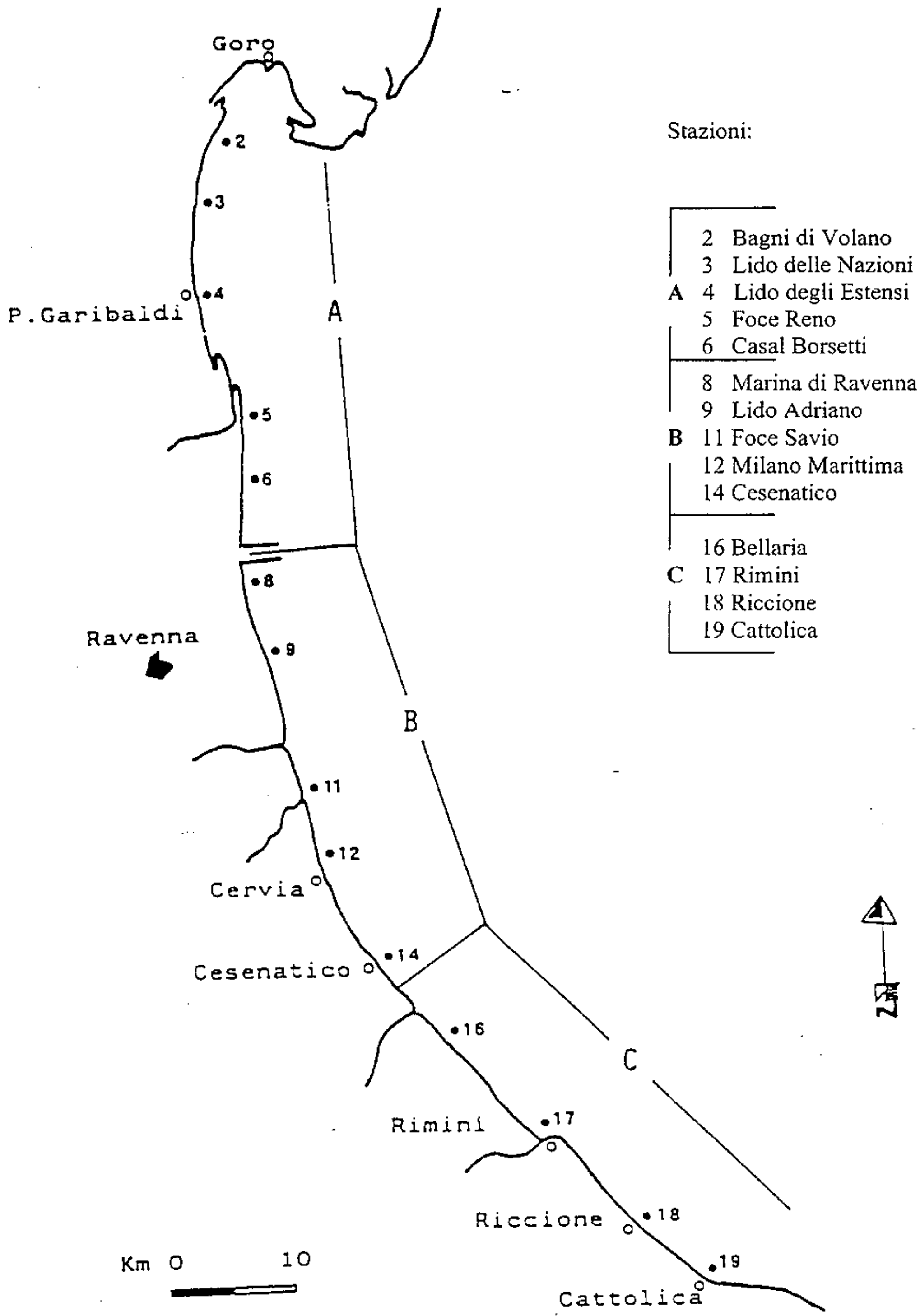


Figura 2. - *Suddivisione in sub-aree della fascia costiera monitorata*

2. *Noctiluca miliaris* appears in springtime in waters even not close to the shore, forming red-orange, big-sized patches. More than 9,000.000 cells/ l and bioluminescence are present;
3. dinoflagellates *Diplopsalis lenticula (Glenodinium lenticula)* and *Prorocentrum micans* appear in springtime. *Diplopsalis lenticula* forms pink strips parallel to the coast, it has more than 30 million algal cells per litre and chlorophyll "A" higher than 20µg/l , whereas *Prorocentrum micans* has levels ranging from 10 to 20 million and gives a red colouring;
4. dinoflagellate *Gonyaulax polyedra* which appears in summertime (July, August) forms patches and then spreads on wide coastal bathing areas within 1000m from the coast. The blooms are red and can exceed 15 million cells / l and 50 µg/l of chlorophyll "a";
5. dinoflagellate *Gymnodinium sp.* appears in late summer through November. This dinoflagellate makes bathing coastal water green, the bloom is so intense that forms a green sludge (called pea soup by the press) with an unpleasant smell which can be smelt from very far and can cause airway irritation.

In their late life cycles, the blooms of *Gonyaulax polyedra* and *Gymnodinium sp.* produce wide anoxic areas in the sea causing benthic fish and bivalve species kills.

After the emergence of these blooms, mostly those of the dinoflagellates of the *Gonyaulax* and *Gymnodinium* genera with millions of cells per litre, one of the aspects that we've been worried by since 1975 hasn't been the health aspect linked to bathing, since tests always showed that water was fit for swimming, but the possibility that microalgae produced toxins. In fact many species, mostly dinoflagellates can synthesise toxins, which if accumulated in bivalves can seriously harm man, and ichthyotoxins which can kill marine fauna.

In order to complete the regional programme and control the effects of microalgal blooms on health, the Department of Health entrusted the Centro Ricerche Marine di Cesenatico with the task of checking the presence of water soluble Paralytic Shellfish Poisoning (PSP) and liposoluble Neurotoxic Shellfish Poisoning (NSP) biotoxins in bivalves fished in the Emilia-Romagna coast.

Research carried out by Centro Ricerche Marine di Cesenatico into the causes of fish kill has always excluded the presence of ichthyotoxin-producing microalgae (Viviani et al., 1985). At the same time, the presence of water soluble and liposoluble biotoxins has been excluded thanks to tests on bivalve and microalgal species themselves.

Our worries were unfortunately confirmed when in the autumn of 1976 dozens of people had to receive medical treatment because of serious neurological disorders due to eating mussels fished in the Spanish Atlantic Coast.

The Centro Ricerche Marine di Cesenatico identified in saxitoxin and some derivative (Viviani et all., 1977) water soluble toxins responsible for this episode, these toxins known as PSP produce neurological disorders. A few milligrams of these compounds which accumulate in big quantities in the edible part of bivalves that filtered phytoplanktonic cells of toxic dinoflagellates, can cause people's death through respiratory paralysis.

Monitoring activities from 1986 to 1997

In the second half of the 80s monitoring on phytoplankton quantity and quality have shown a gradual drop in algal blooms together with a sensible decrease in the number of cells and development of other species that were poorly present in previous years.

Table 2 shows the phytoplanktonic species that have seasonally discoloured water in this period (1986-1997):

1. *Skeletonema costatum* already described;
2. diatoms *Thalassiosira* sp. and *Chaetoceros* spp. appear in the same period as *Skeletonema costatum,* making the water dark yellow and having millions cells per litre;
3. dinoflagellate *Prorocentrum minimum* appears only along the coast in springtime and has some million cells per litre and give the waters a red colouring;
4. dinoflagellates *Scrippsiella trochoidea, Gonyaulaux fragilis* and *Gonyaulax polyedra* appear along wide bathing coastal areas in the summer and make the waters red.

These changes together with a substantial improvement of water quality, even as for transparency, are probably due to a decrease in nutrients which come from the sea; this is due to a series of national and regional laws on the treatment of raw waters coming from public and private businesses as well as to the law regulating the quantity of phosphorus to be used in detergents.

The emergence of mucilage and biotoxin-producing algae are the two important events which happened in the Adriatic Sea in this period.

Mucilage

Mucilages, which are a whitish jelly-like mass, first appeared in the Northern Adriatic Sea in 1988. The phenomenon appeared again, lasted and spread more and reached its maximum extension in late June 1989. Mucilages were reported along the coast up to 20 to 30 km from it. Mucilages tend to stay under the surface at the night and early morning but then surface again in the warmest hours.

The problem becomes more serious when these jelly-like masses get close to the coast, penetrate into harbours and coves causing problems to bathing.

Table 1. - *Most frequent algal blooms along the Emilia-Romagna coast from 1976-1985*

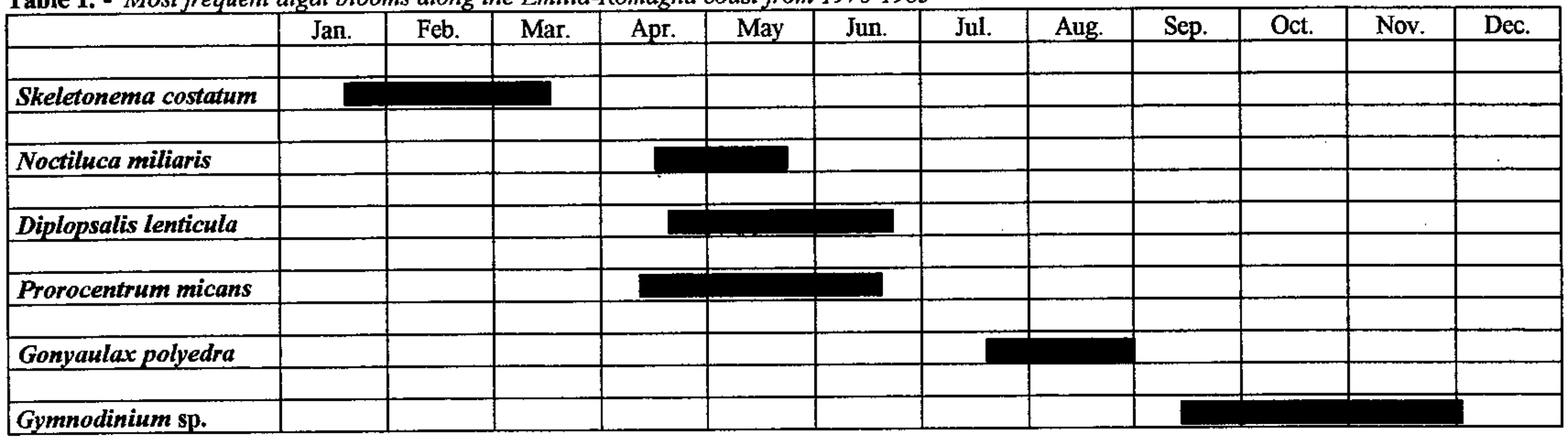
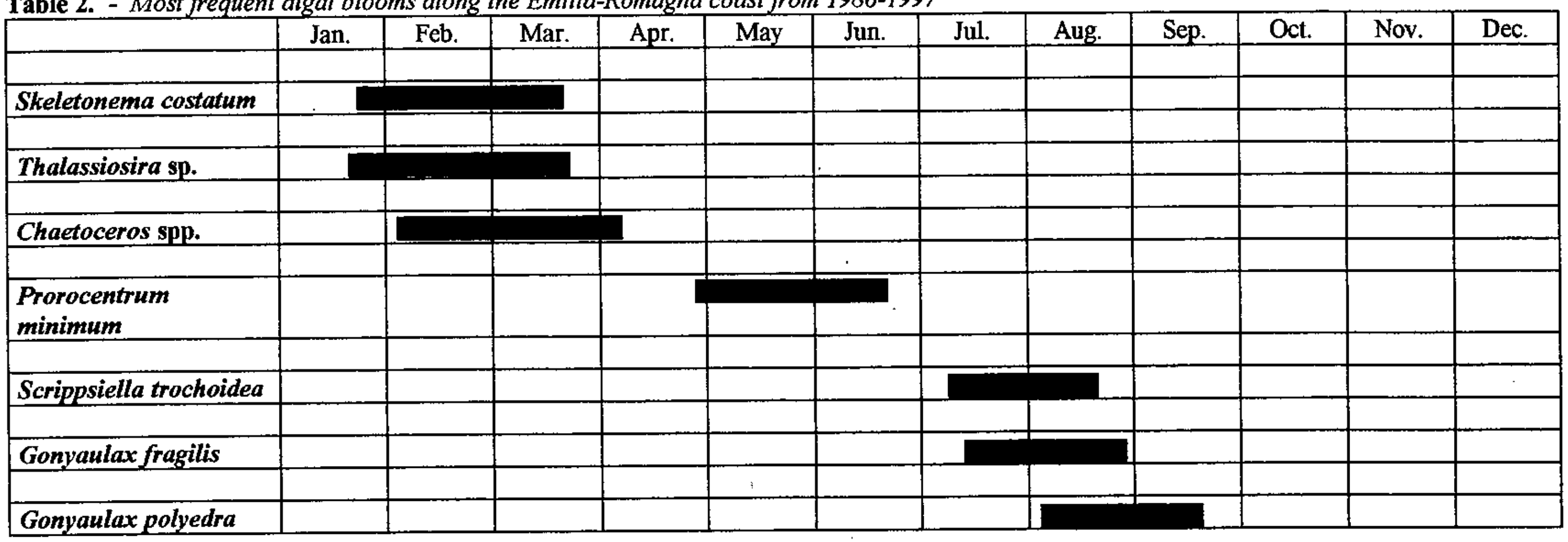


Table 2. - *Most frequent algal blooms along the Emilia-Romagna coast from 1986-1997*



Even fishing is seriously damaged, fish escapes and nets get heavy as they drag these jelly-like masses, as well as bivalves, both farmed and fished in natural banks, wrapped in mucilages, suffer and even die.

The jelly-like material that accumulates in sediments, triggers a decomposition process and can produce anoxic areas. Toxicology tests made by Centro Ricerche Marine on jelly-like masses excluded the presence of marine, water soluble (ADP and PSP) and liposoluble (NSP and DSP) biotoxins (Viviani et all., 1995).

The Centro Ricerche Marine di Cesenatico is conducting a weekly monitoring programme aimed at checking the formation of mucilaginous aggregates.

This event imposed a series of changes in the traditional coastal monitoring activities carried out by the operational structure Daphne; besides chemical, physical and biological parameters, tests with a wire-guided camera on the water column have been introduced in order to check the emergence of mucilage which at first appears in the form of flakes off the coast and under the surface.

Potentially toxic and toxic algae

In June of 1989, regional Health Authorities reported many cases of poisoning characterised by vomiting, abdominal pain and diarrhoea in patients who ate mussels fished in coastal waters.

The Centro Ricerche Marine associated these symptoms with the simultaneous presence in the same molluscs of *Dinophysis sacculus, Dinophysis fortii, Dinophysis acuminata,* which produce DSP (Diarrheic Shellfish Poisoning) biotoxins, in the water and digestive tract.

The liposoluble toxin, responsible for these episodes, was immediately identified by the Centro Ricerche Marine di Cesenatico through the mouse test (Viviani et all., 1990).

This identification was then unambiguously confirmed by chemical tests in HNMR which pointed out the presence of okadaic acid (Fattorusso et al., 1992), a toxin that produces diarrheic effects.

Despite Health Authorities immediately forbade mussel fishing, mussels were still illegally fished and sold, consequently more people received medical treatment. This first episode of toxinfection attributed to mussels fished in our sea, helped to improve controls on bivalves, thanks to the introduction of regular monitoring on waters and molluscs with Governmental Decrees 256 and 257 of 1990.

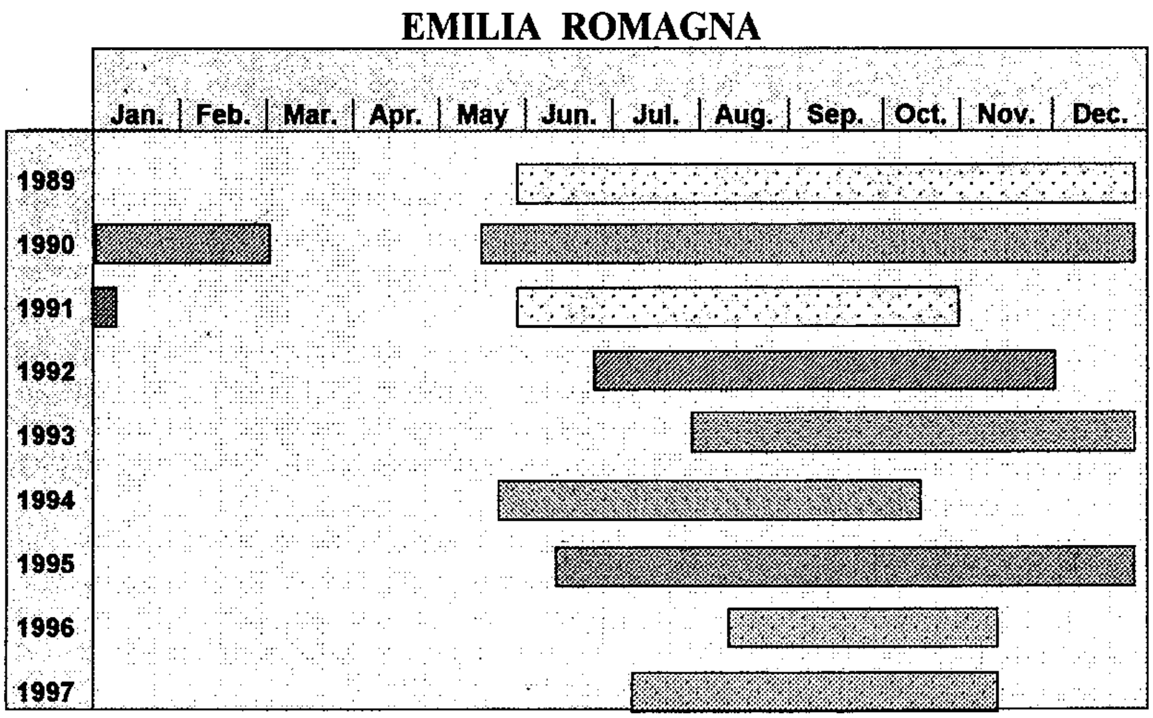
This monitoring allowed the Centro Ricerche Marine di Cesenatico to alert national Health Authorities after PSP first appeared in bivalves in Italy (Emilia-Romagna coast), with a level of toxins of 192,6 μg / 100 p. e., higher than the limits established by the law (Poletti et all.,1996).

Later in December 1992 with legislative decree n. 530 the Italian Government acknowledged Community policy n. 492 of July 15, 1991 which established hygienic rules that regulated the production and sale of live bivalves.

Legislative decree n. 530 confirms and imposes regular monitoring activity on water and bivalves on Health Authorities in order to control the presence of potentially toxic or toxic phytoplankton and the accumulation of water soluble and liposoluble toxins in molluscs.

Space and time distributions of *Dinophysis* spp., a microalgae that produces DSP liposoluble toxins is confirmed as this microalga is present in bivalve species. Table 3 shows the mussel-farming firms which had to close down after DSP biotoxins were found at levels higher than these established in the Italian law (Poletti *et al.*, 1997). In 1997 monitoring activities on mussels showed a high toxicity in summertime due to yessotoxin. This latter toxin has a neurotoxic effect, and it is not produced by *Dinophysis* but by another dinoflagellate. Algal species that can synthesise this new toxin are being looked for.

Table 3. – *Periods of the p prohibition of fishing and selling of mussels during DSP toxicity in the Northwestern Adriatic Sea areas.*



The damage to fishing because of the presence of liposoluble toxins is economically so high that jeopardises the future of mollusc-farming.

Luckily not all bivalve species act the same way; in fact table 4 shows that mussels seem to be hit hardest so far (Poletti et al., 1997).

Table 4. - *Comparison and occurrence of DSP and PSP toxicity in different bivalve species during 1989 - 1996*

BIVALVE SPECIES	N° Samples	DSP		PSP			
		Positive Samples	%	n.d.	µg/100g meat		
					< 40	40 - 80	> 80
Mytilus galloprovincialis	8313	2406	29	8248	24	32	9
Tapes philippinarum	665			662	2	1	
Callista chione	239			239			
Cardium sp.	45			45			
Chamelea gallina	239			239			
Chlamys sp.	38			38			
Crassostrea gigas	85			85			
Pecten jacobaeus	41			41			

As for PSP water soluble toxin-producing microalgae, we've showed the capability of synthetising this kind of toxins only in the *Alexandrium minutum* (Honsell et all., 1996). As Figure 10 shows, this microalgae has a less space and time distribution than *Dinophysis*. Temperature and salinity seem to be the two most important factors controlling growth and distribution of this microalgae. Moreover, this microalgae has its biggest concentration near the mouth of rivers, according to the data of the monitoring in 1994, when the first and only case of ban on fishing mussels in natural banks (coastal breakwater) in the Adriatic Sea was put.

Moreover *Alexandrium* spp. has its highest concentration in the first metres of the water column and to date it has not affected mussel-farming which is done at about 5 metres below the surface.

Experience obtained during these monitoring activities allowed us to identify the months in which toxic algae are more likely to appear in the Northern Adriatic Sea (Table 5), the potential capacity that the most common bivalve species in the Adriatic Sea have for accumulating marine water soluble and liposoluble biotoxins (Table 6) and sea areas in which *Mytilus galloprovincialis* accumulate toxins (Table 7).

All these elements are important to the Health Authorities that have to check the health conditions of fish in order to launch rational monitoring plans.

At the same time, fishing, and especially mollusc-farming, can get important information on both the structure and location of farming sites but also on which bivalve species to farm. In order to favour Health Authorities and mollusc-farmers tasks Centro Ricerche Marine di Cesenatico publishes a weekly report on hygienic conditions of farming as far as marine biotoxins are concerned.

Table 5. - *Potenzialy hazardous months*

	***Dinophysis* spp.** **DSP**	***Alexandrium minutum*** **PSP**
January	●	●
February	●	●
March	●	●
April	●	●●●
May	●	●●●●
June	●●●	●●●●
July	●●●●	●●
August	●●●●	●
September	●●●●	●
October	●●●●	●
November	●●	●
December	●	●

Table 6. *Physiological capabylity to accumulate marine biotoxins*

	DSP	PSP
Mytilus galloprovincialis	●●●●●	●●●●●
Tapes philippinarum	●●	●●●
Callista chione	●	●
***Cardium* sp.**	●	●
Chamelea gallina	●	●
***Chlamys* sp.**	●	●
Crassostrea gigas	●	●
Pecten jacobaeus	●	●

Table 7. - *Physiological capabylity to accumulate marine biotoxins by M. galloprovincialis*

	LONG LINE	**BOTTON**
Offshore	●●●●●	●●●
Reefs	●●●	
Lagoon	●●	●
"Vallette"	●	●

So it is easy to deduce from what has been said the importance of prolonged monitoring activities for both the environment and health. They allow us to monitor even short phenomenon that can later become more serious and substantially modify the environment and heavily affect Italy's economy. In fact the eutrophication phenomena not only negatively affects the biological features of the sea, but can cause serious problems to fishing, tourism and health. Faced with events that concern public health, as the emergence of toxic algal species and fish kill, monitoring systems are useful precautionary means for competent Authorities.

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ALGAL BLOOM DETECTION, MONITORING AND PREDICTION IN THE GALICIAN RIAS (NW SPAIN).

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Introduction

Galician monitoring covers the entire Galician coastline from the Miño River to the Eo River which means 1195 km of coastline.

The morphology of the Galician coastline is highly varied, the most characteristic coastal feature being the Rias. The Rias are a kind of extended bay which reach into the coast in an approximate north-eastern direction. A river flows into the inland extremity of all the Rias.

The general topography of the Rias and the presence of a deeper main channel along the axis of the central zone, the old course of the river mouth, appear to confirm the theories on the origin of the Rias. These theories include the Rias in the general tectonic plate processes as having been formed by the interference of a marine transgression, with a pre-existent relief of fluvial origin where the eroding process of the sea acts on the projecting features, in the shallow spots, causing sedimentation of fine and sandy materials (Vidal Romani, 1984).

The Ria is a fully dynamic, changing ecosystem. In order to understand the processes which take place inside the Ria, a number of factors affecting the biological processes need to be identified, taking into account both the abiotic and the biotic factors, remembering that these covariate in space and time.

The zone may be defined as a rainy oceanic climate. The meteorological conditions basically depend on the position of the Azores anticyclone. In the summer period, northerly winds prevail so that rainfalls are not frequent. In the winter period, this anticyclone becomes more southerly, giving way to stormy weather with predominantly southerly winds and heavy rainfalls.

Within the Rias themselves there is a residual estuarine circulation system (Fraga & Margalef, 1979) which is due to the contributions of freshwater from the rivers leading into the interior part. Freshwater, or at least less dense water, flows on the surface, gradually mixing with the seawater so that, to compensate for this loss, water moves in along the bottom.

This outward movement at surface level preferably occurs at the north–eastern mouth, while the influx takes place on the bottom at the southern mouth due to Coriolis force. The different topographies of the bottoms, however, and the presence of bays, modify the general flow pattern.

One of the more characteristic processes affecting the Galician coastline is upwelling (Fraga, 1981; Blanton *et al.*, 1984). When strong northerly winds prevail, a north to south current forms parallel to the coast, causing coastal waters to leave the Ria on the surface and the upwelling of colder, saline bottom lying waters rich in nutrient salts from the open sea which penetrate the Rias at bottom level. These waters rise up from an approximate depth of 200–300 m to the photic zone, reaching the surface in the more intense events.

When southerly winds blow, a poleward current forms which causes surface water to come in from the platform via the surface level. This phenomenon is known as downwelling and is detected because the water penetrating the Ria is at a higher temperature and has a lower saline content than the water moving in by upwelling, and is poor in cells and nutrients.

Coastal upwelling and estuarine residual circulation are two extremes of an oceanic–continental axis which modifies flow in a Ria. Upwelling is promoted by the wind on the platform and residual estuarine circulation moved by river contributions. Upwelling introduces oceanic water into the Ria ecosystem and the positive estuarine circulation forms a part of the continental water.

Although these processes are clearly differentiated, they do interact. In a positive estuarine circulation model, with strong northerly winds on the platform, upwelling occurs, the effect of which is to reinforce the introduction of bottom waters from the outer Ria, superimposing and accelerating the speeds of positive estuarine circulation.

In the absence of upwelling, flow in the Ria is slowed down, occasionally becoming practically blocked, particularly if this occurs at times of high thermal stratification due to insolation, as occurs in summer.

When downwelling occurs, the surface introduction of water from the platform runs into the outgoing estuarine circulation along the surface. And depending on the intensity of the event, this causes an inversion of the flow, in other words, a negative estuarine circulation or a two–celled circulation.

Upwelling is a characteristic phenomenon of the easterly side of the large anticyclonic rotations governing oceanic circulation in general. Along the coastal strip to the East of the North Atlantic, it is present between parallels 10° and 44°, so the Galician coast lies on the northern limit of the North West Africa upwelling (Wooster *et al.*, 1976).

The richness of the Rias is largely due to the upwelling which provides the nutrients necessary to support the high primary production (Fraga, 1991) which is the basis of all production in the Rias. In this regard, there is a study which directly relates the quality of mussels in the Ria of Arousa with the upwelling indices (Tenore *et al.*, 1982).

There are many species of molluscs marketed on the Galician coast, the most important being mussel, oyster and clam, cockle, scallop, small scallop, razorfish, etc.

Apart from the large number of species present on natural beds, the main semicultures on sandy substrata are the clams (*Venerupis* spp.) and the cockle (*Cerastoderma edule*), floating cultures farm mussel (*Mytilus galloprovincialis*), oyster (*Ostrea edulis*) and scallop (*Pecten maximus*).

The mussel is the basic species of aquaculture in Galicia. The first attempts to farm them in controlled conditions when using the French system of pole hanging proved unsuccessful. In 1945, a private initiative in the Ria of Arousa set up a floating contraption, which proved to be remarkably successful. This method caught on and rapidly developed farming throughout the Rias.

From an administrative viewpoint, these floating culture beds, called rafts, are organised into production areas. There are currently 78 production areas with a total of 3481 floating beds.

Average annual productivity per raft is in the order of 60 tonnes. Spain is the second most important world producer of mussel (after China), and 97% of Spanish mussel production is from the Galician Rias (FAO, 1992).

Current overall production is approximately 200,000 tonnes of mussel, with a first sale value of about 80 million dollars (Durán *et al.*, 1990). Apart from its undeniable economic importance, this sector has outstanding social repercussions as it generates 6,000 direct jobs. A further 15,000 indirect jobs are generated in depuration plants, cooking factories, freezing and canning companies (Miranda, 1996).

The problem of algal blooms associated with toxic episodes is a serious one. Apart from the problems strictly related to health, when the toxicity level detected is over the legal limits, orders are given to close the production area and the extraction of bivalves is forbidden. This situation involves serious economic losses, not only due to the closure itself, but also to the very fact that the time for reopening is unknown, which leads to difficulties in commercial organisation. There are also indirect economic losses due to the negative effect of toxic episodes on health aspects of the product, which create certain mistrust in the consumer, which may lead to a fall in sales.

The incidence of toxic episodes depends on several factors such as the species of bivalve affected, the productive phytoplanktonic species involved and, therefore, on the type of toxin, cellular concentration and the residence time of the toxic stocks in the water, the position of the production area within the Ria affected, as well as sheltered zones where, due to the longer residence time of the water, recovery will take a longer period of time, and the exterior zones will be more affected by the entry processes from outside the Rias.

Some species are specially problematic because they have a very slow desintoxication process, this is the case of the scallop (*Pecten maximus*) (Arévalo *et al.*, 1998) whose toxicity persists for a long time after algal bloom has disappear.

Other species, such as the mussel, store up toxins in the digestive system so that they have a very fast intoxication speed and are used as bioindicator species (Shumway, 1990). The desintoxication process is very complex being implicated several environmental conditions (Blanco *et al.*, 1997) that it is important to known in each moment to an adequate prediction of the toxic event evolution.

Activities of the Marine Environmental Quality Control Centre

The Marine Environment Quality Control Centre, which depends on the Autonomous Regional Government of Galicia, is strategically located at the dock in Vilaxoán, in the heart of the Ria of Arousa.

This Centre started activities in 1992, initially following a line of work focusing on Oceanographic and Phytoplankton Conditions. The departments of Biotoxins, Microbiology and Pathology, Chemical Contamination and Resource Management are also now fully operative.

From 1977 to 1992, Red Tide Monitoring was developed by the "Instituto Español de Oceanografía" in Vigo and La Coruña Centres (I. E. O., 1993) and the sanitary biotoxins control was made by the "Consellería de Sanidade e Consumo" of the "Xunta de Galicia" and the "Sanidad Exterior" ofa the " Ministerio de Sanidad y Consumo" (Alonso Picon, 1990).

The main objective of the Centre (Mariño & Maneiro, 1993) is to run a strict, intensive control system of the characteristics of the marine environment, to meet the legislation in force regarding the regulations applicable to producing molluscs and other marine organisms, to develop an exploitation and commercialisation strategy based on offering a top quality product with all the sanitary guarantees, and finally, to protect and even to improve the quality of our waters in general and in the production area in particular.

The monitoring programme was designed (Mariño *et al.*, 1998) for the extensive and reliable detection of harmful algae and toxins in shellfish while minimising the cost, both in labour and material per unit of information.

Although the basic mission is monitoring, the Centre co-operates with researchers at other centres so that maximum use is made of the information and techniques are kept up to date.

The legislation applicable to biotoxin monitoring gives some idea of its importance in Galicia. Apart from community legislation, which basically means Guideline 91/492 of July, 1991, and two Orders of Council at Spanish State level, namely 345/93 and 308/93, legislation has been developed at Local Galician Government level. This appears in Decree 116/95, Decree 98/97 and the Order of November 14th, 1995 governing the monitoring programme of marine biotoxins in bivalve molluscs and other marine organisms from fishing, shellfish farming and aquaculture.

Monitoring of toxic episodes is conducted in close co-operation between the departments of Biotoxins and Oceanographic Conditions and Phytoplankton.

This legislation has established the sampling points along the Galician coastline, 47 fixed primary points being considered as the most representative in the production areas, as well as being the first to be affected in the event of toxic episodes. A further 128 fixed secondary points are also established which are complementary to the previously mentioned 47.

In accordance with the legislation governing the monitoring programme, different procedural plans are established: A, B1, B2, B3, C1, C2, C3 and D, in terms of the evolution of the toxicity detected in bivalves, and the evolution of the phytoplanktonic community and the oceanographic conditions (Figure 1).

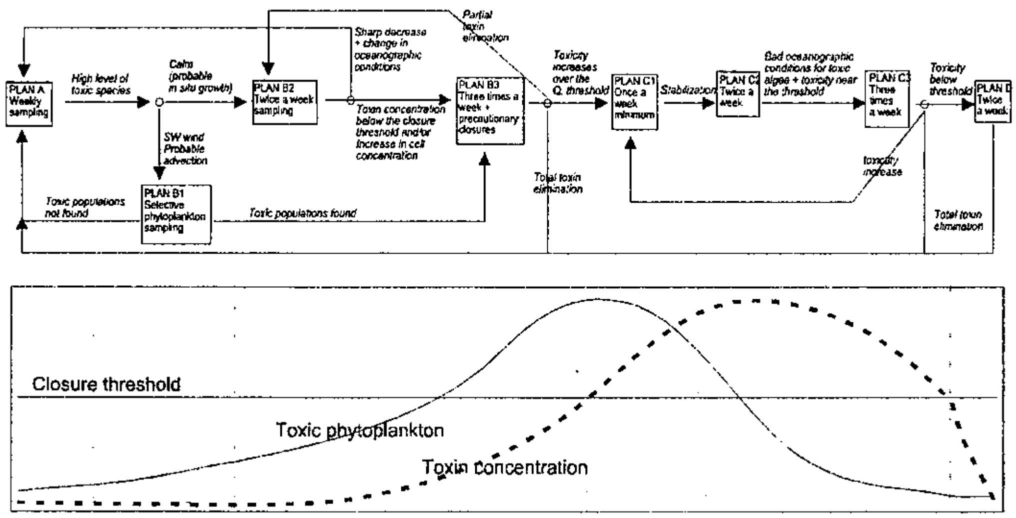

Figure 1.- *Procedure plans in the Monitoring Program*

The step to define the programme is vital for predicting and monitoring since, according to the current programme, sampling frequency varies from 1 to 5 times per week; the geographic extension covering only the primary fixed points or the primary and secondary fixed points; and the intensity analysing the samples, in an integrated manner, of bivalves from levels 1, 5 and 10 metres or as separate samples.

Plan B3 is defined when the oceanographic conditions are favourable for algal proliferation, a significant increase in the concentration of toxic phytoplankton is observed in the water and levels of biotoxins in the bivalves below, although very close to the established legal limits are detected. The programme in this case is intensified to the maximum in extension, intensity and frequency to guarantee a total health control of the molluscs extracted.

This procedure level is identical in plan C3 when oceanographic conditions are unfavourable and a fall in cellular concentrations of toxic phytoplankton in the water and in biotoxin levels is detected, even though it is above the legal limits. In this way, total health control is guaranteed and no zone or production area is kept closed for extraction longer than necessary.

The legislation also establishes that when potentially toxic phytoplankton cells are detected in significantly high concentrations, a Precautionary Closure may be declared for the production area or zone "a priori" of the results of the biotoxin analysis.

In view of this complex, strict procedure, it is essential to carry out a thorough follow-up of the phytoplankton cell concentration along our coastline and of the oceanographic conditions associated with it. But it is also vital to move ahead in predicting, both to detect when a proliferation may appear and as regards the duration of a given episode and the point when it will disappear.

Through an agreement with the Spanish Metereological Office, we have daily reports on rainfall, cloud and trajectory, intensity and direction of the winds.

Through another agreement with the Department of Apllied Physics at the University of Santiago de Compostela, we receive satellite photographs showing the surface temperature of waters off the Galician coast.

The Department of Oceanographic Conditions and Phytoplankton conducts sampling on a weekly basis, at 38 oceanograpic stations covering the main production zones and hydrographically important areas in the Rias and in 23 Coastal Oceanographic Stations, on a fortnightly basis, distributed throughout the rest of the Galician coastline (Figure 2).

The stations in the Rias are sampled on board oceanographic research vessels. The coastal stations are sampled with the kind co-operation of businessmen in the sector.

In the case of hydrographic analysis, vertical profiles with the CTD Sea-Bird 25 sonar are carried out, covering salinity, temperature, oxygen, pH, fluorescence and transmittance in the water column.

At four shallow depth stations, a Valeport sonar was used to collect data on salinity and temperature, and at the coastal stations surface data on salinity, temperature, pH and oxygen were taken using portable equipment.

When a swift sampling of water on the continental platform outside the Rias is called for, we have a system of oceanographic bottles coupled to a rosette specially designed for sampling from a helicopter.

Water column is additionally sampled in three depth intervals from the surface to 5 metres, from 5 to 10 and form 10 to 15 metres by means of a hose following the Lindahl technique (Lindahl, 1986; Sutherland, 1992).

From "this" same sample, all the analyses of pigmentary composition, nutrient salts, organic and inorganic carbon and the quantification of phytoplanktonic cells will be conducted.

The analyses of chlorophylls "a", "b" and "c" in fractions made by filtration of sizes above and below 2.7 μ are conducted according to the spectrofluorometer method by Neveux (Neveux & Panoune, 1987) modified by Zapata (Zapata *et. al.*, 1994). Also, at 4 stations, HPLC is used to characterize the pigmentary profile of the phytoplanktonic community.

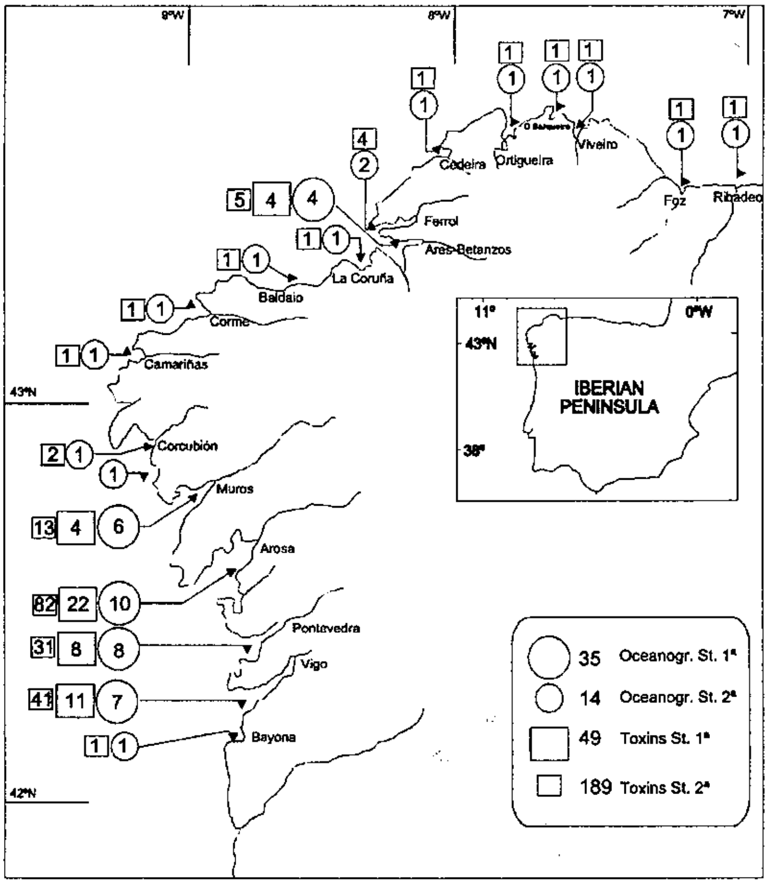

Figure 2.- *Distribution of the Oceanographyc and Biotoxins stations in the Galician Coast.*

The concentrations of nitrates, nitrites, phosphates, silicates and ammonium at the various depth levels are determined with a TRACCS 800 continuous flow analyzer.

The concentrations of total organic and inorganic carbon are determined using a TOC-5000 analyzer.

Samples, which are fixed with lugol, are taken for the quantitative characterisation of the phytoplanktonic community, using the sedimentation technique in Utermöhl chambers. These samples are counted in the inverted microscope.

Also, a net sample is taken with a haul of plankton, which is fixed in formol for qualitative analysis of the phytoplankton stock.

The qualitative net sample is viewed immediately after reception in order to draw up a taxonomic list and to evaluate the possible presence of any potentially harmful algal proliferation.

After the time required for sedimentation, these are graded and the potentially harmful or toxic species are counted first. In this way, the day after the sampling, a

Preliminary Report on Phytoplankton Counts is sent to the various administrative bodies, research centres and business associations in the aquaculture sector.

All the species present in the samples, including the small flagellate counts, are then identified and counted. In the event of any conflicting species from a taxonomic point of view, this problem is solved by a co-operation agreement which we have with the Electronic Microscopy Unit at the University of Coruña. A lot of effort of the monitoring teams has to be devoted to microscopical examination of the plankton samples (Blanco, 1996) waiting for the researches in automatic categorisation are concluded and can to be applied for completed the microscopical examination analysis.

The software for organizing the techniques used has been specifically designed by staff at the Centre itself (Maneiro & Mariño, 1994). All the information gathered by the various teams is centralized in a database which is kept updated and is being widely consulted for use in different research projects.

Once the analyses are completed at the end of each week, all the information for each station is compiled in a Weekly Report covering 50 pages. This is sent by fax and e-mail to all the establishments involved.

All these reports with the accompanying graphics and analysis of evolution over time of the different variables are included in an Annual Report.

This type of graphics makes it possible to have a simultaneous interpretation of all the variables analyzed.

By way of an example, the figure seen here refers to July 19th, 1993. Station VI (Limens) in the Ria of Vigo.

The meteorological data for the days prior to this (19/07/93) show the lack of rainfalls and cloud and a very weak intensity of variable winds. As to be expected in a summer situation, and taking the above into account, in the temperature profile in the figure 3, we can see a very pronounced thermocline with a gradient of 14 to 18°C in the first 5 metres. The maximum fluorescenceand, therefore, the maximum phytoplanktonic biomass, lies in the thermocline at around 5 metres. This maximum coincides with the data on the pigmentary composition analyzed by spectrofluorometry. The nutrients were exhausted at surface level. The phytoplanktonic community was dominated by species of the genus *Leptocylindrus,* and these presented insignificant cellular concentrations of the Ichthyotoxic species *Heterosigma akashiwo*; a significant increase in cells of *Dinophysis acuminata* (1560 cell/l) and a slight increase in concentrations of *Gymnodium catenatum* (160 cell/l). As may be expected, with these cellular concentrations present, all the Rias were closed at the time.

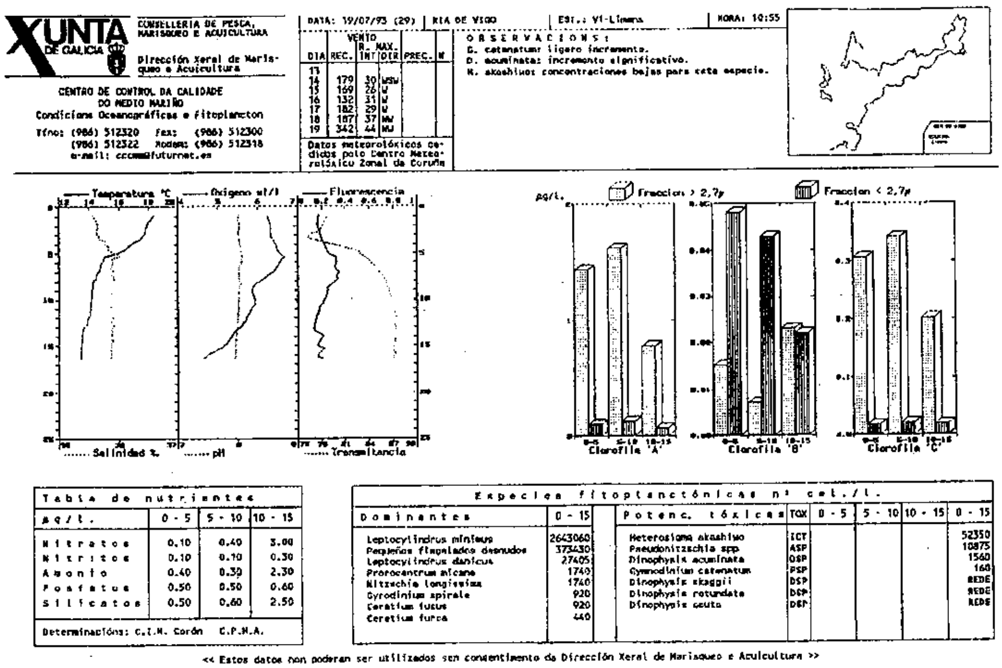

Figure 3.- *Example of weekly report for an oceanographic station in the Ria de Vigo.*

It is in fact very difficult to find a record with these toxic cellular concentration values in the database available in the Galician Monitoring.

Normally, the phytoplankton stocks are made up of non-toxic species. Diatoms, dinoflagellates, other flagellates and ciliates are the principal groups.

Among the diatoms, we find species such as *Chaetoceros* spp., *Rhizosolenia* spp. *Leptocylindus* spp., *Thalassiosira* spp. etc. Some species of diatoms, such as the *Chaetoceros convolutus*, are considered as ichthiotoxic since, when they produce massive proliferations, they may damage the fish gills making them susceptible to infections. *Pseudo-nitzschia* spp. is a genus of diatoms among which are found some species which are responsible for amnesic toxicity. Taxonomic identification is very complex as it is necessary to resort to the optical immersion microscope or even, in some cases, to electron microscopy.

Likewise, the dominant communities of dinoflagellates are innocuous species such as *Ceratium* spp., *Protoperidinium* spp., *Scripsiella* spp., *Prorocentrum* spp., etc. Some interesant species of dinoflagellates developed blooms in the area such *Noctilluca scintillans, Gyrodinium cf. aureolum* (Jimenez *et al*., 1992), *Gyrodinium impudicum* (Fraga *et al*., 1997). *Prorocentrum lima* grow on the macroalges but there are not data on toxics episodes produced by this species in the area (Bravo, 1991). The main dinoflagellates species involved in the episodes of toxicity in the Galician Rias are of the genus *Dinophysis* (Reguera *et al.*, 1990; 1991; 1993a; 1993b), associated with the diarrhetic syndrome (Campos *et al.*, 1982; Fraga *et al.*, 1984; Kumagai *et al.*, 1986; Lee *et al.*, 1989). Occasionally, the presence of the dinoflagellate *Alexandrium lusitanicum* has been detected (Blanco *et al.*, 1985). This species is a paralyzing type toxicity inducer (Rodríguez *et al.*, 1989; Franco *et al.*, 1992). The appearance of *Gymnodium catenatum* has been detected in certain years (Fraga *et al.*, 1988, 1990, Figueiras & Pazos, 1991, Pazos *et al.*, 1995; Figueiras *et al.*, 1998). This cyst-forming species (Bravo, 1986; Anderson *et al.*, 1988) is associated with a paralyzing type toxicity (Lüthy, 1979; Gestal *et al.*, 1980; Anderson *et al.*, 1989; Rodriguez *et al.*, 1989; Casais, 1991).

In the other groups, the most important species from the point of view of monitoring are *Dictyocha speculum* (Prego *et al.*, 1998), *Phaeocystis pouechetii* y *Heterosigma akashiwo* since they are related with ichthiotoxicity problems. These species are developed proliferations in very particular situations of physical stability and supply of nutrients.

Phytoplanktonic communities never comprise one single species. The identification and count of the entire community is vital to be able to characterize these communities in terms of predicting.

When a bloom of a phytoplanktonic species takes place, that phenomenon is called a Red Tide, generally known in Galicia as a "Sea Purge" (Figueiras, 1989), (Fraga, 1989).

In some cases, they are not detected visually at macroscopic level, as is the case of proliferations of species of the genus *Dinophysis*, despite of their producing associated toxicity.

In other cases, the proliferation causes the water to become coloured which can be observed with the naked eye. This may be due to species associated with toxicity, as is the case of *Gymnodium catenatum* or other non-toxic species such are the blooms of *Prorocentrum micans, Noctilluca scintillans, Mesodinium rubrum* or *Gonyaulax spinifera, G. polygranma* which may cause unnecessary social alarm with negative economic effects on the aquaculture and tourist sectors.

Though previous references on innocuous red tides in the Galician Rias exits (Sobrino, 1918; Margalef, 1956; Fraga, 1989), the first important toxic red tide on record in Galicia occurred in 1976. It affected the Rias of Vigo and Pontevedra and was due to a proliferation of *Gymnodium catenatum*, (Estrada *et al.*, 1984) producing the paralyzing syndrome (PSP). Other episodes occurred later, mainly due to *Gymnodinium catenatum* and *Dinophysis acuminata* (Durán *et al.*, 1990; Valcarcel *et al.*, 1992; Valcarcel *et al.*, 1993; Arevalo *et al.*, 1995; Arevalo *et al.*, 1998).

The first scientific articles on the area were developed by Professor Margalef (Margalef, 1952a; Margalef, 1952b; Margalef & Duran, 1953; Margalef *et al.*, 1955; Margalef, 1956). The toxic blooms, particularly the one in 1976, were followed by a bloom of researchers working on the subject in the various institutions and official bodies in Galicia. And in this way, a multidisciplinary approach is underway to increase knowledge of algal proliferations.

Margalef described an ecological succession ranging from diatoms to dinoflagellates, based on a yearly cycle (Margalef *et al.*, 1979). Later it was seen that, by superimposing on this cycle of ecological succession of species which depends on oceanographic conditions, that there are shorter ecological succession cycles (Tilstone *et al.*, 1994; Pazos *et al.*,1995; Blanco *et al.*, 1998) with the duration of a upwelling cycle, and we have gradually located the position of the main toxic species in the area (Figure 4).

When a upwelling occurs, it helps the influx of nutrients along the bottom from the platform (Fraga, 1981) (Prego, 1990) (Alvarez-Salgado *et al.*, 1996). A community is established by small sized pioneer diatoms with a high division rate (for example, *Chaetoceros*) which live at the expense of these nutrients (Figueiras & Pazos, 1991; Figueiras & Rios, 1993).

Following the incipient exhaustion of the nutrients, the community evolves towards long, chain–forming species. These include the species of the genus *Pseudo-nitzschia*, (Maneiro *et al.*, 1997) some of which are associated with the amnesic syndrome.

UPWELLING	Supply of nutrients (along the bottom from the platform)	DIATOMS 1 (*Chaetoceros* spp.) DIATOMS 2 (*Pseudo-nitzschia* spp.) ASP	ECOLOGICAL	SUCCESION
UPWELLING RELAXATION	Deplection of nutrients (surface)	AUTOCHTHONOUS DINOFLAGELLATES (*Dinophysis* spp.) DSP		
NEW UPWELLING	Clean Rías			

DOWNWELLING	Surface introduction of water from the platform	ALOCHTHONOUS DINOFLAGELLATES (*Gymnodinium catenatum*) PSP

Figure 4. - *Ecological position of most important toxic species in the Galician Rias.*

When the northerly winds let up, there is a relaxation in the upwelling process, and flow within the Rias is reduced (Alvarez-Salgado *et al.*, 1996; Rosón *et al.*, 1997). Clines are produced (which are more marked in summer) with an exhaustion of nutrients at surface level.

At this point, the community develops towards dinoflagellates with a lower division rate and adaptational advantages such as the possibility to migrate vertically (Villarino, *et al.*, 1995), taking nutrients by night on the bottom and undergoing photosynthesis on the surface by day; the presence of flagellates which allows them to escape of the depleted microzones of nutrients; as this does not depend on silicate, as occurs with the diatoms for forming frustules; the possibility of optional nutrition (autotroph or heterotroph) in some cases, etc. These constitute what we have named Autochthonous Dinoflagellates, which include species (Reguera *et al.*, 1990; 1991; 1993a; 1993b) of the genus *Dinophysis* associated with the diarrhetic syndrome.

A new strong bloom cleans the Rias, accelerating the flow produced by exportation of organic matter towards the platform (Alvarez-Salgado *et al.*, 1996; Prego, 1993) reintroducing the nutrients whereby a new cycle starts. In this way, a microsuccession cycle is completed which lasts one upwelling cycle.

Irrespective of the upwelling-relaxation cycles of upwelling, with strong winds southerly the downwelling episodes appeared. These episodes as it was commented previously imply the input of superficial water from out of the Rías (Alvarez-Salgado *et al.*, 1996; Rosón *et al.*, 1997).

The downwelling may be oceanographically detected due to its termohaline and nutrient and chlorophylls concentration characteristics. But moreover the downwelling may select phytoplankton communities with species as *Ceratium candelabrum, Ornithocercus magnificus or Prorocentrum rostratum* which we have called Alochthonous Dinoflagellates. One of them is *Gymnodinium catenatum* associated with the paralyzing syndrome (Fraga *et al.*, 1988; 1990; Fraga, 1996; Figueiras *et al.*, 1996; 1998).

Then, to the prediction and control of these toxic species, it is fundamental to distinguish in each moment the precise phase of the upwelling- relaxation cycle of the upwelling or if, in contrast, a downwelling process is occurring.

Based on data supplied by the Spanish Meteorology Office, we can calculate the Upwelling indices, applying Bakun's method (Bakun, 1973), which is a good indicator of the amount of upwelled water per km. along the coast. But the really important information is supplied by the Department of Applied Physics at the University of Santiago de Compostela from satellite pictures (Figures 5-9).

Three days later, on August 31st, upwelling was so intense that water at less than 14°C was observed all along the surface coastal water of Northern Portugal and all along the Galician coastline. There were areas, such as in the Ria of Muros, with surface water at 12°C.

We have a good deal of upwelling pictures in our archives. The downwelling images, however, are more difficult as they coincide with situations of heavy cloud.

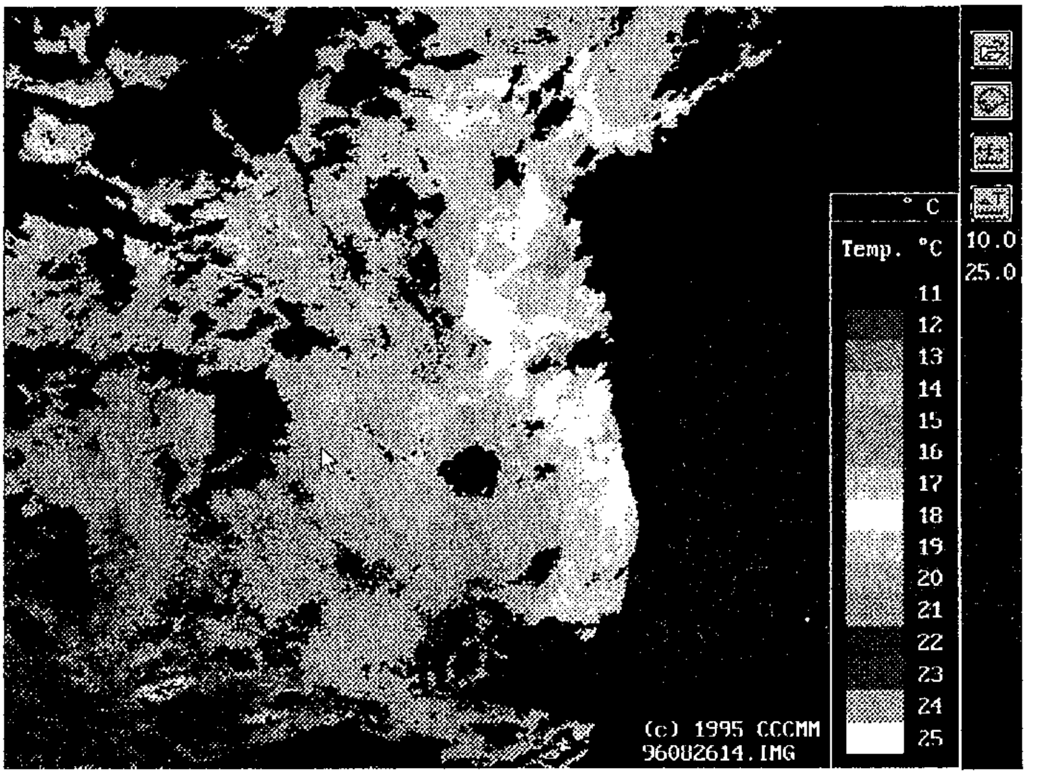

Figure 5. - *Satellite Image of Upwelling 960826 in Galician coastal waters.*

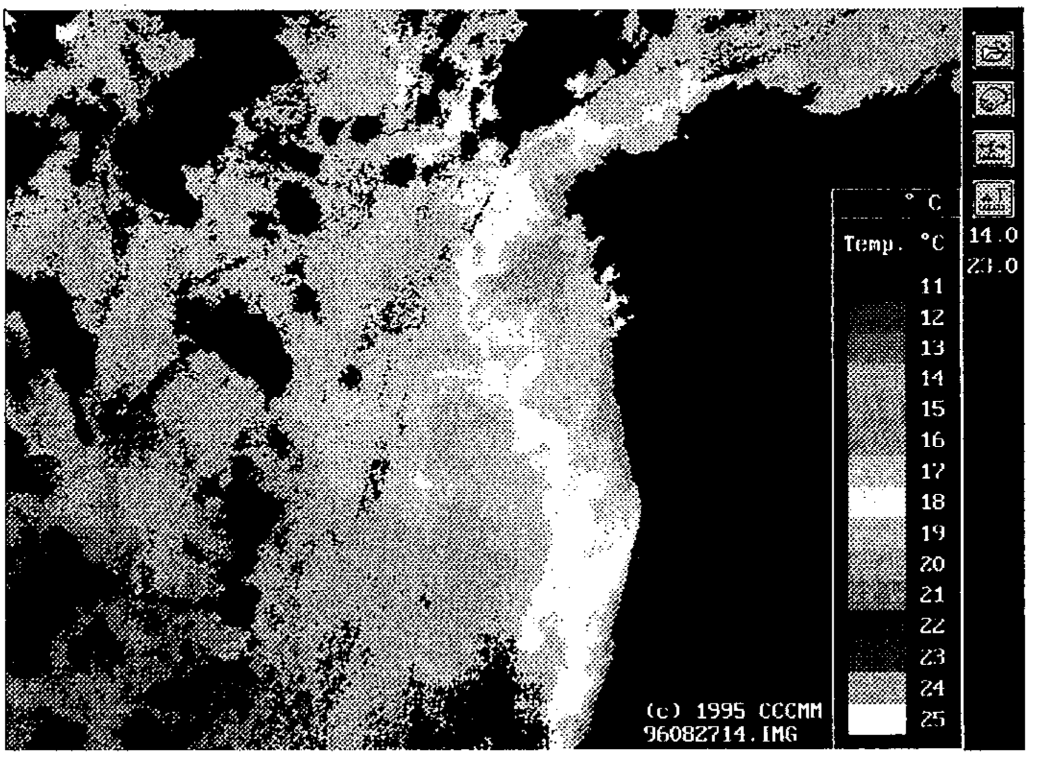

Figure 6.- *Satellite Image of Upwelling 960827 in Galician coastal waters.*

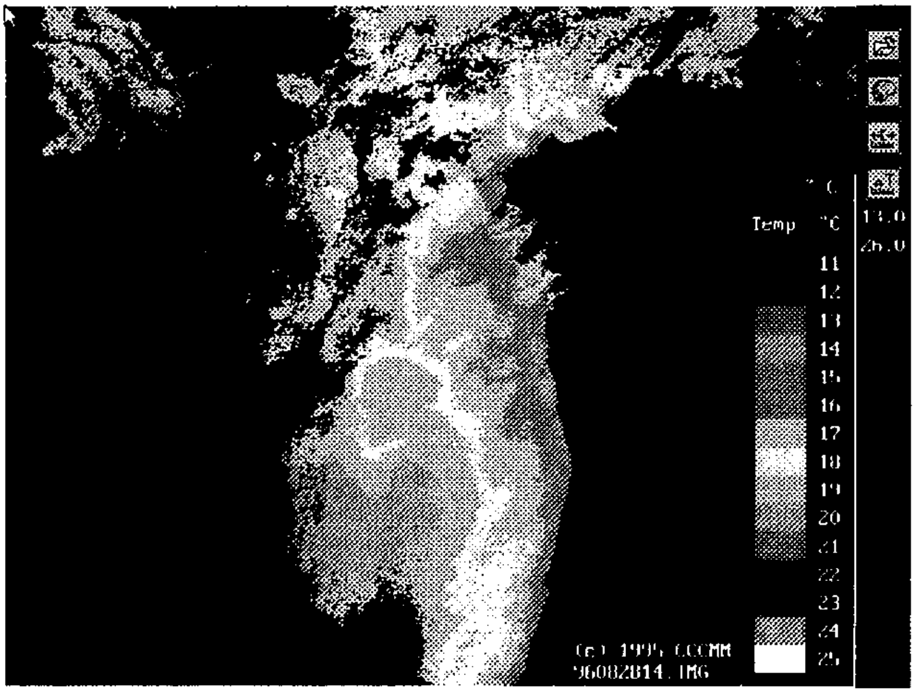

Figure 7 - *Satellite Image of Upwelling 960828 in Galician coastal waters*

On October 12th 1997, with strong southerly winds, the surface temperature in the Rias was around 18°C, but there were areas on the platform with temperatures of up to 22°C in surface water.

These situations, with the influx of warm nuclei, is sampled using the rosetta on board sea rescue helicopters.

The day to day prediction of algal proliferations in Galicia is carried out in terms of the daily analyses of biotoxins and observation of phytoplankton samples in the production zones, according to the programmes mentioned earlier. This is all tied in with the support from the meteorological data and satellite pictures (Figures 5-9). The black background we can see to the right is the Iberian Peninsula coastline (Galicia and Portugal). The black marks in the sea are due to cloud. The colour scale is kept at a constant in all the pictures to help interpretation.

On August 26th 1996, the surface water in the Rias had an approximate temperature of 16-17°C.

The next day, on August 27th, an incipient upwelling was observed in the northern zone of Portugal, with water appearing at 15°C at surface level, close to the coastline. This is shown here in light blue.

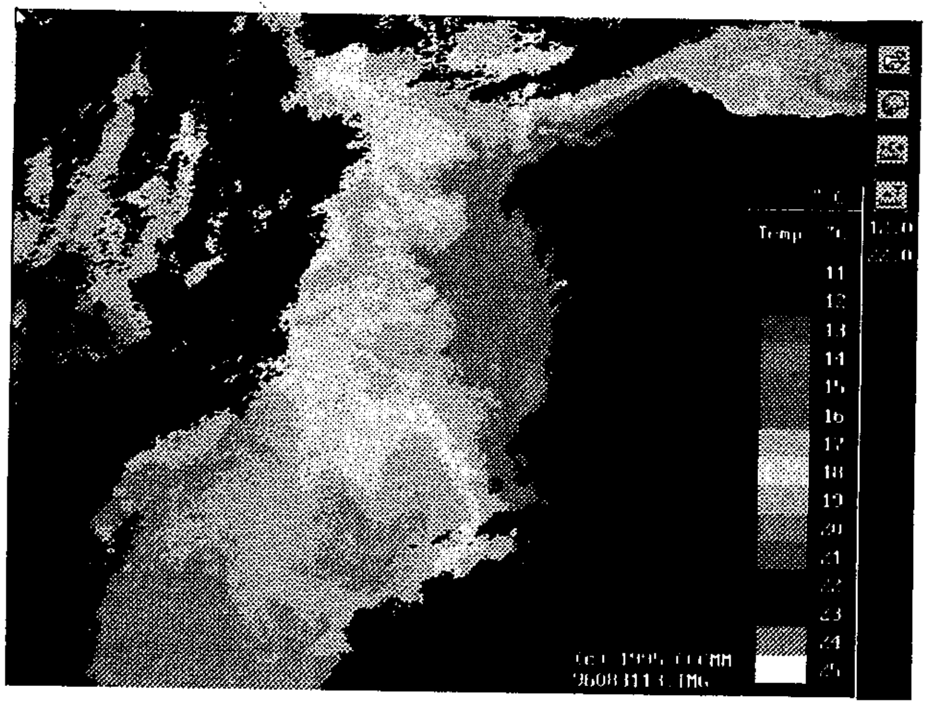

Figure 8.- *Satellite Image of Upwelling 960831 in Galician coastal waters*

The following day, on August 28th, upwelling water at 14-15°C was observed all along the Portuguese coast and in the Finisterre area.

Three days later, on August 31st, upwelling was so intense that water at less than 14°C was observed all along the surface coastal water of Northern Portugal and all along the Galician coastline. There were areas, such as in the Ria of Muros, with surface water at 12°C.

We have a good deal of upwelling pictures in our archives. The downwelling images, however, are more difficult as they coincide with situations of heavy cloud.

On October 12th 1997, with strong southerly winds, the surface temperature in the Rias was around 18°C, but there were areas on the platform with temperatures of up to 22°C in surface water.

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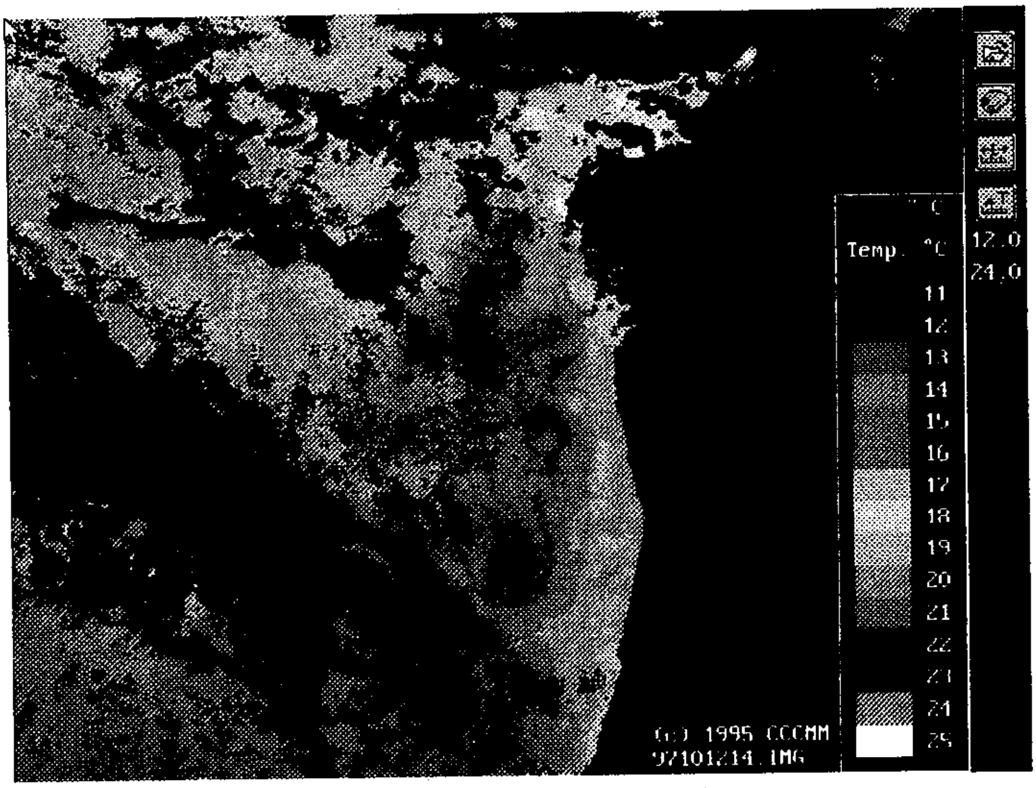

Figure 9. - *Satellite Image of Downwelling 971012 in Galician coastal waters.*

The globalized data on meteorological evolution, the situation of the wind on the platform, thermohaline variability, evolution of concentrations of oxygen, pH, chlorophylls, concentrations of nutrient salts and the evolution of the phytoplanktonic communities at the oceanographic stations is interpreted on a weekly basis.

Finally, the database at the disposal of researchers of the area, makes it possible to predict aspects which are fed back directly into the system. Among them, at the University of Santiago work is underway on setting up an Expert System using multivariant analysis which automatically predicts algal proliferations of toxic species; the intoxication- detoxification model applied (Moroño *et al.*, 1998) to the monitoring data; the trofodinamic models of coastal ecosystems (Barciela, 1997) validates with the monitoring data.

Algal bloom detection, monitoring and prediction in the Galician Rias are possible thanks to the coordinated effort of a number of people and institutions

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