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REPORT OF THE ICES/IOC WORKING GROUP ON HARMFUL ALGAL BLOOM DYNAMICS (WGHABD)

Helsinki, Finland, 17-19 May 1995

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1. WELCOME AND OPENING OF THE MEETING

The ICES/IOC Working Group on Harmful Algal Bloom Dynamics (WGHABD) was convened at the Finnish Institute of Marine Research (Helsinki) following two days of meeting of the ICES/IOC Workshop on Intercomparison of *in situ* growth rate measurements on dinoflagellates. The meeting was chaired by Beatriz Reguera (Spain) .Twenty five scientists from thirteen countries took part and are listed in Annex I. Five more scientists from three countries were not able to attend the meeting and contributed by correspondence. The draft agenda of the meeting was discussed briefly and adopted by the participants with minor changes. This approved agenda is attached in Annex II. Allan Cembella (Canada) was appointed as rapporteur. Major considerations in defining the agenda were to maximize interactions with the Working Group on Shelf Seas Oceanography (WGSSO). In plenary session of the WGHABD, individual participants introduced themselves and their institute and gave a concise description of their major field of research.

2. TERMS OF REFERENCE

At the 82nd ICES Annual Science Meeting (known before as Statutory Meeting) in St John's (Newfoundland, Canada), the Council resolved (C.Res. 1994/2:49) that:

The ICES-IOC Working Group on Harmful Algal Bloom Dynamics (Chairman: Ms B. Reguera, Spain) will meet in Helsinki 17-19 May 1995 to:

a) review the results of the Workshop on Intercomparison of *in situ* Growth Rate Measurements;

b) review ongoing activities in the pilot study areas, and other ICES areas, on physical-biological interactions investigations;

c) develop plans for a future practical Workshop on Modelling using real data obtained in monitoring and projects related with HAB Dynamics;

d) assemble and compile, intersessionally, descriptive information about ongoing monitoring programmes on phytoplankton and phycotoxin monitoring, with a view to its presentation in the Intergovernmental Panel on HABs;

e) define the time and space scales of the physical and biological processes relevant to studies of physical-biological interactions in HAB dynamics;

f) review present knowledge of the abilities of certain harmful algal species to adapt to and modify the microscale physical environment by means for example of vertical migration, mucilage secretion, colony formation, etc.

A Sub-Group consisting of members of the Working Group on Harmful Algal Bloom Dynamics and participants in the 1994 Workshop on "Intercomparison of *in situ* Growth Rate Measurements" will meet under the chairmanship of Ms M.A. Sampayo (Portugal) in Helsinki from 15-16 May 1995 to finalize the workshop report with a view to its publication by ICES (C.Res. 1994/2:49:1).

The Working Group will report to the Hydrography Committee, and the Biological Oceanography Committee, and ACME.

3. REVIEW THE RESULTS OF THE ICES/IOC WORKSHOP ON INTERCOMPARISON OF *IN SITU* GROWTH RATE MEASUREMENTS OF DINOFLAGELLATES

The opening session was followed by a summary review by D. Anderson of the techniques for the determination of phytoplankton growth rates which were considered or employed in the workshop on in situ growth rates held in Aveiro, Portugal in 1994. M.A. de Sampayo, Chairman of the Workshop, presented the recommendations. The report on the review of the results is appended in Annex IV. The overall objective of this excercise has been to develop and compare techniques used for growth rate estimation as applied to toxic species in mixed natural assemblages. The technical problems implicit in establishing species-specific growth rates under these conditions were outlined. Several techniques, e.g. the mitotic index method, are only applicable to a few key species of a certain morphology, whereas in other cases, where molecular probes were tried, inadequate specificity was often indicated. Current methods have not yet been validated for use on a broad suite of HAB species, therefore the workshop discussion focussed primarily on the development of alternative tools rather than the intercomparison of methods.

The group agreed that to have publishable results it was necessary to have a second worshop in 1996. The focus of the follow-up workshop would be on the technical development aspects and would follow an intersessional period with continuous effort dedicated to the subject, to ensure that the technical problems faced in the Aveiro Worshop would be resolved. Specifically, this would include the identification and culture of key target species from the proposed study area, so that cultures might be exchanged among participant researchers for inter-laboratory comparisons and methods development prior to the workshop.

The second workshop was proposed for the Kristineberg Marine Biological Station in September 1996 under the chairmanship of Odd Lindahl (Sweden). This institution has optimum facilities for this kind of exercise, and in the region the natural phytoplankton assemblages are typically dominated by dinoflagellates during September.

4. ONGOING ACTIVITIES IN THE PILOT STUDY AREAS, AND OTHER ICES AREAS ON PHYSICAL-BIOLOGICAL INTERACTIONS RELEVANT TO HABs

4.1 Physical/Biological coupling during *Alexandrium* blooms in the Gulf of Maine (D.M. Anderson)

This presentation highlighted the linkage between populations of the toxic dinoflagellate *Alexandrium tamarense* in the northwestern Gulf of Maine and the behaviour of a buoyant coastal current originating in river outflow. Extensive field surveys were conducted in 1993and 1994 covering 80 stations in a series of parallel transects extending 50 km from shore, spanning an alongshore distance of more than 500 km. Biological, chemical and hydrographic measurements made on approximately bi-weekly cruises during the *Alexandrium* bloom season were supplemented with high-resolution moored measurements within the coastal current. The two field studies documented physical and biological features for one year with extensive PSP toxicity and one year with little or no toxicity. Differences between years reflect event-scale differences in wind and runoff, with upwelling and downwelling winds playing a major role. A "source region" for the initial bloom population was identified at the northern end of the study area near Casco Bay. Localized or in situ growth of cells in that region late in the bloom season apparently provides the cysts that serve as an inoculum for the next year's blooms. A coupled physical/biological model is being used to investigate the aggregation and dispersion of cells within the plume. This also permits examination of the sensitivity of the distribution of cells and freshwater to the timing of forcing events.

4.2 Environmental factors regulating the occurrence of *Dinophysis* spp in a Swedish fjord system (O. Lindahl and B. Andersson)

During 1994-95 high levels of DSP toxicity (>100 µg/100g shellfish tissue) were recorded for mussels on the outer Swedish coast. The net outflow of the Baltic current through the archipeligo produces a low salinity regime, with nutrients relatively enriched within this fjordal system. There is a general counter clockwise circulation of surface water through the fjord system. Typically this results in a high microalgal biomass dominated by diatoms, corresponding to low DSP toxin levels. The occurrence of *Dinophysis* spp. was studied in relation to hydrodynamics, nutrients and phytoplankton species composition in an open-ended fjord system inside the Island of Orust on the Swedish coast. This fjord system is of special interest in the context of *Dinophysis* and its toxicity. The blue mussel (*Mytilus edulis*) is rarely found to be toxic inside the fjord system during periods when high toxicities are recorded in mussels in the outer archipelago. Special attention was directed towards the fjordal mouth areas where strong gradients of toxicity in the mussels is a common feature, and where there is a gradient in surface salinity of 3 to 5 ppt.

In the present study, despite moderate to high concentrations of *D. norvegica, D. acuta,* and *D. acuminata* just outside the mouth areas and an obvious exchange by tidal fluctuations (± 0.15 m), very few cells were found in the fjord water in which diatoms dominated. The maximal cell concentrations of D. norvegica was only ca. 140 cells/L outside the fjord, whereas *D. acuta* concentrations were up to an order of magnitude higher. The highest numbers of *D. acuminata* cells were sometimes found within the fjord. By using diffusion chambers, the hypothesis that *Dinophysis* spp collected outside the fjord could grow in the fjord water was tested. The result was negative because half of the *Dinophysis* cells died within three days, and the caged population became dominated by diatoms, whereas the control was unaffected. The conclusion was that the fjord water was an unfavourable habitat for *Dinophysis* due to higher DSi:DIN and DSi:DIP ratios in the fjord water relative to outside the fjord, thereby favouring diatoms at the expense of dinoflagellates.

4.3 Vertical Profiling the Fine Scale Structure of Algal Exudates (P. Donaghey)

A combination transmissometer-absorbance detection system for determining the fine-scale structure of algal exudates was described. When used for vertical profiling in coastal marine systems this apparatus was capable of yielding a dynamic range spanning six orders of magnitude. During field trials in Washington State, thin layers (sub-metre) comprising a *Pseudo-nitzschia* bloom were resolved. Time-dependent changes in both particulate and dissolved phase material were investigated along the vertical axis. The distribution of particulate material, where dramatic changes were often observed, may be correlated with the profile of dissolved matter, however the converse is not necessarily true. The spectral characteristics of these thin-layer structures, which were associated with high primary production, were clearly distinctive from the surrounding waters.

The importance of understanding the mechanisms underlying the thin-layer concentration phenomenon was underscored in this presentation. It is apparently possible to have relatively stable thin-layers due to low rates of vertical mixing, i.e. the structures are not necessarily homogeneous within a "stratified" layer. The origin of the producing cell population is not always readily apparent, nor is it obvious if this is an *in situ* process or if it is due to a combination of shear and advection.

4.4 A French Project on *Gymnodinium* cf *nagasakiense* (P. Gentien)

The French National Programme on HABs comprises a group of projects which aim to develop an understanding of the ecology of HAB species. The use of a Lagrangian station to determine organic aggregation after sedimentation of particles (mostly >200 µm) for

several hours was described. *Dinophysis* blooms which enter with the tide may be profiting from such organic aggregates, thus occuppying a niche within the pycnocline.

A summary of the achievements of a research programme on *Gymnodinium* cf *nagasakiense* were presented:

1) Taxonomic problems are currently being resolved by using molecular probes (antibodies). In cell cycle studies, Partensky and Vaulot (1989) found that production of "small cells" does not seem to be sexual in character. The division of these small cells (up to 1 div/d) takes place at the beginning of the dark period, in contrast with the division of the large cells that occurs later in the light/dark cycle at a maximum rate of 0.3 div day-1 .

2) Large differences are found between strains isolated 50 miles apart along the coast of Brittany. Maximum cell concnetrations of *G.* cf. *nagasakiense* are found within the pycnocline where irradiance is 5-8% of incident surface levels. There is no evidence of vertical migration although C^{14} assimilation rates indicate the occurrence of rapid growth. Incubation with N15 showed that although NO3- uptake is important at the beginning of population growth, over 90% of N-uptake is due to regenerated N (as NH4+) when cell concentrations were approximately 2 X 106 cells/L. In conclusion, the large standing stocks of *G.* cf. *nagasakiense* can be attained even when levels of inorganic N are very low, and there is no need to invoke N diffusion or vertical migration (Le Corre and L'Helguen, 1993).

3) Complex interactions of this species with other phytoplankton include a link between the spring bloom of diatoms and *G.* cf *nagasakiense* blooms. Substantial *in situ* quantities of polymines, specifically putresceine (at ca. 0.1 µM concentration) have been found, and secretion of allelopathic substances may be an important control factor. Two toxic groups of compounds have been identified from *G.* cf *nagasakiense* - glyco-acyl-glycerol and fatty acids (FA). The effects of these substances are measurable even in early bloom conditions, i.e. at cell concentrations of 10^4 cells/l.

4) Copepods apparently do not graze readily upon *G.* cf *nagasakiense* but the effect upon field populations is largely unknown. Grazing studies with *Calanus*, using an appendage beating recording technique (Gentien and Poulet, in prep) showed that females died after ingesting a few cells (and also became entangled in mucus), whereas males showed a different beating pattern. Entanglement of the appendages due to mucilage production by this dinoflagellate species may affect copepod susceptibility to predation. In exerting its icthytotoxic effect, such mucilage may alter the functional properties of gills by lowering the collision rate of particles due to changes in viscosity. Future research topics include: i)interactions with small scale processes; ii) mode of action of the toxic principle; iii) expansion of the present model to other ichthyotoxic species; and iv) studies of rheological modifications of the medium.

4.5 Environmental Control of Toxin production and accumulation (B.Reguera)

A brief presentation illustrated a conceptual model of studies of the interactions bewteen bivalve shellfish and toxin producing species. Ongoing research is targeted to determing the growth and toxin kinetics of toxic species of interest, including the key parameters of growth as a function of nutrients, such as Vmax, Ks and µmax, based upon work with cultures under controlled conditions, as well as obtaining estimates of *in situ* growth rates.

4.6 Blue-green algae population dynamics in the Baltic Sea (K. Kononen)

The main source of data for this project comes from the unattended monitoring system run by the Finnish Institute of Marine Research and additional cruises in the Baltic Sea. Two

species constitute the main problem, and cause mortality of domestic animals: *Nodularia spumigena* and *Aphanizomenon flos-aquae.* The increase in production of these cyanobacteria is linked to nutrient injections and their capacity for inorganic N fixation. Nitrogen fixation activity, and DIN:DIP changes near the thermocline (aproximately 22 m depth) seem to be significant, as well as oxygen depleted bottom layers connected to P-rich, N- depleted waters. Nutrient distributions in the transects showed a subsurface maximum of P beginning at approximately 40 m and presisting to the bottom due to a thermohaline intrusion.

5. REVIEW PRESENT KNOWLEDGE OF THE ABILITIES OF CERTAIN HARMFUL ALGAE TO ADAPT TO AND MODIFY THE MICROSCALE PHYSICAL ENVIRONMENT BY MEANS VERTICAL MIGRATION, MUCILAGE SECRETION, COLONY FORMATION, ETC.

This session was led by P.Gentien, based on documents received from T.Wyatt, I.R.Jenkinson and E. Granéli, and further comments from the participants in the working group.

Understanding the population dynamics of a single species resides in the ability to formulate phenomenological models which account for the major processes influencing the development of a given population. Considerable attention has been given to eutrophication, grazing control and advection, as important features of HAB dynamics. We consider here subtle, but potentially very important processes which involve adaptation to, or modification of the microscale environment by HAB species. The initial focus is on vertical migration, mucilage production and colony formation. Although any attempts to generalize among all HAB species are doomed to fail, certain mechanisms are potentially important among certain groups. These strategies are highlighted below.

5.1 Vertical Migration

Diurnal vertical migration has been classically viewed as obligate behavior of many dinoflagellates that allows them to maintain high photosynthetic rates in nutrient depleted surface waters by migrating to depth at night to obtain nutrients. Although such behavior has been documented in the field for some species (for example, *Chatonella*, *Dinophysis* cf *acuminata*, *Gymnodinium catenatum*, have been found to migrate actively on a diurnal basis) , there is growing evidence that this behavior is not universal. It has been demonstrated that dinoflagellate vertical migration varies with light, temperature and nitrogen availability. Diurnal vertical migration by *Dinophysis norvegica* (Carpenter et al, 1995) or by *Gyrodinium aureolum* (or *Gymnodinium* cf. *nagasakiense*) has not been observed in the field or in large enclosures (Dahl and Brockmann, 1985;). The typical bloom pattern of this species seems to be an active selection of favorable depths for population development during the early stages of the blooms which often end up at the surface as the blooms culminate. The same pattern is observed with several *Dinophysis* spp. We caution that the concept of favorable environments is not limited to easily measured physiological parameters such as light and nutrient responses. Instead, there is growing evidence that all factors that affect population development or successful completion of life cycles must be considered.Nor should we extrapolate to a whole range of different species on the basis of the nuisances they may induce.

5.2 Colony Formation

Many phytoplankton species appear in the sea as colonies, which may be species-specific characters, but may also be triggered by environmental conditions. The ability to achieve sizes larger than those of individual cells is advantageous purely from physical point of view

by allowing the species to increase biomass while grazing pressure decreases. A further step to colony formation is collision of colonies and individual cells to form aggregates, a process which may be accelerated by excretion of organic substances. Aggregates are possible sites for several microbiological processes modifying the proximate environment of the algal cells on microcales. For example, aggregates of the cyanobacterium *Nodularia spumigena* are colonized by a rich community consisting of bacteria, microzooplankton, algae etc., apparently supporting an intensive recycling of nutrients inside the aggregates. The importance of these processes for bloom dynamics is poorly known and methods for studying these processes are not well developed.

Algal blooms with large-scale mucilage production have been a recurrent phenomenon during the last 200 years in the Adriatic Sea. Although the phenomenon is not new, the extent of the blooms may have increased during recent decades. The tourist industry, mainly on the Italian coast, has suffered substantial economic losses since 1988.

In the mucilage patches, mainly diatom species are found, but dinoflagellates and a large amount of bacteria are also present. Mucilage production has been blamed on phosphorus deficiency in the area. However, recent results (unpublished) show that nitrogen deficiency induces algae to produce more polysaccharides per cell. On the other hand, nitrogen deficiency is the situation where the lowest amount of phytoplankton biomass is produced. However, the polyssacharide production at the levels of so-called P limitation in the area is 10 times higher than under nitrogen limitation. Thus the conclusion is that there are enough inorganic nutrients available in the area to produce large algal biomasses and in consequence production of polysaccharides is high.

5.3 Mucilage Secretion

In the sea and in cultures, many species of algae produce extracellular polymers which have the propensity to increase viscosity and decrease shear stress at the spatial scales of individual organisms. Recent methods to determine the concentrations of ìnon-particulateî organic matter in seawater give typical values for the upper ocean which lie in the range 5 to 10 g.m-3, of which a large proportion is of molecular weight 10 kilodaltons and up. These are concentrations reported in bulk seawater samples. Components of this material from the bulk phase, from the sea surface microlayer, from aggregates or ìsnowî, and from "transparent exopolymer material" (Alldredge et al,1994) have been characterized in various ways, by direct in situ observations, and with the techniques of physical chemistry, histochemistry, rheometry, SEM, and so on. It generally seems to consist of large and complex mixtures of surface-active macromolecules, acidic polyuronides, mucopolysaccharides, some proteins-but it has not been well characterized yet for specific communities in the pelagic environment. The literature is rife with speculations concerning its biological functions (Decho,1990) which are probably very diverse, and in many cases it must affect a variety of processes simultaneously.

In those cases where exopolymers are mostly of algal origin, such as during blooms of species like *Gyrodinium aureolum*, the reported bulk values of their concentrations are minimal estimates since these blooms are restricted to thin layers. Since it is known that the bulk concentrations are already sufficient to cause marked rheological changes in seawater properties, these effects must be even more notable in microlayers with high algal biomass. Jenkinson and Wyatt (1992) analyzed the impact of exo-polymers on various aspects of phytoplankton ecology, including intra- and interspecific relations and the decay of turbulence. In addition, the enhanced cell concentrations in these layers might be expected to promote changes in viscosity directly even in the absence of exopolymers (Wyatt et al, 1993). Once established, the layers can interact further with the physical processes which formed them and cause changes in light penetration, temperature, and mixing rates. As these layers diverge from one another in space, the communities in them will evolve in

different directions. Under these circumstances it is clear that better information on the population dynamics will be achieved by tracking individual layers rather than bulk properties. As cells are effectively stuck together in these layers, they can resist dispersal, and the minimum patch sizes predicted by KISS (**Ki**estead and **S**lobodkin, **S**kellam) models are reduced from scales of kilometers to metres.

At present, we have very little knowledge of how exopolymeric materials behave at ambient shear rates, which are very low, between 0.0003 and 250 s-1. But it is already clear that a Newtonian model of viscosity and of turbulence damping is inappropriate for the spatial scales relevant to phytoplankton ecology. Excess non-newtonian viscosity can be detected in more than 50% of non-bloom samples from coastal waters and elasticity too in some of them. Newtonian materials do not possess elasticity, which means they cannot store energy. The critical parameter at given shear rates is the volume fraction of cells, aggregates, and other extracellular particulate and colloidal materials. This is not only because of the effect of these suspended materials on viscosity, but also because in combination with the shear regime, the rates of processes like the flocculation of cells to form aggregates are affected (Kiorbe, et al, 1994).

Exopolymers and microlayers allow plankton to escape from, or perhaps manipulate, some of the dictates of physics and chemistry (Jenkinson and Wyatt, 1995). They can both couple and uncouple processes which in accordance with purely physicochemical laws would take different courses. Examples are provided by the simultaneous occurrence of nitrogen fixation and oxygenic photosynthesis in *Trichodesmium* bundles-equivalent to division of labour in metazoans-, the coupling of carbon dioxide and nitrogen fixation in microbial consortia, and in a recently reported discovery, the alternating nitrate reduction and sulfide oxidation in *Thioplaca* mats in shelf depths off Chile (Fossing, et al., 1995). Microlayers also provide pastures for myxotrophs and phagotrophs, like e.g., *Dinobryon* in freshwater, and *Dinophysis* in the sea. They can also reduce the problems associated with intraspecific chemical communication, and chemical and other defense mechanisms, for example, against grazing.

A major question of interest in the present context is, what effects do exopolymers and their rheological properties have on the population dynamics of harmful algae? Perhaps a useful general starting point is to consider how exopolymers can alter the spatial-temporal covariance of say nutrients, which constrains growth rates when it is low. Regulation of the rate of turbulent dissipation can effect such an alteration. The effect of scarcity of resources on growth rates is one aspect of the question, and local depletion of resources points to the need to increase bulk flow which can be achieved by reducing dissipation rates. The loss terms in the population budget, principally grazing, are a second aspect, and exopolymers may also be effective in lowering the rates of these losses.

For the last 15 years or so, instruments have existed with which the mechanical properties of seawater (viscosity and elasticity) can be measured at shear rates as low as 0.002 s^{-1}. *In situ* root mean square (rms) shear rates range from less than 10^{-5} in deep-water density discontinuities to about 1 in surface water during winds of Force 4. Because of intermittency in turbulence, at any one time more than 98% of the water experiences shear rates less than the rms value.

Measurements of high-shear-rate drag reduction (induced by elastic effects) in water from a dinoflagellate bloom, and of elasticity and increased and variable viscosity, both correlated to phytoplankton biomass in water from both bloom and non-bloom conditions (Jenkinson, 1993) appear to have confirmed older suggestions (Ostwald, 1902; Hutchinson, 1967; Margalef, 1978) that phytoplankton modifies local viscosity fields. Marked heterogeneity in viscosity and elasticity (Jenkinson, 1993), even within the same samples, indicates a general tendency to flocculation, confirming Morel and Gschwend's (1987) conclusions drawn from

the dynamics of chemical reactions in natural waters, which suggested that adsorption was occurring on previously unsuspected surfaces and flocs, in addition to the well-know marine organic aggregates.

It now recognised that thickening of the sea occurs not only in exceptional events, such as Adriatic *mare sporco* or *Phaeocystis* foam build-up and net sliming. It is also a more general phenomenon, acting over various length scales, and it plays an important role in various types of exchange, including those of heat and gases at the sea surface, also influenced by the viscoelasticity of the surface film.

Progress in the rheology of the bulk phase of seawater has undoubtedly been retarded by the lack of rheological expertise among oceanographers and a corresponding lack of oceanographic knowledge among rheologists which might have allowed them to appreciate the implications for oceanic processes. Interdisciplinary, rheological/oceanographic expertise should be fostered as a priority, particularly among physical oceanographers, geochemists, biogeochemists and plankton ecologists.

Summary

Current modelling of the dynamics of algal populations is concerned mainly with those processes which regulate growth (nutrients,light,...), and losses due to grazing and dispersal. But there are other processes,some of them probably under algal control,which may modulate or even dominate these terms.These additional aspects of algal population dynamics have not so far received sufficient attention.

Each harmful species has evolved its own unique survival strategy.Generalizations covering a variety of distinct species are not in order.

The extrapolation of in vitro experiments to the biological-physical coupling processes of algae in their natural habitat on the relevent temporal and spatial scales cannot yet be realized.

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6. DESCRIPTIVE INFORMATION ABOUT ONGOING MONITORING PROGRAMMES ON PHYTOPLANKTON AND PHYCOTOXIN MONITORING WITHIN ICES COUNTRIES ACCORDING TO THE QUESTIONNAIRE PREPARED BY IOC

The subgroup felt that much progress has been made on this subject due to the IOC-ICES questionnaire exercise which is currently underway. This questionnaire, recently distributed worldwide by IOC and ICES, was devised in response to previous WG recommendations to collate information from ongoing monitoring prgrammes for phycotoxins and harmful algal species. The major aims of the questionnaire are to collate information from countries with

ongoing monitoring in order to advise developing countries currently without such programmes on suitable designs upon which to base new ones, according to the specific needs of their region in terms of the nature of their HAB problems, and to provide a forum for the exchange of information between ICES countries. These tasks may, among other things, be carried out through workshops or symposia, and several members of the WG have agreed to act as advisors for such events. Countries which have not replied will be contacted and reminded of the importance of replying, even if only to report nil returns.

The format to be used for data storage and analysis has not yet been decided, but in order to make the results accessible and useful, it is envisaged that data should be deposited in a computer database which can be consulted by both IOC and ICES. The questionnaires are processed on receipt, and initial results distributed when available. It was confirmed that the raw data in the form of copies of the questionnaire will be available for consultation in these places. It was also suggested that the information could be made available over the Internet, as the IOC Science and Communication Centres on Harmful Algae plan to use the Internet as a means of disseminating data. An initial overview of the questionnaire results is planned for June 1995. The WG agreed that two types o f reports should be prepared - one for administrators (APHAB III, June, 1995) and another for the scientific community, and this should be taken into account when deciding on how the data should be analysed.

Results from the questionnaire provisionally identified as being of particular interest both to countries with ongoing programmes and those in the planning stage are the costs of monitoring in relation to the value of the resource being protected (cost-benefit analysis) and how success of monitoring may be assessed. Of special interest to developing countries will be the initial costs involved in setting up a laboratory for monitoring purposes in addition to the ongoing running costs. It was noted that any recommendations made to developing countries regarding methodology should consider new and proven techniques rather than less appropriate ones adopted in the past, but now which may be now improved based on recent developments. At the next ICES-IOC WG, the results from the questionnaire exercise should be presented and discussed and plans formulated for application of results, e.g. compilation of an inventory of current techniques, guidelines for developing countries, intercalibration of methods, etc.

7. DISCUSS THE POSSIBILITY OF PLANNING A FUTURE PRACTICAL WORKSHOP ON MODELLING USING REAL DATA OBTAINED IN MONITORING AND PROJECTS RELATED WITH HAB DYNAMICS

Plans to develop a practical workshop, using real data, on modelling HABs were discussed, in which the main aim would be to couple biological and hydrodynamic mechanisms regulating the population dynamics of harmful or toxic species, but not the total phytoplankton biomass. Current coupled models are already addressing the latter problem. In the context of species-specific models, it was felt that small scale phenomena are likely to emerge as significant regulating mechanisms, and the appropriate scales of simulation are the meso- and micro-scales.

The suggestion that real data be used in such an exercise was thought to be impracticable, given the limited time available at these kinds of meeting. It was also pointed out that useful models could not be constructed during the course of a short meeting, so that considerable preparation in advance would be necessary for a successful workshop. There was general agreement that such a workshop should deal with the bloom dynamics of selected target species, and not e.g., with toxin dynamics. It was also stressed that models of particular species should not loose sight of the general dynamic features of other algal populations, and that there should be some focus on driving mechanisms other than nutrient fluxes and so on (see above).

Several participants supported the idea of inviting modelling experts to give presentations in the next meeting of the WGHABD. These presentations might for example address the distinctions between biomass and species-specific model requirements, and sensitivity analyses of the different components of model vector.

8. DEFINE THE TIME AND SPACE SCALES OFTHE PHYSICAL AND BIOLOGICAL PROCESSES RELEVANT TO STUDIES OF PHYSICAL-BIOLOGICAL INTERACTIONS IN HAB DYNAMICS

On the afternoon of 18 May, members of the WGHABD and the WG on Shelf Seas Oceanography met in a joint session to discuss issues of fine scale processes and identify specific topics wherein lack of information or understanding is a major impediment to progress. After initial considerations on time and space scales, major features (plumes, fronts) and limitations of existing instrumentation, the discussion focused on water column structure, vertical velocity and distribution of phytoplankton cells. The degree of shear, turbulence, wind mixing, dispersion and resuspension all strongly influence water column structure and the distribution of phytoplankton cells.

Recent observations by Donaghey, Gentien, Dahl, Lindahl, Edler and others on the thin layers of phytoplankton biomass suggests that both biological and physical factors interact to produce fine scale structure. Species differences play a role. Diatoms rely on turbulence or a density gradient to remain in the photic zone while flagellates have the ability to form very sharp boundaries. At the same time, the flagellates, or some of their specific growth stages may be highly sensitive to turbidity and shear. Blooms may settle out of the water column overnight after a storm system passes through the area. In other cases toxin production or cell leakage may differ after physical disturbances. The roles of cell division and growth in layer formation or maintenance are not well understood. Considerations were also given to sampling protocol. How is the sampling method affecting or "creating" the observations of layered structure?

The ideas were numerous, the discussions were vigorous, but the pivotal question, ultimately, was whether there was a sufficient level of interest created to promote joint research. While physicists find it very difficult to measure vertical velocity, small gradients or disturbances, recent developments in instrumentation are able to detect small scale changes in phytoplankton distribution. Perhaps the physicists can use the "effect" to help deterrmine the "cause" while the biologists catchup on 25 years of research on fine scale processes, much of which has not propagated through their literature or been widely appreciated until recently. Much of the early succes of our joint research will be in phrasing questions that can be answered now, helping to cross educate our research patterns and building on advances in each of the fields.

9. NATIONAL REPORTS

The compiled national reports are appended in Annex III. Country members presented a summary of their respective national reports. In a following discussion they all agreed that the information provided in the reports could be very much improved, but so far, it constitute the only available information on toxic outbreaks in the ICES domain, that is quite often consulted by managers and administrators.

To obtain a better information on these events, it was suggested the possibility of dedicating a good session next year to discuss plans for synthesizing the information from the last ten years and even do a general "mapping" that would provide a graphic time series of the events.

Following are some of the summaries given in this session:

CANADA

Shellfish harvesting areas were closed in various regions as a result of unacceptable levels of paralytic shellfish toxins, domoic acid or diarrhetic shellfish toxins. Areas closed due to PST were similar to previous years. Areas affected included Notre Dame Bay in Newfoundland, Bay of Fundy. St. Lawrence Estuary and the coast of British Columbia. Although domoic acid was detected for the first time in Newfounland, closures due to domoic acid were relatively minor in Canada with only one Bay in the Gulf of St.Lawrence closed to harvesting. DST were responsible for six cases of poisoning in Bonavista Bay, Newfoundland. These represent the first recorded incidents of DSP in Newfoundland.

DENMARK

Exceptionally high phytoplankton biomasses were registered in the summer period. This biomass was dominated by *Rhizosolenia fragilissima* and *Prorocentrum minimum.* Exceptionally high concentrations of *Dinophysis acuminata* were registered, and DSP toxins were registered in mussels in three regions in the summer period. Neither PSP nor ASP or fish kills were registered.

NORWAY

The recurrent blooms of *Alexandrium* spp., *Dinophysis* spp., *Gyrodinium* cf. *aureolum* and *Prymnesium* cf. *parvum* were below average, causing minor problems. A mixed bloom of *Chrysochromulina* spp. along the Skagerrak locally reached 5,000,000-10,000,000 cells/l. No effects on farmed fish or wild biota were recorded, but the bloom was slightly toxic to *Artemia salina* in a bioassay.

PORTUGAL

As in 1993, all the Portuguese coast was affected by a PSP outbreak that started in the south and progressively spread to the North. DSP occurred in 1994 but only in isolated zones with the most affected area being the Aveiro Lagoon.

SPAIN

Several areas within the Galician Rias Bajas were subject to the usual occurrence of Dinophysis cf acuminata in several pulses ,between spring and autumn, that caused closures of mussel and other bivalve harvesting, and a moderate PSP outbreak in the autumn (October to December) caused by Gymnodinium catenatum.

Alexandrium taylorii, a non toxic dinoflagellate previously unknown in the area, formed green patches in some Costa Brava (Northern Catalonia) beaches, affecting tourism negatively. A previously unknown Gyrodinium sp bloomed in the Ebro Delta region during winter 1994 causing mortality of fish cultivated in ponds and of natural populations of mussels.

SWEDEN

Along the Swedish west coast, (i.e. The Kattegat and Skagerrak coasts) there were no real large and harmful blooms in 1994. Moderate blooms of *Chrysochromulina* spp., *Prorocentrum micans, P. minimum* and *Ceratium furca* were observed. No adverse effects from these blooms were reported.

DSP was registered along the west coast from August and through December. Values of okadaic acid ranged from 0 to 400 ug/100 g mussel meat. In the Baltic Sea, there were significant blooms of cyanobacteria (*Nodularia spumigena* and *Aphanizomenon "baltica"* during July and August. Samples from Gdansk Bay in the southeast Baltic Sea were toxic. No adverse effects are known from this bloom.

In May there was a large bloom (1.4 million cells.l^{-1}) of the non-toxic dinoflagellate *Peridinella catenata* in the southeast Baltic Sea. In August, high abundance (ca. 100,000 cells.l^{-1}) of *Dinophysis norvegica* and *D. acuminata* were found in the central Baltic Sea. These populations were found at 14-17 m depth, whereas the abundance closer to the surface was very small.

UNITED STATES

1994 was a "normal" year for the U.S. with respect to HAB incidents, with several unusual events. With respect to PSP, harvesting quarantines were issued in areas where this form of shellfish toxicity has often occurred in the past (e.g. Maine, Massachusetts, California, Oregon,Washington, and Alaska). One unusual item of note is that 14 illnesses and 2 human mortalities occurred in Alaska from PSP. That state has no monitoring program for recreational or subsistence harvesters, and the warning that shellfish are always dangerous to eat is often ignored. The state is presently re-evaluating its policies to provide better protection for non-commercial harvesting.

The brown tide of *Aureococcus anophagefferens* bloomed again in several bays of Long Island, with negative aesthetic impacts rather than mortalities of shellfish, larvae, and submerged vegetation as has occurred in the past. A spectacular and unusual brown tide of a related but different chrysophyte species continued to bloom in Texas - the fifth year of this persistant bloom. No toxins are produced, but the dense bloom is altering food web structure and damaging submerged aquatic vegetation.

Domoic acid was detected in the states of California and Washington, but at low levels that did not require management action. Fish-killing red tides of *Gymnodinium breve* occurred in Florida, again an annual phenomenon, as well as to a minor extent in the Pacific northwest (*Heterosigma carterae*). A mass mortality of demersal fishes and crustaceans occurred over a three month period along the coast of eastern Texas and Louisiana. Low oxygen was perhaps involved, but the exact cause of mortality is still not known. Dominant phytoplankton species were *Gymnodinium sanguineum* and *H. carterae*. The investigation of these mortalities was complicated by concurrent mortalities of sea turtles, dolphins, and seabirds.

10. ELECTION OF A NEW CHAIRMAN OF THE WGHABD

After proposals of different candidates and voting by the members of the group, Patrick Gentien (France) was proposed as new the Chairman of the Working Group on Harmful Algal Bloom Dynamics to be appointed in the next Annual Science Meeting (Aalborg, Denmark, September 1995).

11. RECOMMENDATIONS, PROPOSALS OF TERMS OF REFERENCE FOR NEXT YEAR'S MEETING AND OTHER BUSINESS.

The participants agreed that the WGHABD should keep its focus on bloom dynamics, and leave aside toxicological models. There is a plan for a NATO ASI meeting on ecophysiology

of harmful algae to be held in late May 1996 (Chair: D.M. Anderson, USA), and the proposed chairman for the next Worshop on *in situ* growth rate measurements (O. Lindahl, Sweden) would like a planning session during the WGHABD as early in the spring as possible.

For these reasons, the group suggested to meet from 17 to 20 April 1996, with the option to change to May due to time constraints on the WG on Shelf Seas Oceanography.

Nevertheless, as recalled by the ongoing chairman of the WGSSO (H. Dahlin, Sweden), the original plan, when the WGHABD was established, was to form a interdisciplinary group with hydrographers, modellers, and plankton experts within its members. In practise, the interdisciplinar communication has been limited to the joint sessions of the WGSSO with the WGHABD (mainly composed by phytoplankton experts).

The Working Group on Harmful Algal Bloom Dynamics recommends that:

1. Key experts on organismal behaviour related to physical/biological interactions be invited to give presentations and to assist the Working Group members on the analysis of discrete HAB data sets.

2. A theme session on "Small-scale physical biological interactions relevant to HAB dynamics" should be proposed to be held during the ICES Annual Science Conference in 1997, to encourage the presentation of interdisciplinary papers on this subject;

3. A Workshop on "Development of *in situ* growth rate measurements for dinoflagellates" should be held in Kristineberg (Sweden) from 9 to 15 September 1996 under the chairmanship of Odd Lindahl (Sweden), to bring to completion the activities initiated during the previous workshop (see page 20 from annex IV);

4. The ICES/IOC Working Group on Harmful Algal Bloom Dynamics (Chairman: Patrick Gentien, France) will meet in Brest (France) from 17 to 20 April 1996 to:

a) Complete and discuss the logistic planning of the ICES/IOC Worshop on development of *in situ* growth rate measurements (Chairman: Odd Lindahl, Sweden) to be held in Kristineberg (Sweden) from 9 to 15 September 1996, and examine the results of intersessional progress;

b) Continue the development of an understanding of the dynamics of harmful algal blooms, including presentations of recent experimental results;

c) Collate and discuss national reports on HABs and initiate a synthesis of the national reports of the last ten years, and map outbreaks and compile time series of HABs in the ICES area;

d) Review the updating of the ICES Cooperative Research Report nº 181 on the "Effects of HABs on mariculture and marine fisheries" carried out in the intersessional period;

e) Discuss items related to the monitoring of HABs based on the compilation of answers to the IOC-ICES Questionnaire, and considerations by the IOC-FAO Intergovernmental Panel (IPHAB), in order to give advice on further activities, including planning of an international workshop on HAB monitoring and mitigation strategies;

f) Review and discuss recent work on the effect of harmful algae on zooplankton, including discussion of methods to be applied in these studies.

12. CLOSING OF THE MEETING

On Friday 19 May at 1730, the outgoing chairman, Beatriz Reguera (Spain) thanked the participants for their support and collaboration during the last three years, and encouraged them to continue with the same attitude.

ANNEX I: LIST OF PARTICIPANTS

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ANNEX II: APPROVED AGENDA

1. Welcome and opening of the meeting.

2. Appointment of rapporteur.

3. Approval of the agenda

4. Review the results of the ICES/IOC Workshop on intercomparison of *in situ* growth rate measurements of dinoflagellates.

5. Ongoing activities in the pilot study areas, and other ICES areas on physical-biological interactions related to HABs.

6. Review present knowledge of the abilities of certain harmful algae to adapt to and modify the microscale physical environment by means of vertical migration, mucilage secretion, colony formation, etc.

7. Descriptive information about ongoing monitoring programmes on phytoplankton and phycotoxin monitoring within ICES countries according to the questionnaire prepared by IOC.

8. Define the time and space scales of the physical and biological processes relevant to studies of physical-biological interactions in HAB dynamics.

9. Discuss the possibility of planning a future practical workshop on modelling using real data obtained in monitoring and projects related with HAB dynamics.

10. Presentation of National Reports on HAB episodes during 1994.

11. Election of a new Chairman of the WGHABD.

12. Proposals of terms of reference for next year's meeting and other business.

13. Closing of the meeting.

ANNEX III: NATIONAL REPORTS

HARMFUL ALGAL BLOOMS IN 1994 - CANADA

1. Location: Various sites in Bonavista Bay, Newfoundland.

2. Date of occurrence: Late October, 1993.

3. Effects: At least six cases of diarrhetic shellfish poisoning in two seperate incidents involving consumption of blue mussels, collected from wild sites.

4. Management decision: Entire Bay closed for all shellfish harvesting and reopened in August 1994.

5. Causative species: *Dinophysis norvegica* implicated. Other potentially toxic species of dinoflagellate *Prorocentrum* sp. were also present in small numbers.

6. Environment: Water temperature at this time of the year is usually around 6° C and the water column is well mixed and isothermal.

7. Advected population or *in situ* growth: Unknown. *Dinophysis* sp., *Prorocentrum*, and other species of dinoflagellates are usually common in the fall months in this area. Therefore, *in situ* growth is implied.

8. Previous occurrences: This is the first recorded incidence of DSP in Newfoundland, and second only on the Atlantic coast of Canada.

9. Additional comments: The analysis of the toxin showed concentration levels of trace to 4.0 µg/g digestive gland of DTX-1. Presence of trace quantity of Okadaic Acid was confirmed.

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HARMFUL ALGAL BLOOMS IN 1994 - CANADA

1. Location: Various sites in Newfoundland coastal waters

2. Date of occurrence: Various times in 1994.

3. Effects: NA

4. Management decision: No closure for shellfish harvesting, as levels of Domoic Acid were low.

5. Causative species: Unknown

6. Environment: NA

7. Advected population or *in situ* growth: Unknown.

9. Additional comments: The analysis of the toxin showed concentration levels of trace to 6.3 µg/ g shellfish meat. This is the first recorded incidence of ASP in Newfoundland waters.

10. Individual to contact:

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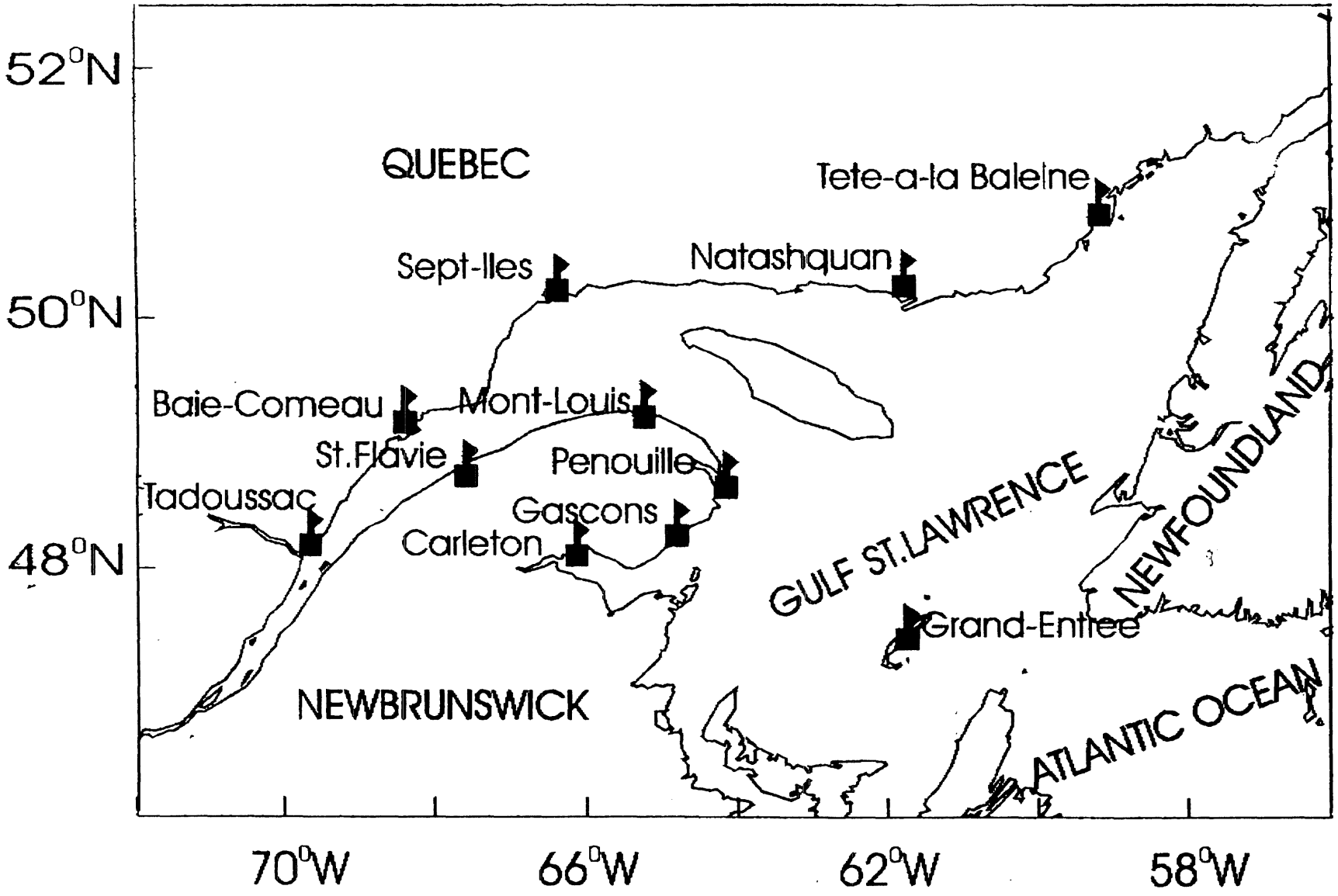
52°N
50°N
48°N
70°W
66°W
62°W
58°W
QUEBEC
Tete-a-la Baleine
Sept-Iles
Natashquan
Baie-Comeau
Mont-Louis
St.Flavie
Penouille
Tadoussac
Gascons
Carleton
GULF ST.LAWRENCE
NEWFOUNDLAND
Grand-Entree
NEWBRUNSWICK
ATLANTIC OCEAN

Preliminary results from the 1994 Harmful Algae Monitoring Programm (Quebec Region). Location and date where maximum concentration of potentially harmful algae were measured.

Species	Station	Date	Abundance
			(cells l^{-1})
Alexandrium spp.*	Ste Flavie	August 10	107,000
	Sept-Iles	July 22	49,000
Dinophysis spp.	Penouille	June 08	17,000
Gyrodinium aureolum	Mont-Louis	Sept. 09	82,000
Prymnesium parvum	Penouille	July 21	4,700

* Include *A. tamarense*, *A. excavatum* and *A. fundyense* and *A. ostenfeldii*

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IML
Mont Joli
Quebec

Harmful Algal Events in 1994 - Canada
Domoic Acid

1. **Location**: Bay of Fundy

2. **Date of Occurrence:** No shellfish harvesting areas were closed due to unacceptable levels of domoic acid in the Bay of Fundy during 1994.

3. **Effects:** None.

4. **Management Decision:** None required.

5. **Causative Species:** *Pseudonitzschia pseudodedicatissima.* Cells were observed throught the year with highest concentrations observed during June and August. Highest concentrations observed during 1994 were 248,064 cells/liter on June 21st at a sampling location in Lime Kiln Bay.

6. **Environment:** Temperature range: 8 - 12° C
   Salinity 32 ppt
   Water Column mixed

7. **Advected Population or insitu Growth:** Insitu.

8. **Previous occurrences:** Shellfish areas were closed to harvesting during 1988, but have not been closed since.

9. **Individual to contact:**

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Harmful Algal Events in 1994 - Canada

1. **Location**: Bay of Fundy

2. **Date of Occurrence:** No shellfish harvesting areas have been closed due to unacceptable levels of DSP toxins in the Bay of Fundy to date.

3. **Effects:** None.

4. **Management Decision:** None required.

5. **Causative Species:** *Dinophysis acuminata*. Highest concentrations observed during 1994 were 640 cells/liter on July 26th at a sampling location near the islands called "the Wolves".

6. **Environment:** Temperature range: 12° C

Salinity	32 ppt
Water Column	mixed

7. **Advected Population or insitu Growth:** Advected

8. **Previous occurrences:** Shellfish areas have never been closed in the Bay of Fundy for DSP toxins to date.

9. **Individual to contact:**

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Harmful Algal Events in 1994 - Canada
Domoic Acid

1. **Location**:Gulf of St. Lawrence - New London Bay

2. **Date of Occurrence:** October 28, 1994.

3. **Effects:** Domoic acid levels of 31.5 ug/g were measured from blue mussels.

4. **Management Decision:** Harvesting was not permitted.

5. **Causative Species:** *Pseudonitzschia pungens*. Highest concentrations observes were 314,000 cells/liter.

6. **Environment:**

7. **Advected Population or insitu Growth:**

8. **Previous occurrences:** Domoic acid was first detected in 1987 in the Gulf of St. Lawrence.

9. **Individual to contact:**

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Harmful Algal Events in 1994 - Canada
PSP

1. **Location**: Bay of Fundy

2. **Date of Occurrence:** The majority of shellfish harvesting areas were closed to harvesting either for some time during or for the duration between late May and mid-August.

3. **Effects:** Levels of PSP toxins in tissues exceeded the safe harvesting level of 80 ug/100g resulting in closures of shellfish areas. Extractions were conducted at the Department of Fisheries & Oceans Inspection Laboratory at Black's Harbour, New Brunswick.
   Highest levels measured were:
   Mya arenaria - 3400 ug/100g - July 5, 1994 - Crow Harbour.
   Mytilus edulis- 4600 ug/100g - July 12, 1994-Beaver Harbour

4. **Management Decision:** Shellfish harvesting areas were closed to harvesting during the time when levels of PSP toxins were greater than 80 ug/100g. The Bay of Fundy is also closed to the harvesting of blue mussels throughout the year.

5. **Causative Species:** *Alexandrium fundyense.* Although cells were observed throughout the region, highest concentrations observed during 1994 were 53,856 cells/liter on June 28th at a sampling location at Deadmans Harbour.

6. **Environment:** Temperature range: 6 - 12° C

Salinity	32 ppt
Water Column	mixed

7. **Advected Population or insitu Growth:** Advected

8. **Previous occurrences:** Shellfish areas are closed in the Bay of Fundy annually (generally during summer months) due to unacceptable levels of PSP toxins.

9. **Individual to contact:**

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HARMFUL ALGAL BLOOMS IN 1994 - CANADA

1. Location: Various sites in Notre Dame Bay, Newfoundland.

2. Date of occurrence: August 1994 to October 1994.

3. Effects: NA

4. Management decision: Entire Bay closed for all shellfish harvesting in August 1994 and reopened for harvesting in October 1994.

5. Causative species: *Alexandrium fundyense* implicated. Other potentially toxic species of dinoflagellate *Prorocentrum* sp. were also present in small numbers.

6. Environment: NA

7. Advected population or *in situ* growth: Unknown. There are several sites on the south and east coast of Newfoundland where hypnozygote of *Alexandrium fundyense* have been found in the sediment. Resuspension and ingestion of these cysts by mussels may also be one of the causes of toxicity.

9. Additional comments: The analysis of the toxin showed concentration levels of trace to 420 µg/100 g shellfish meat.

10. Individual to contact:

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Status of HAB's for Danish waters in 1994

Compiled by Dr. Per Andersen, associated consultant for IOC, Danish Ministry of Fisheries and The Association of the Danish Musselfisheries. Bio/consult as, Johs. Ewaldsvej 42-44, 8230 Aabyhøj, Denmark.
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The phytoplankton situation in Danish coastal waters and fjords in 1994 was characterized by high concentrations and biomasses in the summerperiod, dominated by diatoms e.g. *Rhizosolenia fragilissima* and *Skeletonema costatum* and dinoflagellates *Prorocentrum minimum* and *Prorocentrum micans*. The high biomasses were the result of high external input from run-off from land during the winter and spring period, followed by high internal input of inorganic nutrients from the sediments as a result of oxygen deficiency in the exceptionally calm and sunny summerperiod.

The following toxic and potentially toxic algae were registered in high concentrations:

Dinoflagellates
Dinophysis acuminata

Prorocentrum minimum
Prorocentrum micans

Gymnodinium sanguineum

Noctiluca scintillans

The following toxic and potentially toxic algae were registered in low concentrations:

Dinoflagellates
Alexandrium ostenfeldii
Alexandrium tamarense

Dinophysis norvegica
Dinophysis acuta
Dinophysis rotundata

Gyrodinium aureolum

Diatoms
Pseudonitzschia delicatissima-group
Pseudonitzschia seriata-group

Others
Chrysochromulina spp.
Phaeocystis pouchetii
Nodularia spumigena

No fishkills were registered in 1994.

Harvesting for mussels were closed or restricted in most areas on the east coast of Jutland and in Isefjorden from may to september, and in one area in the Limfjord in most of june as well in two areas in the period from October to November because of high concentrations of *Dinophysis acuminata*, figure 1 and 2. DSP-toxins were registered at the east coast of Jutland (August), possibly in the Isefjorden (July) as well as in two areas in the Danish Waddensea region (August), figure 3 and 4.

PSP and ASP were not registered in 1994.

The concentrations of DSP-toxins in blue mussels (*Mytilus edulis*) were not a simple function of the registered concentration of *Dinophysis acuminata* in the areas, which might be explained by variation of toxicity of *Dinophysis acuminata* (as shown by measurements of the toxicity per cell from concentrated plankton samples using HPLC), as well as high biomasses of other phytoplankton organisms, which might lead to a decrease in the ingestion of *Dinophysis acuminata* by the mussels. Furthermore the hydrographic conditions might lead to a heterogenous distribution of Dinophysis acuminata in the water-column, with the result that the mussels does not "see" the concentrations calculated from the phytoplankton counts.

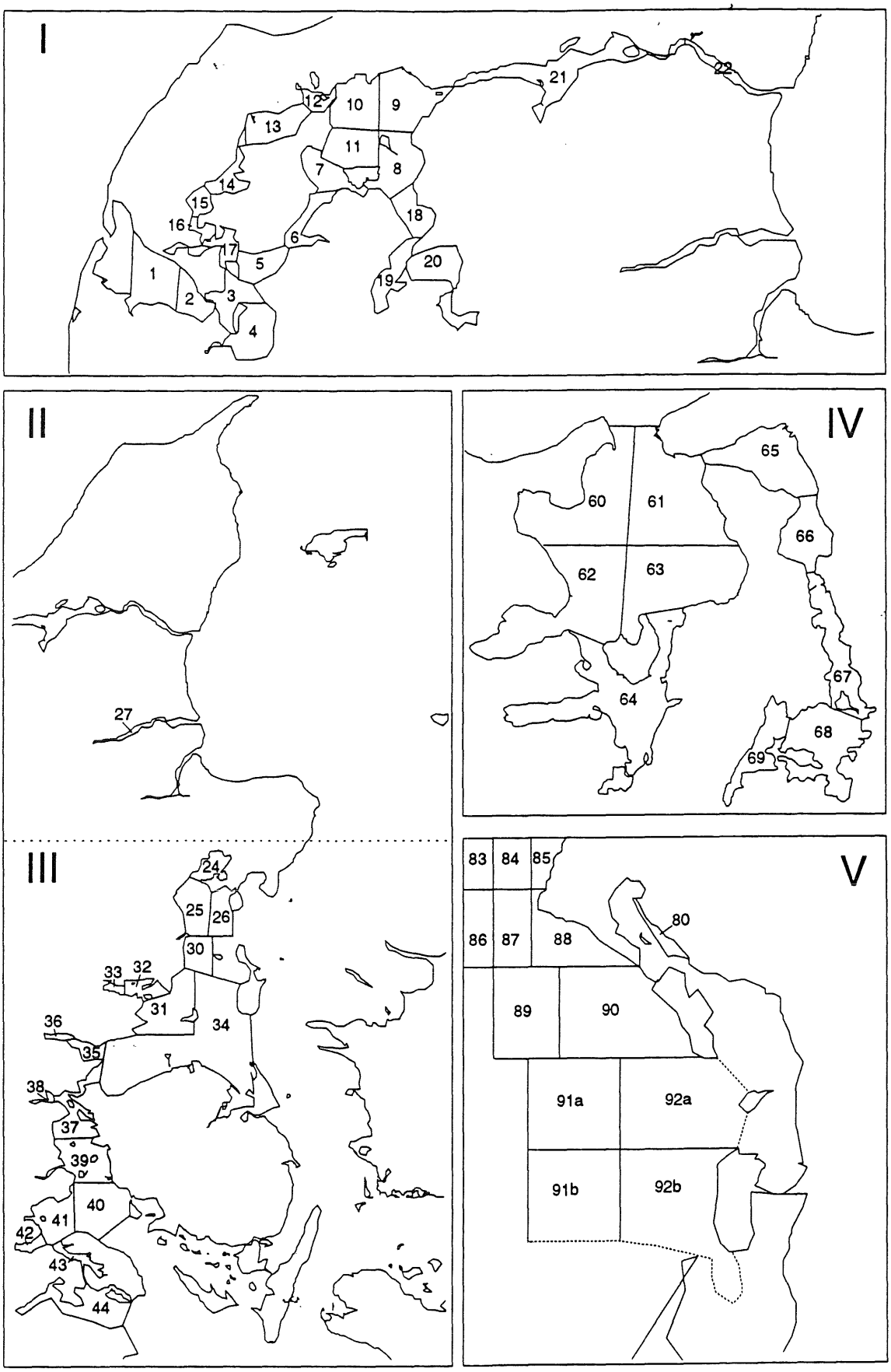

Figure 1. Map showing the different areas used in the monitoring program for toxic algae in relation to the Danish musselfishery in 1994.

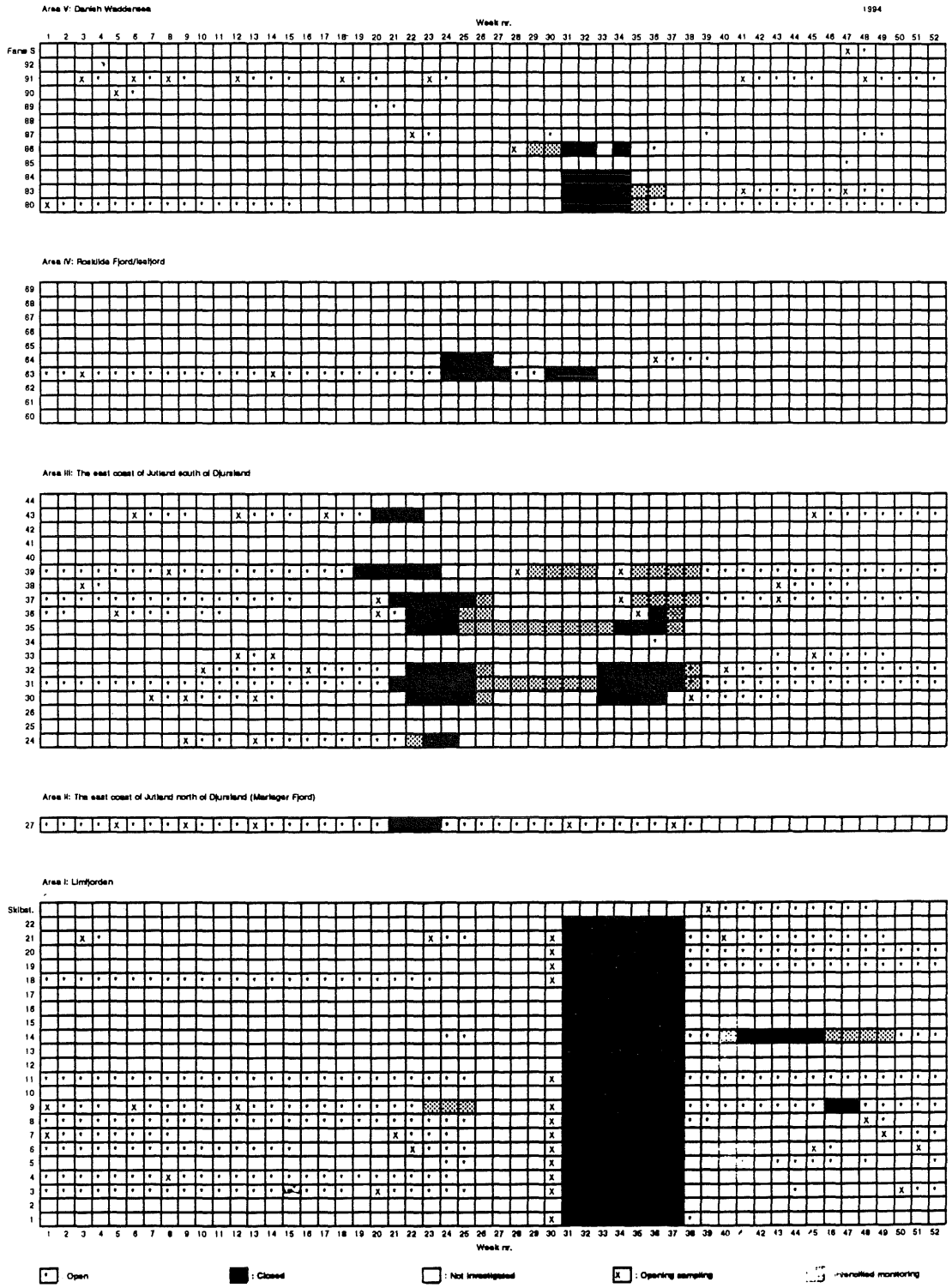

Figure 2. Management status of the Danish musselfishery in 1994. The closed period from week 31 to 37 in The Limfjorden is due to traditional "holiday" closure

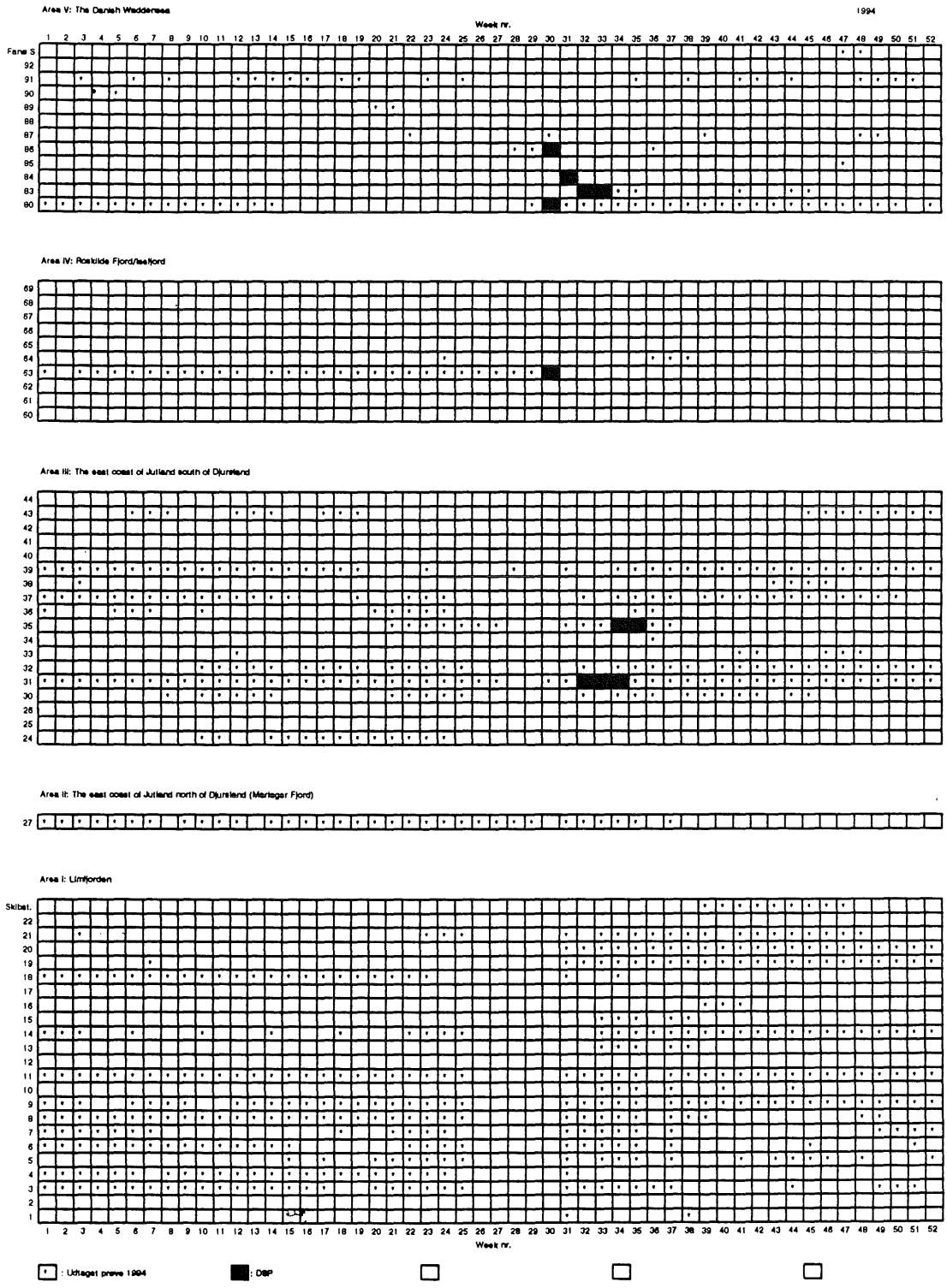

Figure 3. Map showing the temporal distribution of DSP-toxins in mussels in the different areas used in the monitoring program for toxic algae in relation to the Danish musselfishery in 1994.

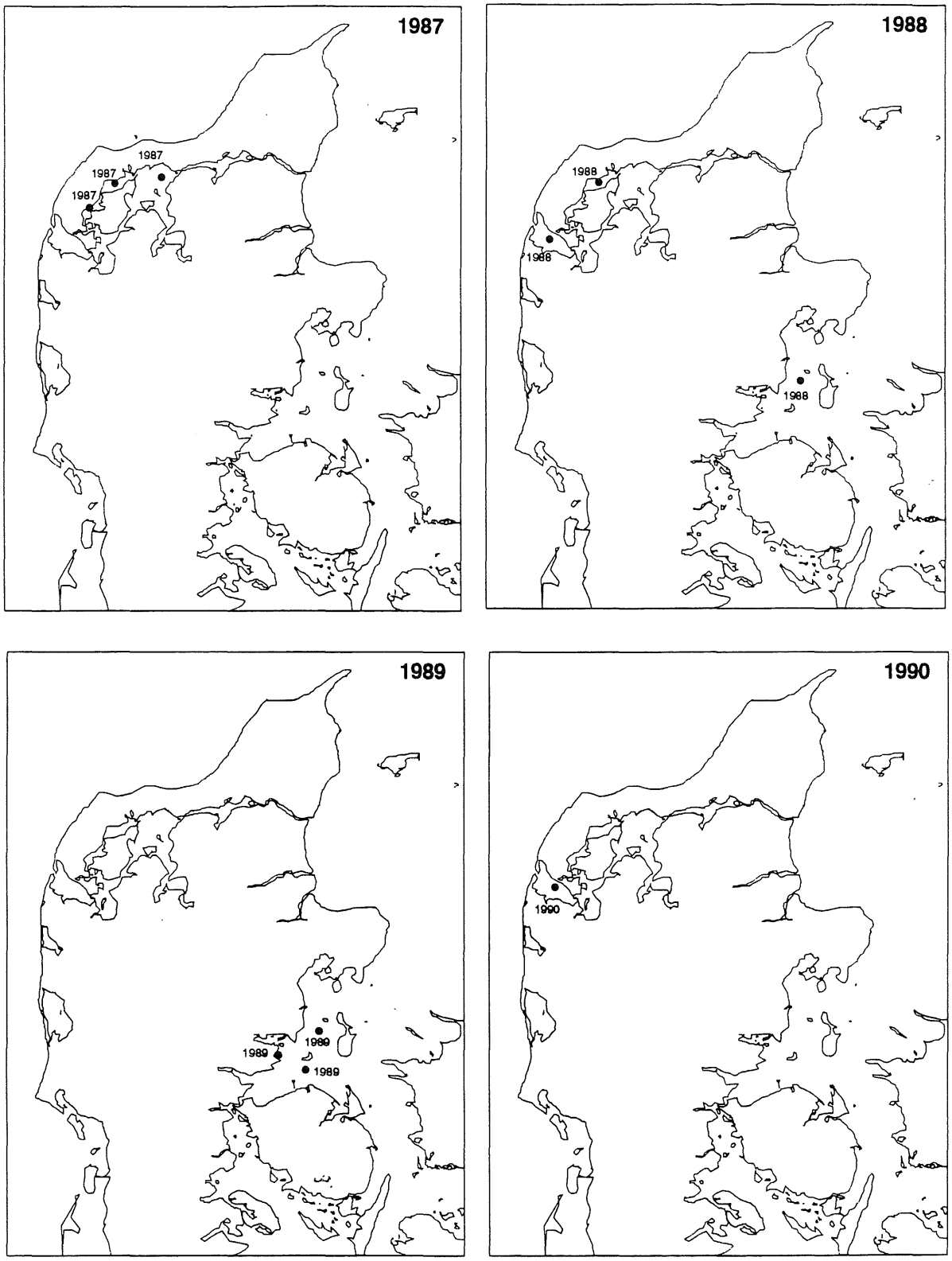

Figure 4a. Maps showing Danish areas with occurrence of DSP-toxins in mussels in the period 1987-1990.

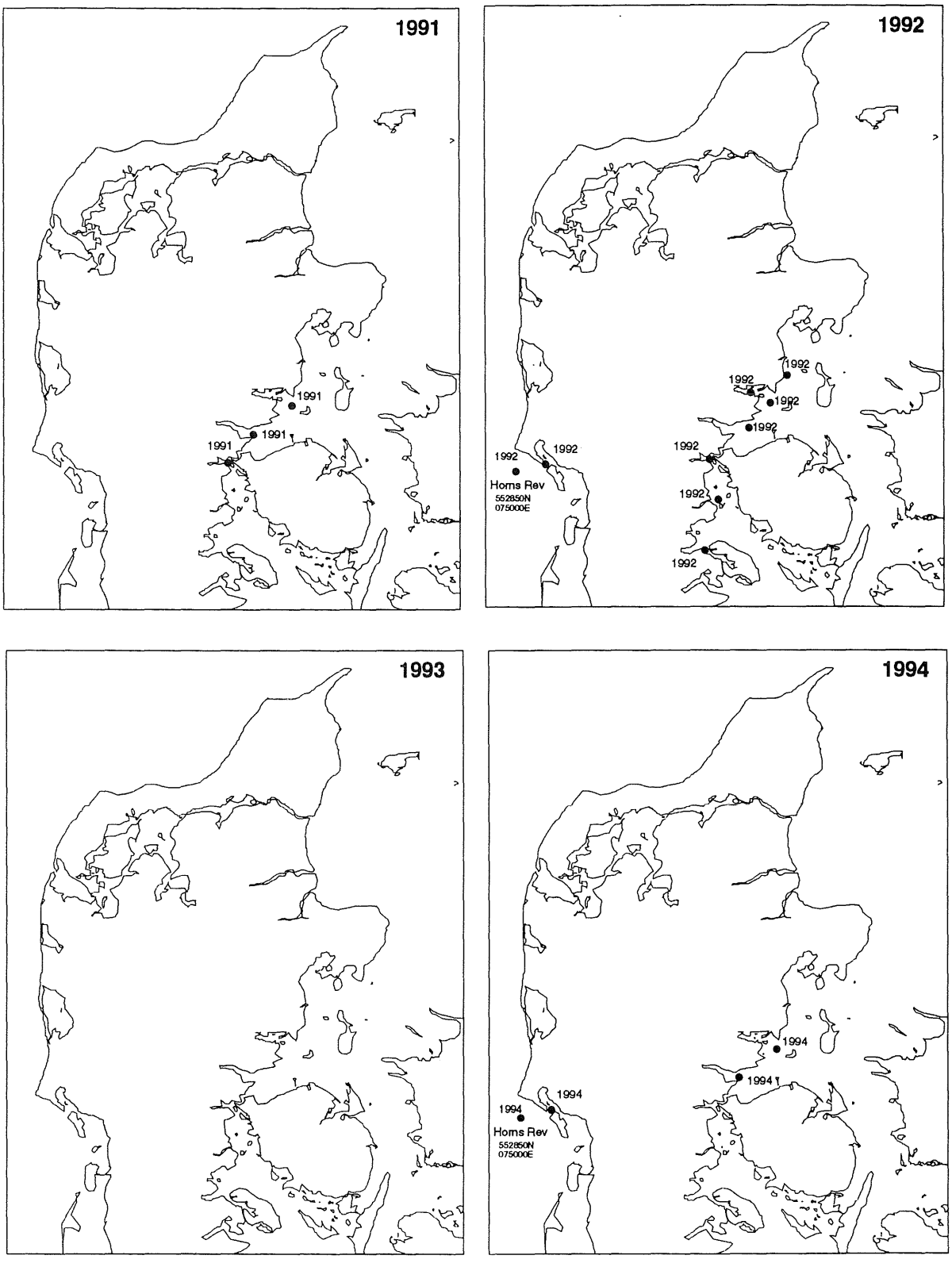

Figure 4b. Maps showing Danish areas with occurrence of DSP-toxins in mussels in the period 1991-1994.

HARMFUL ALGAL BLOOMS IN 1994 - FINLAND

1. Locations: Gulf of Finland, Baltic Proper

2. Date of Occurrence: July-August

3. Effects: not reported

4. Management decision: -

5. Causative species: *Nodularia spumigena*
Aphanizomenon flos-aquae

6. Environment: open sea

7. Advected population or in situ growth: in situ

8. Previous occurrences: yearly phenomena in the Baltic sea

9. Additional comments:

10. Individual to contact:

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MONITORING OF HARMFUL ALGAL BLOOMS
FRANCE - 1994

The French Phytoplankton Monitoring Network (REPHY) has sampling stations along the whole French coast. Sampling water is performed throughout the year, at less twice a month, on certain stations. Other stations are sampled when there is presence of toxic species, and shellfish are collected for toxicity tests (mouse tests in routine, HPLC analysis if confirmation is needed).

The toxic events recorded in 1994 are described in Fig. 1 :

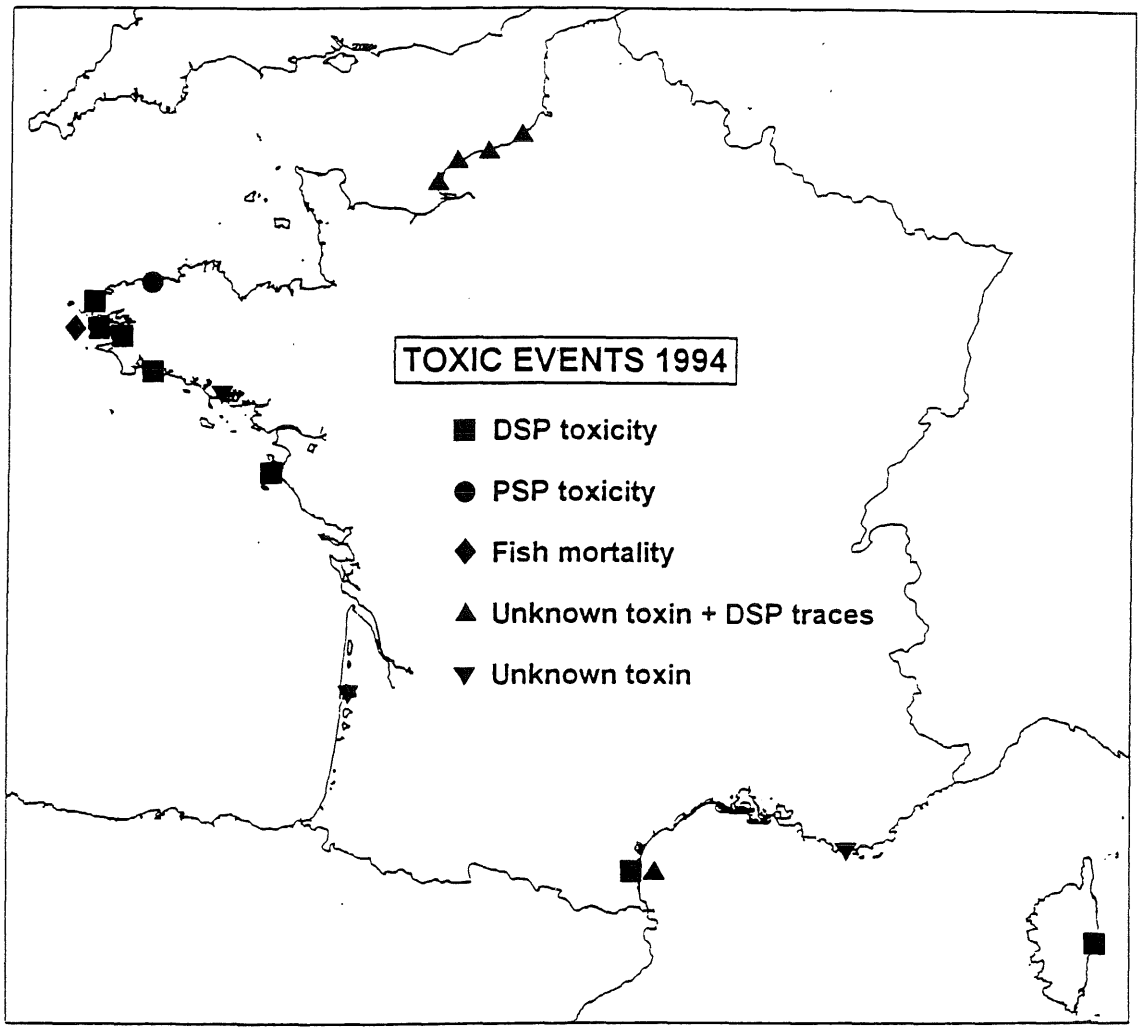
DSP toxicity affected a few areas, rather less than the previous years, and was always linked with presence of *Dinophysis* spp.

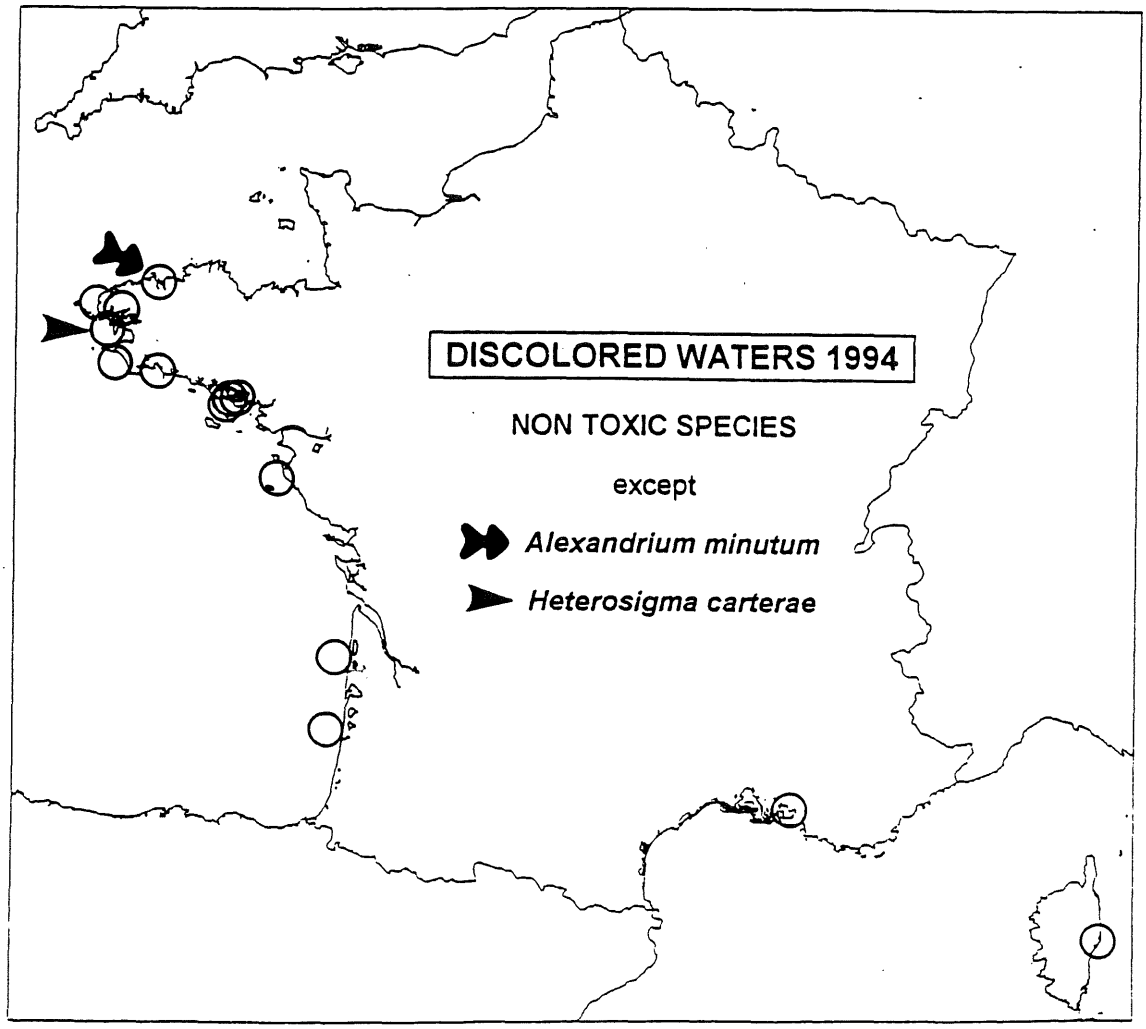
PSP toxicity was recorded in only one area, the same than past years, in northwestern Brittany. The toxic episode succeeded to a bloom of *Alexandrium minutum* (eight millions cells per liter).

Fish mortality was recorded in Camaret bay (western Brittany) in September : ten tons of trouts and one ton of salmon in cages were destroyed. The responsible species, *Heterosigma carterae* (= *H. akashiwo*) had been observed since later August in this area. On September 27-28, the cell counts increased very rapidly and reached 130 millions cells per liter. Rains, followed by sunny weather and absence of wind during a few days, associated with the fast growing of the species, might explain this unexpected increase of concentrations.
Heterosigma carterae (= *H. akashiwo*) was observed a few times before, in Atlantic and Channel waters, but it never reached such concentrations. So it is the first time that this species is associated with fish mortality in France

An **"unknown" toxin** was again recorded in shellfish of a few areas, like in 1993. This toxin, which was neither DSP nor PSP, killed mice in few minutes, and was not linked with a toxic or harmful phytoplankton species in water. In some cases, this toxin was present in shellfish with DSP traces produced by *Dinophysis*.

Discolored waters were primarily recorded along the Atlantic coast (Fig. 2). The main responsible species were *Eutreptiella sp., Alexandrium minutum, Gonyaulax spinifera, Heterocapsa triquetra, Rhizosolenia spp., Heterosigma carterae, Chaetoceros sociale and armatum, Prorocentrum micans, Mesodinium rubrum, Pseudonitzschia seriata, Noctiluca scintillans.*


TOXIC EVENTS 1994
DSP toxicity
PSP toxicity
Fish mortality
Unknown toxin + DSP traces
Unknown toxin


DISCOLORED WATERS 1994
NON TOXIC SPECIES
except
Alexandrium minutum
Heterosigma carterae

National Report: Germany 1994

North Sea:

From the middle to the end of June an increasing number of colonies and cells of *Phaeocystis pouchetii* and *Ceratium furca* respectively were found in the western part of the North Frisian Wadden Sea. At the same time, colonies of *Phaeocystis* were found in high numbers in the East Frisian Wadden Sea. In addition there was a blooming of *Phaeocystis* in the East Frisian Wadden Sea.

Prorocentrum redfieldii was not so abundant as in 1992 and *Gyrodinium aureolum* was not so abundant as in 1993.

During July some "Red Tides" were observed in the Helgoland Bight, caused by *Noctiluca miliaris*.

In the beginning of August there was an increase of *Mesodinium rubrum* around Helgoland with intensive water discolouring. This phenomenon was observed for a fortnight in the south western part of our sampling area.

At the end of July, DSP was detected in mussels (= *Mactra corallina*) about 20 miles offshore (Amrumbank = name of the fishing waters).

At the beginning of August, very high densities of *Dinophysis* species were found mainly in the area of the North Frisian Islands, up to 95,000 cells dm-3. Fishermen closed the area as a precautionary measure.

These exceptional high densities of *Dinophysis* occurred in the East Frisian Wadden Sea too.

But the fishermen didn't collect the blue-mussels, because not enough blue-mussels grew there in 1994. So it wasn't worth collecting them economically. One sample of DSP was detected on the south side of the small island Mellum.

Baltic Sea:

The diatom *Rhizosolenia fragilissima* showed high densities in the fjords and bights during the whole summer, partly causing a reddish colour of the water.

From mid to end of July *Prorocentrum minimum* was abundant in the Flensburg Fjord (up to 5 m cells dm-3). Maximum cell numbers (up to 90 m cells dm-3) were observed in the inner part of the Kiel Fjord in the beginning of September. They caused intensively red water, partly with orange coloured, slimy and oily surface film.

Toxic events were not reported.

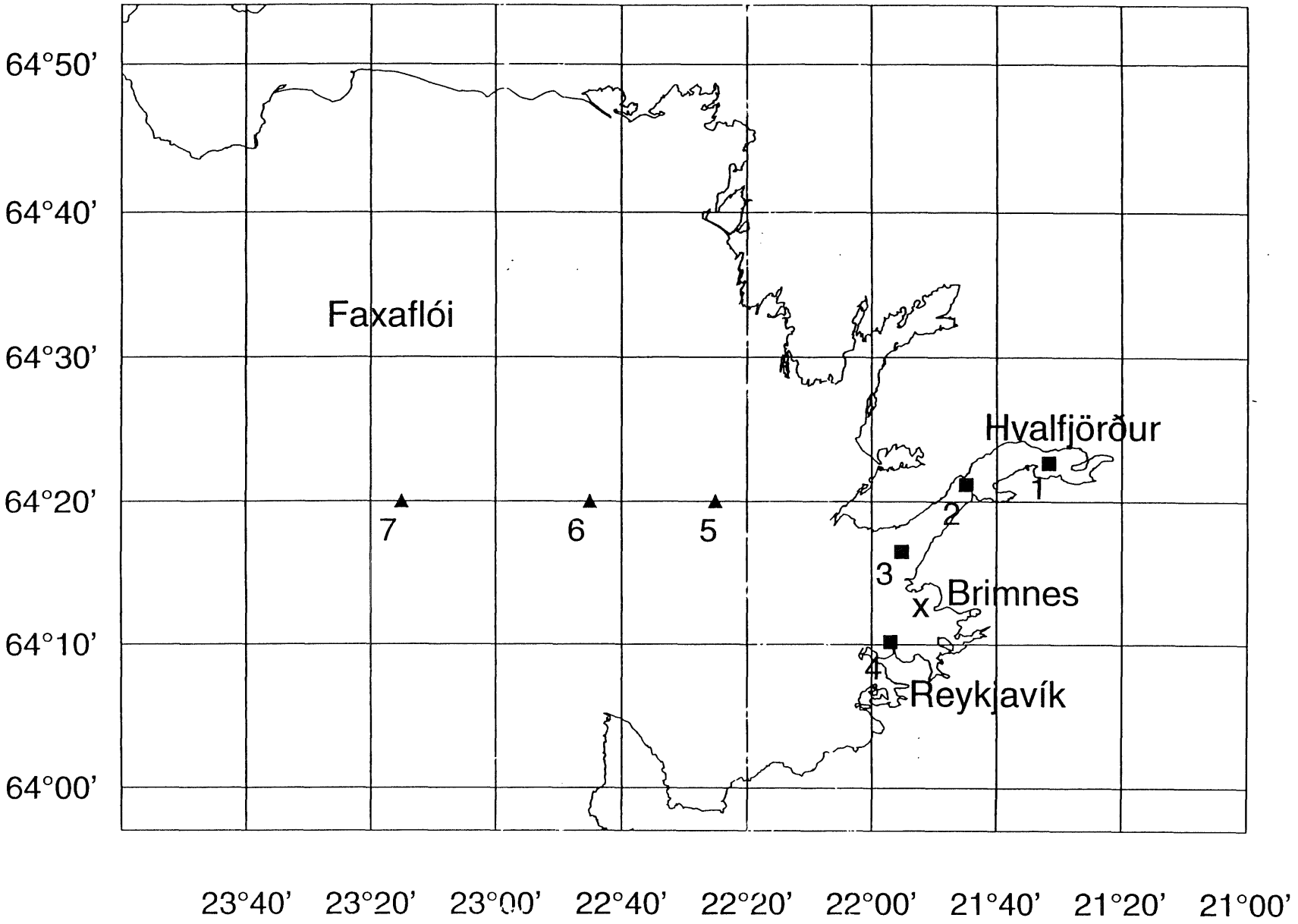


Figure 1. Location of stations where phytoplankton was sampled 1994.

HARMFUL ALGAE IN FAXAFLÓI, ICELAND 1994.

On May 20, in the traditional spring survey of MRI, considerable number of *Alexandrium tamarense* and *A. ostenfeldii* cells were found in netsamples at the stations in the outer part of Faxaflói. Because of these findings it was considered urgent to get samples from the inner area, especially in Hvalfjörður, where there are rich beds of blue mussels. These mussel beds are frequently visited by people collecting mussels. The monitoring of harmful algae was not on the programme in this area and therefore the following information is based on relatively few samples, not systematically collected.

At st. 1-3 (Fig. 1) 25-190 thousand cells pr. liter of *Alexandrium spp* were found in surface samples on May 31. Because of these results it was decided to ban all harvesting of shellfish within the inner part of the bay. Around June 20 the cellnumbers had dropped to several hundreds. Further sampling which was carried out in July (13 and 19) and in August (15 and 31) showed that *Alexandrium* cells were absent in net samples at these stations.

Species of *Dinophysis* found in the area were *D. norvegica, D. acuta, D. acuminata* and *D. rotundata*. Their contribution to the vegetation were small and the pattern of distribution irregular as compared to that of *Alexandrium* species. The highest number of cells recorded was 2.160 cells/l June 23 at station 2, seconded by 1.300 cells pr. l. at the same station August 8.

In the innermost part of Hvalfjörður (st. 1) samples of mussels were taken for mouse testing of PSP and DSP. The sampling was carried out at the following dates: 24/5, 27/5, 6/6, 14/7 and 22/8. In all cases PSP was below the detection limit. DSP was however always present except on June 6. In the blue mussels sample on May 27 the highest level of DSP was found (2 mice dead after 11-24 hours). To the south of the mouth of Hvalfjörður, at Brimnes (Fig. 1), a sample of horse mussel was taken on June 3 at a depth of 15m. No PSP was found but DSP was at a quite high level (3 mice dead in 1-2 hours).

Thórunn Thórdardóttir, Marine Research Institute.
Gudjón Alti Audunsson, Icelandic Fisheries Laboratories.
Skúlagata 4, Reykjavík.

HARMFUL ALGAL BLOOM IN NORWAY 1994

Chrysochromulina spp.

LOCATION	Along the Norwegian Skagerrak Coast
DATES	May 1994
EFFECTS	The bloom was not toxic to fish, but slightly toxic to *Artemia salina* in a bio-test.
MANAGEMENT DECISIONS	Intensivation of monitoring activity
CAUSATIVE	It was a mixed bloom of *Chrysochromulina* spp. Up to 6 000 000 cells/L recorded in the Flødevigen Bay, among them about 50% *C. polylepis*, (confirmed by electron microscopy).
ENVIRONMENT	The *Chrysochromulina* spp. were found in the upper 10m of the water column. This water had a salinity arouind 20 psu. A satellite imagery (AVHRR-data) showed sea surface temperatures between 12 and 13 °C.
ADVECTED POPULATION	The algae seemed to follow the Norwegian Coastal current.
PREVIOUS OCCURRENCE	A harmful bloom of *Chrysochromulina polylepis* in May 1988. Since then monitoring has revealed regular occurrence, about 1 000 000 cells/L, of *Chrysochromulina* spp. each year in May-June.
ADDITIONAL COMMENTS	Due to the meteorological conditions the mixed layer collapsed before any harmful bloom occurred.
INDIVIDUAL TO CONTACT	Einar Dahl, Institute of Marine Research, Flødevigen Marine Research Station, N-4817 His tel. +47 370 10580, fax. +47 370 10515.

HARMFUL ALGAL BLOOM IN NORWAY 1994

Gyrodinium cf. *aureolum*

LOCATION	The southern and south-western coast of Norway.
DATES	September-October 1994.
EFFECTS	Only patches of discoloured water
MANAGEMENT DECISIONS	Intensified local algae monitoring.
CAUSATIVE SPECIES	*Gyrodinium cf. aureolum*, up to 10 million cells per litre were recorded in the Flødevigen Bay.
ENVIRONMENT	The temperature was mainly within 13-15 °C.
ADVECTED POPULATION	The bloom was probaly due to a combination of advected populations and *in situ* growth.
PREVIOUS OCCURRENCES	*Gyrodinium* bloomed in the area in 1966, 1976, 1981, 1982, 1985, 1988, 1990, 1991 and 1992
ADDITIONAL COMMENTS	The meteorological conditions were favorable to avoid a bloom with N-NW winds during the blooming period and no convergence of the Norwegian Coastal Current along the southern and south-western coast.
INDIVIDUAL TO CONTACT	Einar Dahl, Institute of Marine Research, Flødevigen Marine Research Station, N-4817 His tel. +47 370 10580, fax. +47 370 10515.

HARMFUL ALGAL BLOOM IN NORWAY 1994

Prymnesium **cf. *parvum***

LOCATION	Ryfylkefjordene (near Stavanger), south-west coast of Norway
DATES	July-August 1994
EFFECTS	None
MANAGEMENT DECISIONS	Threatend fish farms escaped the area.
CAUSATIVE SPECIES	*Prymnesium* cf. *parvum*, up to 2 million cells/L were recorded.
ENVIRONMENT	Temperature was 16.5-19 °C, a pycnocline present at 2-3m depth and a salinity of about 8 psu in the mixed surface layer.
ADVECTED POPULATION	*In situ* growth in a fjord and some advection of algae and toxic water to nearby waters.
PREVIOUS OCCURRENCE	Blooms re-occurred every summer since 1989.
ADDITIONAL COMMENTS	To detect toxic water-bodies an open well-boat with salmon was tracked in the fjord system.
INDIVIDUAL TO CONTACT	Einar Dahl, Institute of Marine Research, Flødevigen Marine Research Station, N-4817 His tel. +47 370 10580, fax. +47 370 10515.

HARMFUL ALGAL BLOOM IN NORWAY 1994

Paralytic Shellfish Toxins

In 1992 a regular monitoring of algae and control of shellfish toxicity by mouse bioassay along the Norwegian coast were established. The results from this monitoring programme concerning Paralytic Shellfish Toxins in 1993 are summerized.

LOCATION	Along the north-west coast.
DATES	April - May 1994.
EFFECTS	Toxins recorded above the action level (400 ME/100g) according to mouse bioassay.
MANAGEMENT DECISIONS	Harvesting was locally banned. The public was warned against picking toxic mussels.
CAUSATIVE SPECIES	*Alexandrium* spp.
ENVIRONMENT	No information
ADVECTED POPULATION	Mainly due to *in situ* growth ?
PREVIOUS OCCURRENCES	A few historical records, and more or less regular occurrences in the area the recent years, however, the spatial and temporal extent may vary significantly from one year to another.
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HARMFUL ALGAL BLOOM IN NORWAY 1994

Diarrhoeic Shellfish Toxins

In 1992 a regular monitoring of algae and control of shellfish toxicity by mouse bioassay along the Norwegian coast were established. The 1994 results from this programme concerning Diarrhoeic Shellfish Toxins are summerized.

LOCATION	*Dinophysis* spp. were recorded all along the Norwegian coast but most numerous along the south and in the innermost part of the Sognefjord at the west coast.
DATES	From March and troughout the year concentrations of some few hundred cells/L or more were recorded at one or another station. Among the *Dinophysis* spp. occurring along the Norwegian coast, the species *D. acuminata* and *D, acuta* are considered as the most potent for toxicity. The latter has its main season in late summer and early autumn.
EFFECTS	Toxins recorded above the action level according to mouse bioassay at one or another station from March on. Most widespread in early autumn. Harvesting and consumption were banned.
MANAGEMENT DECISIONS	Harvesting was locally banned. The public was warned against picking toxic mussels.
CAUSATIVE SPECIES	Most probably *Dinophysis* spp., with *D. acuminata* and *D. acuta* as the most potent species.
ENVIRONMENT	The problem occur over a wide range of temperatures and salinities.
ADVECTED POPULATION	Along the southern coast there are some evidence that the algae and toxin problems are spread by advection. But along the west coast the "hot spots" seems to be rather patchy which indicate local concentration of the algae and/or *in situ* growth.
PREVIOUS OCCURRENCES	A few more dubious historical records. A yearly, more or less large scale and long lasting phenomenon since 1984 according to mouse bioassay. The phenomenom has never been so extensively monitored as since 1992.
INDIVIDUAL TO CONTACT	Einar Dahl, Institute of Marine Research, Research Station Flødevigen, N-4817 His, NORWAY tel. +47 370 10580, fax. +47 370 10515 Karl Tangen, OCEANOR, Pirsenteret, N-7005 Trondheim, NORWAY tel. +47 73 525050, fax. +47 73 525033

PORTUGAL 1994

PSP

All the Portuguese coast was affected.

1. and 2.- **Location and areas of occurrence:**

- Algarve coast (Faro/Olhão)- June-August (357 ug/100g)
- Formosa Lagoon (Faro/Olhão)- September-October (317 ug/100g)
- Arado estuary (Portimão) - November (146 ug/100g)
- Alvor Lagoon (Portimão)- July (215 ug/100g) ; November (145 ug/100g)
- Sagres coast- July (365 ug/100g)
- Mira estuary (Sines) - October 1994- April 1995 (1 018 ug/100g)
- Sines coast- October-December (411ug/100g)
- Albufeira Lagoon (Setúbal) - September-Novenber (685 ug/100g) and November-December (190 ug/100g)
- Setúbal coast- October 1994-February 1995 (1 690 ug/100g)
- Sado estuary (Setúbal) - October 1994-April 1995 (1 310 ug/100g)
- Lisboa coast- October-December (520 ug/100g)
- Tagus estuary (Lisboa)- October 1994- April 1995 (ug/100g)
- Cascais, Ericeira, Peniche, S.Martinho- October 1994- February 1995 (3 678 ug/100g)
- Nazaré (Óbidos Lagoon) - October 1994- February 1995 (1 801 ug/100g)
- Figueira da Foz Coast - October -December (100 ug/100g)
- Mondêgo estuary (Figueira da Foz) - October 1994-March 1995 (ug/100g)
- Aveiro coast - October-December (108 ug/100g)
- Aveiro Lagoon - October 1994 - February 1995 (789 ug/100g)
- Espinho coast - November - December (105 ug/100g)
- Lima estuary (Viana do Castelo) - November - December (105 ug/100g)
- Minho estuary - November 1994- February 1995 (121 ug/100g)

3. **Effects:**

Almost all the exploited bivalve molluscs from these regions presented PSP toxins:

- Algarve coast (Faro/Olhão)- only *Venus striatula*
- Sagres coast- *Mytilus edulis* and *Crassostrea angulata*
- Arado estuary (Portimão)- *Ruditapes decussata* and *Cerastoderma edule*
- Formosa Lagoon (Faro/Olhão)- only *Cerastoderma edule*
- Sines coast- *Ensis siliqua* and *Donax* spp

- Sines Lagoons - *Cerastoderma edule, Venerupis pullastra , Ruditapes decussata* and *Mytilus edulis*
- Albufeira Lagoon (Setúbal) - *Mytilus edulis* and *Venus verrucosa*
- Setúbal coast- *Ensis siliqua* and *Donax* spp
- Sado estuary (Setúbal) - *Crassostrea angulata ,Scrobicularia plana,Cerastoderma edule, Venerupis pullastra , Ruditapes decussata* and *Venus verrucosa*
- Lisboa coast- *Ensis siliqua* and *Donax* spp
- Tagus estuary (Lisboa)- *Scrobicularia plana, Cerastoderma edule,Venerupis pullastra , Ruditapes decussata* and *Mytilus edulis*
- Cascais, Ericeira, Peniche, S.Martinho- *Mytilus edulis*
- Nazaré (Óbidos Lagoon) - *Spisula solida, Mytilus edulis, Ruditapes decussata, Venerupis pullastra* and *Cerastoderma edule*
-Figueira da Foz Coast - *Spisula solida*
- Mondêgo estuary (Figueira da Foz) - *Scrobicularia plana, Cerastoderma edule* and *Mytilus edulis*
- Aveiro coast - *Spisula solida*
- Aveiro Lagoon - *Mytilus edulis, Ruditapes decussata, Venerupis pullastra* and *Cerastoderma edule*
- Espinho coast - *Spisula solida*
- Lima estuary (Viana do Castelo) - *Cerastoderma edule*
- Minho estuary - *Mytilus edulis*

4. Management decisions:

Bivalve species with PSP values over 80 ug/100g closed to Harvest.

5. Causative species:

The causative species was *Gymnodinium catenatum*.

The highest detected concentrations (cells/l) were:

- Algarve coast (Faro/Olhão)- 1 800 (Aug.18)
- Formosa Lagoon (Faro/Olhão)- 4 500 (Sep.9)
- Arade estuary (Portimão)- 2 000 (Aug.18)
- Sagres coast- 13 000 (Aug.3)
- Sines coast- 392 000 (Oct. 17)
- Sines Lagoons - 24 600
- Sado estuary (Setúbal) - 63 900
- Albufeira Lagoon (Setúbal) - 5 400
- Setúbal coast- 98 000
- Lisboa coast- 102 625
- Tagus estuary (Lisboa)-
- Cascais, Ericeira, Peniche, S.Martinho- 135 000
- Nazaré (Óbidos Lagoon) -205 250
- Figueira da Foz Coast - 3 400
- Mondêgo estuary (Figueira da Foz) - 4 050
- Aveiro coast - 33 000

- Aveiro Lagoon -18 800
- Espinho coast - 13 000
- Lima estuary (Viana do Castelo) - 2 450
- Minho estuary - 2 400

6. Environment:

Temperature range: 14 - 20 °C
Salinity range: 20 - 36.5‰

7. Advected population or *in situ* growth:

A combination of both

8. Previous occurrences:

Since 1986, with a break in 1991, *G. catenatum* has been the responsible species for PSP at the Portuguese coastal zone. In 1993 and 1994 all the coast has been affected beginning by the South and spreading to the North.

9. Individual to contact:

Maria Antónia de M. Sampayo and Maria da Graça Vilarinho
IPIMAR
Av. Brasília 1400 LISBON PORTUGAL

Phone: 351 1 3017361
Fax: 351 1 3015948

PORTUGAL 1994

DSP

DSP toxins were detected at Aveiro, Óbidos and Albufeira Lagoons, Minho and Mondêgo estuaries, Lisbon Coast.

1. and 2. **Location and data of occurrences:**

- Minho estuary: 10 August - 12 September
- Aveiro Lagoon: 12 July - 22 November
- Mondego estuary: 6 April - 10 May and 9 August - 23 November.
- Obidos Lagoon: 13 September - 16 November
- Lisbon coast: 22 August - 30 August
- Albufeira Lagoon: 8 September - 20 November

3. **Effects:**

Most bivalves from these regions presented DSP toxins:

- Minho estuary: *Mytilus edulis*
- Aveiro Lagoon: *Mytilus edulis*
- Mondego estuary: *Mytilus edulis* and *Scrobicularia plana*
- Obidos Lagoon: *Mytilus edulis*
- Lisbon coast: *Ensis siliqua*
- Albufeira Lagoon: *Mytilus edulis*

DSP toxins were determined both by the mouse bioassay and through HPLC.

4. **Management decisions:**

Harvest of affected species closed during toxication.

5. Causative species:

Dinophysis cf *acuminata* and/or *D. acuta* cells/l:.

- Minho estuary: *D. acuminata* 1 350(Oct. 13); *D.acuta* 900 (Oct.24)
- Aveiro Lagoon: *D. acuminata* 1 500(July 20 - 25); *D.acuta* 950 (Sep.14)
- Mondego estuary: *D. acuminata* 700(Jun. 1); *D.acuta* 5 100 (Jul.27)
- Obidos Lagoon: *D. acuminata* 900 (July 20 - 25); *D.acuta* 950 (Sep.14)
- Lisbon coast.- *D. acuminata* 850 (May 25); *D.acuta* 12 850 (Sep.14)
- Albufeira Lagoon:- *D. acuminata* 1 000 (Sep.8); *D.acuta* 1 350 (Oct.12)

6. Environment:

Temperature range: 16 - 19°C
Salinity: 24 - 36‰

7. Advected population or *in situ* growth:

Most probably a combination of both.

8. Previous occurrences:

Since 1987, the year of the first confirmed occurrence, every year, with a break in 1993, we are having the problem. The most affected area was this year as always Aveiro Lagoon.

9. Individual to contact:

Maria antónia de M. Sampayo and Maria da Graça Vilarinho
IPIMAR
Av. Brasília 1400 Lisbon PORTUGAL

Phone: 351 1 3017361
Fax: 351 1 3015948

HARMFUL ALGAL BLOOMS IN 1994 - SPAIN

1. Location: Beach of La Fosca (Costa Brava, Catalonia)

2. Date of Occurrence: July and August 1994

3. Effects: Presence of green patches in the beach, affecting negatively tourism. PSP biotest on mussels: negative.

4: Management Decision:

5: Causative Species: Alexandrium taylori Balech

6. Environment: Calm weather

7. Advected Population or In Situ Growth: probably in situ growth

8: Previous Occurrences: None, it is the first citation of this organism in the Mediterranean Sea.

9. Additional Comments:

10. Individual to Contact: Maximino Delgado
Instituto de Ciencias del Mar
Pº Joan de Borbó s/n
08039 Barcelona
Tel: (93) 221 64 16
Fax: (93) 221 73 40

HARMFUL ALGAL BLOOMS IN 1994 - SPAIN

1. Location: Alfacs Bay (Ebro Delta, Catalonia)

2. Date of Occurrence: From December 1994 to March 1995

3. Effects: Mortatilies of fish in culture ponds (Sparus aurata) and mussels (Mytilys galloprovincialis) in the bay.

4: Management Decision: Monitoring the dinoflagellate concentration before punping of water to ponds.

5: Causative Species: Gyrodinium sp.

6. Environment: Salinity 35-36 psu, temperature 6-17 °C.

7. Advected Population or In Situ Growth: In situ growth

8: Previous Occurrences: None

9. Additional Comments:

10. Individual to Contact: Maximino Delgado
Instituto de Ciencias del Mar
Pº Joan de Borbó s/n
08039 Barcelona
Tel: (93) 221 64 16
Fax: (93) 221 73 40

HARMFUL ALGAL BLOOMS IN GALICIA IN 1994

1.- Location: Some parts of the rías of Pontevedra, Muros and mounth south of the Ría of Arousa and mounth north of the Ría of Vigo.
2.- Date of Occurrence: From the end to May to the beginning to the July.
3.- Effects: Presence of DSP bivalve toxicity.
4.- Management Decision: Harvesting was closed when DSP toxin is present.
5.- Causative Species: *Dinophysis acuminata.* The maximum cell concentration was 3800 cel/l.
6.- Environment: During maximum cell numbers the temperature ranged from 13.5 to 17.8 °C and salinity from 34.5 to 35.6 USP.
7.- Advected Population or *In Situ* Growth: Provably "in situ" growth.
8.- Previous Ocurrences: Blooms of this specie are frecuenty in the rías in spring and summer in the lasts years.
9.- Additional Comments:
10.- Individual to Contact:

J. Mariño; J. Maneiro; Y. Pazos
Condicións Oceanográficas e Fitoplancto
Centro de Control de Calidade do Medio Mariño
Peirao de Vilaxoán D. P. 36600
Vilagarcía de Arousa. Pontevedra. España
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HARMFUL ALGAL BLOOMS IN GALICIA IN 1994

1.- Location: Some parts of the rías of Pontevedra, Muros and mounth south of the Ría of Arousa and mounth north of the Ría of Vigo.
2.- Date of Occurrence: From the beginning of August to the end of September.
3.- Effects: Presence of DSP bivalve toxicity.
4.- Management Decision: Harvesting was closed when DSP is present.
5.- Causative Species: *Dinophysis acuminata*. The maximum cell concentration was 3160 cel/l.
6.- Environment: During maximum cell numbers the temperature ranged from 13.5 to 18.5 °C and salinity from 35.5 to 35.7 USP.
7.- Advected Population or *In Situ* Growth: Provably "in situ" growth.
8.- Previous Ocurrences: Blooms of this specie are frecuenty in the rías in spring and summer in the lasts years.
9.- Additional Comments:
10.- Individual to Contact:

J. Mariño; J. Maneiro; Y. Pazos
Condicións Oceanográficas e Fitoplancto
Centro de Control de Calidade do Medio Mariño
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HARMFUL ALGAL BLOOMS IN GALICIA IN 1994

1.- Location: Rías of Pontevedra, Muros, Vigo and mounth south of the Ría of Arousa.
2.- Date of Occurrence: From the middle of October to the middle of November.
3.- Effects: Presence of DSP bivalve toxicity.
4.- Management Decision: Harvesting was closed when DSP toxin is present
5.- Causative Species: *Dinophysis acuminata*. The maximum cell concentration was 4280 cel/l.
6.- Environment: During maximum cell numbers the temperature ranged from 13 to 15.5 °C and salinity from 33.5 to 35.5 USP.
7.- Advected Population or *In Situ* Growth: Provably "in situ" growth.
8.- Previous Ocurrences: Blooms of this specie are frecuenty in the rías in spring and summer in the lasts years
9.- Additional Comments: This bloom are partially coincident in the time with other of the *Gymnodinium catenatum*.
10.- Individual to Contact:

J. Mariño; J. Maneiro; Y. Pazos
Condicións Oceanográficas e Fitoplancto
Centro de Control de Calidade do Medio Mariño
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HARMFUL ALGAL BLOOMS IN GALICIA IN 1994

1.- Location: Some parts of the rías of Vigo, Pontevedra, Arousa, Muros particularly in the mounths.
2.- Date of Occurrence: From October to December.
3.- Effects: PSP toxicity reaching a maximum concentration of 2652 μg equiv. STX eq. g/ 100 g meat.
4.- Management Decision: Harvesting was closed when PSP toxin content was equal of higher than rechead 80 μg equiv. STX /100 g. meat.
5.- Causative Species: *Gymnodinium catenatum*. The maximum cell concentration was 22720 cells l^{-1}
6.- Environment: During maximum cell numbers the temperature ranged from 13 to 15.5°C and salinity from 33.5 to 35.5 USP.
7.- Advected Population or *In Situ* Growth: Advected population.
8.- Previous Ocurrences: Blooms of this species were recorded in the Rías Baixas in autum in 1981, 1985, 1986, 1987, 1988, 1990 and 1993. In these three latter years, small populations were also found during summer.
9.- Additional Comments:
10.- Individual to Contact:

J. Mariño; J. Maneiro; Y. Pazos
Condicións Oceanográficas e Fitoplancto
Centro de Control de Calidade do Medio Mariño
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ALGAL BLOOMS IN SWEDEN 1994

Prorocentrum minimum

LOCATION	Southeast Kattegat
DATES	August 1994
EFFECTS	No effects observed
MANAGEMENT DECISIONS	-
CAUSATIVE	Mixed bloom. Cell densities of up to 200 000 cells/L of Prorocentrum minimum.

ENVIRONMENT

PREVIOUS OCCURRENCE	Blooms of Prorocentrum minimum occur almost every year since 1981.
ADDITIONAL COMMENTS	-
INDIVIDUAL TO CONTACT	Lars Edler, SMHI Doktorsgatan 9 D, 262 52 Ängelholm, Sweden tel. +46 431 80854, fax. +46 431 83167.

ALGAL BLOOMS IN SWEDEN 1994

Prorocentrum micans

LOCATION	East Skagerrak
DATES	August 1994
EFFECTS	No effects observed
MANAGEMENT DECISIONS	-
CAUSATIVE	Cell densities of up to 120 000 cells/L of Prorocentrum micans.

ENVIRONMENT

PREVIOUS OCCURRENCE	Presence of Prorocentrum minimum every year, but usually not in such high densities.
ADDITIONAL COMMENTS	-
INDIVIDUAL TO CONTACT	Odd Lindahl, Kristineberg Marine Research Station S-450 34 Fiskebäckskil, Sweden tel. +46 523 18500, fax. +46 523 18502.

ALGAL BLOOMS IN SWEDEN 1994

Dinophysis acuminata and ***norvegica***

LOCATION	Central Baltic Sea
DATES	August 1994
EFFECTS	No effects observed
MANAGEMENT DECISIONS	-
CAUSATIVE	Cell densities of up to 100 000 cells/L of Dinophysis norvegica. Dinophysis acuminata in lower abundance.
ENVIRONMENT	Main occurence at 14-17 m depth.
PREVIOUS OCCURRENCE	Presence of these species common but not observed in such high densities.
ADDITIONAL COMMENTS	-
INDIVIDUAL TO CONTACT	Susanna Hajdu, Dept. of System Ecology Box 7050, S-750 07 Uppsala, Sweden tel. +46 18 673155, fax. +46 18 673156.

ALGAL BLOOMS IN SWEDEN 1994

Nodularia spumigena and *Apanizomenon "baltica"*

LOCATION Baltic Sea (see map)

DATES July-August 1994

EFFECTS No effects observed, but moderate toxicity found in population collected in Gdansk Bay, Poland.

MANAGEMENT DECISIONS -

CAUSATIVE Nodularia spumigena and Apanizomenon "baltica"

ENVIRONMENT -

PREVIOUS OCCURRENCE Large blooms of these species common in the summer

ADDITIONAL COMMENTS -

INDIVIDUAL TO CONTACT Susanna Hajdu, Dept. of System Ecology
Box 7050, S-750 07 Uppsala, Sweden
tel. +46 18 673155, fax. +46 18 673156.

Lars Edler, SMHI
Doktorsgatan 9 D, 262 52 Ängelholm, Sweden
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ALGAL BLOOMS IN SWEDEN 1994

Peridinella catenata

LOCATION Southeast Baltic Sea

DATES May 1994

EFFECTS No effects observed

MANAGEMENT DECISIONS -

CAUSATIVE Cell densities of up to 1 400 000 cells/L of Peridinella catenata.

ENVIRONMENT

PREVIOUS OCCURRENCE Presence of Peridinella catenata every year but never observed in such high densities.

ADDITIONAL COMMENTS Toxicity test of this species during the bloom in 1993 was negative.

INDIVIDUAL TO CONTACT Susanna Hajdu, Dept. of System Ecology
Box 7050, S-750 07 Uppsala, Sweden
tel. +46 18 673155, fax. +46 18 673156.

ALGAL BLOOMS IN SWEDEN 1994

Chrysochromulina spp.

LOCATION	Southeast Kattegat
DATES	May 1994
EFFECTS	No effects observed
MANAGEMENT DECISIONS	-
CAUSATIVE	Mixed bloom of *Chrysochromulina* spp. Cell densities of up to 200 000 cells/L.
ENVIRONMENT	Maximum cell density at 10-15 m, i.e. in the halocline.
PREVIOUS OCCURRENCE	A harmful bloom of *Chrysochromulina polylepis* in May 1988. Since then monitoring has revealed regular occurrence of *Chrysochromulina* spp. each year with maximum in May-June.
ADDITIONAL COMMENTS	-
INDIVIDUAL TO CONTACT	Lars Edler, SMHI Doktorsgatan 9 D, 262 52 Ängelholm, Sweden tel. +46 431 80854, fax. +46 431 83167.

ALGAL BLOOMS IN SWEDEN 1994

Ceratium furca

LOCATION	Southeast Kattegat
DATES	September 1994
EFFECTS	No effects observed
MANAGEMENT DECISIONS	-
CAUSATIVE	Cell densities of up to 40 000 cells/L of Ceratium furca.

ENVIRONMENT

PREVIOUS OCCURRENCE	Presence of Ceratium furca every year, but usually not in such high densities.
ADDITIONAL COMMENTS	-
INDIVIDUAL TO CONTACT	Per Olsson, Toxicon Kontorsgatan 20, S-261 35 Landskrona, Sweden tel. +46 418 14550, fax. +46 418 23285.

ALGAL BLOOM REPORTS - ENGLAND AND WALES

1. Location: Weymouth Harbour, Dorset

2. Date of occurence: 01/08/95

3. Effects: None

4. Management decision: Increase sampling frequency. Take samples of mussel flesh for PSP analysis

5. Causative species: *Alexandrium tamarense*.

6. Environment: No data

7. Advected population or in situ growth: no data.

8. Previous occurrences: no data

9. Additional comments: No toxins detected.

10. Individual to contact:

Allan Fraks
NRA Blandford Forum
Tel: 01258 456080

ALGAL BLOOM REPORTS - ENGLAND AND WALES

1. Location: Broad Haven, Dyfed.

2. Date of occurence: 22/8/94

3. Effects: Dead fish (various species) molluscs and echinoderms.

4. Management decision: water samples taken

5. Causative species: *Gyrodinium aureolus.*

6. Environment: no data.

7. Advected population or in situ growth: no data.

8. Previous occurrences: None recorded

9. Additional comments:

10. Individual to contact: Pablo Cotsifis,
NRA Haverford West
Tel: 01554 757031

HARMFUL ALGAL BLOOMS 1994 - SCOTLAND

1. Location: West Coast of Scotland.
   PSP toxins were detected in several lochs in this region and for the first time in samples from sites in Lewis and South Uist. The area south of the Ardnamurchan peninsula was largely unaffected by PSP toxins

2. Date of Occurrence: Toxins detected between:

   Mussels (*Mytilis edulis*): Mid-April - End August
   Scallops (*Pecten maximus*): End June - Early August

3. Effects: Toxins in mussels first appeared in mid-April, but did not peak until early July, where levels reached 2483units. Toxin levels over 2000units were detected in Loch Kishorn, Loch Hourn, Loch Inchard and Loch Kirkaig. Toxins were found until late August. Toxins in scallops reached 1560units in mid-July and were not found after the beginning of August. DSP toxins were detected at 6 locations, the area most severely affected was Loch Greshornish where toxins were found over a four-week period. Levels of okadaic acid of up to 115.8ug / 100g tissue and the presence of DTX1 were found.

4. Management: Voluntary closure agreements were made with affected shellfish farmers and fishermen.

5. Causative species: No water samples were analysed. A new phytoplankton monitoring programme is being set up next year.

6. Environment: No information available.

7. In situ Population or Advected Growth: Not known.

8. Previous Occurrences: Outbreaks of PSP have been previously recorded around Skye and the Inner Sound.

9. Additional Comments: None.

10. Individual to Contact: G. Howard / E. Macdonald
SOAFD Marine Laboratory
PO Box 101
Victoria Road
Aberdeen AB9 80B

HARMFUL ALGAL BLOOMS 1994 - SCOTLAND

1. Location: East Coast of Scotland.
PSP toxins were detected in mussel samples over an area extending from Wick to Fraserburgh and from Aberdeen to the Scottish border. PSP toxins were also found in scallops from the Moray Firth Scallop Grounds. Offshore scallops from Bellrock to Montrose were also affected.

2. Date of Occurrence:

Toxins detected between:

Mussels (*Mytilis edulis)*: Mid-May - End June
Scallops (*Pecten maximus*): End May - Early August

Low levels of toxin detected between:

Brown crab (*Cancer pagurus*): Mid-June (peak 468units)
Lobster (*Homarus gammarus*): Early June (peak 450units)

3. Effects: Toxin levels in mussels first appeared in the Berwick area in mid-May and by early June had peaked at 8982units, then fell to below 400units by the end of the month Toxin levels over 2000units were found in the Eden estuary and at Banff. Levels at Elie in the Firth of Forth, an area where high levels in previous years were regularly recorded, this year did not exceed 200units. Around the Bellrock area, toxin levels of 1198units were detected in offshore scallop gonads in early June. Toxins continued to be detected in scallops from the Moray Firth until early August. DSP toxins were found in mid-June and continued to be detected until mid-July. Levels of okadaic acid up to 80ug / 100g tissue were found.

4. Management: The Dornoch Firth Fishery was subject to a voluntary closure agreement first for PSP and then for DSP, the closure lasting until the 25th of July. Scallop fishing vessels working around the Bellrock area were advised to move to the North of Montrose where levels were lower and scallop processors were required to have End Product Standard Testing.

5. Causative Species: No water samples were analysed. A new phytoplankton monitoring programme is being set up next year.

6. Environment: No information available.

7. In situ Population or Advected Growth: Not known.

8. Previous Occurrences: Regular occurrence every year since 1968 in Forth.

9. Additional Comments: None.

10. Individual to Contact: G. Howard / E. Macdonald
SOAFD Marine Laboratory
PO Box 101
Victoria Road
Aberdeen AB9 80B

HARMFUL ALGAL BLOOMS 1994 - SCOTLAND

1. Location: Orkney and Shetland.
As in 1992 and 1993, Orkney was badly affected by PSP toxins which were detected in a range of different bivalve species. Mussels, scallops and queens were the worst affected, but toxins were also found in razor fish and oysters. All areas of the Orkney Islands were affected, and PSP toxins were also found in the Shetlands.

2. Date of Occurrence:

	Toxins detected between-
Mussels (*Mytilus edulis):*	Mid-April - End August
Scallops (*Pecten maximus*):	End June - Early August
	Low levels of toxin detected between-
Brown crab (*Cancer pagurus*):	End July - Early August (peak 267units)
Lobster (*Homarus gammarus*):	End July (peak 350units)
Razor fish (*Ensis* spp.):	End July (peak 377units)
Oysters (*Crassostrea gigas*):	Early July - End July (peak 365units)

3. Effects: Toxins in mussels first appeared at the beginning of June and by the end of the month levels had reached 1806units, peaking at 4707units in mid-July. Toxins in scallops peaked at 3692units in mid-July and continued to be detected until the end of August. All areas of the Orkney Islands were affected and toxin levels over 1000units were found in Scapa Flow, the Kirkwall, Sanday, Westray and Copinsay areas. DSP toxins were detected in an isolated sample of mussels from Scapa Flow. In Shetland PSP toxins were found in late June, but levels did not exceed 400units.

4. Management: Intially voluntary closure agreements were sought with shellfish farmers, but as the outbreak became more widespread and deeper water scallop ground were affected, closure orders made under FEPA 1985 were introduced. The first order was made on the 20th of July and the final order was revoked on the 19th of August. Further tests were carried out following a resurgence of toxins in scallops in October and November, and processors were required to obtain certification showing that End Product Standards were met.

5. Causative Species: Low levels of *Alexandrium* spp. were found in water samples in May. *Dinophysis* spp. at 500 cells/l were identified in water samples in late April. A new phytoplankton monitoring programme is being set up next year.

6. Environment: No information available.

7. In situ Population or Advected Growth: Not known.

8. Previous Occurrences: Regular occurrence since 1990.

9. Additional Comments: None.

10. Individual to Contact: G. Howard / E. Macdonald
SOAFD Marine Laboratory
PO Box 101
Victoria Road
Aberdeen AB9 80B

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

MAINE

1. **Location:** Tremont, Maine to the Canadian border.

2. **Date of Occurrence:** June to August 1994.

3. **Effects:** PSP in shellfish (*Mytilus edulis, Mya arenaria, Modiolus modiolus, Arctica islandica* and *Placopecten magellanicus*).

4. **Management Action:** Affected areas closed to the harvest of specific species.

5. **Causative Species:** *Alexandrium tamarensis.*

6. **Environment:**

7. **Advected Population or In Situ Growth:**

8. **Previous Occurrences:**

9. **Additional Comments:**

10. **Individual to Contact:** Dr. John W. Hurst, Jr.
Department of Marine Resources
West Boothbay Harbor, Maine 04575

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

MAINE

1. **Location:** Kittery - Stonington, Maine.

2. **Date of Occurrence:** May to September 1994.

3. **Effects:** PSP in shellfish (*Mytilus edulis, Mya arenaria, Spisula solidissima, Modiolus modiolus* and *Euspira heros*).

4. **Management Action:** Affected areas closed to the harvest of specific species.

5. **Causative Species:** *Alexandrium tamarensis.*

6. **Environment:**

7. **Advected Population or In Situ Growth:**

8. **Previous Occurrences:**

9. **Additional Comments:** In February 1994 the entire Maine coast was closed to the harvesting of whelks, due to tetramethyl ammonium (TMA).

10. **Individual to Contact:**

Dr. John W. Hurst, Jr.
Department of Marine Resources
West Boothbay Harbor, Maine 04575

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

MASSACHUSETTS

1. **Location:** Georges Bank, offshore, Area 6

2. **Date of Occurrence:**

3. **Effects:**

4. **Management Action:** The closure of Georges Bank to the harvesting of molluscan shellfish with the exception of sea scallop adductor muscles was continued throughout 1994 because of the risk of paralytic shellfish poisoning.

5. **Causative Species:** *Alexandrium fundyense* and/or *A. tamarense* (variety not yet determined).

6. **Environment:** Georges Bank is an open-ocean environment, 100-200 miles from the nearest land (Cape Cod). Much of the Georges Bank area is very shallow (10-15 m). The region is a rich fishing grounds for shellfish and finfish. Stratification of the waters overyling Georges Bank starts to occur in May, at which time the surface waters are about 10-12°C.

7. **Advected Population or In Situ Growth:** The origin of the offshore toxicity and its relationship with inshore toxicity remain unknown.

8. **Previous Occurrences:** High levels of paralytic shellfish toxins were first observed in Georges Bank shellfish in 1989. Toxin levels increased in 1990. Despite the apparent absence of *Alexandrium* blooms in the Georges Bank region since 1990, the persistence of the toxins in surf clams has resulted in a continuing closure of the Georges Bank surf clam fishery.

9. **Additional Comments:**

10. **Individual to Contact:**

Dr. Alan White
Department of Marine Safety and
Environmental Protection
Massachusetts Maritime Academy
101 Academy Drive
Buzzards Bay, Massachusetts 02532

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

NEW YORK

1. **Location:** Flanders Bay and Great Peconic Bay, on the western end of the Peconic Estuary system. Densities of up to 1.4 x 10^4 cells/ml occurred in Flanders Bay and up to 1.1 x 10^4 cells/ml in Great Peconic Bay.

2. **Date of Occurrence:** Very brief occurrence — the last half of May (5/17-5/24). By 1 June, concentrations were < 200 cells/ml and by 15 June the cells were undetectable.

3. **Effects:** None apparent — the aesthetic effects typically associated with this bloom (water discoloration (brownish) and reduced transparency) are generally not visible until concentrations approach 2.0 x 10^5 cells/ml. Higher numbers than found during 1994 have previously been reported to deleteriously affect shellfish species.

4. **Management Action:** Continue weekly monitoring program.

5. **Causative Species:** *Aureococcus anophagefferens.*

6. **Environment:** Temperature: 14.0°-18.1°C; salinity: 25.71-26.83‰; dissolved oxygen: 7.5-8.2 mg/l; water column stability: mixed.

7. **Advected Population or In Situ Growth:** In situ growth.

8. **Previous Occurrences:** The bloom was present throughout the entire Peconic Bay system from 1985 through 1987, with densities occasionally exceeding 10^6 cells/ml. Cell numbers declined through 1988 and 1989, and were generally undetectable during 1990 with the exception of those from West Neck Bay (Shelter Island). During 1991, densities of up to 2 x 10^6 cells/ml occurred in Flanders Bay and West Neck Bay. During 1992, numbers approached 8.5 x 10^5 cells/ml in Coecles Harbor (Shelter Island) and 10^6 cells/ml in West Neck Bay.

9. **Additional Comments:**

10. **Individual to Contact:**

Dr. Robert Nuzzi
Bureau of Marine Resources
Suffolk County Department
of Health Services
Riverhead, New York 11901

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

NEW YORK

1. **Location:** Moriches and Shinnecock Bays. The bloom was mainly concentrated in eastern Moriches Bay, Quantuck Bay, and western Shinnecock Bay.

2. **Date of Occurrence:** May through June with peak cell densities approaching 2 x 10^4 cells/ml, occurring in mid- to late May. A secondary bloom appeared in early September with counts up to 3.8 x 10^4 cells/ml.

3. **Effects:** Primarily aesthetic — water column discoloration (brownish) and reduced transparency. Effects on various shellfish species have previously been reported.

4. **Management Action:** Increase the frequency of monitoring activities.

5. **Causative Species:** *Aureococcus anophagefferens.*

6. **Environment:** Temperature: 14°-24.8°C; salinity: 26.93-29.65‰; dissolved oxygen: 6.8-9.0 mg/l; water column stability: mixed.

7. **Advected Population or In Situ Growth:** Probably in situ growth in Quantuck Bay, eastern Moriches Bay, and western Shinnecock Bay, with other areas containing advected populations. Both bays are subject to significant tidal flow through ocean inlets.

8. **Previous Occurrences:** 1993: up to 2 x 10^5 cells/ml; 1992: > 10^6 cells/ml; 1991: < 10^3 to 10^6 cells/ml; 1990: < 10^3 to 9.6 x 10^5 cells/ml; 1989: < 1.3 x 10^5 cells/ml in Moriches Bay and < 2.3 x 10^4 cells/ml in Shinnecock Bay.

9. **Additional Comments:**

10. **Individual to Contact:**

Dr. Robert Nuzzi
Bureau of Marine Resources
Suffolk County Department
of Health Services
Riverhead, New York 11901

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

NEW YORK

1. **Location:** Great South Bay. The bloom was present in the central portion of the bay, from the Robert Moses Causeway bridge to the waters off Sayville.

2. **Date of Occurrence:** June through July, with peak concentrations approaching 10^6 cells/ml occurring in late June. A secondary bloom began in mid-August and was still present in late October, when densities up to 1.2×10^4 cells/ml were found.

3. **Effects:** Primarily aesthetic — water column discoloration (brownish) and reduced transparency. Secchi depth readings were less than 0.5 m during peak bloom periods. Effects on various shellfish species have previously been reported.

4. **Management Action:** Increase the frequency of monitoring activities.

5. **Causative Species:** *Aureococcus anophagefferens.*

6. **Environment:** Temperature: 18.4°-28.0°C; salinity: 24.30-29.50‰; dissolved oxygen: 5.8-11.3 mg/l; water column stability: mixed.

7. **Advected Population or In Situ Growth:** Probably in situ growth.

8. **Previous Occurrences:** 1993: $< 10^3$ to 2.6×10^5 cells/ml (January-March, August-November); 1992: 10^3 to 10^6 cells/ml (January-December); 1991: $< 10^4$ cells/ml (January-June); 1990: $< 1 \times 10^4$ cells/ml (May-December); 1989: $< 2.5 \times 10^4$ cells/ml (April-September); 1988: 10^3 to 5×10^5 cells/ml (June-August); 1985, 1986: $> 10^6$ cells/ml.

9. **Additional Comments:**

10. **Individual to Contact:**

Dr. Robert Nuzzi
Bureau of Marine Resources
Suffolk County Department
of Health Services
Riverhead, New York 11901

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

NEW JERSEY

1. **Location:** Raritan Bay, south shore area, to Sandy Hook Bay (centering off East Keansburg).

2. **Date of Occurrence:** June to September 1994 - intermittent blooms peak early August (approximately 3 August).

3. **Effects:** Heavy red to brown water discoloration, flocculent deposits on shore, possible hypoxia from algal decomposition; has been associated with occasional localized fish kills (none in 1994).

4. **Management Action:** Surveillance by NJOEP/EPA and Monmouth County Health Department.

5. **Causative Species:** *Eutreptia/Euglena* spp.; *Chaetoceros* spp., *Thalassiosira* sp., *Skeletonema costatum.* Maximum cell counts of dominant species > 2 x 10^4 ml^{-1}; maximum chlorophyll *a* levels > 115 mgl^{-1}.

6. **Environment:** Secchi disc readings as low as 0.5 m. Water temperature 23-24°C; salinity 20-22.5 ‰ dissolved oxygen 6-9 mgl^{-1}.

7. **Advected Population or In Situ Growth:** In situ growth.

8. **Previous Occurrences:** Chronic annual blooms (at least since the 1960s).

9. **Additional Comments:** Within the past decade, diatoms have dominated for longer periods than phytoflagellates.

10. **Individual to Contact:**

Dr. Paul Olsen
New Jersey Department
of Environmental Protection
Division of Science and Research
Bureau of Water Monitoring, CN422
Trenton, New Jersey 08625

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

NEW JERSEY

1. **Location:** Barnegat Bay (to Little Egg Harbor)

2. **Date of Occurrence:** July to September 1994

3. **Effects:** Intense yellow-green to yellow-brown water discoloration; possible eelgrass die-off.

4. **Management Action:** Surveillance by NJOEP/USEPA, Barnegat Bay citizens watch group/NJ Sea Grant Marine Advisory Service.

5. **Causative Species:** *Nannochloris atomus*; maximum cell counts > 5 x 10^5 ml^{-1}; maximum chlorophyll [a] levels to 30 μgl^{-1}.

6. **Environment:** Water temperatures > 22°C. Secchi disc readings as low as < 0.5 m.

7. **Advected Population or In Situ Growth:** In situ growth.

8. **Previous Occurrences:** Chronic annual blooms at least since 1985.

9. **Additional Comments:**

10. **Individual to Contact:**

Dr. Paul Olsen
New Jersey Department
of Environmental Protection
Division of Science and Research
Bureau of Water Monitoring, CN422
Trenton, New Jersey 08625

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

NEW JERSEY

1. **Location:** Oceanport Creek-Branchport Creek, tributary to Sandy Hook Bay via the Shrewsbury River.

2. **Date of Occurrence:** 8 August, 3 September, 21 November.

3. **Effects:** Intense brown to tea-colored water discoloration; presence of floc from algal decomposition. Numerous dead killies and other dead fish on 13 September.

4. **Management Action:** Investigation by Monmouth County Health Department.

5. **Causative Species:** Diatoms *Navicula* and *Chaetoceros* sp. (8 August); phytoflagellates *Prorocentrum minimum* (dominant), *P. redfieldi, Polykrikos kufoidii*, and several other species.

6. **Environment:** Maximum cell counts of the dominant > 5 x 10^4 ml^{-1}.

7. **Advected Population or In Situ Growth:** In situ population.

8. **Previous Occurrences:** Heavy localized blooms of this nature are not uncommon in this location, which lacks flushing and receiver concentrated runoff from surrounding suburban areas.

9. **Additional Comments:** *P. minimum* is a suspected bather irritant, although this is not normally a bathing area.

10. **Individual to Contact:**

Dr. Paul Olsen
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Trenton, New Jersey 08625

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

FLORIDA

1. **Location:** Florida Bay, Area 16.

2. **Date of Occurrence:** January 1994 to January 1995.

3. **Effects:** Yellow-green to pea-green discolored seawater with decreased water clarity. IN a previous year, sponge mortality coincidental with bloom areas.

4. **Management Action:** None in 1994, although restoration of the bay is part of a long-range interagency plan.

5. **Causative Species:** Cyanobacterium, *Synechococcus elongatus.* Cell concentrations up to 7.1 x 10^6 cells ml^{-1}. Can co-occur with small (< 10 µm) centric diatoms and other cyanobacteria. Chlorophyll *a* levels up to > 30 µg/$liter^{-1}$.

6. **Environment:** Shallow subtropical lagoon with salinities from essentially freshwater to hypersaline (> 50‰) and temperatures from 18.2° to 34.4°C. Resuspension events from winds and tidal action common.

7. **Advected Population or In Situ Growth:** In situ growth within sub-basins of bay. High residency time within sub-basins, but sub-basins flushed by rain and storm events through narrow channels.

8. **Previous Occurrences:** Bloom has been on-going for several years.

9. **Additional Comments:**

10. **Individual to Contact:** Dr. Karen A. Steidinger
Florida Department of Environmental Protection
Florida Marine Research Institute
100 Eighth Avenue, S.E.
St. Petersburg, Florida 33701-5095

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

FLORIDA

1. **Location:** Pinellas to Collier County (1994) Area 16; Collier to Monroe County (1995) Area 16; Monroe to Palm Beach County (1995) Area 15

2. **Date of Occurrence:** 16 September 1994 through February 1995 and on-going (as of 26 February 1995) in southwest Florida.

3. **Effects:** Dead fish — inshore and offshore. Water discoloration brownish-red in some areas. Respiratory irritation from Pinellas to Lee County.

4. **Management Action:** Shellfish harvest bans due to *Gymnodinium breve* red tide — lower Tampa Bay, 21 September 1994 to 25 January 1995; Boca Ciega, 26 September 1994 to 25 January 1995; new pass fall closure due to rainfall, kept closed until 27 January 1995; Lemon Bay fall closure due to rainfall, kept closed until 25 January 1995; Gasparilla fall closure due to rainfall, kept closed until 25 January 1995; Pine Island 17 September 1994-25 September 1995.

5. **Causative Species:** *Gymnodinium breve*. Inshore and coastal surface water samples up to 12 miles offshore had cell concentrations ranging from negative to > 30 x 16^6 cells/liter^{-1}.

6. **Environment:** Occurred in nearshore and shelf waters with wide salinity range (26 to 36 ‰) and temperatures of 16.5 to 28.5°C.

7. **Advected Population or In Situ Growth:** Advected population from offshore waters between Tampa Bay and Charlotte Harbor. In January-February, *G. breve* bloom in southwest Florida offshore shelf waters entrained and transported south. Bloom concentrations found on the Atlantic side in February. An eddy off the Gulf Stream in mid-February delivered above background levels of *G. breve* cell concentrations to coastal waters off Palm Beach County.

8. **Previous Occurrences:** No occurrence in 1993; September 1992-January 1993; January-February 1991; February-March, October-November 1990; March-May 1989; October-December 1988; January/February, May-July, September/October 1987; September-December 1986; September-December 1985; January-March, May-August, 1984; January/February, October-December 1983; January-April, July-October 1982; September/October 1981; January/February, June-November 1980; and before.

9. **Additional Comments:**

10. **Individual to Contact:**

Dr. Karen A. Steidinger
Florida Department of Environmental Protection
Florida Marine Research Institute
100 Eighth Avenue, S.E.
St. Petersburg, Florida 33701-5095

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

LOUSIANA

1. **Location:** Louisiana continental shelf from barrier islands to at least 20 miles offshore south of Terrebonne Bay.

2. **Date of Occurrence:** 7 March 1994 (start of bloom unknown).

3. **Effects:** Water discoloration.

4. **Management Action:** None (over flights planned before bloom dispersed by storm).

5. **Causative Species:** Surface water samples showed concentrations of *Heterosigma* cf. *akashiwo* ranging from 1 x 10^4 nearest shore to 5 x 10^6 cells/liter offshore.

6. **Environment:** 17-19°C, 21-29‰.

7. **Advected Population or In Situ Growth:** Not known.

8. **Previous Occurrences:** Not observed before 1994 at any concentration in Louisiana waters.

9. **Additional Comments:** 1) Bloom dispersed by severe storm and not seen again that year, obviating need for over flights. 2) This species was observed earlier at low levels in Terrebonne Bay estuary, along with possible benthic palmelloid stage.

10. **Individual to Contact:**

Dr. Quay Dortch
Louisiana Universities Marine Consortium
8124 Highway 56
Chauvin, Louisiana 70344
(504) 851-2800

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

LOUSIANA

1. **Location:** Louisiana continental shelf and estuaries.

2. **Date of Occurrence:** All seasons sampled (March-October), peak concentrations occurred in April and October.

3. **Effects:** No monitoring for effects.

4. **Management Action:** None.

5. **Causative Species:** *Pseudonitzschia* spp., present in 64% of samples collected in shelf and estuarine areas. Concentrations up to 1 x 10^7 cells/liter. Species not generally identified, but earlier studies have identified *P. multiseries, P. pungens*, and *P. pseudodelicatissima.*

6. **Environment:** 0.5 to 36‰ (most abundant at 22-32‰), 9 to 32°C (most abundant at 21°C), blooms in moderately well-mixed water. Occurs more frequently and at higher concentrations in coastal zone in comparison with estuary.

7. **Advected Population or In Situ Growth:** In situ growth.

8. **Previous Occurrences:** Occurs annually in very predictable pattern in coastal zone with large blooms in April (based on five-year data). Occurrence in estuary less predictable (based on two-year data). Historical data indicate presence in high numbers back to 1950s.

9. **Additional Comments:** Tests for domoic acid and more detailed taxonomy currently underway.

10. **Individual to Contact:**

Dr. Quay Dortch
Louisiana Universities Marine Consortium
8124 Highway 56
Chauvin, Louisiana 70344
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HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

LOUSIANA

1. **Location:** Louisiana shelf adjacent to Sabine Pass.

2. **Date of Occurrence:** 22 June 1994 (may have been occurring for more than a month prior to this date).

3. **Effects:** Discolored water. A fish kill in Louisiana waters was described by the Texas Department of Parks and Wildlife, but there are no data from Louisiana monitoring agencies.

4. **Management Action:** None in Louisiana. Texas Department of Parks and Wildlife collected samples for identification and environmental conditions and NOAA, NMFS held a workshop to consider relationship between algal bloom, fish kills, and turtle and marine mammal deaths.

5. **Causative Species:** Surface water samples taken from the bloom contained *Gymnodinium sanguineum* at 5.4 x 10^6 cells/liter.

6. **Environment:** 29.8°C, 12.0‰, 15.4 ppm O_2

7. **Advected Population or In Situ Growth:** Not known.

8. **Previous Occurrences:** Previous bloom and fish kill very similar to the 1994 event reported in Harper, D. E., Jr. and G. Guillen. 1989. Occurrence of a dinoflagellate bloom associated with an influx of low salinity water at Galveston, Texas and coincident mortalities of demersal fish and benthic invertebrates. Contributions in Marine Science **31:** 147-161.

9. **Additional Comments:** *Gymnodinium sanguineum* occurs in estuarine and coastal waters of Louisiana at concentrations up to 1 x 105 cells/liter, mostly in summer. Maximum concentrations are observed at low salinities but it can occur at salinities from 0.5 to 36‰.

10. **Individual to Contact:**

Dr. Quay Dortch
Louisiana Universities Marine Consortium
8124 Highway 56
Chauvin, Louisiana 70344
(504) 851-2800

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

TEXAS

1. **Location:** Coastal Texas-Galveston Bay to Sabine Pass, extending to Calcasieu Pass, Louisiana. Includes Chambers and Jefferson Counties.

2. **Date of Occurrence:** 1 May to 1 July 1994.

3. **Effects:** Water discoloration. Mass mortalities of primarily demersal fishes and crustaceans. Not uniformly distributed, with separate blooms occurring at major passes. Low concentrations of dissolved oxygen suspected as major source of mortality.

4. **Management Action:** None taken.

5. **Causative Species:** *Gymnodinium sanguineum* and *Heterosigma* ~~*akashiwa*~~ *carterae*.

6. **Environment:** Coastal waters with blooms concentrating near passes, thus indicating a link with freshwater runoff. During this period abnormally weak, non-westerly, mixed directional surface currents prevailed.

7. **Advected Population or In Situ Growth:** Suggestion of in situ growth, but data lacking.

8. **Previous Occurrences:** Similar occurrence in 1984. Distribution, however, appeared to be more uniform during this time.

9. **Additional Comments:** Picture of mortalities becomes confused with coincidentally high mortalities in sea turtles, dolphin, and seabirds.

10. **Individual to Contact:** Dr. Dean A. Stockwell
Marine Science Institute
The University of Texas at Austin
P.O. Box 1267
Port Aransas, Texas 78373-1267

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

TEXAS

1. **Location:** Upper Laguna Madre, Baffin Bay and Lower Laguna Madre, Texas Nueces, Kleberg, Kenedy, Willacy and Cameron Counties.

2. **Date of Occurrence:** All year — seasonal fluctuation in populations with decline in winter, but always dominant organism.

3. **Effects:** Water discoloration in all of the above areas. Secchi depths range from 0.1-1.0 m and chlorophyll ranges from 20 µg/l to more than 250 µg/l. A major concern is the shading and light limitation on seagrass beds in Laguna Madre. There has been a serious disruption of sports fishing for spotted sea trout and redfish. Loss of seagrass beds becoming apparent.

4. **Management Action:** Shrimp and redfish mariculture hatcheries using alternate water sources where possible. Adjust water intakes to periods of low brown tide standing crop. Grazing studies on zooplankton and larval fish indicate little or no consumption.

5. **Causative Species:** The organism has tentatively been identified as a type III aberrant *Chrysophyte* sp. RNA sequencing, pigment analysis, and polyclonal antibody assays indicate that the organism is similar to *Aureococcus anophagefferens*, but there are some differences.

6. **Environment:** The organism has been found in bay waters ranging from 2-75‰ salinity. The most pronounced accumulation have been observed in Baffin Bay, Texas which has very sluggish circulation. The organism thrives on ammonium nitrogen regenerated from sediments and has little or no uptake of nitrate nitrogen.

7. **Advected Population or In Situ Growth:** In situ growth. Origin not known.

8. **Previous Occurrences:** Brown tide bloom started in December 1989 and has been continuously present in Baffin Bay to the present time. Outbreaks have been observed in Mexico and other Texas embayments (Copano Bay, Nueces Bay, Matagorda Bay) for short times in 1991 and 1992.

9. **Additional Comments:** The bloom was probably triggered by release of nitrogen after a fish-kill caused by a freeze. Other non-point source inputs of nitrogen may be also contributing to the maintenance of the bloom. A viral pathogen appears responsible for its demise in Nueces Bay.

10. **Individual to Contact:**

Dr. Dean A. Stockwell
Marine Science Institute
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Port Aransas, Texas 78373-1267

HARMFUL ALGAL BLOOMS IN 1994 - UNITED STATES

1. Locations: MARIN COUNTY, CALIFORNIA areas affected Drakes Bay, Drakes Estewro, Tomales Bay, Kehoe Beach, Muir Beach

2. Date of Occurrence: January (320 µg/100 g tissue), February (41 µg), March (230 µg), April (680 µg), May (220 µg), August (330 µg), September (930 µg)

3. Effects: Sentinel Bay Mussel (SBM) 930 µg; detectable but below alert levels of PSP in Cultured Pacific Oysters (CPO) were evident.

4. Management Decisions: On January 21, 1994 a quarantine was established for sport-harvested mussels which was lifted on March 7th by the Director of the State Department of Health Services. On March 31 a special quarantine was again established due to the rapid increase in PSP and this continued through the normal quarantine period May 1 to October 31, 1994.

5. Causative Species: Alexandrium catenellum

6. Environment: Warming trend in the sea surface temperatures (SST) following a brief upwelling period.

7. Advected Population or In Situ Growth: In situ growth; high concentrations of A. catenellum were noted each time the PSP concentrations were recorded in the shellfish.

8. Previous Occurrences: 1927, '29, '32, '54, '62, '63, '64, '65, '66, '70, '71, '76, '80, '81, '82, '84, '86, '87, '88, '89, '90, '91, '92, '93, '94.

9. Additional Comments: This area is the aquaculture center for mussels and Pacific oysters and is very well monitored for PSP levels in these shellfish.

10. Individual to Contact:

Dr. Maria R. Ross
Biology Department
University of California at Los Angeles
405 Hilgard Avenue
Los Angeles, California 90024
(310) 206-3528
FAX (310) 559-5120

Ref: State of California Department of Health Services Shellfish Monitoring Program (SDHSSMP) Technical Reports No. 94-03 thru 94-28

CALIFORNIA COUNTIES 1994 PSP CONCENTRATION

DEL NORTE - In September measurable level of PSP was noted in mussels, however it did not exceed the alert level. During the entire year no PSP levels were detected.
Previous occurrences: 1981, '91, '92, '93

HUMBOLDT - No detectable levels of PSP during the entire year except for low measurable PSP concentration during the last two weeks in August.
Previous occurrences: 1969, '71, '73, '89, '92, '93

MENDOCINO - During January, February, March and October PSP levels were measurable but less than 80/ug alert level.
Previous occurrences: 1932, '62, '66, '67, '69, '75, ''82, '84, '89, '90, '91, '92, '93

SONOMA - January PSP level was detectable below the alert concentration. The rest of the months either no dection was noted or no samples were submitted.
Previous occurrences: 1927, '29, '30, '32, '37, '54, '62, '68, '69, 70, '71, '76, '80, '81, '82, '87, '89, '90, '91, '92, '93

SAN FRANCISCO - During the year when samples were submitted no PSP was detected.
Previous occurrences: 1970, '71, '80, '83, '84, '86

SAN MATEO - From June through November measurable concentrations of PSP were noted and in August the level increased to 230/ug. By September the level was below 80/ug.
Previous occurrences: 1970, '71, '82, '83, '84, '86, '87, '89, '90, '91, '92, '93, '94

SANTA CRUZ - There occurred one episode of PSP concentration of 84/ug. In September and November measurable but below the 80/ug alert level.
Ptrevious occurrences: 1971, '84, '89, '91, '92, '93

MONTEREY - PSP levels from not detectable beginning of the year increased to 220 ug in March, 320/ug in April to not detectable until November when the level reached 91/ug.
Previous occurrences: 1988, '89, '94

SAN LOUIS OBISPO - No PSP was detected until October through December when measurable below alert levels were recorded.
Previous occurrences: 1979, '89, '90

SANTA BARBARA - It was free of PSP except for low measurable concentrations in June and December.
Previous occurrences: 1978, '85, '89

VENTURA - Only two episodes of low measurable levels of PSP were reported May and June.
Previous occurrences: 1980, '89

LOS ANGELES - A concentration of PSP of 48 µg/100 g shellfish tissue in January was the single episode for the whole year.
Previous occurrences: 1970, '71, '72, '83, '85, '86, '87, '88 '89, '91, '92, '93

ORANGE - No PSP detected.
Previous occurrences: 1974, '76, '80, '84, '85, '89

SAN DIEGO - Free of PSP.
Previous occurrences: 1985

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Ref:State of California Department of Health Services, Shellfish Monitoring Program Technical Report Nos. 94-03 thru 94-28

CALIFORNIA COUNTIES PSP DATA 1994

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Del Norte	nd	nd	nd	nd	nd	nd	nd	nd	<80	nd	ns	nd
Humboldt	nd	nd	nd	nd	nd	nd	nd	<80	nd	nd	nd	nd
Mendocino	40	39	40	nd	nd	nd	nd	nd	ns	<80	ns	ns
Sonoma	44	nd	ns	nd	ns	nd	ns	ns	ns	ns	ns	ns
Marin	320	41	230	680	220	<80	<80	330	930	<80	<80	nd
San Francisco	nd	ns	ns	ns	ns	nd	nd	nd	nd	nd	ns	ns
San Mateo	nd	nd	ns	nd	nd	<80	<80	230	<80	<80	<80	nd
Santa Cruz	nd	nd	ns	84	nd	ns	ns	nd	<80	ns	<80	nd
Moterey	nd	nd	nd	220	320	ns	nd	nd	ns	nd	91	ns
San Louis Obispo	nd	nd	nd	nd	nd	nd	nd	nd	nd	<80	<80	<80
Santa Barbara	nd	nd	nd	nd	nd	<80	nd	nd	nd	nd	nd	<80
Ventura	ns	ns	nd	ns	<80	<80	nd	nd	nd	nd	ns	ns
Los Angeles	48	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
Orange	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
San Diego	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd

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Ref: State of California Department of Health Services.
Shellfish Monitoring Program Technical Reports
94-06, -08, -11, -13, -15, -17, -20, -23, -25, -27 for 1994
PSP concentrations µg/100 g tissue

DOMOIC ACID/Pseudonitzchia species DATA 1994 - CALIFORNIA COUNTIES

	J	F	M	A	M	J	J	A	S	O	N	D
DEL NORTE	nd	nd	<10	<10	<50	<50	<50	<50	<50	>50	>50	>50
HUMBOLDT	<10	<10	<10	<50	nd	<10	<50	<50	>50	>50	>50	nd
MENDOCINO	<50	nd	<10	<10	<10	<50	>50	<50	<50	nd	nd	nd
SONOMA	<10	<10	<10	<50	<10	nd	<10	<50	<50	>50	<50	<50
MARIN	>50	>50	<50	<10	<10	nd	<10	<50	<50	<50	<50	<50
SAN FRANCISCO	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd
SAN MATEO	<50	<50	<50	<10	<10	nd	<10	<50	<50	<50	<50	nd
SANTA CRUZ	nd	<50	nd	<10	<50	<50	nd	>50	>50	nd	nd	nd
MONTEREY	nd	nd	nd	nd	<50	<10	>50	>50	>50	nd	nd	nd
SAN LOUIS OBP	nd	<50	<50	<50	>50	>50	>50	>50	>50	<50	<10	<50
SANTA BARBARA	>50	<10	<10	<50	<50	<50	>50	<10	<50	<10	<50	nd
VENTURA	nd	<10	nd	<50	<10	<50	<10	<10	<10	nd	<50	nd
LOS ANGELES	nd	<50	nd	>50	>50	nd	nd	nd	nd	nd	<10	nd
ORANGE	<10	<50	<50	<10	<10	<50	nd	nd	nd	nd	nd	nd
SAN DIEGO	<50	<50	<10	<10	>50	>50	<10	nd	<10	nd	<50	<10

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Ref: State of California Department of Health Services,
Shellfish Monitoring Program Technical Reports 94-03 thru 94-28
Cell counts expressed as percentages (%)

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

OREGON

1. **Location:** Oregon coast from Cape Lookout near Netarts to the Columbia River (approximately 44.4-46.7°N). This area is called the Clatsop Beaches.

2. **Date of Occurrence:** Toxicity in shellfish began in August 1992. Mussels peaked at 4365 µg/100 g in September 1992 and did not drop below detection until May 1993. Razor clams were 298 µg/100 g in late October 1993. Older, bigger clams had higher PSP than younger ones. PSP in razor clams still 38-60 µg/100 g in December 1994.

3. **Effects:** Processing of razor clams for food slowly returning. Tourist facilities hurt because no clam diggers for three years (PSP and domoic acid). Intensified testing o razor clams has also increased costs of collection and laboratory testing for the Shellfish Program.

4. **Management Action:** Commercial and recreational harvest of razor clams was re-opened on Clatsop Beaches on 1 November for the first time since November 1991.

5. **Causative Species:** Not confirmed, thought to be *Alexandrium catenella.*

6. **Environment:**

7. **Advected Population or In Situ Growth:** Not known.

8. **Previous Occurrences:** The last Oregon PSP alert began on 25 September 1991 on the central beaches in mussels, peaking at 150 µg/100 g and declining to less than 50 µg/100 g by 30 October 1991.

9. **Additional Comments:** No human illnesses confirmed.

10. **Individual to Contact:** Dr. Deb Cannon
Shellfish Program Specialist
Oregon Department of Agriculture
635 Capitol Street, NE
Salem, Oregon 97310

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

OREGON

1. **Location:** Oregon coast from Cape Lookout near Netarts to the Columbia River (approximately 44.4-46.7°N). This area is called the Clatsop Beaches.

2. **Date of Occurrence:** Toxicity in razor clams began in November 1993 when domoic acid levels increased to 10 ppm. This was the first time levels exceeded 6 ppm since November 1992. Mussels were 1 ppm, the first time detected since November 1992. DA levels rose to 24 ppm in January and declined to less than 5 ppm by April. Levels less than 5 ppm in bimonthly sampling for the rest of 1994.

3. **Effects:** The razor clam fishery was already closed.

4. **Management Action:** Commercial and recreational harvest of razor clams was re-opened on Clatsop Beaches on 1 November for the first time since November 1991.

5. **Causative Species:** Not confirmed.

6. **Environment:** Typical late summer and fall weather conditions of water temperatures 10-13°C, salinity 25-30 ‰ or greater. Fall rains began in early November which is later than usual.

7. **Advected Population or In Situ Growth:** Not known.

8. **Previous Occurrences:** The last Oregon domoic acid alert began on the northern beaches in November in razor clams and continued to exceed 5 ppm until July 1992.

9. **Additional Comments:** No human illnesses confirmed.

10. **Individual to Contact:**

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Salem, Oregon 97310

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

OREGON

1. **Location:** Entire Oregon coast from California border to Columbia River. North coast bays affected were Netarts, Tillamook, and Nehalem.

2. **Date of Occurrence:** South: 27 April at Bandon, mussel PSP = 62 µg/100 g peaking at 282 µg/100 g on 23 June with levels less than 40 µg/100 g by mid-August. North: 7 May at Roads End with 37 µg/100 g, peaking at 199 µg/100 g on 6 July, levels less than 40 µg/100 g by early September. Toxicity in bay clams ranged from 73 µg/100 g in Nehalem Bay cockles on 5 July to 98 µg/100 g in Tillamook Bay on 6 July.

3. **Effects:** A large mussel shipper was closed for three weeks before lot sampling assured safety of product. Commercial clam divers in northern bays were closed for three weeks. Limited oyster production in northern bays monitored by lot sampling.

4. **Management Action:** Commercial and recreational harvest of shellfish was closed on the north coast on 6 March; southern beaches on 10 June; entire coast closed by 22 June; northern bays were closed on 8 July. South coast reopened on 16 August; north coast bays on 29 July, and north coast beaches, except for razor clams on 30 August.

5. **Causative Species:** Not confirmed, but thought to be *Alexandrium catenella.* Field staff trained to examine species recorded an increased abundance on the south coast prior to toxicity in mussels.

6. **Environment:** Warmer than normal spring temperatures occurred in April and May. July was very hot.

7. **Advected Population or In Situ Growth:** Not known.

8. **Previous Occurrences:** The last Oregon PSP alert began on the northern beaches in August 1993 in mussels and continued to October 1993.

9. **Additional Comments:** No human illnesses confirmed.

10. **Individual to Contact:**

Dr. Deb Cannon
Shellfish Program Specialist
Oregon Department of Agriculture
635 Capitol Street, NE
Salem, Oregon 97310

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

WASHINGTON

1. **Location:** Allyn, Washington at the north end of Case Inlet, Puget Sound.

2. **Date of Occurrence:** late September 1994.

3. **Effects:** Wild fish mortalities.

4. **Management Action:** None.

5. **Causative Species:** *Heterosigma carterae.*

6. **Environment:** Temperature ranged from 20.5°C at the surface to 15.2°C at the bottom; salinity ranged from 26.3‰at the surface to 29.6‰at the bottom; nitrate was low at 0.22 µM, and phosphate was 1.26-1.4 µM.

7. **Advected Population or In Situ Growth:** Probably in situ growth.

8. **Previous Occurrences:** Not known.

9. **Additional Comments:** Fewer than 100 fish died; most were chum salmon, but a few chinook and coho salmon also died.

10. **Individual to Contact:**

Dr. Rita Horner
University of Washington
School of Oceanography
Box 357940
Seattle, Washington 98195-7940

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

WASHINGTON

1. **Location:** Case Inlet, southern Puget Sound, Washington.

2. **Date of Occurrence:** late September through October 1994.

3. **Effects:** PSP toxin in mussels higher than 80 µg/100 grams shellfish meat.

4. **Management Action:** Harvest closure.

5. **Causative Species:** Unknown, presumably *Alexandrium catenella.*

6. **Environment:** Not known.

7. **Advected Population or In Situ Growth:** Probably in situ growth.

8. **Previous Occurrences:** No previous closures in that area of southwestern Case Inlet.

9. **Additional Comments:**

10. **Individual to Contact:**

Dr. Mary McCallum
Office of Shellfish Programs
Washington Department of Health
P.O. Box 47824
Olympia, Washington 98504-7824

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

WASHINGTON

1. **Location:** Southern Hood Canal, Washington.

2. **Date of Occurrence:** November-December 1994.

3. **Effects:** Domoic acid levels in mussels about 10 µg/grams.

4. **Management Action:** None.

5. **Causative Species:** *Pseudonitzschia pungens* f. *multiseries.*

6. **Environment:** Not known.

7. **Advected Population or In Situ Growth:** Presumably in situ growth.

8. **Previous Occurrences:** Not previously known for the area, but few samples collected there.

9. **Additional Comments:** Domoic acid levels in phytoplankton samples collected from the area on 29 November 1994 ranged from < 1 to about 15 µg/ml sample (DA analyses by National Marine Fisheries Service, Seattle).

10. **Individual to Contact:**

Dr. Rita Horner
University of Washington
School of Oceanography
Box 357940
Seattle, Washington 98195-7940

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

ALASKA

1. **Location:** Perryville at Humpback Bay. Humpback Bay is a bight 5 miles across on the south coast of the Alaska Peninsula 12 miles ENE of Stepovak Bay (55°52'N, 152°20'W).

2. **Date of Occurrence:** 30 March 1994.

3. **Effects:** 7 people with symptoms and two people still symptomatic on 31 March 1994.

4. **Management Action:** Village put out a general warning not to eat cmals, State will put out a press release.

5. **Causative Species:** Unknown at this time.

6. **Environment:** Protected area of the Alaska Peninsula.

7. **Advected Population or In Situ Growth:** Razor clams.

8. **Previous Occurrences:** 14 August 1982 — PSP levels of 5028 µg/100 grams with mussels. Five people ill and medivaced to Anchorage.

9. **Additional Comments:** Razor clams sampled for PSP results. Razor clams with viscera, shucked and without siphon tips — 528 and 562 µg/100 grams.

   Razor clams canned, eviscerated (siphon tips removed) — 129 and 263 µg/100 grams. Broth from canned product — 136 and 249 µg/100 grams.

10. **Individual to Contact:**

Dr. Michael J. Ostasz
Shellfish Coordinator, Seafood Program
Division of Environmental Health
State of Alaska
Dimond Center
800 East Dimond Blvd., Suite 3-455
Anchorage, Alaska 99515

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

ALASKA

1. **Location:** Mayflower Beach (Kodiak Island); ~ 57°38'45"N, 152°25'45"W.

2. **Date of Occurrence:** 25 May 1994

3. **Effects:** One person consumed butter clams (2 raw and 3 cooked) and after 1 hour she developed numb lips, followed by numbness of hands; no nausea, vomiting, ataxia, weakness, dysphagia, dysarthria, or respiratory arrest (hospitalized).

4. **Management Action:** Press release.

5. **Causative Species:** ***Alexandrium***

6. **Environment:** No data.

7. **Advected Population or In Situ Growth:**

8. **Previous Occurrences:** General area has had PSP episodes.

9. **Additional Comments:** Butter clams collected had a PSP level of 1701 µg/100 grams.

10. **Individual to Contact:**

Dr. Michael J. Ostasz
Shellfish Coordinator, Seafood Program
Division of Environmental Health
State of Alaska
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800 East Dimond Blvd., Suite 3-455
Anchorage, Alaska 99515

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

ALASKA

1. **Location:** Chiniak Bay area on Kodiak Island (57°42'N, 152°20'W). 13-mile indentation on the NE coast of Kodiak Island.

2. **Date of Occurrence:** 25 May 1994

3. **Effects:** Three individuals ate raw mussels. Consumption varied from 12 mussels (> 12, but less than 50 mussels and greater than 50 mussels). Two individuals hospitalized. One went into respiratory arrest and was then transferred to Providence Hospital in Anchorage. Still intubated and paralyzed (5/25/94) at this time.

4. **Management Action:** Samples to be taken and press release to be issued.

5. **Causative Species:** Suspect ***Alexandrium.***

6. **Environment:** N/A.

7. **Advected Population or In Situ Growth:**

8. **Previous Occurrences:** General vicinity has had PSP occurrences and morbidity in the past.

9. **Additional Comments:** Mussels from implicated beach sampled on 5/26/94 had toxin levels of 18,684 µg/100 grams of shellfish tissue.

10. **Individual to Contact:**

Dr. Michael J. Ostasz
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Division of Environmental Health
State of Alaska
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Anchorage, Alaska 99515

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

ALASKA

1. **Location:** Monashka Bay on Kodiak Island (57°50'N, 152°25'W).

2. **Date of Occurrence:** 26 May 1994

3. **Effects:** One person hospitalized after consuming 20 cooked mussels. Symptoms after 4 hours included dizziness, numbness of lips and hands; symptoms resolved after 20 hours and patient was discharged.

4. **Management Action:**

5. **Causative Species:** ***Alexandrium***

6. **Environment:** 3-mile long bay.

7. **Advected Population or In Situ Growth:**

8. **Previous Occurrences:** Area has had PSP episodes in the past.

9. **Additional Comments:** Mussels collected had PSP levels of 1778 µg/100 grams.

10. **Individual to Contact:**

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800 East Dimond Blvd., Suite 3-455
Anchorage, Alaska 99515

HARMFUL ALGAL BLOOMS IN THE UNITED STATES — 1994

ALASKA

1. **Location:** Old Harbor (Kodiak Island), on west shore of Sitkalidak Strait, 56 miles SW of Kodiak (57°12'15"N, 153°18'00"W).

2. **Date of Occurrence:** 29 May 1994

3. **Effects:** Two people ill after consuming mussels from a beach at Old Harbor. Two of the three people became ill while third person was unaffected. One person ate 10 raw and 3 cooked mussels. Symptoms developed within 1 hour of paresthesia, vomiting and ataxia, following onset of dysphagia and respiratory arrest. Medivac to Kodiak Hospital with coarse ventricular fibrillation. Death occurred within 6 hours of consuming mussels. Other person ate 4 or 5 cooked mussels and symptoms of tingling occurred in 1 hour. Evacuated to hospital in Kodiak and released.

4. **Management Action:** Press release and State of Alaska via Emergency Powers closed beach to harvesting.

5. **Causative Species:** ***Alexandrium.***

6. **Environment:** No data.

7. **Advected Population or In Situ Growth:** Small beach area.

8. **Previous Occurrences:** Unknown.

9. **Additional Comments:** Mussels collected on 31 May 1994 had PSP levels of 19,418 µg/100 grams (toxin levels highest ever recorded in the State of Alaska).

10. **Individual to Contact:**

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DURATION OF TOXIC EPISODES

TYPE OF TOXICITY (PSP, DSP, ASP, NSP, ETC.): PSP

YEAR	area		code	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Maximum toxicity (ug/100g)
1994	KODIAK		26					X	X							19,418 ug/100 GRAMS
																WITH MUSSELS

This table should be used to indicate the duration of the toxic episodes and the maximum level of measured toxicity.

HUMAN INTOXICATIONS

YEAR	MONTH	AREA (CODE)	COMMENTS
1994	MAY	26	KALSIN BAY ON KODIAK, AK. THREE SICK. 18,684 ug/100
			grams w/ mussels is the highest ever recorded in the
			State for PSP
	MAY	26	MAYFLOWER Beach ON KODIAK, AK. 1 SICK. ATE BUTTER
			CLAMS 1701 ug/100 gm level of PSP.
	MAY	26	MUSSELS - ~~MONAS~~ Monashka BAY on kodiak, 1 SICK
			and PSP level of 1778 ug/100 GRAMS
	MAY	26	OLD HARBOR ON KODIAK ISLAND. 2 SICK which
			ATE MUSSELS. ONE PERSON DIED. SAMPLE
			TAKEN 2 DAYS LATER ON SAME Beach Showed
			19,418 ug/100 gm OF PSP TOXIN.

ANNEX IV

REPORT OF THE MEETING ON THE ICES/IOC WORKSHOP OF INTERCOMPARISON ON *IN SITU* GROWTH RATE MEASUREMENTS (DINOFLAGELLATES)

Helsinki, Finland, 15 - 16 May, 1995

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1. OPENING THE MEETING

The meeting was opened by the chairman, Maria Antonia Sampayo. The participants (see Annex 1) were welcomed by the Kaisa Kononen from the Finninsh Institute of Marine Research, which hosted the workshop.

1.1 Approval of the agenda and rapporteur

The agenda was approved by the meeting. Dr. Odd Lindahl was appointed as rapporteur.

1.2 The purpose of the meeting

The meeting was held according to Council Resolution 1994/2:49:1. The purpose was to report on the workshop on the intercomparison study of *in situ* growth rates of dinoflagellates which was held in Averio, Portugal, 25 - 29 July 1994, in order to support the study of harmful algal blooms. Good estimates of population dynamics parameters, such as growth rates, are essential to providing the means to quantify the detailed structure and processes which lead to a capability to model algal populations and bloom development. During the meeting we were aware that most of the techniques used have not been fully developed for dinoflagellate growth rate studies. We think that the obtained results are not adequate for an ICES Co-operative Research Report as the methods need further development to fulfil the purpose to measure *in situ* growth rates of HAB dinoflagellates.

2. SITE INFORMATION

2.1 The Ria de Aveiro system

Aveiro is situated 240 km north of Lisbon (N 40° 38.5', W 8° 44'). The Ria de Aveiro is a shallow lagoon with a wet area of 43-47 km². The Lagoon has a complex topography with three main channels radiating from the mouth, several branches, islands and mudflats (Annex1, map 1). Nutrient concentrations from 1992 and 1993 were presented, clearly demonstrating that the Ria is eutrophic. Organic pollution levels are high mainly from spring to autumn. Along its main channels and at some of the mudflats there is an important bivalve molluscs exploitation, mainly *Mytilus edulis*, *Cerastoderma edule* and *Venerupis pullastra*, which present almost yearly problems of PSP and DSP related with the presence respectiviely of *Gymnodinium catenatum* and *Dinophysis* spp. From the phytoplankton monitoring programme it was shown that a rich variety of diatoms and dinoflagellates are generally found in the area. During this time *Dinophysis* cell numbers were low (<1000 cellsl^{-1}) in the Ria, however, 1.8 - 3.7µg OA/g hep. and DTX-2 were found in *Mytilus edulis* between 14 and 29 of July.

2.2 Logistics

The Aveiro laboratory was well equipped with the basic analytical equipment which was needed for the workshop as well as a helpful staff. Two research vessels were moored at fixed stations (Annex1, map 2) for the sampling and the incubation studies: R/V MESTRE COSTEIRO (27 m) from Lisbon (IPIMAR) at the mouth of the Ria and R/V JOSE MARIA NAVAZ (14 m) from Vigo, Spain (IEO), in the commercial harbour. Two small boats were used for transfer between the laboratory and the vessels.

3. SUMMARIES

3.1 Enclosed water column measurement (E. Dahl and M.A. Sampayo)

In enclosed water columns the same waterbody including its organismns can be studied over time. During the first few days after enclosing a waterbody, the enclosure may represent a more or less natural

ecosystem. The enclosures were plastic bags mounted on aluminium frames (1m in diameter) (Brockmann et al. 1977). Five experiments/fillings were performed (see Section 4.2).

Dinophysis acuminata increased in numbers during the first day after enclosure, with approximate growth rates of 0.1-0.2 divisons per day. However, when the entire experimental period (3-7 days) was considered *D.acuminata* showed negative growth rates, as did another prevalent dinoflagellate, *Ceratium fusus*. Two other dinoflagellates, *Helgolandinium subglobossum* and *Prorocentrum micans* prevalent in the bags showed variable growth rates according to the cell counts. Even if the concentrations of the other *Dinophysis* species were too low to obtain growth data one got the general impression that the heterotrophic species, *Dinophysis rotundata* , showed somewhat better survival in the bags than other *Dinophysis* spp during the experiment. The addition of nutrients (nitrate, phosphate and iron) did not stimulate growth of *Dinophysis* spp. during the following two days, while phytoplankton biomass measured as chlorophyll increased significantly during the same period.

Accompanying diatoms in all the bags were mainly *Leptocylindrus danicus, Thalassionema nitzschioides, Pseudonitzschia* sp., *Thalassiosira* spp. and *Asterionella glacilis.* Their chlorophyll content per cell decreased until nutrients were added to the bags. After the addition of nutrients the chloroplasts recovered and the diatom population looked healthier, and *Leptocykindrus danicus* increased in numbers. Together with the immediate increase in chlorophyll biomass after addition of nutrients this indicates nutrient limitation during the first four days of the experiment. The macro-nutrient concentrations during this initial period were, however, rather high (Table 17) and could hardly have been physiologically limiting to algal growth. During the experiment, encystment of different species of dinoflagellates was recorded. This supports the impression that the growth conditions in the bags were unfavorable to dinoflagellates.

3.2 RNA and DNA Measurements as Indicators of Growth Rate (D.M. Anderson and D.M. Kulis)

Given the potential utility of RNA:DNA ratios and DNA measurements as indicators of growth or physiological condition, an approach was pursued during this workshop to obtain simultaneous measurements of RNA and DNA in the same cell (see Section 4.7). The approach that was pursued involved double-labeling of cells with DNA-specific stains, used in conjunction with fluorescently-labeled ribosomal RNA probes. The latter are short segments of synthetic DNA designed to bind to the rRNA of target organisms. Since rRNA represents the vast majority of total RNA (Kemp et al., 1993), this provides a useful estimate of the RNA content in a cell and avoids the dificulty of finding a stain for total RNA that does not bind to DNA (Danzynkiewicz et al., 1987). In an ideal case, the rRNA probe can be species-specific, and thus serve two purposes: identifying the target species and simultaneously quantifying its rRNA. With respect to *Dinophysis*, and the vast majority of dinoflagellates, no species- or genus-specific rRNA probes yet exist, so a "universal" probe (Giovannoni et al., 1988) that binds to rRNA of all organisms was used instead. Discrimination of the species or genus of interest was thus to be based on the bright orange phycoerytherin fluorescence of *Dinophysis*, in combination with size information from 90° or forward angle light scatter measurements determined by flow cytometry. Since it was not known whether simultaneous RNA and DNA measurements would be possible for individual dinoflagellate species within a mixed assemblage, a "fall-back" position was to measure DNA content alone and to use the distributions of cells passing through mitosis to calculate growth rate (e.g. Carpenter and Chang, 1988).

No growth rate measurements were obtained in this sub-project, although techniques were developed which could have led to estimates of *Helgolandinium subglobosum* growth rate had there been sufficient interest in that organism to justify the expense and time. Reasons for the lack of results include the following:

- Cells of *Dinophysis* species were not abundant. Had they been more numerous, their distinctive orange fluorescence from phycoerythrin pigments would have been used as a distinguishing character to identify cells for which RNA and DNA per cell would be measured. Instead, it was necessary to use chlorophyll

content, size, and labeling intensity (using fluorescent lectins) to distinguish alternative dinoflagellate species such as *H. subglobosum*. Given the need to preserve chlorophyll content as a marker, an untested preservation technique was employed that was ultimately found to degrade ribosomal RNA or to inhibit its labeling.

- An antibody that was supposed to label only *Prorocentrum micans* (another alternative species for study) cross reacted with numerous other phytoplankton in the Aviero samples. This made it impossible to distinguish that species from other organisms, so automated detection of RNA and DNA was again not possible.

- A procedure was developed which allowed *Helgolandinium* cells to be identified using a fluorescent lectin, in conjunction with size information and chlorophyll content, all determined using the flow cytometer. It was then possible to measure the DNA per cell distribution for cells of that species in a sample, and theoretically at least, to calculate growth rate using the mitotic index method of Carpenter and Chang (1988). This approach was not pursued further, however, because *H. subglobosum* was not a species that other participants were investigating, and because of time and money constraints for the many flow cytometric analyses required. Furthermore, the separation of *Helgolandinium* species using lectin labeling needed further verification.

Several conclusions are suggested by this sub-project:

1. The technique of using either RNA:DNA ratios, or RNA or DNA content alone as indicators of growth rate or physiological condition remains an attractive option that deserves further study.

2. These methods are likely to be automated using a flow cytometer, and thus should be focused on species which can be distinguished optically using either distinctive size and pigment characteristics, or which can be labeled with species-specific probes. Development of such probes for *Dinophysis* species and other HAB organisms should continue to be a high priority.

3. Preservation methods must be carefully considered, and several different fixatives used on aliquots of the same samples, in order to adequately preserve nucleic acids and other identification characters such as pigments.

4. Methods must also be developed to concentrate target dinoflagellates when they are present in low concentrations, while simultaneously removing co-occurring diatoms and other unwanted organisms. As with most other methods being evaluated for *in situ* growth rate measurements, low cell abundance is a serious constraint.

3.3 Calculations of *in situ* growth rates from fractions of cells undergoing mitosis (I. Bravo, E. Garcés and B. Reguera)

The frequency of cells with paired nuclei, paired cells and just divided cells (50%, i.e., those missing the lower sulcal list) of *Dinophysis cf acuminata* were monitored during 36 hours in integrated net haul samples (0-10m) to estimate the *in situ* specific growth rate (μ) applying a mitotic index approach (see Section 4.6). Sampling frequency was more intense (every half an hour) during the time of the day when cellular division was suspected to take place.

Concentration of *Dinophysis* in the samples was very low and combined with high concentration of diatoms and detritus. Therefore, it was not possible to observe the amount of cells necessary for an accurate determination of μ . A rough estimation of μ (0.13 day $^{-1}$ the first day, 0.21 day^{-1} the second, equivalent to 0.19 and 0.3 div.day -1 respectively) was obtained by applying the equation of Vaulot (1992) were the calculation of T_D is not required. T_D calculated as twice the distance (h) between the peaks of maximum frequency of paired-nuclei cells and just divided cells was 5.0 $\pm$ 1h. The division rate are

comparable with those obtained in mesocosm bags during the first couple of days. Some preliminary results and conclusions follow:

1. Fixation of the net haul slurry with cold methanol and further storage of the samples in the deep freeze gave the best results for posterior staining of the cells with the DNA-specific fluorochrome DAPI and examination of the nuclei by epifluorescent microscopy.

2. Enumeration of paired nuclei cells in *Dinophysis* cf *acuminata* has proven to be a very time consuming task subject to a high percentage of error if cells are not individually tipped-over, because the two nuclei are disposed parallel to each other and can be interpreted as only one.

3. Induction of sexuality, under various forms of environmental stress, in a significant percentage of the population could constitute an additional source of problems, that have not been tested with this species, because nuclear fusion could be misinterpreted as nuclear fission.

4. Enumeration of recently divided daughter cells in *Dinophysis* is a much easier task as the absence of the lower sulcal list is a conspicuous morphological feature.

5. The application of the mitotic index, based on morphological observations, is time consuming and requires a significant knowledge of the cell cycle peculiarities and life history of each individual species before applied routinely. Once this is achieved, a next step would be to try to automatize the microscopic observations (i.e. image analysis). In species where there are still uncertainties about their biology and life cycle, microscopic observations during cell cycle studies is essential if progress is to be achieved.

6. In field studies, the concentration of cells will always be a limiting factor if the accompanying species can not be easily separated by sieving in a short period of time that does not interfere with the reproduction process.

7. It is possible that container effects started to manifest themselves in *Dinophysis* spp after incubation had proceeded more than 24 h and that the values from the first 24 h provide a better model of what was occurring in the unrestrained habitat.

3.4 Diffusion Chamber Method (M. Varela)

Estimation of growth rates in enclosed natural populations were made according to the method of Furnas (1991) that uses acrylic cylindrical chambers with Nitex mesh (1μm) to close the two ends for *in situ* incubations during a variable period of time. In this exercise, two chambers were carefully filled with samples taken with Niskin bottles at 0 and 5m, and moored at the same depths for 48 h incubation. We made some modifications to the Furnas method by using a 20 μm mesh instead a mesh of 1 μm (see Section 4.3).

All dinoflagellates species exhibited a drastic decrease in numbers, and high concentration of detritus was observed after 48h. It seems that the chambers acted as detritus traps that prevented growth, and caused damage to the surviving cells, which did not look very healthy. It is possible that some of the smaller sized species, like *Prorocentrum micans*, were able to cross through the meshes. It seems that the larger size mesh is not recommendable. This technique could be improved further if some previous tests of the hydrodynamics of the area were to be performed with the same chamber filled with some dye, e.g. rhodamine, that would allow an estimate of the diffusion rate inside the chambers.

3.5 Single cell ^{14}C uptake method (M. Varela, B: Reguera and I. Bravo)

This method based on that Rivkin and Seliger (1981) and later modified by Granéli et al (1992) consists of a typical ^{14}C productivity incubation, using much higher concentrations of radioactive carbon

to make possible estimates of uptake in small numbers (25-50) of cells of a single species isolated by micro manipulation after the incubation period (see Section 4.5). It was planned to apply this technique to *Dinophysis* cf *acuminata*, but the very low concentration of this species made it impossible, and *Prorocentrum micans* was used instead.

The values of ^{14}C fixation obtained at the two incubation depths (0 and 5m) were extremely low, indicating practically zero growth. These values relate well with those obtained in the diffusion chamber. Nevertheless, we have mentioned that the radioactivity of *P. micans* cells was not measured immediately after the incubation period. This was partly due to the fact that the same experts had to deal with several techniques at once to cover for the absence of several key participants. Therefore, the well rinsed phytoplankton population was kept in a refrigerator, and the isolation of cells and measurement of radioactivity was carried out a few weeks later. The possibility of leakage of radioactivity from *P. micans* cells during this interval cannot be discounted, and would have led to an underestimate of the real carbon uptake.

4. TECHNIQUES, MEASUREMENTS AND RESULTS

4.1 Current meter measurements (P. Silva and J. Dias)

Instituto Hidrográfico (Lisbone, Portugal) collected current meter data in two stations at Ria de Aveiro (stations 1and 2) at three different levels in the water column (1 m above the bottom, middle water column depth and at 1 m below the surface). The following results were achieved:

Station 1 (near mouth of Ria de Aveiro):

1. The currents observed were highly related to ocean tidal wave, as expected.
2. The velocity of the current near the mouth was approximately constant in he vertical axis, although the values were greater near the surface. The maximum values occurred in ebb situations. The ebb mean time was longer than the flood mean time, 5h 40 and 6h 30 respectively.
3. The maximum velocities were observed at intermediate tide (2 h after the high and low tide), which showed that the tidal wave in the Ria, at least near this location, was a mixture of a progressive and stationary wave.

Station 2 (Commercial Harbour):

1. In flood situations the velocity currents had a significant value while in the ebb situations the velocity was almost zero. This showed that the harbour could be considered as a reservoir that filled fast end emptied slowly during the tidal cycle.
2. The currents were not constant in the vertical axis; they were more intense near the surface and decreased with depth.

4.2 Enclosed water column measurements (E. Dahl, M.A. Sampayo and H. Cavaco)

INTRODUCTION

With the enclosed water column method the same waterbody including its organisms can be studied over time. During the first few days after enclosing a waterbody the enclosure may represent a more or less natural ecosystem, however, after this initial period the conditions in the enclosures may differ from nature because important physical processes such as turbulence and advection are excluded from the enclosures. Even then rate measurements after some days among species which have survived the enclosure conditions may have some relevance to nature.

MATERIAL AND METHODS

The enclosures were plastic bags mounted on aluminium frames (1m in diameter, Brockmann et al. 1977).

Table 1: An overview of the enclosed water column measurements.

Bag number	Iniated	Filling technique	Zoopl. removed	Nutrients added	Number of samplings	Terminated
Bag 1	22 July	Pump	Yes	No	4	25th
Bag 2	22 July	Pump	Yes	on 27 July	12	29th
Bag 3	24 July	Pump	No	No	10	29th
Bag 4	24 July	Pump	Yes	on 27 July	10	29th
Bag 1-II	25 July	Enclosure	No	on 27 July	9	29th

Five experiments/fillings were performed (Table I). All the bags were filled and placed in the commercial harbour (Annex 2, map 2). The depth of the bags was aproximately 2m. When filled by pumping, water from 2 m depth in the bay was pumped into the bags using a Pumpex GA 200. On 25 July a natural water column was enclosed in Bag 1-II by lowering the flattened plastic bag mounted on the frame to 2m depth, and then enclosing the upper 2m water column by raising the bag to the surface. Zooplankton was removed by sieving the water from the pump through a 140 µm mesh. On 27 July nutrients (nitrate, phosphate and iron) were added to three bags (Table 1). Sampling in the bags was carried out with a tube to obtain integrated samples. When the nutrients were added, the water in the bags was mixed by raising and lowering a disc, this was repeated before the last three samplings.

Parameters measured in the bags during the experiment were nutrients (nitrate, nitrite, ammonium, phosphate and silicate), chlorophyll and phaeopigments, particulate carbon and nitrogen, and phytoplankton composition with emphasis on selected species. Phytoplankton counting under the microscope was performed by different persons after concentration by different methods e.g. filtration, sedimentation and centrifugation (see the results).

RESULTS

The results from counting of *Dinophysis* spp. on filters in microscope with epifluorescence attachment are shown in Tables 2 to 6 /Annex 2). From each sampling, two or three subsamples of 50 ml were concentrated by filtration and counted. This method should theoretically detect concentrations of *Dinophysis* spp. down to 10 cells/l. Only *D. acuminata* was present in numbers high enough to get indicative data on their concentration and growth rates. In all bags cells of this species increased in number during the first 24 hours, but showed negative growth rates when the entire experimental period was considered. From data in Tables 2 to 6 during the first 24 hours of the experiment, the following growth rates for *Dinophysis acuminata* can be calculated according to the formula of Eppley and Strickland (1968):

$k = 3.32 \cdot (\log n_t - \log n_o) / (t - t_o)$

Bag no.	Div./day
1	0.09
2	0.11
3.	0.23
4	0.40
1-II	0.5-1.1

k = growth rate as divisions per day (24 hours)
t_o and t = point of time for two different measurements of cell concentration (unit= days)
n_o and n_t = the corresponding concentration of cells; log = $\log_{10}$

After about 24 hours, however, the concentration of *Dinophysis acuminata* decreased in all bags. Even if the concentrations of the other species of *Dinophysis* were too low to get data on growth rates from Table 2-6, one may get the general impression that the heterotrophic species, *Dinophysis rotundata* , showed somewhat better survival in the bags than *D.acuminata* during the experiment. The addition of nutrients (nitrate, phosphate and iron) at 11.00 h on 27 July to bag 2, 4 and 1-II did not stimulate growth of *Dinophysis* spp. during the following two days, whereas phytoplankton biomass measured as chlorophyll increased significantly during the same period.

The counting of *Dinophysis acuminata* in a Palmer Maloney (PM) chamber after concentration by centrifugation (limit of resolution 50 cells/L) supported the impression of a negative growth rate when the entire experimental period was considered. Counting of other prevalent dinoflagellates from the bags in a PM chamber showed a negative growth rate for *Ceratium fusus* and variable population growth rates for *Helgolandinium subglossum* and *Prorocentrum micans* (Annex 2,Tables 7 to 11).

Accompanying diatoms in all the bags were mainly *Leptocylindrus danicus, Thalassionema nitzschioides, Pseudonitzschia* sp., *Thalassiosira* spp. and *Asterionella glacilis* (Annex 2,Tables 12 to 16). Only *Leptocylindrus danicus* showed a positive growth rate. The content of chlorophyll per cell decreased until July 27 when nutrients were added to the bags. After the addition of the nutrients (Annex 2, Tables 12 to 16), the diatom population looked more healthy (increased pigmentation) and *Leptocylindrus danicus* in particular increased in numbers. Together with the immediate increase of chlorophyll biomass after addition of nutrients this indicates nutrient limitation during the first few days of the experiment.

The macro-nutrient concentrations present during the first few days of the experiment were, however, rather high (Annex 2, Table 17). Dissolved inorganic nitrogen (DIN), phosphorus (DIP) and reactive silicate were in the range 1.5-5.5, 0.1-0.9 and 2.6-5.7 µmol/l respectively, and unlikely to have been physiologically limiting to algal growth. If nutrients happened to be limiting, would possibly be micronutrients, for instance iron. The DIN/DIP ratios in the bags were rather low, 3.4-9.9 (atomic ratios) which indicated that nitrogen would become limiting before phosphorus.

During the experiment, encystment of different species of dinoflagellates were recorded. This supports the impression that growth conditions in the bags were unfavorable to dinoflagellates. By the end of the experiment the sediment in each bag was qualitatively checked for the presence of deposited algae, and the preliminary results revealed a rather strong sedimentation in the bags during the experiment, especially of diatoms. Sub-samples of the sediments will also be carefully examined for dinoflagellate cysts by Portuguese experts in cooperation with University of Oslo..

4.3 Diffusion chamber method (M. Varela)

INTRODUCTION

Phytoplankton production is translated into population growth through increases in cell numbers by binary fission. General approaches have been taken to measure or, usually, estimate *in situ* growth rates of phytoplankton species or communities. One of these approaches is to enclose natural phytoplankton assemblages in containers that are incubated *in situ* or in simulated *in situ* conditions. The method used here to estimate growth rate of several dinoflagellate species, is based on that described by Furnas (1991). Incubation chambers made of clear acrylic plastic were fitted with Nitex mesh (20 µm) as the diffusion membranes. The use of permeable bags *in situ* has several advantages. The exponential growth phase can be extended for several days in natural waters, even when nutrient concentrations are very low. There is

a gradual transition from exponential to the stationary growth phase, which permits to measure most of growth rates lower than the maximum rate (Sakshaug, 1980).

MATERIAL AND METHODS

Sampling was carried out at a fixed station in the Ria de Aveiro (Annex 2, map 2) in the R/V José María Navaz. Samples were taken with Niskin bottles of 5 l volume capacity, at the surface (0m) and 5m depth. A subsample from each depth (T_0 sample) was inmediately preserved with Lugol's iodine solution for microscope counting, to estimate initial numbers of cells of different dinoflagellate species. Another subsample was poured into the diffusion chamber and incubated *in situ* for 48 h (T_{48}), after which the chamber contents were poured into a plastic bottle and preserved with Lugol's solution. Samples for microscope examination were kept in darkness until time of analysis. Six dinoflagellate species were selected for estimating growth rates from cell counts: *Dinophysis acuminata, D. acuta, Helgolandinium spp, Prorocentrum micans, Ceratium fusus* and *C. tripos*. Counts were performed on a Nikon inverted microscope with Nomarski optics following the Utermöhl technique. At least 4 replicates were counted for each sample, and only healthy cells were taken into account. Daily growth rates (Guillard, 1973) of dinoflagellate species were calculated from differences in concentration between T_{48} and T_0.

RESULTS AND DISCUSSION

Preliminary counts of the dinoflagellate populations at time zero (T_0) and after 48 h incubation (T_{48}) incubated at 0 and 5 m depth, showed that all phytoplankton species exhibited a drastic decrease in numbers. Table I summarizes the results of cell counts and growth rates for each taxon.

The content of the diffusion chamber included a very high proportion of detritus that prevented growth and caused damage to the surviving cells that did not appear healthy. This was probably due to the high content of detritus in Ria de Aveiro. Figures 1 and 2 (Annex 2) show the initial (T_0) and final (T_{48}) cell concentration for the six species of selected dinoflagellates. Results clearly show an important decrease in cell density for all species except for *Helgolandinium* and *D. acuta* at 5m. The decrease was dramatic for *P. micans* at surface samples and for *C. fusus* at 5m depth.

Table I. Growth rates for selected taxa of dinoflagellates.

Taxa	depth	T_0 cells l^{-1}	T_{48h} cells l^{-1}	μ (div. day^{-1})
P. micans	0 m	7760	624	-1.82
P. micans	5 m	2304	760	-0.80
C. fusus	0 m	10592	3560	-0.79
C. fusus	5 m	12700	980	-1.85
D. acuminata	0 m	166	56	-0.78
D. acuminata	5 m	240	72	-0.87
C. tripos	0 m	464	120	-0.98
C. tripos	5 m	270	70	-0.97
D. acuta	0 m	64	40	-0.34
D. acuta	5 m	40	32	-0.16
Helgolandinium spp	0 m	3660	1640	-0.58
Helgolandinium spp	5 m	1880	1730	-0.06

4.4 ^{14}C incubation method *in situ* (O. Lindahl and L. Davidsson).

INTRODUCTION

One of the purposes of the workshop was to compare the "old" ^{14}C-method with newly developed methods. The ^{14}C-method is known to give relatively good estimates of the gross production of the whole phytoplankton community in the experimental bottle (Williams, 1993). Thus, in this workshop the community growth rates were to be compared with growth rates of single species measured by both ^{14}C-uptake and by other methods, obviously a difficult task. However, according to existing regional data sets on phytoplankton the summer phytoplankton flora in the Ria de Aveiro is often dominated by a few species. Therefore a comparison between community and single species growth rates might be possible. The ^{14}C measurements were performed in the traditional way by taking water from different depths with a water-bottle and incubating in a single glass bottle (125 ml) at each depth for 2 to 4 hours (BMB, 1976). 10 µCi of ^{14}C was added to each bottle. Immediately after the incubation three parallell subsamples of 10 ml were transferred from of each bottle into a scintillation vial, then acidified and bubbled with air for 15 minutes. The carbon uptake of the whole phytoplankton community was thus measured.

^{14}C-measurements *in situ* are time consuming and may introduce errors due to transfer of water from different depths to the deck of the ship and then back again. In particular cells which are adapted to darkness may be disturbed by this handling. To reduce this problem Dandonneau (1993) developed an automated sampling and incubation device which closes while being lowered. This closing system is suitable for homogenous and clear waters. However, in coastal stratified waters with low visibility and a high abundance of thin subsurface chlorophyll and production maxima, an *in situ* incubator should contain water representative of a certain depth or a thin layer. An *in situ* incubator which should meet these needs has been constructed (Lindahl and Haamer, unpubl.) and is still under development. This incubator is similar to a small water-bottle made of acrylic plastic and kept horizontal when deployed. The closing is triggered by a small hydraulic plunger after approximately 5 minutes. ^{14}C is added from a syringe after the incubator is closed. After incubation the *in situ* incubator and sample content are treated like an ordinary ^{14}C-bottle. Some parallel measurements were made with this *in situ* incubator.

RESULTS

Three measurements on 26 July and one on 27 July were carried out at the station situated just inside the channel mouth of the Ria. Due to very strong tidal currents, the depth of sampling and incubation chosen were not appropriate, and only the samples incubated close to the surface (0.5 m) were accurate.

Day	Time	Chlorophyll *a*	Prim. prod. 0.5 m	Chl./Pp.
		µg l^{-1}	mgC $l^{-1} \cdot h^{-1}$	
26	08.15 am	11.6	128	10.8
	11.10 am	9.6	55	5.5
	14.30 pm	15.1	131	8.5
27	08.15 am	no data	107	no data

Both the chlorophyll *a* concentration and the primary production were high, i.e. within a range typical for an eutrophic area. There is no explanation other than patchiness to the variation in chlorophyll and productivity among the different measurements. To avoid the strong currents at the channel mouth an incubation was carried out at the raft with the bags during the afternoon on 27 July. One bottle was incubated at each 0.5 m down to 4 m depth . Light inhibition at the surface led to a maximum productivity of 340 µgC l^{-1} h^{-1} found at 0.5 m depth (Annex 2, Figure 3). This was a very high value. However, at 2 m depth the productivity was approximately 200 µgC $l^{-1} \cdot h^{-1}$ and at 4 m (just above bottom) a productivity of 22 µgC l^{-1} h^{-1} was measured (as a comparison a high spring bloom value may reach 75 µgC l-1 h and high summer values are approximately 25 µgC l^{-1} h^{-1} in Scandinavian coastal waters). When integrated

over depth the productivity was 699 mgC m^{-2} h^{-1} and the daily production was estimated by the light factor method (BMB, 1976) as 7700 mgC m^{-2} d^{-1}, which indicated that primary production was very high on this occasion. Unfortunately, no chlorophyll samples were taken during this day. The Secchi depth was 1.5 m when all primary productivity measurements were made .A comparison of the results from the two sites shows that the primary productivity at 1 m depth was almost three times as large in the commercial harbour as at the channel mouth.

4.5 Single cell ^{14}C uptake method (M. Varela, B. Reguera and I. Bravo)

INTRODUCTION

Primary production experiments are carried out routinely to estimate growth of total phytoplankton populations. However, this method can be used to measure growth rates for individual species, if cells are isolated from the rest of phytoplankton population and ^{14}C uptake measured.

The method used here is based in that of Rivkin and Seliger (1981), later modified by Granéli *et al.* (1992) to estimate growth rates of initial cells of dinoflagellate species. The purpose of the experiments was to conduct a typical ^{14}C productivity incubation, in chambers of sufficient size that cells are not disturbed. In the present study, we measured growth rates of *Prorocentrum micans* in a station in Ría de Aveiro. This species was one of most abundant dinoflagellates in the samples.

MATERIALS AND METHODS

Sampling was carried out at a fixed station in the Ria de Aveiro (Annex 2, map 2) in the R/V José María Navaz. Samples were taken with Niskin bottles of 5 l volume, at surface (0 m) and 5 m depth. Polycarbonate bottles of approximately 1 l volume were used. Water samples from 0m and 5m were gently poured into these bottles. Two replicates samples for each depth were inoculated with ^{14}C at a concentration of 1 μCi per ml. Inmediately after ^{14}C was added and mixed, an aliquot was taken from each bottle to measure the true activity added to the samples, and placed in a scintillation vial with a 1 ml of Carbo-sorb E, to avoid losses of radioactivity. After 24 h incubation, the samples were poured through a large sieve (130 μm) into a beaker. The material collected was then poured through a second 20 μm sieve, followed by at least 2 liters of filtered seawater, to rinse away residual inorganic ^{14}C. The sieve contents were washed into a small tube, which was placed in a beaker on ice in a cooler and kept in darkness.

Subsamples were taken from this suspension and placed on slides for cell isolation. Cells were washed thoroughly through transfers to drops of filtered seawater before placing them into scintillation vials, while noting the exact number of cells isolated. Around 50 cells were isolated into each vial to yield statistically valid observations. As controls 50 samples of background water (i.e. no cells) approximately equal in volume to the amount included with each cell isolated were prepared. Controls were also placed in scintillation vials for counting. Two drops of 5% HCl were added to each vial to remove all inorganic ^{14}C. Vials were filled with 10 ml of Insta-gel II, and activity was measured with a Wallac 1409 Liquid Scintillation Counter. To estimate growth rate, it is necessary to estimate the amount of carbon in *P. micans* cells. Therefore, we estimated the abundance of *P. micans* in samples at 0 and 5m and the cell dimensions were measured to estimate cell volumes. Cell volumes were converted into carbon content using the equations of Strathmann (1967).

RESULTS AND DISCUSSION

Table I summarizes the results obtained in the single cell ^{14}C uptake experiment. Initial cells density was 7760 and 2034 cells. l^{-1} for 0 and 5 m, respectively. The carbon content per cell, estimated from cell volume was 921 and 915 pg C $cells^{-1}$ for 0 and 5 m depth samples. The^{14} C uptake after 24 h incubation for both depths was very low. Consequently, the increase of biomass was minimal yielding a growth rate of nearly zero.

Table I.- Cells. l^{-1}, pg C $cell^{-1}$, Initial biomass (mgC m^{-3}), ^{14}C uptake after 24 h (mgC m^{3}), final biomass after 24 h (mg C m^{-3}) and growth rate (μ, doublings day^{-1}) of *P. micans* at 0 and 5 m depth.

depth	cells l-1	pg C $cells^{-1}$	Initial biomass	^{14}C uptake	Final biomass	μ
0m	7760	921	7.15	0.05	7.20	0.010
5m	2034	915	2.12	0.02	2.14	0.014

Microscopic examination of samples showed very high densities of phytoplankton. Bacteria and microflagellates were very abundant. Under these conditions it is possible that ^{14}C activity measured was not only the result of a direct uptake of *P. micans* but a consequence of associated material (bacteria, flagellates....) attached to the cell wall. The low values of ^{14}C uptake relate well to those obtained in the diffusion chambers, where a strong decline in the *P. micans* population was observed after 24 h.

4.6 Calculation of *in situ* growth rates from the fraction of cells undergoing mitosis (I. Bravo, E. Garcés and B. Reguera)

INTRODUCTION

The main objective was to calculate the potential specific growth rate (μ) of *Dinophysis* cf *acuminata* from the fraction of cells undergoing mitosis, i.e. the mitotic index method (McDuff and Chisholm, 1982; Vaulot, 1992). *D.* cf *acuminata* is the main agent of DSP outbreaks in the west coast of the Iberian Peninsula (Galicia, Portugal), where it usually proliferates in several pulses between spring and autumn, but concentrations are highly variable and determined mainly by tidal and upwelling regimes.

During the development of the intercomparison exercise in Ría de Aveiro, the phytoplankton community was composed mainly of a thick biomass of diatoms, and there was a high amount of detritus that rendered observation of the samples a difficult task. Dinoflagellate concentrations were moderate to low. Cells of *Dinophysis* spp were not abundant (300-1000 cells/l), and there were suggestions about focussing the effort on other species. The most abundant dinoflagellates were *Ceratium fusus*, *Helgolandinium* sp., and *Prorocentrum micans*. *C. fusus* was judged to be unsuitable by some participants because it has shape an inappropriate for flow cytometry measurements. *Helgolandinium* sp was identified in the samples after thecal plate examination during the exercise. Nevertheless, when examining the whole phytoplankton population this species can be easily confused with *Protoperidinium* spp. Furthermore, it does not show any remarkable morphological features that allows the recognition of daughter cells after cytokinesis. For *P. micans*, only one of the two daughter cells exhibits the apical spine, a morphological character quite difficult to distinguish under the light microscope with field populations. The main reason this species was rejected as a candidate for the application of the mitotic index was because of its heart-shaped and permanently split nucleus. Besides that, in the course of cell cycle studies with cultures of *P. micans,* Bhaud et al (1988) have shown that under environmental stress, a very high proportion of the population exhibits a double nucleus that is the product, not of vegetative division, but of sexual reproduction. These binucleated cells are morphologically indistinguishable from vegetative cells. In the case of *Dinophysis* spp. previous observations in field populations (Reguera et al, 1995) suggest that potential gametes and cells undergoing sexual reproduction usually comprise a very small percentage of the population (less than 5%), but we can not discount the possibility that at certain times of the growth season and under certain hydrographic constraints this proportion might be much higher, as has been reported by MacKenzie in New Zealand (1992). Nevertheless, in the case of *Dinophysis* spp there seems to be an anisogamous sexual conjugation, and the "small cells" can be easily distinguished from normal vegetative cells.

Although the number of cells examined were clearly unsufficient for statistically reliable determination of μ, the observations on *Dinophysis* cf *acuminata* would provide valuable descriptive information and a rough estimation of the specific growth rate during the two cycles. This information can

be used to encourage discussion with the other participants on the technical problems encountered during *in situ* studies of a real "species of interest" for many phytoplanktologists and aquaculturists all over the world.

METHODS

Samples were collected within a period of 36 h by means of vertical hauls of a 20 μM plankton net in the upper 10m. Frequency of sampling was every two hours from noon to 8.00 pm, every hour from 8.00 pm to 6.00 am and even every half hour between 6.00 am and 10 am, the period when the phased division is expected, and when the processes of cytokinesis and sucal list regeneration can take place quite rapidly. The net hauls were screened through a 150 μm sieve to eliminate large zooplankton, the effluent rinsed through a 20 μM mesh to concentrate the microphytoplankton, and the slurry obtained resuspended in cold methanol. This fixed material was kept in the deep freeze until stained. For observation of the nuclei by epifluorescent microscopy, subsamples of the methanol-fixed material were centrifuged in Eppendorf vials 5 min at 10000 rpm, the supernatant was aspirated away and the pellets were stained with the DNA -specific fluorochrome DAPI at a final concentration of 1 $\mu g.ml^{-1}$ and kept in the refrigerator for at least 24 h.

Frequencies of paired-nuclei, paired cells and recently divided cells (only the cells missing the lower sulcal list) were plotted against time to estimate division time (Td), and division rates based on the mitotic index approach. The division time of *Dinophysis* populations was calculated from the distance (time) between the peak of paired nuclei cells and the peak of recently divided cells multiplied by two, following the description of Carpenter and Chang (1988).

RESULTS AND DISCUSSION

Figure 4 (Annex 2) shows the distribution of frequencies (percentage over the total) of dividing (4A, 4C) and half the number of recently divided cells (4B, 4D) (only those missing the lower sulcal list). In some samples, the abundance of *Dinophysis* was so low that only a few cells were detected after examining three aliquots (the examination of one aliquot often took more than one hour under the epifluorescent microscope). These results, then, are very unaccurate, but we can extract some qualitative information, and give some rough estimations of μ_{min} following the equation of Vaulot (1992):

$$\mu_{min} = 1/1day \,.\, \ln (1 + f_{max})$$

(f_{max} = maximum frequency of of dividing cells; μ_{min} = minimum growth rate).

As it has already been observed in other *Dinophysis* populations from the Mediterranean coast of Spain (Delgado et al., 1995; Garcés et al., in prep.) and from the Galician rías (Reguera et al, 1995), division is in phase and takes place during a defined window of time that can vary in different locations, seasons and hydrographic conditions. In the case of Ría de Aveiro, the window for nuclear division was between 3.30 and 8.00 GMT. The maximum frequency of just divided cells in Aveiro appeared both days at 7.30 (in the Galician rías with hourly intervals for sampling in early June it was between 6.00 and 7.00, n=1000).

The peak frequency of binucleated cells at 4.00 on the second day (fig. 1C) is very unreliable because of the low number of cells observed (n=16). If we assume that the peak is in the middle of the plateau of this figure, i .e. at 5.30 (n=157 in that sample), as a very rough estimate we can calculate a distance of about 2-3 hours between the peaks of maximum frequency of paired nuclei and maximum frequency of recently divided cells. Therefore, we can give an aproximate value of T_D (Tpaired + Trecent) of 5.0 $\pm$ 1 h. Division time of *Dinophysis sacculus* in Mediterranean coastal waters was estimated as 6h, from 3.00 to 9.00 h (GMT) (Garcés et al, in prep.).

Because we sampled every 0.5 h during this critical interval, the duration of mitosis is far greater than the interval during which the increase in frequency of dividing cells occurs. Maximum frequency of recently divided cells on the second day (fig. 4D) was 0.24. We noted that the observation of paired nuclei in *Dinophysis* can have a high percentage of error. Quite often in lateral view there seems to be only one nucleus, but in frontal (girdle) view, two paralell nuclei can be seen. The observations are extremely time consuming if it is necessary to shift the orientation of each cell. Observation of cells exhibiting only the upper sulcal list is much easier, less subject to errors and subjective differences, and is more reliable when trying to estimate maximum frequency of cells that have gone through mitosis. Another detail worth mentioning is that paired cells, just before cytokinesis, can be easily seen shortly before the appearance of recently divided cells if the samples are gently manipulated. The link between daughter cells of *D. acuminata* seems to be very labile, and is largely destroyed with centrifugation prior staining. Given the above comments, and if we apply the approach of Vaulot (1992), we can give an approximate value of μ_{min} of 0.13 the first day and 0.21 the second day of the exercise.

4.7 RNA and DNA Measurements as Indicators of Growth Rate (D. M. Anderson and D. M. Kulis)

INTRODUCTION

RNA and DNA measurements can be used in different ways to obtain estimates of phytoplankton growth rates. For example, the ratio of RNA:DNA is used extensively in studies of fish, fish larvae, and other larger marine organisms as an indicator of physiological condition. The concept has been explored for marine bacteria (Delong et al., 1989) and phytoplankton (Dortch et al., 1983). For some of these micro-organisms, it is clear that the ratio varies systematically with growth rate (e.g. Dortch et al., 1983; DeLong et al., 1989). Nevertheless, considerable work remains, especially with micro-organisms, to determine whether the environmental variables that limit growth affect the ratio in different ways (Dortch et al., 1985; Berdalet et al., 1992, 1994).

With respect to toxic or harmful dinoflagellates, relatively little is known about the utility of the RNA:DNA ratio as an indicator of physiological condition or growth rate. One of the objectives of this subproject within the workshop was to investigate how this ratio might vary in a *Dinophysis* population. Another potentially useful measurement would be of DNA alone, as shown by Chang and Carpenter in a series of papers (Çhang and Carpenter 1988, 1991, 1994; Carpenter and Chang 1988). For this technique, DNA-specific stains are used to quantify the amount of DNA in individual cells through time, which can then be used to estimate growth rate using the mitotic index approach (McDuff and Chisholm 1982; Weiler and Chisholm 1976).

Given the potential utility of RNA:DNA ratios and DNA measurements by themselves, an approach was pursued during this workshop to obtain both types of data. In order to obtain simultaneous measurements of RNA and DNA in the same cell, double-labeling with DNA-specific stains (7-AAD, propidium iodide, DAPI, or Hoechst) were used in conjunction with fluorescently-labeled ribosomal RNA probes. The latter are short segments of synthetic DNA designed to bind to the rRNA of target organisms. Since rRNA represents the vast majority of total RNA (Kemp et al., 1993), this provides a useful estimate of the RNA content in a cell and avoids the problem of attempting to find a stain for total RNA that does not bind to DNA and does not vary stoichometerically due to conformation of the rRNA (Danzynkiewicz et al., 1987). In an ideal case, the rRNA probe can be species-specific, and thus serve two purposes: identifying the target species and simultaneously quantifying its rRNA. With respect to *Dinophysis*, and the vast majority of dinoflagellates, no species- or genus-specific rRNA probes yet exist, so a "universal" probe (Giovannoni et al., 1988) that binds to rRNA of all organisms was used instead. Identification of the species or group of interest was to be based on the bright orange phycoerytherin fluorescence of *Dinophysis*, in combination with size information from 90° or forward angle light scatter measurements from flow cytometry. Since it is not known whether simultaneous RNA and DNA measurements would be possible for individual dinoflagellate species within a mixed assemblage, a fall-back position was to measure DNA content alone and to use the distributions of cells passing through mitosis to calculate growth rate . Alternatively, if an organism other than *Dinophysis* was to be analyzed for RNA:DNA or

DNA alone, attempts would be made to specifically label the target organism using fluorescently labeled lectins (Costas et al. 1993), or to otherwise distinguish it using size and chlorophyll content.

METHODS

All experiments were conducted in the Ria de Aveiro lagoon, Aveiro, Portugal, as described in the introduction to this report. For the RNA:DNA analysis, nonquantative, integrated plankton samples were collected every two hours for 36 hours by lowering a 20 mM plankton net to within 2 meters of the bottom of the water column and raising it vertically twice in succession. The sample was then screened through a 130 mM Nitex sieve and the effluent was rinsed through a 20 mM sieve to concentrate dinoflagellate species. Cells were preserved in 2.5% formaldehyde, and stored at 4°C in the dark until analysis.

For analysis, a 1 ml subsample was removed and resuspended in 3 ml filtered sea water. This subsample was then passed through 80 and 35 μm Nitex sieves to further purify the sample. The 35-80 μm fraction was resuspended in approximately 1 μm filtered sea water, and the cell slurry injected through a tuberculin syringe into 10 ml of ice cold methanol and stored at -20°C for several hours to facilitate chlorophyll extraction. One ml of the subsample was centrifuged at 2000 x g for 5 minutes, the supernatant aspirated, and 0.5 mL hybridization buffer containing 5X SET (750 mM NaCl, 100 mM tris-HCl, 5 mM EDTA, pH 7.8), 0.1 mg/L polyadenylic acid, 0.1% Tergitol NP-40, 10% formamide was added to the cell pellet. The sample was prehybridized at 37°C for 30 minutes. 50 μl of a FITC conjugated universal or negative shipworm bacterium control rRNA probe (final conc. 5 ng/l, Distel et al., 1991) were added and the sample was incubated for an additional 2 hours at 37°C. The sample was then centrifuged as described above and the cell pellet washed in 0.2X SET buffer for 10 minutes at 37°C. Following the wash, the sample was again centrifuged, the supernatant aspirated, and the hybridized pellet was resuspended in 5X SET, and 5μl of each fluorescently labeled lectin was added. The lectins used were: Concanavalin A (Con A), *Dolichos biflorus* agglutinin (DBA), peanut agglutinin (PNA), *Ricinus communis* agglutinin (RCA120), soybean agglutinin (SBA), *Ulex europaeus* agglutinin I (UEA I), and wheat germ agglutinin (WGA) (Vector laboratories Inc., Burlingame, CA). Con A was added at a concentration of 67 mg/ml, while the other six lectins were used at a concentration of 6.7 mg/mL. The samples were allowed to incubate in the dark for 1 hour at room temperature and then washed twice with 1.5 mL 5X SET. 75 ml of the DNA specific stain, 7-AAD (7-aminoactinomycin D, 50 mg/ml, Molecular Probes Inc., Eugene, OR) was then added and the samples analyzed on an Epics flow cytometer to quantitate the rRNA and DNA fluorescence.

RESULTS

Due to the low numbers of *Dinophysis* encountered in theAveiro samples, effort was focused on two dinoflagellate species that were considerably more abundant: *Helgolandinium subglobosum* and *Prorocentrum micans*. The first attempt was to focus the study on *Prorocentrum*, as a polyclonal antibody targeting its cell surface proteins was available to label and identify these cells (Vrieling 1993). However, problems with cross reactivity of this antibody to other phytoplankton species were quickly apparent, precluding use of this technique for species identification (L. Peperzak pers. comm.).

Helgolandinium subglobosum, a large, round, thecate dinoflagellate, approximately 45 μm in diameter was therefore the only remaining study organism. Post-collection size fractionation of the plankton samples yielded a relatively pure sample of *Helgolandinium*. Since *Helgolandinium* has neither the characteristic phycoerytherin autofluorescence of *Dinophysis*, nor a species-specific antibody probe, an attempt was made to find a lectin which only labeled this species. A suite of seven lectins were initially screened to determine if one or more of them were specific towards extracellular sugars of *Helgolandinium*.

Examination of the lectin-treated samples by fluorescence microscopy revealed that all had an affinity for *Helgolandinium* as well as the majority of the plankton in the sample, including *Prorocentrum*. The labeling intensity was, however, less intense in *Helgolandinium* than that in similarly sized cells of

other species in the processed sample. In effect, the labeling worked in reverse -- the target species was detectable due to its *lack* of intense labeling fluorescence. This phenomenon was especially true for the lectin Con A, which was selected as the label of choice. The combination of reduced lectin staining of the cell surface, cell size information (as measured by forward angle light scatter on the flow cytometer) and residual chlorophyll autofluorescence was used to distinguish this dinoflagellate from the rest of the sample population. (Annex 2, Figure 5A,B,C,D) Even though chlorophyll was extracted prior to analysis by methanol in the fixation procedure, this process is not completely efficient. Consequently, residual chlorophyll signal could still be used as a marker to help distinguish *Helgolandinium* from the rest of the population. The vast majority of the other phytoplankton cells in the same size range were heterotrophic and had no chlorophyll autofluorescence.

After the *Helgolandinium* population had been identified using the flow cytometer, a sample labeled with Con A and 7-AAD was analyzed. A broad DNA distribution of this gated population was obtained, but to accurately access which of the peaks represent cells in G1, S, or G2+M cell cycles stages, further analysis of the time series samples would be required. This information, integrated with cell density and photoperiod data could then be used to establish when the cells were undergoing division, as indicated by an elevated percentage of S and G_2 +M phase cells. At this stage, analysis of the samples was terminated for reasons given below.

DISCUSSION

Cell Preservation

This project highlighted the need for further research on which method of cell preservation and storage best retains rRNA in phytoplankton cells. Ongoing experiments suggest that a quick formalin fixation followed by low temperature storage in methanol is a suitable choice. However, storage of the cell pellet in methanol extracts most of the chlorophyll-a, a pigment that can be useful in the identification of the target organism. Phycoerythrin, the dominant pigment in *Dinophysis* is not affected by this preservation method. As samples were being taken during the workshop, it was realized that *Dinophysis* concentrations in the Ria de Aveiro lagoon were quite low, and were predicted to remain low throughout the study. The decision was thus made to preserve the samples in formalin without methanol extraction, in order to preserve chlorophyll-a as an identification character for the autotrophic plankton.

In retrospect, this was a bad decision, as RNA analysis proved to be impossible given the manner in which the cells were preserved. We now believe that formalin preservation followed by long term storage at 4°C in the dark either destroys rRNA or renders it inaccessible by excessive cross-linking of proteins and alkylation of amino groups of bases in single-stranded nucleic acids (Keller and Manak, 1989).

Species Identification

A specific set of unique characteristics for species identification is a prerequisite for an automated approach to enumerating phytoplankton and determining their physiological properties. Traditionally, lectins, antibodies, or nucleic acid probes have been used as specific markers for cell identification. In this study, the distinctive size and pigmentation of *Dinophysis* was to be used as as the distinguishing marker for this group of organisms. Due to a shortage of this cells of this genus in the lagoon, however, *Prorocentrum* was the next choice. Identification of this species was to be accomplished using an antibody to *P. micans*, but it was tested and found to cross react with other phytoplankton.

The final option was to use lectins to differentially label the dinoflagellates in the samples. The fluorescently labeled lectin, concanavalin A provided an indirect method to distinguish *Helgolandinium* from the rest of the plankton in the sample. By utilizing the reduced fluorescent signal of this lectin on *Helgolandinium* as compared to other cells of similar size, this organism could be distinguished from the other phytoplankton. Once a unique identifying characteristic for an organism is determined, it was then

necessary to concentrate it and eliminate as many of the co-occurring organisms as possible, since the ultimate objective was to use flow cytometry in the analyses. Simple size fractionation by washing the sample through an appropriately sized series of Nitex sieves was the means by which this was easily accomplished.

Dual Labeling

In this study, the outer cell surface of *Helgolandinium* was labeled with an FITC-conjugated lectin and the nucleus stained with the DNA-specific fluorochrome 7-AAD. An important consideration when using multiple fluorescent probes in the same cell is the possible interference the flurochromes may have on one another. This concern may be minimized by selecting fluors which are spectrally separated, such as FITC and 7-AAD, and by understanding which membranes or organelles the probes are targeting. For instance, in the toxic dinoflagellate *Alexandrium fundyense,* an rRNA probe conjugated with FITC could not be detected when the nucleus was simultaneously stained with propidium iodide. Presumably the FITC emission (which has a wavelength maximum of about 520 nm) is quenched by the propidium iodide in the nucleus (which is maximally excited by 535 nm light). If, however, the FITC labeled rRNA probe was used in combination with the nuclear stain 7-AAD, which has an excitation maximum of approximately 550 nm, the two flurochromes are both detectable. Even though the emission of FITC can and probably does excite the 7-AAD in the nucleus, only a very small amount of the emission is quenched (unpub. data).

Growth Rate Measurements

Had *Dinophysis* been present in high numbers, plankton samples would have been preserved in a quick fix of formalin, followed by storage in ice-cold methanol. In the absence of abundant *Dinophysis*, the decision was made to preserve samples so that chlorophyll could be used as a character for identification on the flow cytometer. Subsequent to this decision, we learned that the rRNA in these samples was either destroyed or rendered inaccessible due to this preservation method, precluding the use of RNA:DNA ratios for analysis.

It would be still possible, however, to pursue the mitotic index approach (Chang and Carpenter 1988, 1991, 1994) on *Helgolandinium* spp by other workshop participants. Furthermore, this is still, as yet, an unproven method of automated species identification which needs to be verified by studies which would prove that only the target *Helgolandinium* cells and none of the other organisms in the sample are included in the population analyzed on the flow cytometer.

Future Considerations

Unanticipated problems were encountered in the attempts to analyze RNA:DNA ratios in specific dinoflagellate species. Some of these have been remedied and others will continue to be investigated. Most importantly, it remains to be seen whether the approach of simultaneously quantifying RNA using an rRNA probe and DNA using a DNA-specific fluor will provide useful information on growth rate or physiological condition. The ratio alone does not provide a growth rate estimate. It is either necessary to calibrate a target organism using laboratory cultures, or to use the patterns of rRNA or DNA analysis to infer a division rate.

In addition to specific methodological obstacles, there were several aspects of this study that could be improved upon so that future workshops on the intercomparison of growth rate measurements could have a better chance of success. Some of these are:

- Choose a field location where *Dinophysis* or the intended study organism has a predictable record of high population densities, without elevated abundances of other co-occuring phytoplankton species. This would allow for easier sample preparation, and more reliable data due to fewer interfering organisms.

- Have laboratory cultures available that are growing at known rates, which could then be used by all participants as controls to confirm the measurements made by each of the techniques under study. Without this, the growth rates measured in the field will always be suspect even though there may be agreement between methods.

- Collect sufficient biomass and store it in several different ways to allow methods to be attempted that might not have been anticipated at the start of the study.

- Overall, the ambitious goal of obtaining in situ growth rates for s single dinoflagellate species in a mixed assembalge were not achieved in this project or in others that were attempted during the workshop. As methods development continues on this important issue, and as workers realize the changes in procedures that are needed to avoid the problems encountered during the first workshop, it should be possible to conduct a more successful, second intercalibration exercise int he near future.

4.8 DNA/PCNA cell cycle method (E. Carpenter and S. Lin).

We measured growth rates of phytoplankton using a cell cycle technique. Basically, we obtained the growth rate by sampling the phytoplankton at 2 hr intervals over a 24 h period, then determining the percentage of cells which are dividing. From this information and a determination of the length (duration) of the division phase (or some other "terminal event"), growth rate was calculated. A terminal event is defined as a marker occurring at the end of the cell division cycle. It can be a microscopic observation of the number of paired cells, a measure of cells with 2x DNA or the presence of a chemical which might only be present at one stage of the cell cycle or some other type of observation. We used two methods for determining the percent which will divide, DNA and PCNA.

For the DNA technique, we collected phytoplankton and preserved them in methanol. The methanol serves to remove photosynthetic pigments which might fluoresce and also preserves the cells. Next we added the DNA- specific fluorochrome DAPI. DAPI fluorescence is proportional to DNA content, and we measure DNA in single cells using a TV-computer-based microscope system. After the DNA content of about 300 cells of a selected species is saved on the computer, we can plot a histogram of the DNA profile of the population. By examining profiles at 2 hr intervals through the day we can see how the population progresses through the cell division cycle. Equations are then used to deconvolute the histograms and extract each of the cell cycle phases: G, S, G_2+M. The G, S, G_2+M phases are used as the "terminal event" and we calculate growth rate by comparing those which are dividing (with a "terminal event") with those that are not.

Since the above method is time consuming and involves a lot of expensive equipment, we have developed an antibody method to substitute as the "terminal event". The presence of the cell cycle protein PCNA (proliferating cell nuclear antigen), a cyclin compound is used as the event. All that is required is to add fluorescent labeled antibodies to PCNA to a sample and then to visually examine the sample using a standard epifluorescence microscope. This way, the investigator can visually examine the species composition of the whole phytoplankton population and obtain growth rates for all species. Sample collection and formulas for determining growth rates are identical to that used for the DNA method.

No results were achieved due to deficient samples conservation.

4.9 Monoclonal antibodies, species specific diel DNA measurements and bioassay (L. Peperzak)

METHODS

1. Collection of *Dinophysis* spp, to be used for the production of monoclonal antibodies (Vrieling et al, 1994).

2. 48 hours of sampling for flowcytometric species and DNA measurements. Samples will be labelled with a species specific label and a DNA dye. The species label will trigger the f.c.m. that will then measure the amount of DNA present. Growth rates can then be calculated with the Carpenter-cell cycle method (Chang and Carpenter, 1988). (Species labels: *Prorocentrum micans, Alexandrium tamarense, Pseudonitzschia pungens f. multiseries*).

3. Samples for bioassay experiment were incubated in bottles that were moored *in situ*. The following additions were made: 1.) none, 2.) growth factors, 3.) pH lowering, 4.) chelator, 5.) PEP-Si growth medium with extra vitamins, 6.) All (6 bottles in duplicate). Effects were measured as *in vivo* chlorophyll fluorescense and cell (*P. micans, Dinophysis spp*) concentration.

Because *Dinophysis* spp abundance was low, there was no opportunity to collect enough cells for monochlonal antibody production. The 48 h sampling programme was reduced to 36 h. Two vertical net hauls were taken with a 20 µm plankton net at two stations. *s.*

RESULTS

It was calculated that All and PEP-Si were significantly higher than the rest (tested as a group). Looking at a number of individual species however, the effects seem negligible. There is one exception: the number of *Polykrikos schwartzii* cysts is significantly higher in the bottles with chelator added (EDTA). The incubation itself had a negative effect on the abundance of *P. micans*, *C. fusus* and *D. acuminata*. *H. subglobosum* increased slightly in most bottles. The abundant diatom *Leptocylindrus* increased too in all bottles. All in all however, these data cannot be used for the calculation of growth rates of dinoflagellates. In a number of cases these would be negative, as I already showed during the workshop.

The conclusion for the measurement of *in situ* growth rates of (toxic) dinoflagellates is that incubations should be avoided; the dinoflagellates counted all performed worse then the diatoms. Maybe some of these species of phytoplankton are especially vulnerable to (long) bottle incubations (*Dinophysis* spp. versus *Helgolandinium*). If growth rates are relatively small this effect leads to net negative values ("death rates").

Sampling, fixing and determining some kind of terminal event (DNA cycle, PCNA, number of dividing cells, etc.) to calculate growth rate seems therefore more preferable than incubations. This may perhaps also apply to short term (hours) incubations

5. CONCLUSIONS AND RECOMMENDATIONS

1. The WG highlighted the availability of current methods for determining *in situ* growth rates of HAB species. Although significant progress in the comparison of algal growth methods was achieved, some of the applicable techniques are not yet fully developed and evaluated for dinoflagellates. Given the crucial importance of growth rate estimates in understanding algal bloom dynamics, we propose the planning and convening of a second technical workshop to bring this to completion. The early fall of 1996 is a possible target date.

2. The study site selected should have well-defined hydrographic conditions and it would be advantageous to utilise a mesocosm facility in order to reduce the complications which advection or other physical processes may introduce.

3. The study site should host commonly occurring HAB species that can be cultured. Cultures will be available prior to the workshop for preliminary studies and throughout the workshop as a complement to the growth rate measurements on the natural dinoflagellate community.

4. We recommend that the workshop be located at the Kristineberg Marine Research Station, situated on the Swedish west coast, since the above important criteria, as well as laboratory facilities and housing for the participants, can be provided there.

5. During the intersessional period, we encourage investigations which attempt to overcome methodological difficulties identified in the first workshop. This should include work of cultures on a selected set of HAB species. Careful co-ordination of tasks and responsibilities by a technical planning committee appointed by the WG is necessary so that appropriate measurements are made with optimal precision and accuracy.

6. One day of the 1996 ICES working group meeting on Harmful Algal Bloom Dynamics should be devoted to detailed planning of the workshop. Key investigators should attend the Working Group meeting.

7. Dr Odd Lindahl, Kristineberg Marine Research Station, should chair the workshop, including the intersessional planning and preparation with the help of a planning committee.

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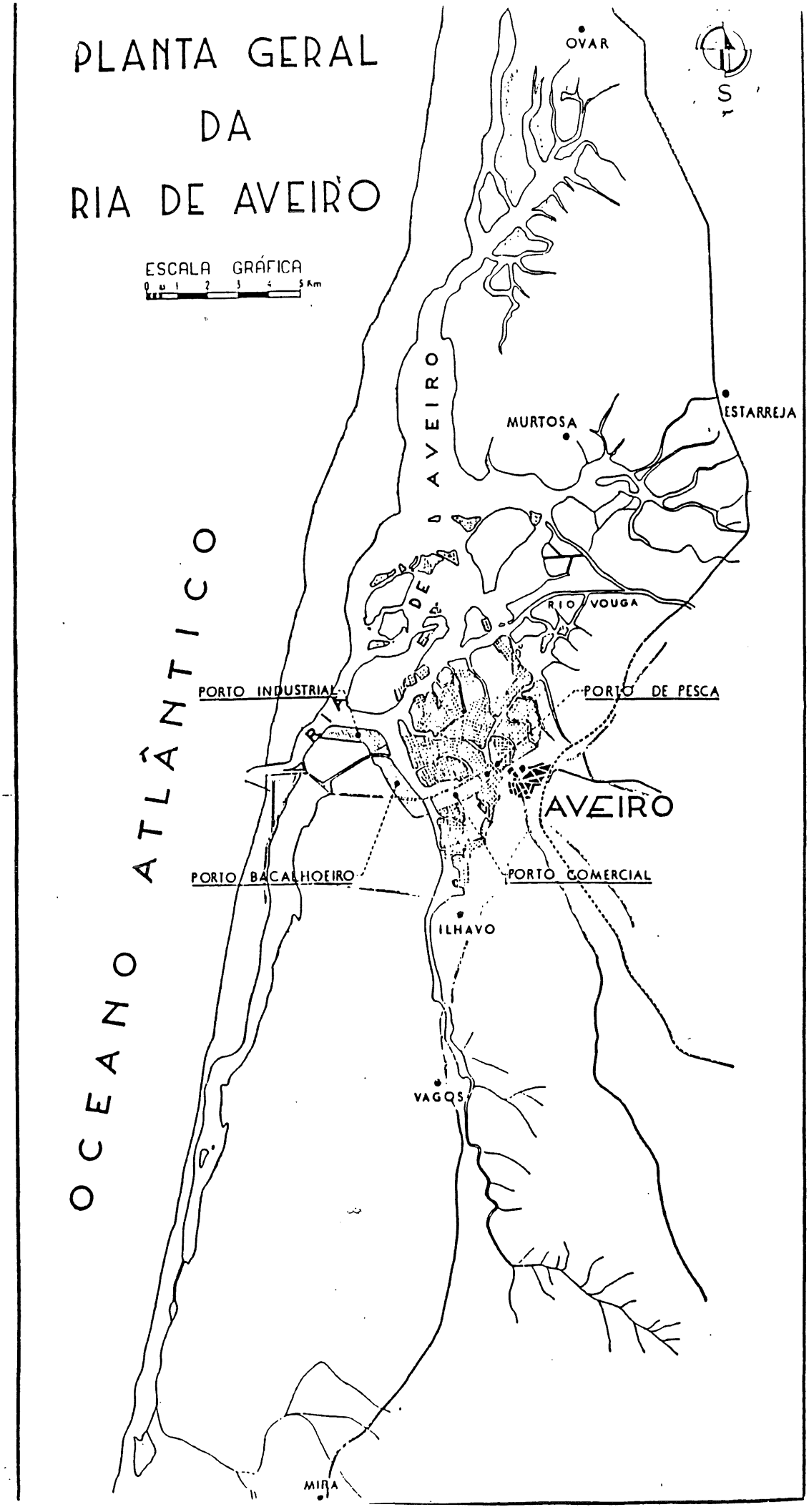
ANNEX 2: MAPS, TABLES AND FIGURES

Annex 2
Map 1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PORTO COMERCIAL
ILHAVO
VAGOS
MIRA

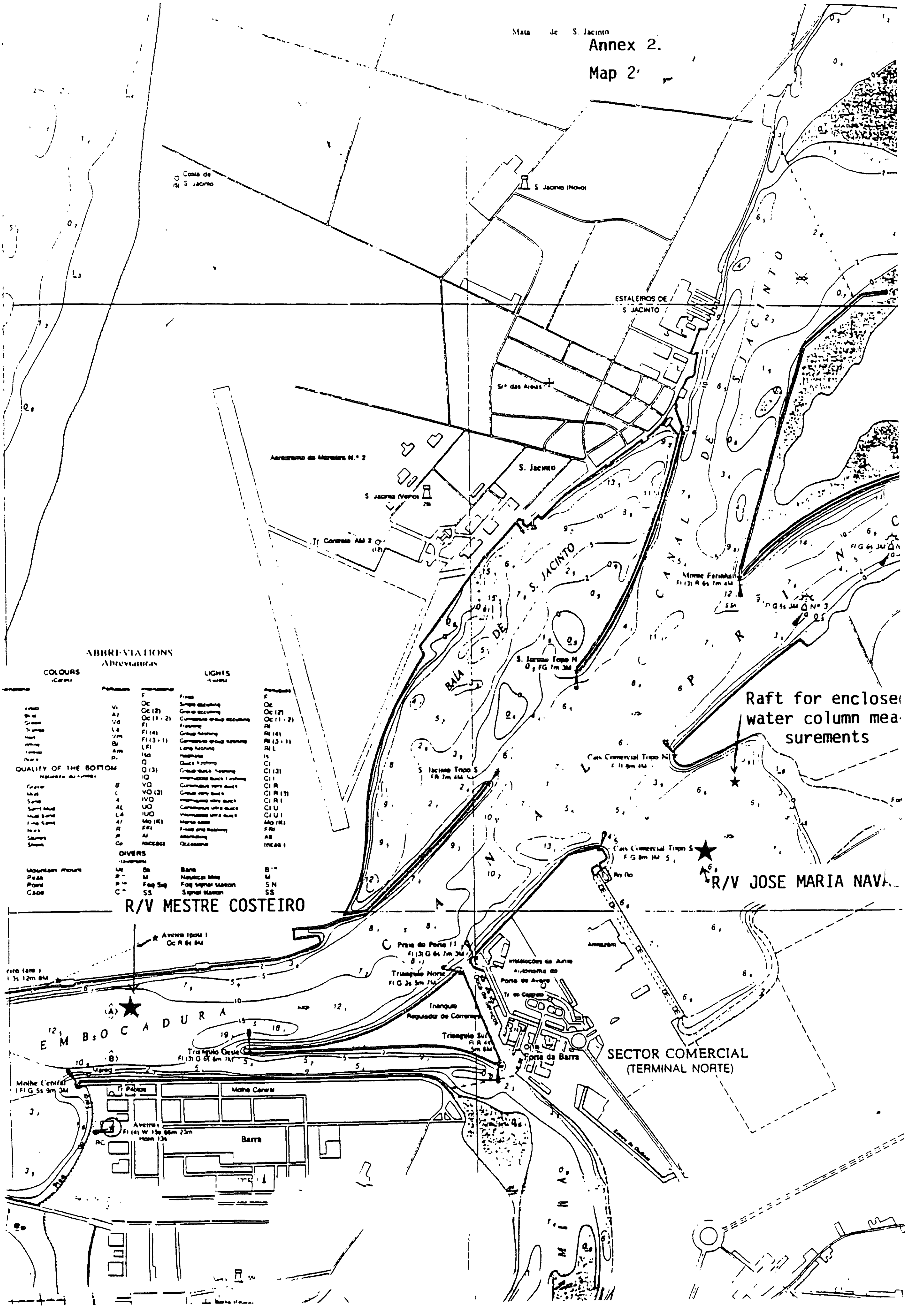

Annex 2.
Map 2
Mata de S. Jacinto
S. Jacinto (Novo)
ESTALEIROS DE S. JACINTO
Srª das Areias
Aeródromo de Manobra N.º 2
S. Jacinto
S. Jacinto (Velho)
BAÍA DE S. JACINTO
CANAL DE S. JACINTO
Monte Farinha
S. Jacinto Topo N
S. Jacinto Topo S
Cais Comercial Topo N
Cais Comercial Topo S
Raft for enclosed water column measurements
R/V JOSE MARIA NAVA
R/V MESTRE COSTEIRO
ABBREVIATIONS
COLOURS
LIGHTS
QUALITY OF THE BOTTOM
DIVERS
EMBOCADURA
Triangulo Norte
Triangulo Sul
Triangulo Oeste
Tanque Regulador de Correntes
Porto de Aveiro
SECTOR COMERCIAL
(TERMINAL NORTE)
Forte da Barra
Armazém
Molhe Central
Aveiro
Barra

Table 2. Occurrence of Dinophysis spp. (cells/l) and chlorophyll (µg/l) in bag 1

Date	Time	Hours	D. acuminata	D. acuta	D. caudata	D. rotundata	D. tripos	Chloroph.	Phaeopig.
22 July	1800	0	1070	30	0	20	20	6.39	2.57
23 July	1200	18	1120	60	0	20	60	7.10	2.67
24 July	1000	40	1060	160	0	60	30	5.56	1.91
25 July	1300	63	800	30	10	30	0	3.98	1.34

Table 3. Occurrence of Dinophysis spp. (cells/l) and chlorophyll (µg/l) in bag 2

Date	Time	Hours	D. acuminata	D. acuta	D. caudata	D. rotundata	D. tripos	Chloroph.	Phaeopig.
22 July	1800	0	1020	40	0	30	20	6.70	2.32
23 July	1200	18	1080	50	20	30	0	7.26	2.60
24 July	1000	40	1060	100	10	50	0	5.34	1.74
25 July	1300	63	760	40	10	40	0	2.25	0.68
26 July	700	81	640	70	10	130	10	3.80	1.97
26 July	1300	87	400	40	0	70	0		
26 July	1900	93	280	10	0	50	0		
26 JUly	2400	98	210	0	0	40	0		
27 July	700	105	300	10	0	60	10	2.64	1.16
27 July	1400	112	280	10	0	90	0	2.62	0.69
28 July	1200	134	200	20	0	30	0	14.51	3.38
29 July	1400	160	90	10	0	90	0	14.16	3.06

Table 4. Occurrence of Dinophysis spp. (cells/l) and chlorophyll (µg/l) in bag 3

Date	Time	Hours	D. acuminata	D. acuta	D. caudata	D. rotundata	D. tripos	Chloroph.	Phaeopig.
24 July	1000	0	700	40	0	100	10	5.73	2.22
25 July	1300	27	840	20	0	60	0	7.31	2.08
26 July	700	45	610	60	0	160	20	4.58	1.93
26 July	1300	51	650	30	0	80	20		
26 July	1900	57	570	40	0	110	0		
26 July	2400	62	430	40	0	150	0		
27 July	700	69	520	100	0	140	0	4.09	3.48?
27 July	1400	76	540	40	0	150	20	5.64	1.58
28 July	1200	98	180	0	0	110	10	4.80	2.24
29 July	1400	124	40	0	0	150	0	2.60	0.83

Table 5. Occurrence of Dinophysis spp. (cells/l) and chlorophyll (µg/l) in bag 4

Date	Time	Hours	D. acuminata	D. acuta	D. caudata	D. rotundata	D. tripos	Chloroph.	Phaeopig.
24 July	1000	0	820	80	0	50	0	7.16	2.26
25 July	1300	27	1120	20	0	70	30	11.03	2.90
26 July	700	45	670	120	0	50	10	5.68	2.14
26 July	1300	51	860	30	20	50	10		
26 July	1900	57	400	0	0	50	10		
26 July	2400	62	320	40	0	70	0		
27 July	700	69	370	50	0	20	30	6.65	2.46
27 July	1400	76	180	10	0	110	0	6.08	0.34
28 July	1200	98	240	30	0	40	0	18.21	4.07
29 July	1400	124	130	30	0	70	0	18.60	3.36

Table 6. Occurrence of Dinophysis spp. (cells/l) and chlorophyll (µg/l) in bag 1-II

Date	Time	Hours	D. acuminata	D. acuta	D. caudata	D. rotundata	D. tripos	Chloroph.	Phaeopig.
25 July	1800	0	670	160	0	30	10		
26 July	700	13	690	90	0	20	40	15.32	3.64
26 July	1300	18	1180	90	10	10	0		
26 July	1900	24	950	20	0	30	0		
26 July	2400	29	-	-	-	-	-		
27 July	700	36	640	100	0	70	30	12.49	3.80
27 July	1400	43	870	80	0	0	0	5.29?	3.37
28 July	1200	65	870	70	0	40	0	22.33	6.00
29 July	1400	91	270	50	0	30	10	19.80	3.36

Table 7. Occurrence of most abundant Dinoflagellates (cells/l) in Bag 1

Date	Time	Hours	C. fusus	H.subglobosum	P. micans
22-Jul	18:00	0	20000	1500	10000
23-Jul	12:00	18	20000	3000	11000
24-Jul	10:00	40	11000	10000	9000
25-Jul	13:00	63	10000	7500	18000

Table 8. Occurrence of most abundant Dinoflagellates (cells/l) in Bag 2

Date	Time	Hours	C. fusus	H.subglobosum	P. micans
22-Jul	18:00	0	20000	1000	9000
23-Jul	12:00	18	20000	1500	9000
24-Jul	10:00	40	20000	5000	8000
25-Jul	13:00	63	13000	1000	12000
26-Jul	7:00	81	20000	3000	12000
26-Jul	19:00	93	13500	2000	9000
27-Jul	7:00	105	20000	7000	8000
27-Jul	14:00	112	14000	2000	17000
28-Jul	12:00	134	20000	4000	12500
29-Jul	14:00	160	14500	1000	20000

Table 9. Occurrence of most abundant Dinoflagellates (cells/l) in Bag 3

Date	Time	Hours	C. fusus	H.subglobosum	P. micans
24-Jul	10:00	0	21000	6000	4000
25-Jul	13:00	27	20000	1000	5000
26-Jul	7:00	45	20000	5000	7000
26-Jul	19:00	57	17000	10000	9000
27-Jul	7:00	69	20000	10000	20000
27-Jul	14:00	76	21000	10300	14000
28-Jul	12:00	98	13000	14000	10000
29-Jul	14:00	124	12000	9000	8000

Table 10. Occurrence of most abundant Dinoflagellates (cells/l) in Bag 4

Date	Time	Hours	C. fusus	H.subglobosum	P. micans
24-Jul	10:00	0	20000	5000	4000
25-Jul	13:00	27	16000	3000	20000
26-Jul	7:00	45	14000	5000	9000
26-Jul	19:00	57	25000	7000	12000
27-Jul	7:00	69	11000	7500	4000
27-Jul	14:00	76	10500	5000	10000
28-Jul	12:00	98	11500	10000	10000
29-Jul	14:00	124	30500	9500	27000

Table 11. Occurrence of most abundant Dinoflagellates (cells/l) in Bag 1-II

Date	Time	Hours	C. fusus	H.subglobosum	P. micans
25-Jul	18:00	0	20000	2000	10000
26-Jul	7:00	13	21000	5000	12000
26-Jul	19:00	24	7000	7000	15000
27-Jul	7:00	36	10000	7000	12000
27-Jul	14:00	43	10000	5000	10000
28-Jul	12:00	65	13000	6500	20000
29-Jul	14:00	91	17000	8000	18500

Table 12. Occurrence of most abundant Diatoms (cells/ml) in Bag 1

Date	Time	Hours	Leptocylindrus danicus	Thalassiosira spp	Pseudonitzschia spp	Asterionella glacialis	Th.nitzschioides
22-Jul	18:00	0	940	50	40	60	270
23-Jul	12:00	18	1930	140	220	160	180
24-Jul	10:00	40	2140	50	220	56	140
25-Jul	13:00	63	1930	20	160	34	20

Table 13. Occurrence of most abundant Diatoms (cells/ml) in Bag 2

Date	Time	Hours	Leptocylindrus danicus	Thalassiosira spp	Pseudonitzschia spp	Asterionella glacialis	Th.nitzschioides
22-Jul	18:00	0	780	90	100	270	60
23-Jul	12:00	18	2740	260	150	140	130
24-Jul	10:00	40	2750	40	130	80	120
25-Jul	13:00	63	1580	23.5	60	17	40
26-Jul	7:00	81	2640	20	100	30	20
26-Jul	19:00	93	1850	100	100		60
27-Jul	7:00	105	2120	2	90		90
27-Jul	14:00	112	1470	10	120		10
28-Jul	12:00	134	2440	6	130	8	20
29-Jul	14:00	160	2230	8	100	5	100

Table 14. Occurrence of most abundant Diatoms (cells/ml) in Bag 3

Date	Time	Hours	Leptocylindrus danicus	Thalassiosira spp	Pseudonitzschia spp	Asterionella glacialis	Th.nitzschioides
24-Jul	10:00	0	1040	12	50	100	50
25-Jul	13:00	27	3190	12	90	65	100
26-Jul	7:00	45	5050	40	220	36	310
26-Jul	19:00	57	2980	38	160	28	98
27-Jul	7:00	69	3520	32	80	20	130
27-Jul	14:00	76	3810	30	140	10	200
28-Jul	12:00	98	2990	23.5	90	2	137
29-Jul	14:00	124	2660	27	120	5	140

Table 15 Occurrence of most abundant Diatoms (cells/ml) in Bag 4

Date	Time	Hours	Leptocylindrus danicus	Thalassiosira spp	Pseudonitzschia spp	Asterionella glacialis	Th.nitzschioides
24-Jul	10:00	0	1640	37	90	3	100
25-Jul	13:00	27	3920	40	90	23	290
26-Jul	7:00	45	3720	50	220	70	290
26-Jul	19:00	57	3250	20	140		210
27-Jul	7:00	69	2040	18	70	8	90
27-Jul	14:00	76	3130	20	100	6	40
28-Jul	12:00	98	5150	19	110	5	83
29-Jul	14:00	124	3210	20	110	6	70

Table 16 Occurrence of most abundant Diatoms (cells/ml) in Bag 1-II

Date	Time	Hours	Leptocylindrus danicus	Thalassiosira spp	Pseudonitzschia spp	Asterionella glacialis	Th. nitzschioides
25-Jul	18:00	0	1930	40	210	54	313
26-Jul	7:00	13	3740	54	230	65	425.5
26-Jul	19:00	24	3210	39	300	90	500.5
27-Jul	7:00	36	4570	60	170	130	443.5
27-Jul	14:00	43	2740	30	90	50	245
28-Jul	12:00	65	2830	23.5	70	29	212
29-Jul	14:00	91	3720	20	50	31	143

Table 17. Nutrients inside the Bags

Nutrients in Bag 1 (uatg/l)

Date	NO3	NO2	SiO2	PO4	NH4
23	0.5	0.05	3.3	0.53	1.27
24	0.6	0.03	2.6	0.45	1.38

Nutrients in Bag 2 (uatg/l)

Date	NO3	NO2	SiO2	PO4	NH4
23	0.9	0.04	4	0.45	1.31
24	0.4	0.03	2.6	0.47	1.13
26	1.3	0.01	2.6	0.18	0.47
27 07h00	0.8	0.01	6.1	0.5	1.26
27 13h00	>20.0	0.02	3.1	>4.00	1.53
28	>20.0	0.04	3.2	>4.00	0.91
29	>20,0	0.06	3.6	>4.00	0.72

Nutrients in Bag 3 (uatg/l)

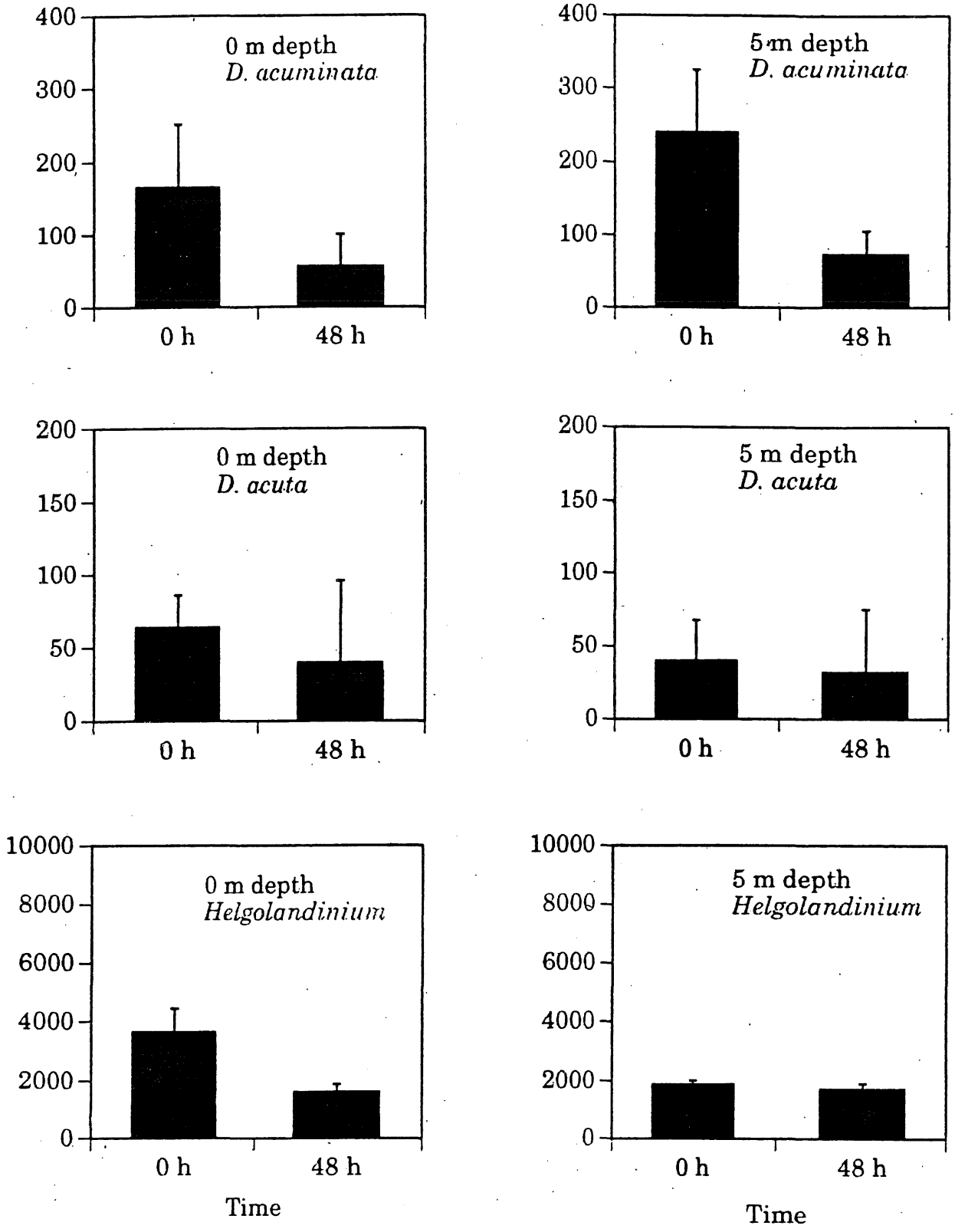
Date	NO3	NO2	SiO2	PO4	NH4
24	1.3	0.02	3.4	0.72	2.99
26	1.1	0.01	2.6	0.39	0.4
27 07h00	4.8	0.01	0.1	0.1	<0.50
27 13h00	4	0.01	4.6	0.39	1.54
28	3	0.02	1.7	0.58	0.65
29	7.3	0.04	3.5	0.73	0.61

Nutrients in Bag 4 (uatg/l)

Date	NO3	NO2	SiO2	PO4	NH4
24	1.5	0.03	4.1	0.88	3.7
26	1	0.03	5.7	0.6	1.17
27 07h00	0.9	0.01	0.6	0.13	<0.50
27 13h00	>20.0	0.02	3.7	>4.00	1.54
28	>20.0	0.06	2.5	>4.00	0.99
29	>20.0	0.07	0.9	>4.00	0.62

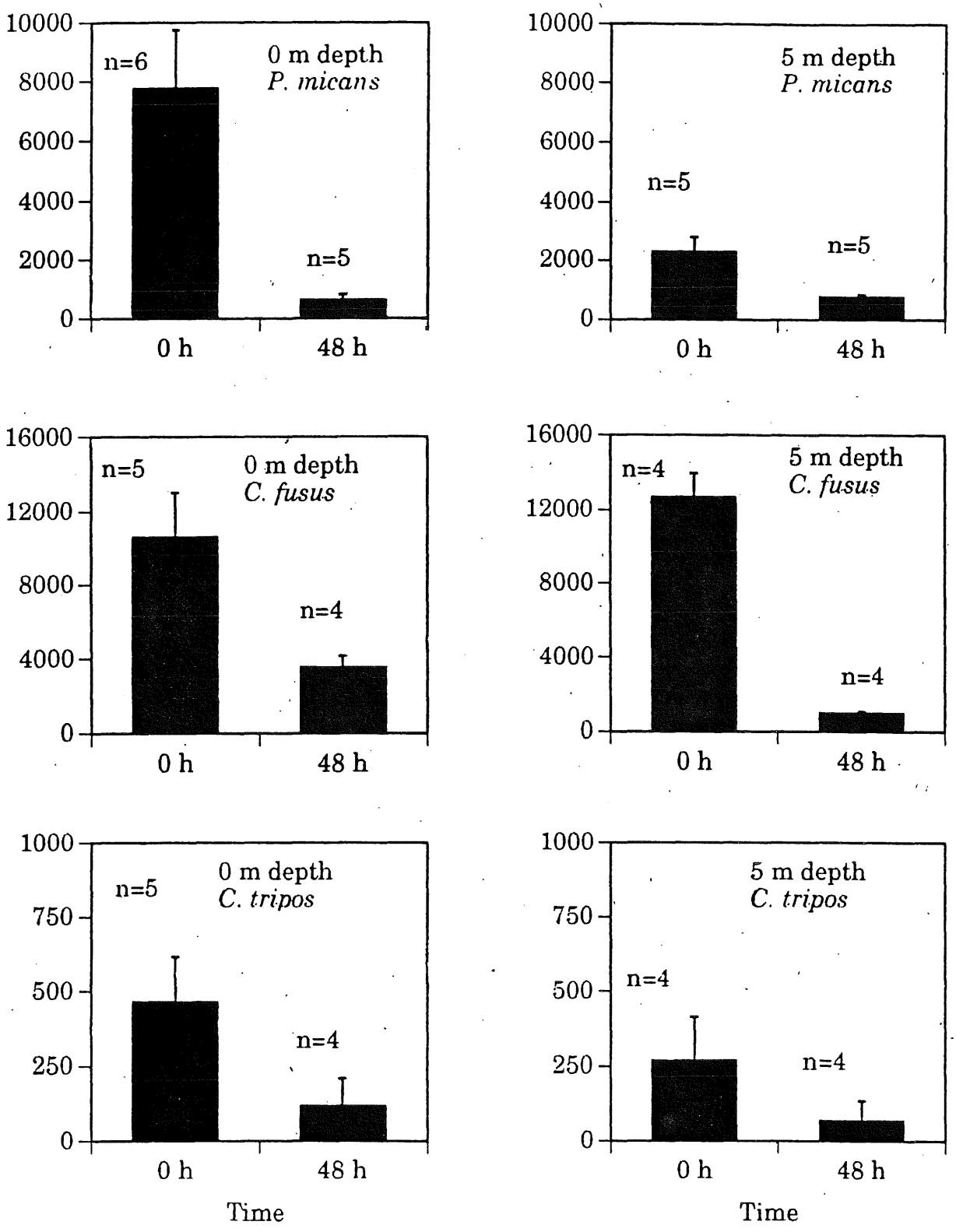
Nutrients in Bag 1-II (uatg/l)

Date	NO3	NO2	SiO2	PO4	NH4
25	2.2	0.03	5.4	0.87	1.87
26	1.1	0.03	4	0.51	1
27 07h00	0.8	0.01	5	0.4	0.9
27 13h00	>20.0	0.02	5.5	>4.00	1.73
28	>20.0	0.03	6.8	>4.00	1.46
29	>20.0	0.05	4.9	>4.00	1.49



Annex 2

Figure 1.- Cell densities of *D. acuminata*, *D. acuta* and *Helgolandinium* spp at 0 m and 5 m depth, before (0 h) and after (48 h) incubation in the diffusion chambers.



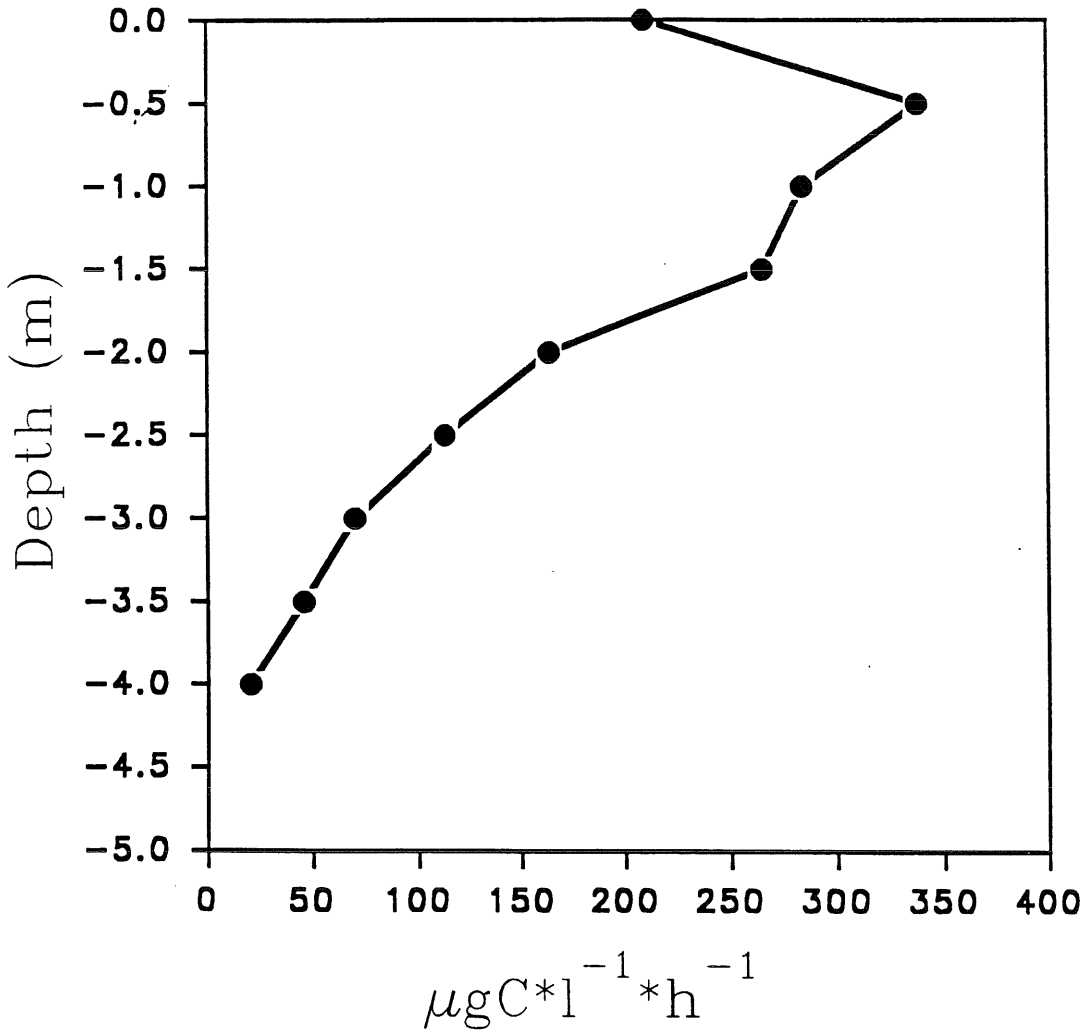
Annex 2

Figure 2.- Cell densities of *P. micans*, *C. fusus* and *C. tripos* at 0 m and 5 m depth, before (0 h) and after (48 h) incubation in the diffusion chambers.

Primary productivity

"Einars raft"*, Ria de Aveiro

94.07.27



* the raft where the enclosed water column measurements were carried out.

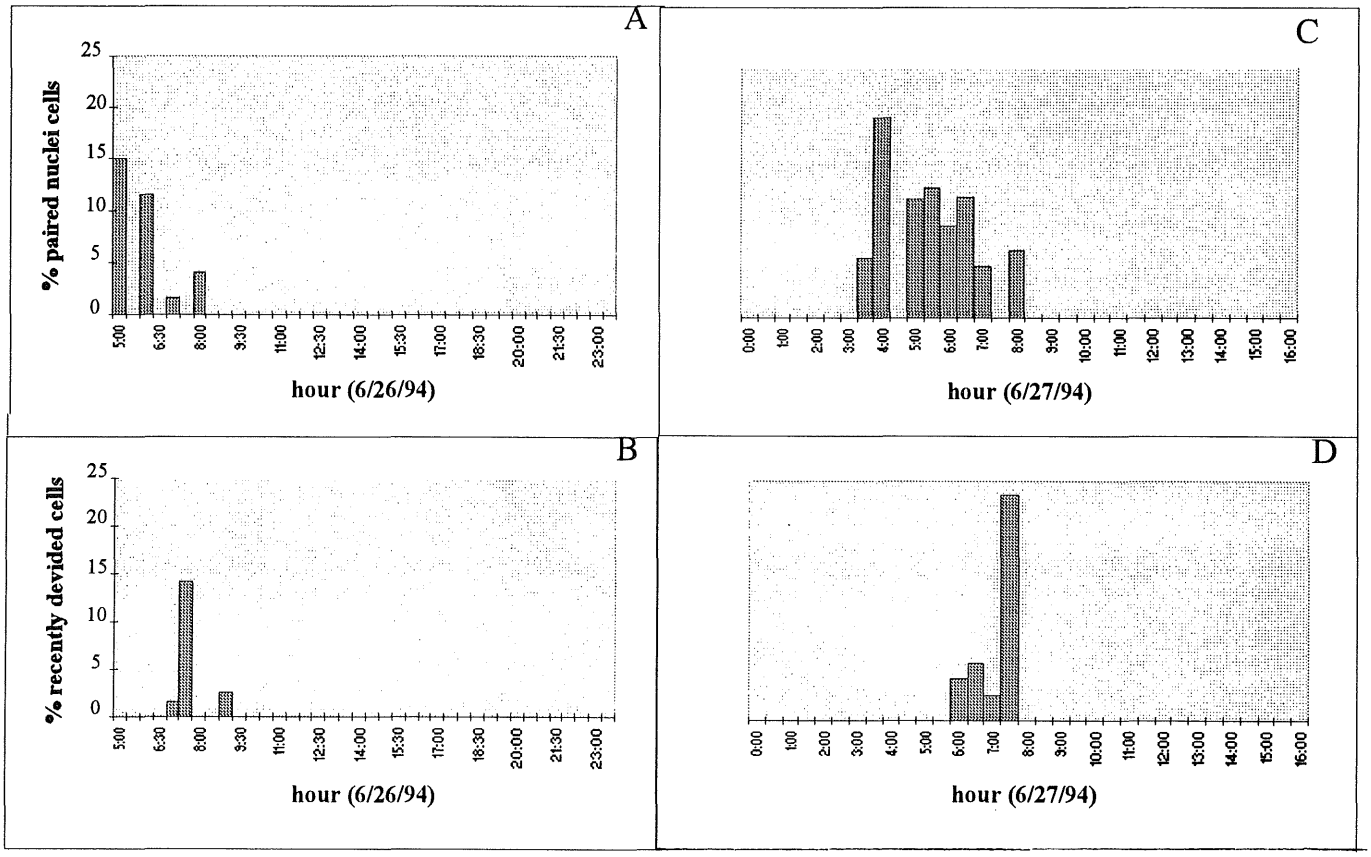


Fig.4.- Distribution of frequencies (percentage over the total) of dividing (4A, 4C) and half the number of just divided cells (1B, 1D) (only those missing the lower sulcal list).

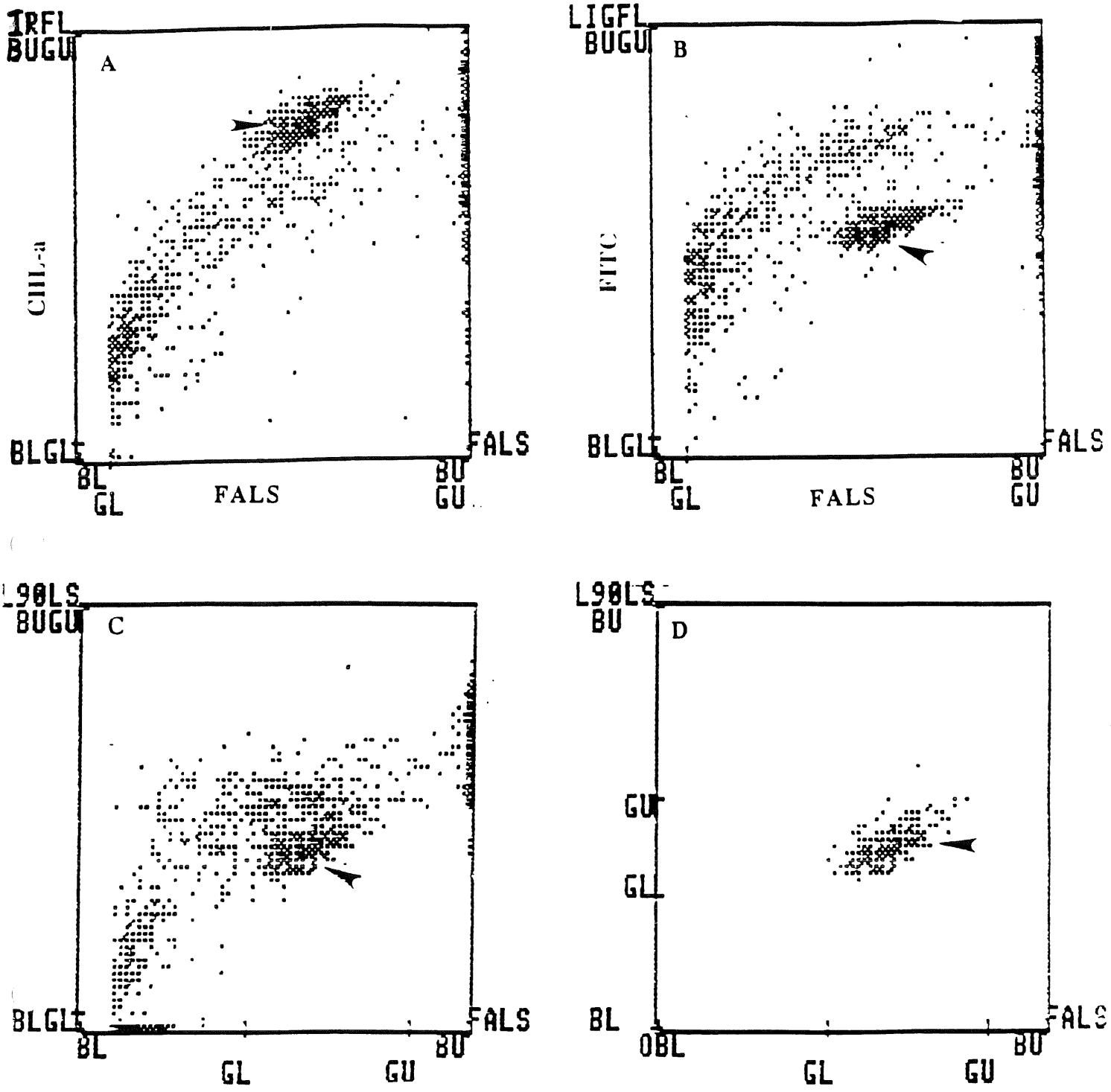


Fig. 5 Flow cytometric analysis of *Helgolandinium subglobosum* from the Ria de Aveiro lagoon samples. Arrows in panels A, B, and C show the approximate location of the *Helgolandinium* population. **A.** Residual chlorophyll (red) fluorescence versus forward angle light scatter (approximate cell size). **B.** Lectin Con A green (FITC) fluorescence versus forward angle light scatter. **C.** Ninety degree light scatter versus forward angle light scatter . **D.** Isolation of the *Helgolandinium* population by gating on forward angle light scatter and linear green (FITC) fluorescence and forward angle light scatter and linear red fluorescence.