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REPORT OF THE ICES STUDY GROUP ON THE "DYNAMICS OF HARMFUL ALGAL BLOOMS IN COASTAL WATERS"

Vigo, Spain, 7-9 April 1992

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General Secretary ICES Paægade 2-4 DK-1261 Copenhagen K DENMARK

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Vigo 7-9th April 1992

ABSTRACT

This Study Group discussed questions relevent to the population dynamics of harmful algal blooms in coastal seas, and the operational problems to be encountered in field studies designed to improve our understanding of them. Emphasis was placed on the need to build bridges between the ecological problems and the physical oceanographic background on a site-specific basis, and on the value of collaboration between the two disciplines.

Three regional pilot studies were proposed: Gulf of Maine, Skagerrak-Kattegat, and Western Atlantic Iberian Shelf), as well as a proposal in favour of the complementary value to these field studies of Mesocosm Experiments, especially in relation to nutrient and grazing problems. The common elements of these different proposals were identified, and growth, grazing, and physical-biological interactions were agreed to be the main problem areas for the population dynamic approach. The problems posed by vertical migration, encystment and excystment, and toxin prpoduction in relation to the different phases of population growth were also summarized.

1. OPENING OF THE MEETING

1.1 The meeting was opened by the chairperson, Ms Beatriz Reguera at 0930 hours on the 7th of April 1992, who welcomed the participants as Director of the Centro Oceanográfico de Vigo. The draft agenda for the meeting (Annex I) and a list of participants (Annex II) are appended to this report.

1.2 At the 79th Statutory meeting of ICES (La Rochelle, September 1991), the Biological Oceanography and the Hydrography Committees recommended that " A Study Group on the Dynamics of Harmful Algal Blooms will be established with B. Reguera (Spain) as chairperson and will meet in Vigo, Spain in 1992 for 3 days to plan and propose a programme for investigating the dynamics of harmful algal blooms in the coastal ocean. IOC should be asked to co-sponsor this Study Group".

Justification: This Study Group is considered necessary in view of the importance and urgency regarding the problem of harmful algal species, and the fundamental necessity of clarifying their dynamics and development in order to understand and predict timing, location, intensity, and detrimental effects. IOC co-sponsorship is required in order to ensure close collaboration with the IOC (OSLR) programme on Harmful Algal Blooms (HAB). 1.3 The meeting was co-sponsored by IOC which was represented by Dr. T. Osborn. He stressed the need to formulate the plan in such a way as to achieve more interdisciplinary research, particularly to attract greater participation by physical oceanographers. The relation of the IOC to the ICES harmful algal bloom programme was described. IOC has been involved in a 5 to 6 year development of a programme on harmful algal blooms beginning with a meeting in Takamatsu in 1987, followed by one in Paris (1990) and another in Newport (1991). At present, the IOC programme is divided into operational, scientific, and educational elements with each of these elements composed of several components (see IOC Workshop Report No. 80 "Programme on Harmful Algal Blooms", pp. 7 and 14). One of the components of the scientific element of the IOC programme is "Ecology and Oceanography", and it is with these aspects of harmful algal blooms that the ICES Study Group is concerned. IOC has various subsidiary groups such as WESTPAC which may also propose programmes to study harmful algal blooms in other regions of the world. SCOR is also concerned about the harmful algal bloom situation and is organizing a two year study on the physiological ecology of harmful species which will result in a published report describing the state of knowledge concerning this problem and identifying the areas of future research.

It was stressed that neither ICES nor IOC are funding agencies, but that a well designed international programme coordinated by such agencies would be effective in eliciting the financial support of various national governments.

2. ADOPTION OF THE AGENDA

The agenda was adopted and is attached as Annex I.

3. ELECTION OF RAPPORTEUR

Dr John Smith agreed to act as rapporteur.

4. A POPULATION DYNAMICS APPROACH TO HARMFUL ALGAL BLOOMS

Discussions began with a presentation on a population dynamics approach to the study of harmful algal blooms (D. Cushing). Although it was recognized that physical (e.g., diffusion, advection) and population parameters are equally important in the dynamics of harmful algal blooms, for the purposes of discussion it was assumed that the boundaries and dynamics of the system being studied were known and estimates of the fluxes in and out of it available. The rate of change of a population within such a system is the difference between growth and loss terms; losses are due to sinking or grazing.

If it were possible to know both the maximum growth rate (μ_{max}) under the prevailing conditions and the actual net growth rate, then a significant difference in these values suggests that loss terms are significant and must be examined. It is possible to estimate the maximum division rate μ_{max} using the Eppley relation between μ_{max} and temperature (Eppley, 1972) (which assumes light saturation), but for particular cases μ_{max} should be measured directly as a function of light and temperature.

It is also necessary to have an algorithm to account for nutrient limitation such as the Michaelis-Menten or Droop formulations (Droop, 1974). Another algorithm is also needed to account for self-shading as the population grows. This should permit estimates of μ_{max} .

To measure the net growth rate it is necessary to count the organisms, but this is difficult to do in real time at sea. An estimate of division rate can be obtained at sea by the Furnas difusion method (Furnas, 1990), but this does not work for some phytoplankton species. Ways of estimating loss terms due to sinking and grazing as well as the effects of vertical migration were also suggested. This approach can be used in a defined system, given sufficient observations in time and space, to describe the population dynamics of a harmful algal bloom as it develops.

These ideas were used as a framework for a general discussion of how to plan a field programme to investigate the dynamics of harmful algal blooms. The mathematical formulation is attached as Annex III.

4.1 General comments raised by this approach.

Following this presentation the following points were raised:

i) Many bloom-forming harmful algal species are capable of swimming and this coupled with a stratified water column might make such an approach difficult to apply in practice,

ii) Logistical problems might be very great in the field but such an approach might be suitable for mesocosm studies,

iii) The nutrient status of vertically migrating organisms is difficult to define,

iv) Construction of a model of harmful algal dynamics should be an aim of the programme but there are many practical problems associated with present methods of measuring the requisite parameters; e.g., diffusion chamber methods have not worked well in many circumstances. It is important to estimate growth rates but new methodologies are required. It is possible to carry out photosynthesis-irradiance studies on single cells and this might be a possible approach. Vertical migratory behaviour should be incorporated into a model; light and nutrients are very important in regulating this behaviour,

v) Attention was drawn to the possible partial heterotrophy or mixotrophy of dinoflagellates and other harmful organisms,

vi) The Eppley relation is based on the maximum instantaneous division rate, which is perhaps a more relevant parameter, and most work is done on diatoms which are generally held to have higher division rates than dinoflagellates. Nevertheless, the lower generation times of dinoflagellates are based on studies of only a few organisms, and some species (e.g. *Alexandrium lusitanicum, Amphidinium spp*) may divide twice a day, and there can be rapid population growth followed by a crash in cell numbers without reaching nutrient limitation, vii) Under natural conditions, the prior light and nutrient histories of a cell can be quite different from those at the time samples are taken. This may cause large discrepancies between the actual and the expected growth rate that would not be due to loss terms. Therefore, laboratory data may be difficult to apply in the field,

viii) The importance of having good physical data was stressed. Turbulence may affect division in the algal species of interest and this may be a problem with certain methods of measuring growth,

ix) Mitotic index methods (Weiler and Chisholm, 1976; Mc Duff and Chisholm, 1982) work well with only certain types of organisms. A modification of this approach using DNA stains and quantitative microfluorometry (Chang and Carpenter, 1988a, 1988b and 1990; Antia et al, 1990) is a promising technique but requires a considerable capital investment and is quite labor intensive.

Another method which may offer some hope of estimating *in situ* growth rates is the single cell ¹⁴C uptake method ((Rivkin and Seliger, 1970). This is very difficult to use at sea, and requires an accurate estimate of a species cell carbon if a growth rate is desired. A not yet developed method based on the levels of cell cyclins (proteins involved in the cell division process) has been described by Chang and Carpenter (1990). Autoradiographic techniques might also be appropriate. It was generally agreed that the utility of these and other possible methods needs to be evaluated.

4.2 Blooms suitable for population dynamics approach.

Some participants felt that the group should be concerned with 'unusual blooms'. But for the purposes of this study, it is necessary to study blooms from their very earliest stages and hence it is necessary to utilize blooms which are 'expected' and for which timing, location, etc. are well known. The recent 'unusual' *Chrysochromulina* and *Aureococcus* blooms were well underway when recognized as important phenomena and were not then (nor have they since proved to be) suitable subjects for bloom dynamics studies.

4.3 Relations between population dynamics and toxicity.

The next question raised was when in the growth cycle the cells actually become toxic; it is necessary to know cell numbers, the toxicity per cell and the timing of toxin production in the cell cycle. *Alexandrium* blooms are toxic in their early stages, toxicity increases to a maximum and then declines. PSP toxins are constitutive, i.e., always present in the cell. The same thing has not been demonstrated for DSP toxins, and their presence in *Dinophysis spp*. In cultures of *Prorocentrum lima*, maximum DSP toxicity occurs in late exponential phase, but the toxins are always present (Bravo and Fernández, unpubl.). In *Nitzschia pungens* f. *multiseries* domoic acid production begins in the postexponential phase and is very rapid; domoic acid production may continue well into stationary phase depending on the nature of the nutrient limitation (Bates et al, 1989; Smith, unpubl.).

The constitutive toxins may be thought of as primary metabolites (in a microbiological sense), whereas domoic acid is clearly a secondary metabolite produced as

a consequence of slowing growth. Nitrogen and phosphorus limitation affect the levels of PSP per cell differently.

There are also considerable interclonal variations in toxin levels in those species for which this information is available. Clearly, these variations in the timing and level of cell toxicity need to be taken into account in attempts to model the dynamics of toxic blooms.

4.4 Functions of toxins and grazing

The role of toxins in the food web and their possible effects on grazing were discussed. It is necessary to identify which components of the food web are vulnerable to the toxins. Fish are known to be affected. Toxins may reduce grazing and this could be examined by observing the effects of toxins on grazing estimated by observing filtration rates and gut contents. For such studies it is necessary to use proper controls, i.e., a nontoxic clone of the toxic organism being studied. Further, grazing may not be reduced by a chemical message conveyed by the toxin but by actual physiological impairment of grazers caused by ingestion of toxic cells. Thus the effect on grazing rate may be difficult to quantify.

Grazing can also have positive effects on blooms through population compositional shifts; in mesocosm studies ctenophores have been observed to eat the copepods which were grazing on algae thus allowing the algae to bloom. Grazing studies should clearly be a necessary feature of a bloom dynamics study, but it should be recognized that such studies have considerable uncertainty.

4.5 Vertical migration and advection.

Red tides can sometimes appear suddenly with population growth exceeding realistic cell division rates. Rapid mechanisms involving vertical migration and advection are known (Seliger et al, 1979; Tyler and Seliger, 1978 and 1981). It is not always possible to separate the effects of vertical migration from advection unless careful physical measurements are taken. Vertically migrating organisms appear to have an advantage over diatoms in stratified systems and this behaviour can have important consequences for sampling and interpreting cell distribution patterns. Also, water mass movements are important for seeding toxic populations into new areas. There does not always appear to be vertical migration in *Dinophysis* and *Gyrodinium*, but these species are nevertheless often found growing in very thin layers.

4.6 Role of cysts.

Benthic life history stages were briefly discussed. Cyst counts can give good estimates of the size of potential blooms in freshwater or in enclosed embayments, but it was not agreed that this was necessarily the case in the open sea. There were additional comments that we do not know the life histories of many important harmful algae, and that this is an important field for future study. Bloom size cannot be predicted from cyst counts but timing and location can. This concluded the plenary discussion of the population dynamics approach to harmful algal blooms.

5. PILOT STUDY PROPOSALS AND OTHER PRESENTATIONS.

5.1 Pilot study proposals.

The Study Group then heard six proposals for pilot studies. These are collected together as Annex IV with brief introductions here, and the names of the people who presented them.

i) Harmful Algal Bloom Pilot Study in the Gulf of Maine. (D. M. Anderson)

This proposal was for a study of *Alexandrium* dynamics in a coastal current. In the sothwestern Gulf of Maine, the outflow from several rivers forms a buoyant plume that can extend for hundreds of kilometers as a coastal current. A strong association between the buoyant, reduced salinity water mass and the toxic Alexandrium populations has been documented. Patterns of nearshore shellfish toxicity match the movement of the coastal current as influenced by local wind stress and rainfall. There may even be a link between the coastal current and PSP toxicity well offshore on Georges Bank. A pilot study in the Gulf of Maine could be appropriate for many reasons. The toxic Alexandrium species to be studied are of great importance to other countries affected by thesse organisms; coastal currents are common features of numerous other regions impacted by harmful blooms; the mechanism underlying this association with distinct water masses are not known, bur are of great importance; Alexandrium blooms are highly regular seasonal events, so a field programme could be conducted with a high possibility that a bloom would occur. The toxins affect several different levels of the food chain; and the area is well studied oceanographically, and is readily-accesible from several marine research institutions.

ii) Harmful Algal Bloom Mesocosm Experimental Study. (T. Smayda).

The objective of this proposal is to study *Heterosigma akashiwo* in the mesocosms at the Marine Ecosystem Research Laboratory at the University of Rhode Island. In addition to the arguments advanced in the written proposal, attention was directed to the suitability of mesocosms for grazing studies (although *Heterosigma* seems to be harmful to most food web components); the work should show how nutrients and nutrients combined with grazing affect growth. Mesocosms can also be used for studying the effects of altered nutrient regimes on blooms. The need to combine mesocosm studies with field observations was noted, as was the fact that the approach is equally applicable to other organisms such as *Phaeocystis*.

iii) Dynamics of Pyrodinium bahamense var. compressum (T. Smayda).

This is a PSP producing organism which has caused severe problems in the Indo-Pacific. A non-toxic variety of this organism exists with many other bloom-forming dinoflagellates in Bahia Fosforescente in Puerto Rico all the year round. The rationale for this proposal is that an area is needed where investigators could conduct a method development/intercalibration study on a continuous dinoflagellate bloom in preparation for larger field studies of harmful organisms.

iv) Studies of Harmful Algae in Areas Influenced by Eutrophication : Skagerrak-Kattegat (L. Edler/E. Graneli)

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There are long term records of nutrients from this area and changes in nitrogen and phosphorous levels appear to be associated with the appearance of blue green algae on the Swedish east coast and dinoflagellates on the west coast. Ongoing efforts to clean up sewage effluents and other inputs to coastal waters here have resulted in changes in nutrient levels and ratios which constitute, in effect, a large scale experiment; N/P ratios in sewage receiving waters are less than the Redfield ratio. There are long term data sets on nutrient ratios and grazing and on the utilization of organic nitrogen (by *Ceratium*).

In these areas diatoms appear to be grazed preferentially, favouring the production of dinoflagellates. UV-B radiation appears to break down the abundant humic materials yielding organic nitrogen compounds which are utilized for growth by organotrophic dinoflagellates.

v) Harmful Algal Bloom Pilot Study in the Kastela Bay. (I. Marasovic).

Because Kastela Bay is not in the ICES geographical area, this proposal was directed primarily to the IOC component of the group. A long data series is available for this site and the rôle of temperature in initiating blooms of *Lingulodinium polyedra*, the organism of concern, is established. The effects of wind forcing on the resuspension of cysts and intensification of the blooms have also been described.

vi) IBERIA: A Harmful Algal Bloom pilot study along the Atlantic coast of the Iberian Peninsula (T. Moita/B. Reguera)

It was pointed out that the region has a wide diversity of harmful algae and related toxicity problems. There are toxic blooms of *Dinophysis acuta* and *D. acuminata* within the rías of Galicia and the Portuguese coast which result in closures of the mussel culture industry (Galicia) and bivalve extraction industries and which are probably influenced by anthropogenic factors. A unique feature of this proposal relative to the others presented at the Study Group is the fact that there are annual offshore proliferations of *D. acuta* and *D. tripos* and almost annual blooms of the PSP producer *Gymnodinium catenatum* which are clearly associated with the intensity and duration of upwelling events. The complex interactions of different water masses, currents, fronts, bottom topography and upwelling with the diversity of toxic species, each with apparent preferences for particular combinations of abiotic environmental factors, would appear to present unusual opportunities to understand harmful algal blooms both under apparently natural conditions in the offshore and under human and terrestrial influences in the inshore.

There also appear to be unusual opportunities for in situ study of the basic biology of several members of the genus *Dinophysis*, a group which has not been amenable to laboratory culture so far. It was pointed out that it would be possible to study the life history of *Dinophysis* and the relations of various morphotypes of the different species, to measure growth rate and to investigate changes in cellular toxicity at different times in the growth season. Vertical migration, mixotrophy and organotrophy are other subjects which could readily be explored in this system. Other positive features of this potential study area are the existing data base, monitoring programmes, local expertise and infrastructure, as well as the strong government support and obvious sense of community.

5.2 Other presentations.

Six further presentations were then made. These were not proposals for pilot studies, but rather in the nature of notes and comments intended to flesh out the preceding discussions in both their theoretical and technical aspects. Part of them are collected in Annex IV too, together with other documents sent by mail from authors who were not able to attend the meeting.

i) Ecological implications of size in phytoplankton (T.Wyatt)

Most red tide organisms are members of the microplankton (> 20μ m in diameter), their growth is mainly nitrate based (or new production) and grazing pressure could take several weeks to develop. Such grazing could be studied by the Strickler method. Ultraplankton (nano- and picoplankton) are much smaller and live on regenerated nutrients. These have not been associated with harmful blooms until quite recently but problems such as *Chrysochromulina* and *Aureococcus* are now emerging in the group. Few grazing studies have been carried out for ultraplankton.

ii) Predictability of the occurrence of harmful species (M. Estrada)

This presentation was concerned with the ability to predict blooms and interannual variation, and with the structure of the community with respect to the different niches provided by various combinations of abiotic factors. The success rate of predicting the arrival of a community to which a toxic species belongs is likely to be higher than attempts to predict the appearance of a particular species.

iii) Harmful algal blooms in Dutch coastal waters. (G.C. Cadée).

The effects of eutrophication off the Dutch coast on *Phaeocystis* blooms were discussed as were the importance of nutrient ratios. N/P ratio decreases were correlated with increased size of *Phaeocystis* blooms while the duration of these blooms has increased also threefold. There is already interesting information reported about the European STEP project on *Phaeocystis* (Lancelot et al, 1991a and 1991b).

iv) Nitzschia pungens blooms in eastern Canada (J. Smith).

These blooms occur in shallow estuaries that are well mixed during the fall bloom period. The blooms depend on turbulent mixing and the shallow depth to obtain enough light for growth and generally appear to be nitrogen limited, depending to a large extent on terrestrial nutrient sources. The blooms are strongly influenced by advective forces, depending on a favourable combination of nutrient supply and calm weather for full development. They are very easily dispersed to sea by strong winds which are common at that time of year.

v) Harmful phyoplankton of the northern Gulf and estuary of the St. Lawrence River. (M. Levasseur).

This area has many similarities to the Gulf of Maine with respect to the dynamics of *Alexandrium*. There is a buoyant coastal current (Gaspé Current) on the south coast of the estuary and PSP appears to be associated with this water masses. *Dinophysis* species are ubiquitous in the area, blooming in June but with no toxicity recorded to date. Cysts of *Alexandrium* appear in the sediments associated with plumes of large tributaries to the St. Lawrence; following stratification in June, the vegetative cells become entrained in the Gaspé current. There are no good data on population growth within the current. This raises the question of whether there is enhanced *in situ* growth in the plume or whether there is an entrainment concentration mechanism. Interannual variation experienced along the plume are similar to that observed in the Gulf of Maine; the source of this variation may reflect very large scale regional effects.

vi) Influence of runoff of the River Rhone on offshore phytoplankton biomass and nutrient levels in the Gulf of Lions. (A. Cruzado).

A summary of this work was presented. It was found that while phytoplankton biomass was enhanced in the vicinity of the river mouth, the seaward influence of the plume was less than formerly thought. The Gulf of Lyon itself appears to be a sink for nutrients rather than a source.

5.3 Clarifying comments

Following these presentations, a number of clarifying comments were made. It was emphasized that the population dynamics equation which had formed the basis of earlier discussions (Agenda item 4) is nothing more than a basic conservation equation, and states that the rate of change of some quantity in a system equals the input from sources less the output to sinks. Sources are from excystment cell division, and transport in, losses are due to grazing, encystment, sinking and transport out. It is helpful to state problems in such a way so as to attract collaboration from physical oceanographers. Attempts to quantify advective effects and to differentiate these from growth might be an example of this. Hypotheses and statistics are means to avoid population dynamics and it is necessary to relate questions concerning harmful algal blooms to processes rather than hypotheses. The processes are related to the terms of the equation: growth (bloom initiation, excystment, vegetative growth), physical interactions (advection, upwelling, vertical migration) and grazing. It might already be possible to provide a good estimate of grazing pressure on harmful algal blooms through a synthesis of the available literature and a few targeted laboratory studies if required. Eddy diffusion terms are taken into account in the advection terms.

6. COMMON ELEMENTS IN THE PILOT STUDY PROPOSALS

The problem is illustrated by three tiers of complexity based on the degrees of interaction to be considered. The lowest level is concerned with species and itself contains three categories based on levels of interaction. The first category at this level is that of single species ecophysiological studies; these included studies of autotrophy, mixotrophy,

heterotrophy, life cycles, potential growth rates in relation to nutrient levels and ratios, light and temperature, toxin production, cyst formation and germination, and grazing. The second category at the species level is concerned with mesocosms; these may be used to study growth rates, species interactions, nutrient level and ratio effects, toxin production, life cycles and grazing under what are hopefully relatively natural conditions; species particularly attractive for initial pilot studies (because they are relatively certain to cause problems each year) are *Alexandrium spp*, *Gymnodinium catenatum*, *Heterosigma akashiwo*, *Gyrodinium aureolum* and *Dinophysis spp*. The third category at the species level is grazing; this includes heterotrophy, mixotrophy, phagotrophy, grazing of toxic species, effects on grazers and the role of grazers in controlling harmful events.

Ecosystems



These categories increase in complexity from single species studies to multispecies interactions. In moving from level to level in the three-tiered scheme, we move from the species level to the ecosystem level. The second level studies are concerned with *in situ* physical/biological interactions: the processes to be studied include coastal currents, plumes and fronts, stratification, entrainment, mixing and diffusion, upwelling and advection; concentrating processes, bloom initiation processes and vertical migration; cyst dynamics, toxin dynamics, *in situ* growth rates, subsurface maxima, sedimentation, and¹ population dynamics.

Finally, third level studies are directed to modelling the system: these include subprocess studies, interactions between subprocesses, time series analyses, prediction, ecosystem understanding and species population dynamics. This three-tier summary drew several brief comments. Attention was directed to an early model of red tide outbreaks in the literature (Wyatt and Horwood, 1973) which has not been exploited. There was a short inconclusive discussion of the merits (or otherwise) of mesocosms; it was suggested that their utility needs to be further assessed. The importance of cyst studies, the resupply of cysts and the desirability of involving sedimentologists and palynologists in such work were also discussed.

Discussion groups were then formed to consider the means by which we could advance our understanding of the processes governing harmful algal blooms. Three groups were formed to discuss respectively how to measure:

- i) Growth
- ii) Grazing
- iii) Physical/Biological interactions.

Participants in each subgroup are listed on Annex V.

7. DETAILED DISCUSSION IN SUBGROUPS

7.1 Growth Measurements

- How is growth to be measured?

It is assumed (as a simplification for the discussion) that there are no inputs to or losses from the system, i.e. that population changes are due only to growth. It is necessary to distinguish between cyst formers and non-cyst formers, including epibenthic stages. In cyst-forming species, the initiation of the bloom has to be through cyst germination (in the place where the bloom develops or somewhere else before hand). The rate of excystment has to be measured: it is a birth or growth term.

If resources are available, cyst distribution maps should be prepared; methods for this are straitforward and well known (coring, counting, etc.). Cyst distribution maps indicate where cysts are concentrated, but not necessarily where they will bloom: e.g. *Alexandrium tamarense*. The size of a bloom is not closely related to the magnitude of excystment. Conditions for cell division afterwards are more important.

- How do we establish if cysts are germinating?

i) One approach is to make use of cyst germination characteristics, i.e., for some species, cysts about to germinate make chlorophyll, which fluoresces and can be detected by epifluorescence microscopy. But in some species such as *Scrippsiella*, cysts are always fluorescent, probably because maturation intervals are short and essentially continuous excystment may occur; in other species fluorescence is aperiodic. For each species, we need to measure in the laboratory the effect of temperature and light on germination. Once the optimal temperature-window is established, we must establish how long it needs

to be at each temperature to germinate. These processes influence the recruitment to the "seed stock" or inoculum.

ii) We may be able to use the morphological characteristics of newly hatched cysts (or newly encysted cells) or inmunotechniques to establish excystment rate. For example, in some species, such as *Gymnodinium catenatum*, recently excysted cells can be recognized morphologically. In other species, the hatchlings have extra flagella.

To summarize, the germination rate of excysting cells is an important growth parameter needed to establish seed stock recruitment and to distinguish this from physical accumulation and losses due to advection. Seed stock provides cells for population growth which require separate measurements. Field studies may need to include cyst distribution surveys if the same population originates from cysts within the boundaries of the system being studied/modelled.

Population growth is difficult to measure because of factor interactions. Growth is influenced by irradiance, temperature, turbulence, nutrients and their ratios, and sometimes by excystment rates. Endogenous growth and excystment rythms have to be taken into account. Losses are due to sinking, advection, grazing and en cystment. Thus we need to know why cysts form and the cyst induction trigger.

- Direct measurements of growth:

i) Paired cell technique

Can be used only in the case of species where it is easy to distinguish the recently divided daughter cells. For example, in *Dinophysis acuta* and *D. acuminata* the newly divided cells exhibit complementary parts of the wings sulcal lists. If the ogives of numbers of *paired* and *separated* cells of *Dinophysis* are plotted on time, the separation of the two peaks measures the division rate.

ii) Single cell ¹⁴C labelling

Incubation of isolated cells with $H^{14}CO_3$. Measure two increments in equal time intervals:

 $\frac{P_2 - P_1}{P_1 - P_0} = \frac{P^1 (expnl^{-1})}{P_0 (expnl^{-1})} = \frac{P_1}{P_0}$ $\ln \frac{P_1}{P_0} = \ln t \qquad (see in Skagerrak/Kattegat programme)$ $\frac{P_2 - P_1}{P_0} = \frac{P^1 (expnl^{-1})}{P_0} = \frac{P_1}{P_0}$

iii) Cage or diffusion culture techniques.

iv) Mitotic index (paired nuclei).

v) DNA microfluorometry and mitotic index.

- Indirect measures of growth:

Establish u_{max} in laboratory experiments and apply a Michaelis-Menten approach to calculate expected growth rate as a function of irradiance, temperature and nutrients. Experimentally derived constants can be obtained using semi-continuous culture and chemostat procedures.

7.2 Grazing

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The significance of grazing as a regulatory mechanism controlling toxic phytoplankton blooms is a poorly understood phenomenon. Nevertheless, herbivory is a potentially important loss term in the species-specific net production equation.

Zooplankton predators upon phytoplankton blooms may be crudely subdivided into pure suspension filter-feeders, such as rotifers and tintinnids, and raptorial species, including copepods, capable of seizing prey from suspension. The little evidence that does exist from zooplankton gut content analysis of field populations reveals that certain copepods, rotifers, tintinnids, and other ciliate micrograzers are capable of ingesting toxic algal species, such as *Alexandrium*, and can occur in high abundance during toxic blooms. As a general rule, prey selectivity tends to relax somewhat as the concentration of particles in the appropriate size range drops below a critical threshold, but rejection of toxic species merely on the basis of their toxicity has not been adequately studied. The impact of grazing upon phytoplankton community structure when toxic species are present, and the physiological effects of toxin ingestion on predator feeding behavior in natural assemblages, are largely unknown and merit further attention.

Molluscan filter-feeders are particularly efficient at grazing upon and assimilating phytoplankton, including toxic species, over a wide range of particle sizes. The extent to which toxic bloom dynamics are affected by size- or species-specific selection in areas with dense bivalve populations, such as intertidal zones and intense shellfish aquaculture sites, must be established. Under such high density circumstances, it is clear that food demand often exceeds supply.

Possible interactions between the population dynamics of toxic species and grazing are multivariate: a) the presence of a toxin may lead to chemotactic rejection of a toxic prey species, i.e. selective avoidance response; and b)physiological incapacitation of the predator by the toxin may reduce grazing pressure. Grazers which actively select against the toxic species may alter the phytoplankton community structure and favor toxic bloom intensification through selective removal of phytoplankton competitors for growthlimiting resources.

Small-scale flask experiments on grazing are useful in estimating several key parameters required of bloom dynamic models and toxin budget estimates, but small closed systems have severe limitations. For micrograzing experiments, the filtration rate F can be calculated as: $F = V \ln(C_0/C_1)/t_1-t_0$, where V = volume of water and C_0 and C_1 are the concentration of prey cells (or other biomass indicator) at times t_0 and t_1 , respectively. This calculation assumes that F is constant, whereas this is true only for small changes in prey density, i.e. over short time intervals in a small-scale batch incubation. Such limited-volume experiments are prone to a variety of other flask effects, including induced high predator mortalities and physiological perturbations. The time scale and prey density in batch culture grazing experiments must be carefully chosen to avoid artifacts, such as nutrient depletion, which may affect *in situ* growth rates. The accumulation of toxic metabolites inimical to either prey or predator must also be considered. For operational reasons, in such small volumes it is usually desirable to maintain a relative homogeneity in the experimental incubation vessel by agitation or bubbling; therefore, realistic estimates of loss rates of both predator and prey species due to sinking and vertical migration must necessarily be ignored.

In spite of these drawbacks, flask experiments can be employed to determine a number of toxic prey-predator interactions. Specifically, the capacity of the predator to ingest the toxic putative prey species can be established - is the predator/prey size ratio, nutritional mode, and structure of the feeding apparatus adequate for grazing to occur? Techniques such as gut pigment fluorescence and fecal pellet analysis, optical or electronic particle counting of prey cells, and incorporation of radioactively-labelled food particles are effective in confirming and quantifying feeding activity. If a suitably sensitive analytical method for the specific toxin is available, toxin levels in both prey and predator species can be used as a chemical tracer to determine toxin uptake, assimilation and detoxification kinetics.

It is well known that micrograzers exhibit a sophisticated array of chemosensory and tactile behaviors to optimize food quality while minimizing the expenditure of metabolic energy. Prey selectivity is normally mediated on the basis of size, shape, ease of capture, and olfactory and gustatory factors. In the case of toxic prey species, presence of a toxin may be a criterion for rejection by certain predators. Limited-volume flask experiments, where the direct toxic effects on filtration and clearance rates can be readily determined, are suitable for the study of these phenomena.

Once ingestion of the toxic species has occurred, the toxin becomes potentially available to the next trophic level. Nevertheless, there may be a post-ingestion feedback effect inhibiting further toxin uptake, due to the incapacitation of the predator. The physiological effects of toxic prey ingestion can be evaluated by measuring changes in respiration rate (microrespirometry), cardiac rhythms, swimming and filtration rates, and predator mortality over time. In flask experiments, microvideo recording has been shown to be a useful tool for monitoring predator behavior.

Some of the difficulties inherent in using small-scale closed incubation vessels for grazing experiments can be alleviated through the use of flow-through systems. Such quasi-steady state experiments attempt to replicate more natural conditions, by ensuring that the inflow prey density remains roughly constant over time, and that prey depletion and nutrient limitation do not occur. Concentration gradients determined by comparing biomass indicators at the inflow and outflow of the system can yield good estimates of filtration rates. Small-scale recirculating and non-recirculating flow-through systems have been successfully employed in studies of toxin kinetic parameters with filterfeeding bivalve species known to accumulate toxins from marine algal blooms. For micrograzer experiments, where total filtration rates are much lower, flow-through systems may not be practical.

On a larger spatial scale, mesocosms offer significant advantages over small flask batch systems in the level of complexity that may be addressed in grazing studies, with some loss of resolution and control. The attendant reduction in "flask effects" generally enhances the survival and reduces manipulative stress on both the prey and predator species, i.e. inherently a more "natural" system. The relaxation in survival pressure on the prey species allows for the use of complex multispecific assemblages, either by mixing cultured isolates in various proportions, or by introducing natural *in situ* assemblages into the mesocosm. Thus the interaction due to interspecific competition among prey species can be compared with the relative impact of selective grazing pressure on species survival. A variety of manipulations, including the pulsed addition of nutrient enrichment. which may influence these competitive outcomes by affecting species-specific growth rates (μ) , can be assessed. By size-fractionating the predators within the natural assemblages in the mesocosm, the size structure of potential predators upon a given toxic species can be determined. A gradient in predator numbers within each size fraction can be established by addition or removal of particular predators within a size class, e.g. adult copepods versus tintinnids. Finally, mesocosm-scale grazing experiments permit the study of secondary grazing pressure by carnivorous zooplankton and larval stages upon herbivorous grazers, thereby yielding insight into the efficiency of toxin transfer within food chains.

At the ecosystem level, even within a defined large water mass, predator/prey interactions are so complex that valid measurements of grazing component variables which could be incorporated into a population dynamic model of toxic algal blooms are difficult to achieve. Nevertheless, some clues as to the potential importance of grazing on toxic bloom dynamics in natural ecosystems may be gleaned from standing stock estimates of inter-annual and seasonal co-fluctuations in potential predator and prey abundance. For example, a decrease in grazing pressure by ctenophores and carnivorous larval fish as upper echelon zooplankton grazers may lead to an overabundance of herbivorous microzooplankton, hence increased grazing on the phytoplankton standing crop. Some of these relationships could be re-defined by using the co-occurrence of phycotoxins in different size fractions as temporal evidence that grazing upon particular toxic species has actually taken place. Similarly, diurnal vertical migrations by both prey and predator species should be followed in the field, to determine whether or not avoidance mechanisms and prey-targeted predator aggregations should be considered in bloom dynamic models.

Regardless of the spatial scale of the experimental system adopted for the grazing experiment, the importance of providing appropriate non-toxic control prey organisms cannot be overemphasized. In some cases, the correct interpretation of grazing experiments on toxic species has been confounded by the selection of a taxonomically unrelated species as the non-toxic control - is the rejection response due to the presence of the toxin, or to some other species-specific factor unrelated to the toxin? Fortunately, nontoxic or low toxicity analogues which could serve as controls for many of the most important toxic algal bloom species are available in culture collections. A promising recent approach which could be incorporated into grazing studies on toxic microalgae involves the use of synthetic microspheres, essentially as selectively neutral particles for various discrete size ranges. The effect of a toxin, either adsorbed to the surface of the beads or micro-encapsulated, could then be evaluated in isolation from the matrix effect of the toxic algae. These measurements are crucial in interpreting the role of prey selection by grazers in marine food webs.

7.3 Physical / Biological Interactions

Two aspects were discussed:

1. The growth equation around which the initial discussions were centred does not explicitly invoke gains and losses in population numbers due to physical processes. In fact it assumes that the population is closed. In reality of course, the boundaries conditions must be established so that we can estimate the contributions of advection and other physical processes to changes in population numbers. We may also need to know the dynamics of water movements, both advective and turbulent, within these boundariess. In both cases we need to call on physical oceanographers. These requirements are of course site specific. The problem is to define the scales within which exponential growth models can be used.

2. The problems posed by vertical migration were discussed, and in particular the ways in which behaviour interacts with physical features and the dynamics of the environment. It is often assumed that vertical migration in flagellates is a strategy designed to optimize occupancy of the high light/low nutrient mixed layer above the thermocline and the harvesting of nutrients in or below the thermocline.

Nevertheless, some migratory species, e.g. Gyrodinium aureolum, Dinophysis, remain in the thermocline most of the time, and their vertical movements are not diurnal. It was generally agreed that vertical migration is a part of life history strategy in these organisms, but that we still do not know what its functions are. In some cases it may simply solve the problem of maintaining the cells at a preferred depth in the face of mixing processes tending to remove them from that depth. There is also some evidence that vertical migration plays a role in swarming at the surface associated with sexual processes and encystment.

8. RECOMMENDATIONS

8.1 The design of field programmes to study HABs should be driven by the fundamental population equation (section 4) so that the magnitudes of gain and loss terms are explicitly estimated.

8.2 Population gains and losses due to hydrodynamic processes should be brought to the attention of physical oceanographers during the design phase of field programmes.

8.3 Plans should be made for 3 to 5 year studies to begin in 1993 if possible.

8.4 Laboratory and mesocosm studies should be undertaken to illuminate life history features with significant impacts on growth and loss terms (e.g. effects of toxins on grazing rates, vertical migration, encystment and excystment, changes in nutrient ratios,..). Nevertheless, the utility of mesocosm experiments should be critically assessed,

with a view to estimating to what extent their findings are relevant to the interpretation of field data.

8.5 Field studies should be targeted on harmful species whose appearance is already predictable (e.g. *Alexandrium* in Gulf of Maine and St. Lawrence, *Dinophysis spp* in Iberia and Kattegat-Skagerrak, *Gymnodinium catenatum* in Iberia, *Gyrodinium aureolum* in Scandinavia and Britain, *Heterosigma akashiwo* in British Columbia)

8.6 The cyst phase in the life history of those harmful species which have one should be examined in more detail, in collaboration with palynologists.

8.7 A catalogue of harmful species should be prepared, with indications for each species of when and where physical or sedimentological processes may play important roles in modifying population gain and loss terms.

8.8 The study group should be reconvened next year with a more prominent participation of oceanographers, and an agenda designed to resolve outstanding operational aspects of HAB pilot studies.

8.9 In order to capitalize on a broader range of expertise, the implications of HAB's need to be presented within a larger forum, such as an ICES mini-symposium or a special meeting.

9. TERMS OF REFERENCE

The Study Group on the Dynamics of Harmful Algal Blooms should meet for 3-4 days in early 1993 in a place to be decided (Chairperson Ms Beatriz Reguera) with the following terms of reference:

1. To continue and if possible finalize discussions on a programme for investigating the dynamics of harmful algal blooms in the ICES area. Methods to be used should be discussed and the time-tables for the development of pilot studies outlined.

2. To discuss algal population dynamics in relation to hydrodynamic processes.

3. To assess the utility of mesocosm experiments for the understanding of harmful algal population dynamics.

4. To examine the cyst phase in the life histories of relevant, potentially harmful algae.

5. To co-ordinate work on harmful algal blooms with the activities of the Programme of Harmful Algal Blooms suggested by the Joint ad hoc IOC-FAO Intergovernmental Panel on Harmful Algal Blooms.

Physical and chemical oceanographers should be invited to take part in the meeting. Scientists from other countries than those covered by ICES are welcomed to participate.

IOC should be invited to continue its co-sponsorship for the Study Group.

Justification: Harmful algal blooms and the problems related to them, e.g. fish death, damage to ecosystems and/or problems in relation to human health, are becoming increasingly important. Improvement and co-ordination of research carried out on these problems is urgently needed both within the ICES area and in co-operation between ICES and experts in other parts of the world.

10. PILOT PROGRAMME DRAFTS

Finally the group divided again to prepare proposals for actual pilot studies. The subgroups were Skagerrak/Kattegatt, Mesocosm Studies, Gulf of Maine, and Iberian Peninsula. The draft of these programmes is presented in Annex VI.

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ANNEX I

STUDY GROUP ON THE DYNAMICS OF HARMFUL ALGAL BLOOMS

7-9 April 1992

Instituto Español de Oceanografía, Vigo, SPAIN

Agenda

- 1. Registration
- 2. Welcome to participants
- **3. Opening of the meeting**
 - 3.1. Why the study group was established.
 - 3.2. Terms of Reference.
 - 3.3. Relation with IOC programme on Harmful Algal Blooms.
 - 3.4. Objectives of the meeting.

4. Introduction of participants

5. Approval of the Agenda

5.1. Designation of a rapporteur.

6. **Population dynamics approach to Harmful Algal Blooms: initiation, growth,** mortality...

7. Pilot studies proposals and other presentations

- 7.1. Identification of hypothesis to be tested in each regional case.
- 7.2. Innovative suggestions
 - a) Role of grazing; b) Role of toxicity

8. Identification of common elements in pilot proposals

- 8.1. Discussion of the common elements.
- 8.2. Common elements with monitoring programmes.

9. Instruments and capabilities to answer the questions poses. (Contributors to identify themselves)

- 10. Separation in subgroups discussing subjects of study.
- 11. Discussion of subgroup Reports and Development of the programme proposal.

ANNEX II

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LIST OF PARTICIPANTS

NAME	AFFILIATION
Donald M. Anderson	Biology Dept. Woods Hole Oceanographic Institution Woods Hole MA 02543 - USA Tel: 1 508 457 2000 Ext: 2351 Fax: 1 508 457 2169
Juan Blanco	Centro Experimental de Vilaxoán. Consellería de Pesca. Xunta de Galicia. Aptdo 208. 36600 Vilagarcía de Arousa - SPAIN Tel: 34 86 500155 - 86 500161 Fax: 34 86 506788
Isabel Bravo	Instituto Español de Oceanografía. Aptdo 1552. 36280 Vigo - SPAIN Tel: 34 86 492111 Fax: 34 86 492351
José M. Cabanas	Instituto Español de Oceanografía. Aptdo 1552. 36280 Vigo - SPAIN Tel: 34 86 492111 Fax: 34 86 492351
Gerhard C. Cadée	Netherlands Institute for Sea Research PO Box 59 1790 AB Den Burg Texel THE NETHERLANDS
Allan Cembella	National Research Council Institute for Marine Biosciences 1411 Oxford Street Halifax, N.S CANADA Tel: 902 426 4735 Fax: 902 426 9413
Antonio Cruzado	Centre d'Estudis Avançats de Blanes Cami Sta. Barbara 17300 BLANES - SPAIN Tel: 34 72 33 61 01 Fax: 34 72 33 78 06

M. Emilia Cunha	Instituto Nacional de Investigação das Pescas Av. Brasilia 1400 Lisboa - PORTUGAL Tel: 01 301 08 14 Fax: 01 301 59 48
David H. Cushing	198 Yarmouth Road Lowestoft, Suffolk UNITED KINGDOM
Hans Dahlin	SMHI S-60176 Norrkoping - SWEDEN Tel: 46 11 158305 Fax: 46 11 158350
Ian Dundas	University of Bergen NORWAY
Lars Edler	Swedish Meteorological and Hydrological Institute Doktorsgatan 9D, S-26252 ANGELHOLM SWEDEN Tel: 46 431 80854 Fax: 46 431 83167
Henrik O. Enevoldsen	IOC 1, Rue Miollis 75014 Paris - FRANCE Tel: 33 1 45 68 40 16 Fax: 33 1 40 56 93 16
Marta Estrada	Institut de Ciencies del Mar P. Nacional, s/n 08039 Barcelona - SPAIN Tel: 34 3 3106450 Fax: 34 3 3199842
Santiago Fraga	Instituto Español de Oceanografía Aptdo 1552, 36280 Vigo - SPAIN Tno: 34 86 492111 Fax: 34 86 492351
Patrick Gentien	IFREMER/Centre de Brest BP 70 29280 Plouzane - FRANCE Tno: 98 22 4324 Fax: 98 22 4548

Edna Granéli	Marine Ecology Dept. University of Lund Box 124, S-22100 Lund - SWEDEN Tel: 46 46 152984 Fax: 46 46 104003
Maurice Levasseur	Maurice-Lamontagne Institute Department of Fisheries and Oceans Mont-Joli, Quebec - CANADA Tel: 418 775 0608 Fax: 418 775 0542
Odd Lindahl	Kristineberg Marine Biological Station S-45034 Fiskebackskil - SWEDEN Tel: 46 523 22280 Fax: 46 523 22871
Elspeth Macdonald	SOAFD Marine Laboratory P.O. Box 101 Victoria Road, Aberdeen AB9 8DB UNITED KINGDOM Tel: 0224 876544 Fax: 0224 295511
Ivona Marasovic	Institute of Oceanography and Fisheries P.O. Box 500, 58000 Split - CROATIA Tel: 3858 46688 Tel: 3858 46593
Teresa Moita	Instituto Nacional de Investigação das Pescas Av. Brasilia, 1400 Lisboa - PORTUGAL Tel: 01 3010814 Fax: 01 3015948
Thomas Osborn	IOC 1, Rue Miollis 75014 Paris - FRANCE Tel: 33 1 45 68 40 16 Fax: 33 1 40 56 93 16
Helle Ravn	National Environmental Research Institute. Department of Marine Ecology and Microbiology. Postbox 358 DK-4000 - DENMARK Tel: 45 46 301263 Fax: 45 46 301114

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Beatriz Reguera	Instituto Español de Oceanografía Aptdo 1552, 36280 Vigo - SPAIN Tel: 34 86 492111 Fax: 34 86 492351
María A. de M. Sampayo	Instituto Español de Investigação das Pescas Av. Brasilia, 1400 Lisboa - PORTUGAL Tel: 3017361 Fax: 3015948
Ted Smayda	Graduate School of Oceanography Univ. Rhode Island Kingston, RI 02881 Tel: 1 401 792 6171 Fax: 1 401 792 6682
John C. Smith	Dept. of Fisheries and Oceans Gulf Fisheries Centre Moncton, N.B., CANADA E1C 9B6 Tel: 506 851 3827 Fax: 506 851 2079
Zoila Velásquez	Centre d'Estudis Avançats de Blanes. Cami de Santa Barbara 17300 Blanes - SPAIN Tel: 3472 336101 Fax: 3472 337806
Timothy Wyatt	Instituto de Investigaciones Marinas Eduardo Cabello, 6, 36208 Vigo - SPAIN Tel: 34 86 231930 Fax: 34 86 292762

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ANNEX III

A NOTE ON THE POPULATION DYNAMICS OF A PLANKTON BLOOM

BY

DAVID CUSHING

By the time the tide is red, it is a little late to study it as a population. As some dinoflagellates grow rather slowly, the population study should start early. It is a truism that the same population must be sampled all the time, but that depends on the physical regime.

The population dynamics are simple in principle, if perhaps a little awkward to put into practice.

 $dP/dt = (\mu - l)P$

Where P is the stock of algae in numbers, carbon or nitrogen:

" μ is the algal division rate;

" l is the loss rate of algae.

Then, in a short time interval, t, $P_1 = P_0^* \exp((\mu - l)^*t)$.

Further, $(\mu - l) = (1/t) * ln(P_1/P_0)$

and $l = \mu - (1/t)^* ln (P_1/P_0)$

or $\mu = 1 - (1/t)^* \ln (P_1/P_0)$

It can be shown that the quantity produced, P_r , in the interval of time, t, is:

 $P_r = \{ \mu / (\mu - 1) \}^* (P_1 - P_0).$

Similarly, the quantity lost, L, in the interval of time, t is:

 $L = \{ l/(\mu - 1) \}^* (P_1 - P_0).$

Consider the two exponents

1.- The algal division rate, μ .

Fasham et al. (1990) use Eppley's (1972) dependence of maximal division rate on temperature; it was assumed that all observations were made at P_{max} , the maximal rate of photosynthesis as a function of irradiance. If such information is not available for a particular dinoflagellate, it could be obtained very readily, the maximal division rate in terms of irradiance and temperature.

Further, the algal division rate can be estimated at sea with the double diffusion method devized by Furnas and others.

Nutrient limitation is best estimated with the Droop function (Droop, 1968):

$$\mu = \mu' * (1 - K_q/Q)$$

Where μ ' is the division rate at infinite cell quota; K_q is the subsistence quota at which uptake is zero; Q is the cell quota, the quantity in store available for use.

$$\mu_{\rm max} = (1 - K_{\rm q}/Q_{\rm m})$$

Where Q_m is the maximal cell quota.

A self-shading function should be used, which might have to be developed specially for the red tide organism. The densities of red tide organisms are very high.

Thus, it should be possible to develop estimates of algal division rates which take into account irradiance, temperature, nutrients and self shading and the rates can be checked by observations of the algal division rates at sea.

2,-1, the loss rate.

Dinoflagellates may migrate vertically and may sink. Consider sinking first. In the interval of time, t, at the same depth, z_1 ,

$$P_1 = P_0 * exp(\mu - g - s_1) * t$$

Where g is the mortality rate due to grazing;

 s_1 is the sinking rate of the algae.

At z_2 (> z_1),

$$P'_1 = P_0 * \exp \{ \mu - g + (s_1 - s_2) \} * t.$$

Recall that P_0 is the initial number at z_1 .

Let us assume that (μ - g) is constant during the time interval, t, and in the depth interval ($z_2 - z_1$).

Then $(1/t) * \ln (P_1/P_0) = \mu - g - s_1$.

And $(1/t) * \ln (P'_1/P_0) = \mu - g + s_1 - s_2$.

Then $\mu - g - s_1 - \mu + g - s_1 + s_2 = 2 s_1 - s_2$.

So $(1/t) * \ln (P_1/P_0) - (1/t) * \ln (P'_1/P_0) = 2 s_1 - s_2$.

With this method, sinking rates could be estimated and a negative rate would estimate the rate of vertical migration less that of sinking. If such rates are averaged for the depths sampled, s,

$$(1/t) * \ln (P_1/P_0) = (\mu - g - s) * t.$$

and $g = (\mu - s) - \ln (P_1/P_0)$.

If the red tide organisms are not eaten, then g = 0, but it has then been shown.

I have left out the most important loss rates, diffusion, because it must be particular to the area sampled. The area must be sampled for a fairly long time and this is also peculiar to the area sampled. Physical oceanographers will have two jobs to do, establish the identity of the patch sampled and to estimate the loss rate from it by diffusion.

The point of this system is that it can be used in an area with frequent observations in time and in depth to describe the population dynamics of the patch as it develops.

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ANNEX IV

HARMFUL ALGAL BLOOM PILOT STUDY IN THE GULF OF MAINE

BY

D.M. ANDERSON A.W. WHITE

In many ways the Gulf of Maine offers an excellent site for a multi-investigator study of issues common to numerous harmful algal blooms. Perhaps the best way to evaluate this site relative to others is to compare its characteristics with respect to the list of criteria established at the recent IOC Workshop on Harmful Algal Blooms in Newport, Rhode Island.

Common or Globally Distributed Species

The causative species of paralytic shellfish poisoning in the Gulf of Maine are Alexandrium tamarense and A. fundyense. The taxonomy of the local species has been well established. Studies on Alexandrium in the Gulf of Maine thus would be relevant to blooms of these species or closely related species in many other parts of the world where PSP is common. Since both A. tamarense and A. fundyense include a dormant, cyst stage in their life cycle, a Gulf of Maine study would provide an opportunity to document in detail the quantitative contribution of encystment and excystment in overall bloom dynamics. In this region, the cyst stage is essential to bloom initiation, since the Alexandrium species are absent from the water column during the cold winters. Studies of this nature would be greatly facilitated by previous studies that have mapped the horizontal and vertical distribution of Alexandrium cysts throughout the Gulf of Maine.

Harmfulness

Paralytic shellfish toxins are among the most potent biotoxins known and have serious consequences for fisheries resources, aquaculture and human health around the world. In the Gulf of Maine, the annual blooms of *Alexandrium tamarense* necessitate closures of vast stretches of productive shellfish areas, recently encompassing offshore as well as inshore resources. Despite surveillance programs, occasional incidents of PSP still occur. Through food web transfer events, these toxic blooms have caused massive kills of fish and even marine mammals; larval and juvenile stages can also be effected. The total impact of *Alexandrium* blooms on the biota of the Gulf of Maine is unknown, but the repercussions of these blooms may be substantial and increasing, especially considering the apparent recent spread of the blooms hundreds of miles offshore, resulting for the first time in high levels of the toxins in animals on Georges Bank, one of the richest fishing grounds in the world.

Significant Features

<u>Hydrography</u>. Recent research suggests that in the southern Gulf of Maine, blooms of *Alexandrium* are closely linked to the development and movement of a buoyant coastal current. Such coastal currents are thought to be important in harmful bloom dynamics in coastal regions in many areas of the world, so studies in such a hydrographic feature should be a high priority in a global program. In addition, in northern waters of the Gulf of Maine, the blooms are more likely linked to tidal fronts of the type first described by Pingree, Simpson and Holligan, which are also important features for detailed study in a global program. Finally, offshore PSP toxicity on Georges Bank may be caused by a third hydrographic mechanism, namely the intrusion of water from either the nearshore region or from offshore, possibly an eddy from the Gulf Stream. Clearly, a field exercise in the Gulf of Maine would permit investigation of harmful blooms and their association with hydrographic features of several different types.

<u>Pollution and Nutrients</u>. Southern waters of the Gulf of Maine are soon to be the site of a massive "experiment" in possible nutrient enhancement of algal blooms. In 1995 the first phase of a sewage effluent diversion will take place, releasing up to 1 billion gallons per day of primary treated effluent into Massachusetts Bay, near the path of the coastal current that carries *Alexandrium* populations. In the past, this effluent has been released directly into Boston Harbor, from where it gradually enters the Bay. By 1999, the effluent will receive full secondary treatment. This plan offers an excellent opportunity to study the effects of altered nutrient loading on harmful bloom species, especially *Alexandrium*.

Logistical Support

The Gulf of Maine is easily reached from several different marine research institutes, that are supported by both large and small research vessels. Equipment for sediment sampling and water column characterization are also readily available, including box corers, Carib corers, CTD systems, pump profiling systems, etc. Considerable field work has been conducted in this region to establish baseline information on *Alexandrium* populations, so there is good experience with respect to choosing the correct vessel for different types of surveys. In addition, the proximity of several marine laboratories means that land-based investigations are also possible in conjunction with the field surveys. Other important logistical support comes from a remote sensing node at the Woods Hole Oceanographic Institution, capable of providing near-real time infra-red images twice each day to help guide field efforts and to help in the interpretation of field results. A remote sensing capability should be a requirement in any pilot study planned for a global research program on harmful blooms.

Background Information

Blooms of *Alexandrium* have been studied in the Gulf of Maine for many years. These studies provide valuable background information on the timing of blooms, the distribution of the toxic cells, and the regularity of the outbreaks. In this latter context, it should be noted that toxicity has occurred along the coast of the Gulf of Maine every year since 1972 when the problem first began in the region. The pattern of the toxin outbreaks has varied somewhat from year to year geographically, but this could be accommodated in a field program through careful interaction with shellfish monitoring programs along the coast and a somewhat flexible cruise schedule. Even if cruise scheduling has to be rigid, experience suggests that a bloom will be present between late May and mid-June in the waters between Southern Maine and Massachusetts. The only question is how far the bloom will have progressed down the coast. Information on the vertical distribution of cells and the manner in which they are best sampled is also readily available for this region. It should be noted that although the Gulf of Maine blooms have been studied in reasonable detail, many issues remain to be clarified. These include: the manner in which the coastal current (i.e. entrainment, vertical migration, enhanced growth through micronutrients, etx.); the general nutritional status of the cells throughout the blooms; the quantitative role of cysts in bloom initiation and decline; the impact of grazing on bloom magnitude; the link between *in situ* dinoflagellate populations and the genetic characteristics of regional vs. local populations.

Summary

One of the objectives in selecting a site for a multi-investigator pilot study should be to maximize the chances of learning as much as possible about possible aspects of harmful bloom dynamics. This requires that: 1) the causative bloom species be one of interest to many countries; 2) the characteristics of the blooms and the region be known with sufficient accuracy so that field exercises can be scheduled in specific places at specific times with good likelihood of encountering a bloom; 3) vessel, equipment, and other logistical support facilities be readily available within the region; 4) major hydrographic features be of broad applicability to many bloom types in other regions; and 5) the site be accessible without serious transportation constraints. It is our belief that the Gulf of Maine would be an excellent site for a pilot program based on these considerations.

HARMFUL ALGAL BLOOM MESOCOSM EXPERIMENTAL STUDY

ΒY

TED SMAYDA Graduate School of Oceanography University of Rhode Island Kingston, RI 02881

No single program or pilot study on harmful algal blooms will fulfill the objectives of the proposed IOC/SCOR initiative to develop an international, multi-disciplinary scientific program. The great diversity in types of bloom occurrences, triggering environmental factors, bloom species and their biotoxins, requires a multi-pronged scientific approach to establish both the commonalities of bloom events and the unique features of specific types of blooms. Studies of bloom events in both open coastal waters associated with hydrographic features such as frontal zones, buoyant current systems, pycnocline structure, upwelling, etc., as well as blooms typical of shallower, nearshore embayments, estuaries, etc., where nutrient and grazing conditions may be important control factors, should be included within the IOC/SCOR initiative. Large scale experimental studies at the population and community levels are also needed to supplement such descriptive field studies and laboratory investigations of cellular level, physiological and toxicological features. Such large scale experiments should evaluate the regulation of bloom dynamics over complete bloom cycles, including (where applicable) induction of dormant stages and subsequent germination, and use representative bloom species grown in experimentally manipulated mesocosms. These mesocosms experiments should be designed for extrapolation to naturally occurring blooms. Such an experiment is proposed here, using the raphidophycean Heterosigma akashiwo.

Heterosigma akashiwo: Suitability for experimentation

Heterosigma akashiwo increasingly has been implicated in red tide blooms, sometimes of prodigious magnitude, globally in nearshore waters: Japan, Korea, China, Taiwan, New Zealand, Spain, U.S. (Narragansett Bay, Puget Sound), Canada (British Columbia) and Chile. Taxonomic issues are largely resolved; frequent past confusion with Olisthodiscus luteus is not a problem, since the latter taxon is now recognized to be a benthic-dweller; H. akashiwo is planktonic, with an epibenthic stage in its life cycle.

Significant mortality of cultures has resulted from *H. akashiwo* blooms. In Japan, total economical loss over a 16-year period has been about 2 billion yen. In Puget Sound (U.S.) and Chile, extensive mortality and economic loss have accompanied its blooms. *H. akashiwo*, in fact, may be the leading, present cause of catastrophic fish kills in high-value fish aquaculture, replacing *Chattonella* spp. in this regard. *H. akashiwo* has been shown experimentally to be antagonistic or lethal to diatoms; microzooplankton (tintinnids, rotifers), copepods, benthic larvae, and certain shellfish, among others. Thus, *H. akashiwo* is a broad spectrum antagonist or lethal to several trophic levels within the food web. The nature of its presumed toxin has not been established.

Numerous autecological studies have been carried out on *H. akashiwo*; it is easy to culture, and many geographical, clonal isolates are available. We have considerable knowledge as to its basic cellular properties with respect to the effect of temperature and salinity on its growth rate, its phosphorus metabolism, photosynthetic properties, phototaxic properties, etc. In addition, a 25-year data set from Narragansett Bay, based on weekly quantitative measurement, provided insight into interannual variations in its bloom dynamics and associated environmental conditions. Numerous field studies have been carried out in Japan as well. Thus, an extensive autecological data base on cellular processes of *H. akashiwo* and its *in situ* occurrences is available to design and interpret large-scale experimental manipulation of this flagellate to assess its population dynamics.

Experimental setup and site

It is proposed that the mesocosm experimental facilities at the Marine Ecosystems Research Laboratory (MERL) at the University of Rhode Island, be used. The experimental facility consists of contemporary laboratory facilities and 14 outdoor mesocosm tanks, each ca 5.5 m in height, 1.8 m internal diameter and containing 13 m³ of water (30 $^{0}/_{00}$). The mesocosms can be operated in batch mode, or with continuous flow input of unfiltered water from Narragansett Bay. The mesocosms also have vertical mixing capability, with adjustable rate settings, temperature regulation to within 1°-2° C of the adjacent bay waters; both pycnocline and thermocline structures can be induced and maintained, and the mesocosms can also be operated with, or without bottom sediments in place. Local waters are very favorable for *H. akashiwo* growth. Narragansett Bay has been shown to be one of the major bloom areas for *H. akashiwo* within its distributional range. The excellent toxicological laboratory facilities of Dr. Yuzuru Shimizu are also available nearby.

Important issues within bloom ecology include the role of nutrients as regulators of bloom events, in species selection, and the effect on bloom events of the observed changes in nutrients occurring in many global coastal waters. Such issues are not quantifiable from culture-flask experiments, nor readily tractable from descriptive field studies, but are approachable via mesocosm experimentation.

A prospective nutrient experiment

Mesocosms could be enriched with nutrients in various combinations, doses and ratios, different mixing (turbulence) regimes, and inoculated with large volumes of cultured *H. akashiwo* to start the experiments, with or without added competing species (exclusive of entrained flora); with or without benthic filter feeders; with or without variable microzooplancton and copepod communities, and the relationships between nutrient loading (solely or interactively with the other manipulated parameters) and *H. akashiwo* bloom dynamics evaluated over a complete bloom cycle. Measurements of key physiological and ecological processes, such as nutrient uptake, growth, productivity, species competition, succession, etc. would be carried out concurrently. Variations on, and expansions of this illustrative type experiment are evident. For example, the influence of differing nutrient ratios on diatom/dinoflagellate/rhaphidophycean bloom species selection (hypothesized to occur) can also be evaluated in mesocosms, as can the relationship between nutrient loading and anoxia-producing blooms be examined, etc.

The proposed mesocosms are not offered as an experimental panacea, but are very suitable experimental tools useful in seeking answers to some fundamental ecological questions, particularly with regard to the role of nutrients, grazing and turbulence in the regulation of bloom events, bloom species selection and in situ toxigenesis. Field studies are usually *a posteriori* studies beginning after a bloom has begun, whereas mesocosms can allow both experimental assessment over a complete bloom cycle, i.e. from inception to termination, and environmental manipulations.

Summary

Experimental studies at ecologically meaningful levels are widely acknowledged to be essential to quantification of harmful bloom events. Ecological studies were identified at the Fifth International Conference on Toxic Marine Phytoplankton as being of high priority. Nutrient regulation of bloom events is poorly understood, with present knowledge inadequate to evaluate the impact on harmful blooms of the widespread, ongoing changes in the chemical environment occurring in coastal waters globally. *H. akashiwo* is a particularly prominent and harmful rhaphidophycean representative of this group which, together with other phytoflagellates, generally appear to be increasingly involved in bloom events. The reasons for this emergence are obscure. The proposed mesocosms studies are relevant to a variety of harmful bloom issues, would employ needed multi-disciplinary, collaborative approaches, and are needed in addition to descriptive field studies and laboratory experimentation.
COMPARATIVE STUDIES OF THE FISH-KILLING FLAGELLATE HETEROSIGMA AKASHIWO BLOOMS IN THREE DIFFERENT LOCATIONS

BY

F.J.R.TAYLOR Department of Oceanography University of British Columbia Vancouver, B.C., Canada V6T 1Z4

Blooms of the fish-killing chloromonad flagellate *Heterosigma akashiwo* (commonly misidentified as *Olisthodiscus luteus* which has not been linked with fisk kills yet) are a regular feature of widely scattered geographic locations. They have been studied paticularly well in Osaka Bay, Japan (eutrophic), the Strait of Georgia, British Columbia (estuarine, stratified) and Narragansett Bay, Rhode Island (less estuarine). Similarly, the general oceanography of these waters, including most nutrient levels, has been well studied. A major characteristic of the blooms in these areas is their great regularity: they are annual events, beginning in late May or early June in British Columbia, appearing abruptly when the surface temperature reaches 15°C off Vancouver (observations of the last 3 years).

Heterosigma is an excellent example of an exploiter of stratified conditions, using vertical migration to take up nutrients below the seasonal pycnocline. It can bloom as long as the weather allows it to. Some studies indicate allelopathic properties. Studies in Japan have shown that it can be limited by micronutrients.

The mechanism of fish kill (exotoxin released in the water? polyunsaturated fatty acids with toxic superoxides formed? mucus release on the gills causing suffocation?) is unknown since it is difficult to kill fish with cultures. In British Columbia the organism is of major economic importance because of its kills of cultivated salmon. In the Inland Sea of Japan it is its close relative, *Chattonella*, that is the main fish killer.

These events in different parts of the world offer the opportunity to intercalibrate research methodologies, compare limiting factors under different circumstances, study genetic heterogeneity, and compare ameliorating methods, among other opportunities.

I propose that the three regions be compared by focussing on one per year for three years, involving personnel from all three regions. I am sure that other benefits can accrue from this. N.B. In marked contrast to the Gulf of Maine these are very calm areas.

HARMFUL ALGAL BLOOM FIELD STUDY IN BAHIA FOSFORESCENTE

BY

TED SMAYDA Graduate School of Oceanography University of Rhode Island Kingston, RI 02881

Blooms of the dinoflagellate, Pyrodinium bahamense

Toxic (PSP) blooms of the dinoflagellate, *Pyrodinium bahamense* var. compressum, have scourged and spread within the Indo-Pacific region since a 1972 outbreak in New Guinea; unexpectedly developed off the Guatemalan coast in 1987, and have devastated aquacultural activities in the Philippines. Toxic blooms of this species are estimated to have been responsible for more than 1000 human illnesses and 60 deaths resulting from the consumption of toxic shellfish and planktivorous fish such as sardines and anchovies. This epidemic has resulted in two workshops dedicated to *Pyrodinium* bloom issues convened in Brunei (1989) and Singapore (1984). These workshops led to a series of research recommendations on the biology, ecology and toxicology of *Pyrodinium*. This proposal, which partly responds to these recommendations, outlines an ecological field study of *Pyrodinium bahamense* var. bahamense blooms in Bahia Fosforescente, Puerto Rico, well known for its bioluminescence.

Significant features

More or less continuous blooms of Pyrodinium bahamense occur in this ca 20 ha, relatively shallow (mean depth ca. 4 m) environment surrounded by a mangrove ecosystem and open to the sea by a narrow (~150 m) breachway. Pyrodinium co-occurrence dominates in successional changes and competition with a number of other major dinoflagellates, including Ceratium spp., Gymnodinium splendens and Cochlodinium polykrikoides, all of which are bloom species. Thus, Fosforescente Bay appears to be primarily a dinoflagellate biotope, particularly suited for year-round investigation of dinoflagellate blooms, and having a continuous availability and succession of diverse dinoflagellates relevant to toxic bloom problems. Toxic blooms of P. bahamense var. bahamense in this area have not been reported, in marked contrast to the virulence of P. bahamense var. compressum. Whether this is a reflection of the relatively low use of shellfish in this area, or whether it reflects divergent toxigenesis between these two Pyrodinium varieties, including genetic differences and absence/presence of environmental triggering factors, can be cleared up in the proposed study.

Additional features of this embayment which make it particularly amenable logistically for the proposed study result from its relatively small area and narrow outlet into offshore waters. This facilitates the logistics of following, on a regular basis, perturbations in water exchange rates, flushing patterns, nutrient levels, turbidities and distributions, successional and growth patterns of species, and allowing species emigration/immigration and phototaxic behavioral properties to be established. A common feature at all sites of the *Pyrodinium* red tides in the western Pacific has been the presence

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of mangroves. The mangrove, *Rhizophora mangle*, dominates the periphery of Bahia Fosforescente.

The predictable and abundant occurrence of *Pyrodinium* and other dinoflagellate populations in Bahia Fosforescente facilitates *in situ* and laboratory experimentation during the field studies. The availability of the University of Puerto Rico marine laboratory, located nearby, provides access to a field station. The circulation patterns and basic biological features of Fosforescente Bay have been established, principally by Seliger and Margalef. This provides a data resource facilitating design of the field experiment.

Objectives of study

The objectives are two fold. One is to use Bahia Fosforescente as a site for the development of collaborative, multi-disciplinary, integrative strategies and approaches needed for the IOC/SCOR initiative, as well as to provide an inter-calibration opportunity.

The other objective is to carry out a quantitatively based, experimentally driven, field study on the physical, chemical and biological factors specifically regulating *Pyrodinium bahamense* blooms in this embayment, and suitable for potential extrapolation to the toxic Indo-Pacific *Pyrodinium* blooms, but also carried out on a first principles basis as well. The latter approach would facilitate more general extrapolation of the results to bloom dynamics of the inshore type which differs from open coastal blooms.

Seliger has hypothesized that *P. bahamense* var. *compressum* blooms may be spreading in the Indo-Pacific area by sequential colonization of eutrophicated, invasion sites, followed by emigration and dispersal to new sites. That is, increased nutrients along the involved coastlines permit increased growth rates and population accumulations to compensate for dilution losses and enhancing emigration over longer distances. Such coupling between physical oceanographic processes and nutrification in regulating *Pyrodinium* can be partly assessed in Fosforescente Bay. Core samples for cyst analyses can also be collected to put into focus variability in such bloom events over geological time. Such information is relevant to current discussions of the role of natural variation vs. anthropogenic stimulation in bloom events. An organic geochemical component evaluating mangrove exudate chemistry could also be accommodated and would be a valuable concurrent activity, given the provocative association between *P. bahamense* blooms and mangrove-lined ecosystems.

Summary

Pyrodinium bahamense blooms are a major tropical and sub-tropical neritic event and among the most prominent of dinoflagellate blooms. The pronounced toxicity causing significant human illness and death and regional spreading of this species in the Indo-Pacific region identify *P. bahamense* blooms as ones which should be given very high priority for, and require multi-disciplinary, collaborative and integrated study. Bahia Fosforescente and its dinoflagellate community dominated by *Pyrodinium bahamense* are particularly suited for such studies, including intercalibration efforts and technique development needed for use in the IOC/SCOR international program on harmful bloom studies.

STUDIES OF HARMFUL ALGAE IN AREAS INFLUENCED BY EUTROPHICATION

ΒY

L. EDLER, E. GRANÉLI, O. LINDAHL AND H. DAHLIN

In the Skagerrak and Kattegat sea areas several harmful phytoplankton events have taken place. It is generally accepted that many of the blooms have been triggered by eutrophication. For the study of the influence of eutrophication on harmful algal blooms this area together with the Adriatic Sea would be excellent as a cooperative study site. With the variety of phytoplankton species involved in the harmful events, the Skagerrak and Kattegat have the possibility to cover many aspects of bloom dynamics. The area is surrounded by three countries, Denmark, Sweden and Norway, all playing an active role in the study and management of harmful algal blooms.

Phytoplankton Species

The species causing problems of different kind and strength in the Skagerrak and Kattegat are widespread and common in many parts of the world. They have all been reported to cause trouble at several locations in temperate waters. They include Dinophysis spp., Gyrodinium aureolum, Ceratium spp., Alexandrium tamarense and Chrysochromulina polylepis.

Harmfulness

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Outbreaks of DSP, caused by *Dinophysis* spp., are annual phenomena along the Swedish and Norwegian Skagerrak coast. Nearly every year mussel farms are hit by DSP which necessitates closure of mussel harvesting in the autumn.

The annual blooms of *Gyrodinium aureolum* are transported as subsurface populations to the coasts of Norway and Sweden, where nearly every year they cause considerable loss of cultured fish.

Considerable blooms with cell densities of more than one million cells per liter of *Ceratium* spp. are annual events in early autumn. When sinking they contribute to the recurring oxygen depletion in the deeper layers of the stratified parts of the area.

PSP, caused by *Alexandrium tamarense*, occurs in the spring and may cause closure of mussel harvesting.

Chrysochromulina polylepis blooms develop every year during spring in the area, although the size of the bloom, the large distribution and the toxicity and effects were unusual in 1988. During the 1988 bloom all sorts of marine plants and animals were affected.

Significant features

Hydrography

Not only the coastal currents from the Baltic and the German Bight entering the Skagerrak and Kattegat area, influence the hydrography and hence the biological processes, but also water masses from the south and central North Sea. The nutrient rich currents may transport phytoplankton, developing into blooms. The currents transporting water of a quite different origin cause the establishment of very sharp haloclines, especially in the Kattegat. The pycnoclines and the fronts are important features of the hydrodynamics of the Skagerrak and Kattegat and are factors known to be of importance for bloom developments.

The dynamics of the Skagerrak has recently been thoroughly studied in a large scale ICES project "SKAGEX". Considerable amounts of data, both hydrographic and biological, are now emerging from this study. Among the interesting features found during SKAGEX was the widespread very active subsurface fluorescence peaks.

Eutrophication and nutrients

The hydrography of Skagerrak and Kattegat has been studied for many years and long time series of nutrients are available. The Swedish Environmental Protection Agency funded a large eutrophication project (see Ambio vol 19, no 3, 1990) which may serve as a background for new studies in the area. In Sweden the treatment of sewage is well developed. Sewage treatment plants along the coast have reduced phosphorus for many years and at present nitrogen reduction is developing. Reductions of nutrient effluent of rivers and diffuse sources are also underway.

Logistics

The Skagerrak and Kattegat area is surrounded by a number of marine institutes at universities and field stations. Several well equipped research vessels are available for cooperative studies and at the field stations there are good opportunities to carry out experimental work.

HARMFUL ALGAL BLOOM PILOT STUDY IN THE KASTELA BAY

ΒY

IVONA MARASOVIC Institute of Oceanography and Fisheries. Split Croatia

Kastela Bay is the largest semi-enclosed bay in the middle Adriatic and one of the most eutrophic bays in the Mediterranean Sea.

Common species

The causative species of red tide in the Kastela Bay is Lingulodinium polyedra. The first red tide outbreak was in 1980 and lasted for two weeks. Red tide events have been observed there regularly every summer for the last 11 years, however for the last six years they have lasted the whole summer. In 1980, 1985, 1987, 1989 and 1990 summer blooms in the Bay were followed by mortality of demersal fish and shellfish. From November to April L. polyedra motile cells are absent from the water column, but cysts of L. polyedra are present in the surface layer of the bottom sediment.

For the past few years during the L. polyedra blooms, we have found Alexandrium tamarense cells and cysts.

Harmfulness

L. polyedra is a suspect toxic organism, but its toxicity has never been established with certainty. We believe the mortalities of marine organisms in Kastela Bay were due to the very low oxygen levels in the water that occurred as the phytoplankton biomass decomposed.

A. tamarense is a PSP organism but in the Adriatic Sea did not cause toxic effects during the bloom (Emilia Romagna coast). In Kastela Bay A. tamarense is never present in large numbers.

Significant features

Results of our analyses show that *L. polyedra* blooms are due to a rapid and strong process of eutrophication, whereas temperature of the sea water is the bloom triggering mechanism. Even at the time when the Bay was not so heavily polluted, it was a highly productive basin. One small river discharges into the eastern part of the Bay and several submarine springs are located in the Bay. The eastern part of the Bay also receives large quantities of untreated municipal and industrial effluents. An agricultural area extends along the northern coast of the Bay, and the southern side is occupied by a pine forest, so that precipitation waters also carry appreciable quantities of nutrients.

Water circulation in the Bay is generated mostly by the local wind and exchange with the open sea is more intense during the winter than during the summer. It has been observed that the eastern part of the Bay is weakly coupled with the rest of the basin during the summer and it was also documented by the different composition of the phytoplankton community in that part of the Bay.

Logistical support

Kastela Bay has been a kind of experimental basin for the Institute of Oceanography and Fisheries owing to the position of the Institute in the Bay. Field research is performed by Institute's own research vessels (large - 30m and small - 10).

Background information

A long term increase in biological activity in the Bay has been noted from a primary production time series. Changes in abundance and diversity of species have shown the significant increase of eutrophication also. Blooms of L. polyedra have been studied for the past twelve years and we determined the factors which are most important for initiation, temporal variations and termination of blooms.

Oceanographic studies, which were started in the early 30's, have explained a number of processes occurring in the environment and in biota. However, there is still a lack of knowledge of physical and biological, and particularly of chemical processes.

Therefore, we propose Kastela Bay and this red tide bloom for a multi-investigator pilot study of IOC.

ATLANTIC COAST OF IBERIAN PENINSULA AS PILOT REGION FOR THE STUDY OF PSP AND DSP PROBLEMS RELATED WITH HARMFUL ALGAL BLOOMS

IBERIA

Instituto Español de Oceanografía Instituto Nacional de Investigação das Pescas Conselleria de Pesca. Xunta de Galicia.

General history of the problem in the region

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The Atlantic coast of the Iberian Peninsula offers an excellent opportunity for the simultaneous study of toxic algal blooms which can cause both PSP and DSP. The threat of both syndromes is annual in Portugal and Galicia, and an interdisciplinary programme aimed at increasing our knowledge of bloom dynamics in this region would contribute to worldwide knowledge of the phenomena involved. Portugal and Galicia lie at the northern limit of a typical eastern boundary current, and thus experience seasonal upwelling which blesses them with rich fisheries, and in Galicia with the largest mussel cultivation industry in the world. The impacts of harmful algal blooms therefore cause serious socioeconomic problems to the shellfish industry, to aquaculture, and to public health.

The toxic species which cause these problems in Portugal and Galicia are Gymnodinium catenatum and Alexandrium lusitanicum (both associated with PSP), and various species of Dinophysis. G.catenatum has occurred almost every year since 1985 -it may be an introduced species- and causes problems in Japan and Tasmania, as well as in Iberian waters. A.lusitanicum has caused problems in Italy, and the closely related A.minutum in Mediterranean waters (Greece and Cataluña coasts in Spain) and in Atlantic France (Brittany). This species also occurs in Tasmania.

Blooms of these species have been studied in Iberian coastal waters since 1985 and have provided valuable information on the timing of blooms, the distribution of the toxic species and the regularity of the outbreaks. Spatial variation of toxic outbreaks from year to year has been noticed and is always recorded through the monitoring programmes carried out by Spain and Portugal. In any case, DSP toxicity is almost a constant in the northwestern region between Nazaré and Cape Finisterre from July till November. The only question is if it will be only DSP or PSP plus DSP. All the problem species produce resting forms (to be confirmed in the case of *Dinophysis* spp) which can be the seed for outbreaks. Previous studies have revealed a higher abundance of cysts in the northwestern Iberian region.

Relevant environmental features

Despite the possible influence of anthropogenic factors on these coastal waters, the causative mechanisms of bloom development in the region seem to be closely related to natural environmental factors where the confluence of tropical and temperate waters as well as nutrient enrichment by coastal upwelling are of prime importance. There is evidence that in the open sea area some different ecosystems are well marked, and the

region between Cape Finisterra and Cape Roca is the one favorable for the yearly occurrence of toxic episodes.

The development of harmful phytoplankton species in the region has been related to the persistence, intensity and duration of the upwelling season. The influence of a poleward surface slope current off the western Iberian Peninsula and its interaction with coastal upwelling appear to be of basic importance in the development of these blooms.

Knowledge of the location of the initial populations from which autumnal blooms develop (planktonic forms in coastal waters, planktonic forms in warm slope waters and/or benthic resting cysts) as well as the physical causes of blooms (advection of warm surface slope water to the coast, coastal downwelling, the influences of the plumes of River Minho and River Douro) are still to be answered. It is also important to clarify several features like the position of the toxic species in the successional cycles of phytoplankton related with the variability of coastal upwelling; the sources and availability of nutrients and micronutrients and their link with toxicity; the relations between *in situ* toxic dinoflagellates and shellfish toxicity; the transfer of toxins (PSP and DSP) through the food chain and the possible genetic relations between the different populations; the mechanisms affecting bloom dispersion and disappearance.

Logistical support

An international effort and cooperative research program in this region has a great chance to be successful since it is very easily reached from several different institutions in Europe that have the necessary requirements including both small and large research vessels, sampling equipment, remote sensing capabilities, etc. There is baseline information on the problem species since considerable field work has been conducted in the area, that is affected simultaneously by PSP and DSP problems every year. The ongoing monitoring programmes in Portugal (INIP) and Galicia (autonomous government) will provide continuous information about the phytoplankton populations in the area. Both national institutions, INIP and IEO, have culture collections of dinoflagellates .

Rationale

In order to optimize the chances of increasing knowledge of the dynamics of harmful blooms and their effects, a joint multi-disciplinary programme focused in a region where simultaneous problems of DSP and PSP occur will maximize the results. The causative species in the region are of interest to many countries all over the world and there is sufficient knowledge on the species occurrences to schedule field exercises at specific places and specific times, with good probabilities of encountering a bloom, namely of *Dinophysis* (highest concentrations registered in Europe) that are not yet available in culture collections. Based on these considerations it is believed that the Atlantic West Coast of the Iberian Peninsula is an excellent site for a pilot program that will help to understand harmful algal blooms, contributing to a possible mitigation of their effects as well as to develop models of prediction.

ECOLOGICAL CLASSIFICATION OF HARMFUL ALGAL BLOOMS: THE LIMITATIONS FOR THEIR PREDICTION

Timothy WYATT

1. Classical phytoplankton studies were concerned mainly with that fraction of the phytoplankton larger than 20 um in size, the "net plankton" or *microplankton*. Very broadly, two subgroups of this community are recognized which correspond to the turbulence gradient. Well mixed waters are typically characterized by a diatom dominated community, stratified waters by dinoflagellates and cyanophytes. The seasonal trend from the first to the second in temperate waters is a manifestation of succession. The species which grow early during succession are relatively inefficient at nutrient uptake, hence more dependent on external nutrient sources, and turbulence is more important to them than molecular diffusion.

The time lag between production and consumption of microplankton in temperate waters is measured in weeks. In shallow seas, individual algal species may spend only a few weeks (two to six) growing in the photic zone, and the rest of the year in some state akin to diapause, often on the bottom. Seasonal variations in the abundance of microplankton biomass, whether of individual species or total microplankton, are often over three or four orders of magnitude, the community is far from a steady state, and the production for which it is responsible is "new".

2. The existence of *nanoplankton*, 2-20 um in size, has been known at least since the pioneering work of Lohmann about 100 years ago, but its ecological role has been a long standing source of confusion and dispute. Even smaller organisms belonging to the phytoplankton, 0.2-2 um in size, have been discovered very recently (Johnson and Sieburth, 1979), and are now usually called *picoplankton*, and together with the nanoplankton as *ultraplankton*. There is a "profound discontinuity" between microplankton and ultraplankton (Fogg, 1991) which goes a long way to explain the confusion and dispute just mentioned. The picoplankton is comprised chiefly of heterotrophic bacteria, photosynthetic procaryotes, and flagellates, and its taxonomic composition seems to be rather similar in all regions so far as we know.

This community is in dynamic equilibrium and is driven by trophic relationships rather than dependence on external nutrients and turbulence. The time lag between production and consumption is measured in hours, molecular diffusion is important, and biomass variations are small, of one order of magnitude (Yamaguchi and Shibata, 1982). As a first approximation, we can think of micro and ultraplankton communities as independent, while noting that the latter can be "short-circuited" (Sherr and Sherr, 1990; Flood et al, 1992). In the present context, the most important difference between them may lie in their distinct responses to nutrients and turbulence, and their very different turnover times.

3. We anticipate that a significant increase in nutrient input to the microplankton community will lead to an increase in stock, which may in turn, after some weeks, lead to increased secondary production. But if grazing rates fail to respond with sufficient rapidity, senescence, parasitism, or sedimentation may intervene. This decoupling of production and consumption can perhaps be recognized in some intermittently hypertrophic or dystrophic areas, and frequently leads to anoxia and mortalities. The elementary expectations of this scenario are diatom problems in mixed waters (e.g. "mare porco" in northern Adriatic) and dinoflagellate problems in stratified waters (e.g. exceptional *Ceratium* blooms in New York Bight).

4. Increased nutrient inputs to the ultraplankton however should actuate the feedback mechanisms and increase flux rates, rather than lead to accumulation of biomass (Fogg, 1991). On the basis of this simple argument then, we predict that most algal blooms problems will be due to microplankton species, which on present evidence is true. But if the feedbacks involved in maintaining ultraplankton stability can also be rendered temporarily ineffective, problem blooms can be expected here too. The causes of brown tides due to the picoplanktonic *Aureococcus* between Rhode Island and New Jersey since 1985 was attributed to just such a combination by Sieburth (1989), when lawn fertilizers may have increased nutrient inputs, and pesticides may have eliminated grazers. The nanoplanktonic *Chrysochromulina* event in Scandinavian waters in 1988 was similarly attributed to the combination of nutrient changes (silica exhaustion) and grazer inhibition, through toxic secretion in this instance (Maestrini and Granéli, 1991).

5. At some scales, the importance of turbulence is relatively clear. It is a parameter in the classical model of Sverdrup (1953), and distinguishes the two broad divisions of microplankton (Margalef, 1979). Since the ultraplankton is found in almost all environments, and in any case lives at very low Reynolds numbers, we can assume that it is relatively indifferent to changes in turbulent patterns. At finer scales, changes in turbulence can alter the form of functional responses (e.g. Marrasé et al, 1990; Rothschild, 1991), and hence the time courses of production and consumption. Thus altered patterns of turbulent decay might bring about changes analogous to those caused by pesticides or toxins, but this is very speculative. They might also select for species able to alter turbulent patterns in their own microzones, for example to regulate grazing parameters (Jenkinson and Wyatt, in prep.).

HARMFUL DINOFLAGELLATES IN THE BAYS OF THE EBRE DELTA

ΒY

M. Delgado, J. Camp and M. Estrada Institut de Ciències del Mar, P. Nacional, s/n 08039 Barcelona - SPAIN

The Ebre delta bays are two semi-confined masses of sea water separated from the open Mediterranean by sandy bars (Fig. 1). Alfacs bay, in the south, with a surface of 49 km² and an average depth of 3.13 m, is the largest and most important from the point of view of aquaculture. Between April and October, the northern edge of the bay receives freshwater inputs, via several channels and lagoons, of about $275 \times 10^6 \text{ m}^3$ per year. Due to the needs of rice cultivation, these inputs are very reduced during the rest of the year. This freshwater brings considerable quantities of inorganic nutrients and organic matter into the bay.

The hydrographical structure of the bay presents generally a salinity dominated stratification, with a superficial water layer (0 to 2-3 m depth) of low salinity (30-35) flowing outwards and a deep, salty (36-38) layer flowing into the bay. Total mixing of both layers is rare and occurs in association with strong northwesterly winds. Stratification sets in again within 48 hours after the wind drops. This causes an export of the mixed water and an entrance of sea water from outside the bay. In the absence of strong winds, the estuarine circulation produces a renovation of the bay waters in about 15 days. Additional exchange mechanisms between the bay and the open sea include episodes of dilution by freshwater with a periodicity of about 10 days.

Chlorophyll concentration in the Alfacs ranges generally between 0.5 and 5 mg/m³, but during intense blooms can reach up to 50 mg/m³. Phytoplankton abundance is very variable, both spatially and temporally, and seasonal patterns are not very marked. Typically, diatom blooms occur in spring and autumn, as in the neighbouring open waters, but they can appear also at other times, following strong mixing. Small flagellates and dinoflagellates tend to be very abundant during a large part of the year and present frequent population maxima.

The first documented occurrence of a toxic planktonic organism in the Ebre delta bays took place in May 1989. Following a red tide in the harbour of Sant Carles de la Ràpita (located within Alfacs bay), PSP was detected in mussels from raft cultures in the bay. The responsible organism was identified as *Alexandrium minutum*. Meteorological and hydrographical conditions during the red tide were typical of similar events elsewhere. Mixing by strong winds at the end of April caused nutrient enrichment. The following period of calm weather favoured the accumulation of dinoflagellates, which were eventually dispersed by strong winds at the end of May.

Since its first detection in 1989, A. minutum has been found at concentrations of 0.1-7 cells/ml in Alfacs bay during the winters of 1990, 1991 and 1992, apart from sporadic presence on other dates. The 1991 and 1992 appearances were accompanied by positive PSP tests, although the toxin level was always below the limits allowed for human consumption. The analysis of the hydrographical parameters and phytoplankton community composition

associated with the A. minutum occurrences is under way. Further studies will address the possible presence and role of cysts in the sediments.

Several species of *Dinophysis* have been frequently recorded in the Ebre delta bays and neighbouring marine areas. In summer 1991, relatively high concentrations (up to 10000 cells/l) of *D. acuminata* were detected in both bays. However, no okadaic acid was detected by HPLC, and no DSP toxicity was found with the usual mouse test. *D. acuminata* was first recorded in appreciable numbers in March, and its abundance increased until July, in parallel with the total number of dinoflagellates.

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STUDY GROUP ON THE DYNAMICS OF HARMFUL ALGAL BLOOMS

BY

G.C. CADÉE & R. RIEGMAN Netherlands Institute for Sea Research P.O. Box 59 1791 AB Den Burg (Texel) THE NETHERLANDS

Suggestions:

The traditional approach in studying harmful algal blooms is the performing of extensive field research programmes in order to identify those environmental conditions that specifically are responsible for the triggering of these harmful blooms. Apart from logistic reasons (initial conditions are difficult to trace because when the bloom is just recognised it is already there) one might evaluate whether it is likely that only one specific set of conditions may be responsible for all types of harmful blooms. The logistic problems may largely be solved when a study area is choosen in which harmful blooms show a predictable cycle like for example the blooming of <u>Phaeocystis</u> in Dutch coastal waters.

The urgency to study harmful blooms comes from their evident increase in frequency and intensity. Furthermore, there is an increasing number of reports in which a close link is suspected between these blooms and human activities. This point of view is interesting since anthropogenically induced harmful blooms have the potention to be reduced when proper measures are taken. This is in contrast with harmful blooms that are triggered by natural causes, i.e. intrinsic to the particular unaffected ecosystem.

One suggestion might be to focus on those mechanisms that are expected to be involved when anthropogenic disturbances induce harmful blooms.

Disturbances

Possible mechanisms involved

1. Fishery activities. - Enhanced nutrient exchange from sediments by turbation.

- Increased nutrient load from fish farms.

- Shift in inorganic/organic nutrient ratios due to fish farming.

- Stimulated grazing pressure on phytoplankton leading to poorly edible (toxic?) species. (fisheries leads to higher mesozooplankton biomass?).

2. Eutrophication. - Higher nutrient load gives higher algal biomass, gives oxygen depletion under certain conditions.

- Higher algal biomass lead to higher specific grazing pressure: potential shift towards poorly edible species.

- Shifts in nutrient ratios affect type of controlling nutrient, new, possibly toxic algal species may appear. Principle: Ability to compete for the growth rate limiting nutrient is species dependent.

3. Micropollutants. - Direct impact on algal growth potential at natural contaminant concentrations hardly known.
- Potential possibility of inhibition of zooplankton activity and/or biomass: reduced grazing pressure inducing higher algal biomass.

It would be useful if a detailed list of potential mechanisms were constructed. Research should be designed in such a way that the mechanisms involved could be quantified. This allows the construction of ecosystem models that are essential for the evaluation of the possible effects of water quality measures.

At the NIOZ a choice was made to use three different approaches:

1.- The monitoring and detailed research (process studies) of the yearly occurring <u>Phaeocystis</u> blooms.

2.- The performance of laboratory culture experiments to quantify possible mechanisms involved like competition under different nutrient limitations. Also 800 liter mesocosms are intended to be used to study the impact of eutrophication on the structure and functioning of the pelagic food web.

3.- Ecosystem models are constructed and used to evaluate the impact of eutrophication on coastal areas of the North Sea. Data from experimental and field research are used to improve these models.

STUDIES OF HARMFUL ALGAE IN THE BAY OF FUNDY

ΒY

JENNIFER MARTIN

The Bay of Fundy is located on Canada's Atlantic coast -indicated by Figure 1. Water flows into the Bay of Fundy from the Gulf of Maine eddy (located south of the Bay to the east of Maine, U.S.A. and southwest Nova Scotia). The waters in this system are dynamic with tides ranging from 8 m at the mouth and 16 m at the head.

Paralytic shellfish poisoning has a long history in the Bay of Fundy, with shellfish harvesting areas being closed annually (generally during summer months). The primary causative species is <u>Alexandrium fundyense</u> with <u>A. tamarense</u> observed to a lesser degree. Although PSP accumulation in shellfish is the more common event, fish kills from PSP have also occurred -in the late 1970's- when hundreds of tonnes of herring died after accumulating toxins through the food chain.

More recently, in 1988, domoic acid was detected in shellfish from the Bay of Fundy. <u>Nitzschia pseudodelicatissima</u> was determined to be the primary source of the toxin.

Other organisms that have been observed annually and been associated with problems in other areas include: <u>Dinophysis</u> spp., <u>Ceratium</u> spp., <u>Gyrodinium aureolum</u> and <u>Chaetoceros</u> sp. Although we have no confirmed cases of diarrhetic shellfish poisoning from the Bay of Fundy, DSP toxins were detected at a mussel farm in Nova Scotia during 1991.

The bay of Fundy has a long history of research on <u>A. fundyense</u> and PSP. Studies have included the overwintering cyst populations as well as the resulting summer excysted horizontal and vertical distributions. Results indicate the currents and water movements play a key role in dispersing cells from cyst seed bed areas.

A relatively new and rapidly expanding industry in the region is salmonid aquaculture. There have been considerable concerns as to what effect the industry might have on the environment. A study was initiated in 1987 to study phytoplankton populations and other physical/chemical data (including nutrients). From this work we are establishing baseline data and trends and patterns for the phytoplankton populations of the area. ì



RED TIDES IN GREEK WATERS

ΒY

DR. GOTSIS-SKRETAS National Centre for Marine Research Hellenikon, Athens - GREECE

Eutrophication and the consequent undesirable phytoplankton blooms have emerged in a number of Greek gulfs, which receive urban and industrial wastes and effluents from rivers and agricultural activities. The most polluted gulfs in Greece are Saronikos and Thermaikos Gulf, situated in the Central and Northern Aegean Sea respectively. Several appearances of red tides which result from excessive production of phytoplankton organisms, most commonly dinoflagellates, but not always, have been recorded in Greek waters.

In August 1978, a fish-killing, red brown phytoplankton bloom which occurred in the Gulf of Saronikos was due to <u>Gymnodinium breve</u> with concentrations of 10^7 cell/l (Satsmadjis and Friligos, 1983). The presence of this species in this region has been observed since 1972. From 1978 since 1988, <u>G. breve</u> has appeared constantly in Saronikos Gulf, fluctuating seasonally, with its blooms during the stratification period, over a temperature range of 22.0 - 27.0 °C (Gotsis-Skretas, 1985; Pagou, 1990). Other species which were responsible for red tides in various parts of Saronikos Gulf are: <u>Scrippsiella trochoidea</u> (3x10⁷ cell/l; August 1987), <u>Pyraminomonas sp.</u> (6.5x10⁸ cell/l; June 1990), <u>Gymnodinium catenatum</u> like species (2.9x10⁷; September 1987), <u>Prorocentrum dentatum</u> (1.1.5x10⁸ cell/l; July 1991) and <u>Noctiluca scintillans</u> (1.8x10⁶; April 1985) (Gotsis-Skretas, unpublished).

In July 1987, a red tide phenomenon was recorded (Friligos and Gotsis-Skretas, 1989), when a red-brown patch occurred off the harbour of Volos in Pagassitikos Gulf (Central Aegean Sea). The most abundant species were a <u>Gymnodinium catenatum</u> like species $(1.1 \times 10^7 \text{ cell/l})$ and <u>Cachonina niei</u>. In August 1986, the brown-red colour in Kavala Gulf (North Aegean Sea) came from the proliferation of <u>Gonyaulax sp.</u> which reached a concentration of $1.2 \times 10^7 \text{ cell/l}$ near the harbour. A reduction was also noticed with the distance from the coast and depth (Panayiotidis et al., 1987).

In addition, the phenomenon of dense, amorphous, gelatinous masses, similar to those which often appear in the N. Adriatic during summer, was quite often recorded in several Greek gulfs during the hot months at Thermaikos (1982 until now, almost every summer), and in gulfs of Central Greece such as Euboikos (1972, 1983), Saronikos (1985), Korinthiakos (1988, 1990) and Pagassitikos Gulf (1982, 1983). In most cases, this phenomenon was of a limited character, except in June and July 1983, where the gelatinous mass covered a big area along the east coasts of Central Greece.

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REPORT OF DE L. BONI Universitá di Bologna ITALIA

DSP events in Italy occurred in 1989 for the first time. Only blue mussels, not oysters, clams, etc. were toxic. DSP was detected in the Gulf of Trieste and off the coast of Emilia-Romagna and Marche. Mussels from artificial and natural banks were examined. No *Dinophysis* bloom was detected and the maximum cell number was 3000/1, but toxicity was found also with lower cell numbers. In Emilia-Romagna and Marche water and mussels samples were collected at the surface, middle and bottom; in the Gulf of Trieste only at the surface. Toxicity analyses had been carried out according to Italian law (Yasumoto's fluorescence HPLC method modified by IFREMER), but in Trieste tests were also carried out by the immunological ELISA technique. The involved species were *D. fortii*, *D. sacculus*, *D. tripos*, *D. acuta*, *D. acuminata* and some others. In 1991 we had no DSP even though the same species were present (but *Dinophysis spp*. had been always present in the Adriatic sea without DSP).

ANNEX V

4

PARTICIPANTS OF EACH SUB-GROUP

1. GROWTH

- D.M. Anderson
- I. Bravo
- L. Edler
- M. Levasseur
- I. Marasovic
- B. Reguera
- M.A. Sampayo
- T. Smayda (chairperson)

2. GRAZING

- J. Blanco
- A. Cembella (chairperson)
- E. Cunha
- D. Cushing
- H. Enevoldsen
- E. Granéli
- E. Macdonald

3. PHYSICAL/BIOLOGICAL INTERACTION

- J.M. Cabanas
- G.C. Cadée
- A. Cruzado
- H. Dahlin
- M. Estrada
- S. Fraga
- P. Gentien
- O. Lindahl
- T. Moita
- T. Osborn
- J. Smith
- Z. Velásquez
- T. Wyatt (chairperson)

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ANNEX VI

POPULATION DYNAMICS OF PSP-PRODUCING DINOFLAGELLATES IN A BUOYANT COASTAL CURRENT: A PILOT STUDY IN THE GULF OF MAINE.

I. Geographic Area

The objective of this pilot study is to understand and model the population dynamics of blooms of paralytic shellfish poison (PSP)-producing Alexandrium species in a buoyant coastal current. The southwestern Gulf of Maine has been chosen as the project location since it is a hydrographic and ecological system that has direct relevance to many coastal areas with similar currents and toxicity patterns in other parts of the world. The proposed studies address fundamental mechanisms underlying bloom dynamics of species for which blooms are highly seasonal and for which toxin propagation is associated with long-distance or alongshore transport in discrete water masses. This type of system is one of several that should be investigated in a series of coordinated, multidisciplinary pilot studies, with the overall goal of resolving the different mechanisms controlling harmful algal blooms throughout the world.

II. Species

The organisms responsible for PSP in the Gulf of Maine are dinoflagellates of the genus Alexandrium. These species are responsible for blooms in many other parts of the world where PSP is common. The general geographic distribution of the toxic Alexandrium species is now reasonably well-defined, but a quantitative understanding of bloom dynamics in any of these locations remains a distant goal. An important characteristic of these blooms is that they are highly seasonal, due to the presence of a dormant cyst stage in the organism's life history (Dale, 1977; Anderson and Wall, 1978). These resting cysts permit Alexandrium species to survive through periods of stress, with germination providing the inoculum cells for subsequent blooms. A Gulf of Maine pilot study thus provides an opportunity to document in detail the growth and development of a population that originates from a small number of cells during a discrete and predictable interval of time. It also is an excellent location to examine the quantitative contribution of encystment and excystment in dinoflagellate bloom dynamics, an area where little is known at present. The pilot study will focus on Alexandrium species, but it should be noted that other potentially harmful Dinophysis species (DSP producers) that co-occur in these waters can be investigated with little additional effort.

III. Oceanographic Regime

Recent research suggests that in the southwestern Gulf of Maine, blooms of *Alexandrium* are closely linked to the development and movement of a buoyant coastal current (Franks and Anderson, 1992 a). The current originates with the outflows of several rivers that enter the Gulf and travel alongshore in a coastally-trapped buoyant plume (Figure 1). The coastal current is seasonal and thus has a major influence on the timing of regional *Alexandrium* blooms and the resulting toxicity (Franks and Anderson, 1992 b). It varies with the magnitude and timing of both snowmelt and rainfall and is influenced in a dynamic, though predictable manner by local wind stress. The area

affected by the current is large, encompassing both the alongshore zone of the southwestern Gulf and the rich fisheries resources of Georges Bank that lie several hundred kilometers "downstream". The ecosystem to be studied thus incorporates a variety of important hydrographic features common to many coastal areas subject to recurrent blooms of harmful species. These include plume and coastal fronts, long-distance transport, and stratified buoyancy-driven alongshore flow that is affected by wind-driven upwelling and downwelling.

This is <u>not</u> a bloom phenomenon presently affected in any obvious way by eutrophication. However, southern waters of the Gulf of Maine are soon to be the site of massive "experiment" in the possible nutrient enhancement of algal blooms. In 1996 the first phase of a sewage effluent diversion will take place in Massachusetts Bay, releasing up to 1 billion gallons per day of primary treated effluent into the path of the coastal current that carries *Alexandrium* populations from the north. By 1999, the effluent will receive full secondary treatment. In the past, effluent has been released directly into Boston Harbor, from where it gradually enters Masssachusetts Bay. The proposed pilot study thus offers an excellent opportunity to evaluate the effects of altered nutrient loading on harmful bloom species, especially *Alexandrium*.

A very important point with respect to the planning and execution of major field programs targeting specific blooms is that toxiciy has occured along the coast of the Gulf of Maine <u>every year</u> since 1972, when PSP first spread into the region. Experience suggests that a bloom will be present between late May and mid-June in the waters between southern Maine and Massachusetts, and thus that a field program can be planned with a high probability of success. There is considerable inter-annual variability in the magnitude of the blooms, however, so a multi-year study is needed if the mechanisms underlying this variability are to be understood.

IV. The Problem

1. Paralytic Shellfish Poisoning

Paralytic shellfish toxins are among the most potent biotoxins known (Shimizu, 1987)). PSP toxins, which are accumulated primarily by filter-feeding bivalves and other organisms, have serious consequences for the exploitation of fisheries resources, aquaculture and human health around the world. In the Gulf of Maine, annual blooms of *Alexandrium* species necessitate closures of vast stretches of productive shellfish beds, recently encompassing offshore as well as inshore resources. The situation is much the same in over a dozen other countries scattered throughout the world. Despite surveillance programs in many of these countries, occasional incidents of PSP still occur. Through food web transfer events, these toxic blooms have also caused mortalities of fish and even marine mammals. Larval and juvenile stages of fish can also be affected. The total impact of *Alexandrium* blooms on the biota of the Gulf of Maine or other areas is unknown, but the repercussions of these blooms are substantial and increasing. This is especially apparent in the Gulf of Maine considering the apparent spread of the blooms hundreds of miles offshore, resulting for the first time in high levels of the toxins in animals on Georges Bank, one of the richest fishing grounds in the world.

At present, PSP problems in the Gulf of Maine and elsewhere are managed by shellfish monitoring programs. This approach in large measure protects the public health, but adds little to the development or refinement of resource management strategies, nor does it address the approaches that must be pursued to restrict the further expansion of the toxic species. All efforts to reduce the impacts of harmful blooms through effluent restrictions, harvesting quarantines, or bloom reduction (through human intervention) require an understanding of bloom dynamics.

On a global scale, the Gulf of Maine provides a model ecosystem to potentially resolve questions regarding the long-term effects of environmental changes, such as effluent additions or a rise in sea surface temperature, on the frequency and duration of toxic algal blooms and shifts in the species composition of phytoplankton communities. A dynamic model, based up on reliable empirical information aquired in the course of this proposed bloom study, could serve to address these issues.

2. Present Understanding and Gaps in our Knowledge

We presently have a reasonable understanding of the characteristics of the coastal current in the Gulf of Maine and its association with *Alexandrium* cells (Franks and Anderson, 1991a). The *Alexandrium* cyst distribution has also been mapped in the region (Anderson and Keafer, 1985). The sea surface temperature signature of the water mass has been established, and capabilities have been developed to monitor it on a near real-time basis through remote sensing (Keafer and Anderson, in press). There is excellent meterological coverage for the region, as is also true for PSP monitoring in shellfish alongshore. A reasonable number of laboratory ecophysiology, toxinology, and chemotaxonomy studies have been completed on North American *Alexandrium* species (e.g. Anderson et al, 1984; Boyer et al, 1987; Cembella et al, 1987; Cembella and Taylor, 1986; Anderson et al, 1990 a, b). Finally, 3-dimensional numerical models are either under development or are operational to describe the circulation of the southwestern Gulf of Maine (Lynch et al., 1992).

There are many gaps in our knowledge. First and foremost is that we have no understanding of the details of *Alexandrium* bloom dynamics in the Gulf of Maine or elsewhere. We have data on *Alexandrium* biomass levels during blooms, but no knowledge of the underlying rates of growth or grazing, or of the advective losses and inputs that generate the observed cell concentrations. Suprisingly, there are no nutrient measurements that coincide with documented blooms. We do not understand the nature of bloom initiation -- whether it is truly localized near the river mouths, and whether it is driven by a pulse of excysted cells or as a continuous supply of cells supplied by *in situ* growth of a localized population. Most importantly, perhaps, we have no knowledge of the reason the cells are localized within the buoyant water mass. They could be growing faster there due to a unique chemical environment associated with the freshwater runoff, or they could be localized within the plume through a combination of swimming behavior and physical circulation. A quantitative relationship has yet to be determined between *Alexandrium* growth rate and ambient nutrient concentrations or cell quotas.

Overall, the level of knowledge of *Alexandrium* ecology, physiology, and toxinology is not sufficient at present to permit formulation of a working model of the

population dynamics of this organism in a coastal current. However, it is adequate for the design of an efficient, targeted field and laboratory research program.

3. Understanding to be Developed

The objective of this pilot study is to understand and model the population dynamics of blooms of PSP-producing *Alexandrium* species in a buoyant coastal current. A model of population dynamics is essential in order to determine the relative importance of growth and the potential loss factors (e.g. grazing, sinking, lateral advection, encystment) during bloom development. The model will allow for a general understanding of the importance of physical processes (dilution, mixing, cross frontal exchange) on the initiation, development and decline of harmful blooms, not only in the Gulf of Maine, but in other systems where populations are contained within, and travel with, discrete water masses.

The pilot project will also increase our understanding of the physiology of *Alexandrium* and related species. The laboratory efforts to obtain the specific nutrient, light, and temperature requirements of *Alexandrium* will fill important gaps in our knowledge of how these species respond to the external environment. Important field measurements will include the *in situ* growth rate of the species of interest - a measurement of critical importance to the bloom dymanics model, but which has never been measured before in natural blooms. Species-specific grazing losses will also be determined. These are all key issues with respect to the spatial and temporal distribution and abundance of *Alexandrium* species around the world, yet they have poorly characterized and never included in a population dynamics model.

Determination of the factors responsible for the natural variability of the rate processes used in the model (e.g. relationship between growth rate and temperature or light) will make the model useful for a variety of environments, both real and hypothetical. Thus it should be possible to examine the possible effects of effluent discharge strategies or other human alterations in the nutrient environment.

V. Proposed study.

1. Approach

The experimental design of pilot study will be formulated with the ultimate objective of producing a dynamic model of bloom propagation in the Gulf of Maine, which may be more generally applicable. The change in toxic cell numbers (ΔN) integrated over time (Δt) in a given defined ecosystem can be expressed as:

$$\Delta N/\Delta t = A-L$$

where A = the species-specific accumulation of cells and L = loss of cells from the system. The components of A that must be determined include a) *in situ* growth and b) introduction of cells through physical/biological processes, such as horizontal and vertical advection, excystment, or migration. The loss term L includes grazing, cell death and encystment, and dispersion through physical processes, including sinking, advection, and small-scale diffusion through the system boundaries. Provided that appropriate techniques

for the measurement of a key factor, μ , the *in situ* growth rate can be applied, the relative importance of each of the L components in determining the standing stock of the toxic species can be evaluated.

One of the major focal points of the proposal study, and one that makes this program unique with respect to past work, is the effort to develop and use methods for estimating the *in situ* growth rate of *Alexandrium* populations. The rationale for this enphasis comes from several directions. First, given the necessity to develop a working understanding and model of the bloom dynamics of the major toxin-producing algae, it is evident that the major population accumulation and loss terms and their range of variability must be known with confidence. Another motivation is that biomass estimates alone give no information about the rate of transfer of toxin into the food chain from the dinoflagellate. We need to know the rate of production of that toxin, which requres knowledge of growth rates. In an analogous fashion, one cannot estimate carbon fluxes without rate measurements - hence the need to measure rates of primary production in other phytoplankton studies.

As fundamental as the *in situ* growth rate is in this context, there have never been any attempts to estimate it in *Alexandrium* blooms. The reason is autecological in nature in that the species of interest in typically a minor fraction of the total plankton biomass. Bulk carbon or nutrient uptake rates or changes in biomass of the whole community are of little value when our concern is one species. Fortunately, methods for measuring speciesspecific *in situ* growth rates are being developed (discussed biefly elsewhere in this report). These will be refined in laboratory and field studies and applied to *Alexandrium* populations in the Gulf of Maine.

The manner in which these measurements will be directed needs some elaboration as well. It would be naive to assume that *Alexandrium* growth rates are constant over both time and space during the bloom. We believe there is an "initiation site" near the Androscoggen and Kennebec rivers that must be carefully characterized to provide the boundary conditions for the model. *In situ* growth rates are needed in that zone to demonstrate whetheir the input of cells is in the form of an initial pulse (presumably from cyst germination) or is a continuous supply to the coastal current, supported by a growing population. Note that this also addresses the important issue of how an initiation site can continue to supply inoculum cells (that travel south and out of the system, never to return) year after year without replenishment of the cyst "seedbed". A localized population in the initiation zone where the rivers enter the Gulf Could complete an entire bloom cycle and re-deposit cysts if *in situ* growth balances losses of cells entrained into the coastal current.

In addition to *in situ* growth rate measurements at the initiation site, two other areas must be scrutinized carefully. Since *Alexandrium* and many other dinoflagellates can migrate vertically in order to maintain optimal light and nutrient conditions, one might hyhpothesize that the growth rate within a water mass will be relatively constant. That rate must be determined, and compared to similar measurements at the boundary of the system (i.e. in the water mass adjacent to the coastal current). This would provide data to test the hypothesis that the reason *Alexandrium* cells are most abundant within the coastal current is that the chemical conditions are better for growth than is the case in the adjacent non-plume waters. If growth rates are the same on each side of the alongshore front formed on the east flank of the coastal current, we must then seek other explanations (probably physical and behavioral in nature) for the localization and persistence of cells within the buoyant water mass that comprises the coastal current.

2. Research Plan.

A 5 year study is envisioned that will include both laboratory and field investigations and that will have sufficient detail to accomodate interannual variability. The initial assumption is that this will be a regional exercise with participation of US and Canadian scientists, with some limited help from other countries. However, if the decision is made to implement a series of successive multi-national pilot studies in various sites throughout the world, the field effort described below can be shortened by several years, with much more information to be gathered during the cruises due to more active foreign participation.

The field studies will involve extended, 5-7 day cruises on 30-40 m vessels as well as shorter cruises on very fast, small vessesls (10 m) that can collect samples and data rapidly over a large geographic area. This approach has been used successfully in this region in the past (Franks and Anderson 1992 a). The general plan is for field work in years 1 and 3 to focus on defining population distributions, nutrient fields, *in situ* growth rates, and general circulation characteristics. Laboratory efforts prior to those cruises will develop methods for *in situ* growth rate determinations, quantify the relationship between ambient nutrient concentrations and growth rate, determine growth rate as a function of irradiance and temperature, and begin development of a population dynamics model that can be incorporated into existing numerical models of nearshore Gulf circulation.

Alternate years (2 and 4) will involve field measurements that focus on specific physiological, behavioral, or physical processes within the bloom, driven by the need to define parameters in the population dynamics model. Year 5 is planned for final data analysis and publication.

The general research plan can be briefly outlined as follows. Years 1+3

Laboratory studies

- In situ growth rate methods development

- Nutrient/growth kinetics
- Growth as a function of irradiance and temperature
- Model development

Field Studies

A. <u>Bloom Initiation Phase</u> (April)

1) Fast, regional mapping of cell distributions and hydrography (2-3 day cruises, small vessel). Define initial conditions.

- Measurements:

physical: CTD, transmittance. *chemical*: nutrients. *biological*: cell counts.

- 2) Characterize initiation site (2-3 days, larger vessel)
 - Measurements:

physical: CTD, transmittance, irradiance. *chemical*: nutrients, toxicity. *biological*: cell counts, chlorophyll <u>a</u>, CHN, *in situ* fluorescence, grazing rates.

B. Bloom Development Phase (May)

- 1) Re-characterize initiation site; detailed examination of developing bloom "downstrean" (large vessel, 5-7 days)
 - Measurements:
 - physics, chemistry.
 - cell concentrations, *in situ* growth rate measurements at different sites (initiation site, inside/outside plume)
 - nutrient pertubation experiments
 - grazing experiments.

C. Bloom Decline or Washout Phase (June)

1) Population characterization along bloom path (initiation site to offshore, Georges Bank). Fewer stations, more geographic coverage. (Large vessel, 5-7 days).

- Measurements:

- physics, chemistry.
- cell concentrations, in situ growth rate measurements.
- nutrient pertubation experiments.
- grazing experiments.

Years 2+4

Laboratory Studies

- refine methods
- continue model development
- reduce and analyze data

Field Studies (7 days, large vessel)

Drogue to follow population.

- vertical migration
- in situ growth rate
- grazing rates
- Acoustic Doppler Profiler (ADP) and detailed cell distributions at interfaces.
- Georges Bank

3. Resources Needed

It is difficult to estimate the logistical details of this project without some indication of the scale of the funding or of the degree of collaboration between countries. This is especially true with respect to personnel and vessel costs, since some investigators will require funds for salary while others will not; likewise some vessels must be hired wheras others are free to their investigators. Nevertheless, the following very rough estimates are provided assuming mainly US and Canadian participation.

a) Vessels

Two vessels are required. One is a very fast, small boat (10 m) that will be used for rapid, large-scale mapping (as described in Franks and Anderson (1991 a). This would be used 10-12 days in each of years 1 and 3, costing about \$1000 per day.

A second, larger vessel would be needed for the more detailed mapping and experimentation on longer 5-7 day cruises. A likely choice is the RV Argo. Maine a 30 m vessel which costs about \$2500 per day. This vessel would be used approximately 17 days in each of Years 1 and 3, and 7 days in each of years 2 and 4.

b) Personnel

This is exceedingly hard to estimate. At the technical level, this project could support 2 graduate students who could do much of the work for their graduate theses, but there is still a clear need for at least one full time technician. Principal Investigator time should be 6 months or more per year for a biologist (assuming collaboration with others who require no salary support) and a 3-4 months per year for a physical oceanographer. This is an absolute minimum projection, as more investigators would do much to insure the measurements are made at the level of detail neded for adequate model formulation and verification.

c) Instruments

The project would use standard oceanographic instruments readily available to most investigators, including CTD's with transmissometer and <u>in situ</u> fluorometer, a hose pumping system and water bottles for sample collection, light meters, plankton nets, deck incubators, etc. Two items should be purchased specifically for the project. One is a nutrient Autoanalyzer (\$30,000) which could be dedicated to the project. (alternatively this amount of money could be budgeted to pay for a subcontract to have analyses performed by another laboratory). Another useful purchese would be of ARGOS drifters (\$2500 each, 3 or more meeded) which would be released and tracked by satellite on a daily basis to reveal the behavior of the coastal current.

Another capability fundamental to the success of this project is remote sensing of sea surface temperature on a near-real time basis. This is needed to guide cruise activities so that important hydrographic features are sampled, but also to allow interpolation of the behavior of the coastal current and its associated *Alexandrium* populations between cruises. A NOAA coastwatch node has recently been established in the Woods Hole laboratory of D.M. Anderson that will permit two sea surface temperature images to be

downloaded each day and analyzed at no cost to the project other than personnel time (Keafer and Anderson, in press).

Finally, it is evident that an extraordinary number of samples will need to be processed for cell counts. Ideally, these should be completed rapidly so the data can be used to design subsequent experiments and cruise transects. Manual cell counting is an obvious constraint in this respect, so methods are needed to automate the counts. Some promising techniques are under development that could accelerate cell counting considerably, including cell surface monoclonal antibodies that are specific for <u>Alexandrium</u> species (Sako et al, in press).

4. Time duration

(See attached time line).



Figure 1. The Gulf of Maine region, with arrows showing the path of the buoyant coastal current (from Franks and Anderson, 1992 a)

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POPULATION DYNAMICS OF PSP-PRODUCING DINOFLAGELLATES IN A BUOYANT COASTAL CURRENT: A PILOT STUDY IN THE GULF OF MAINE

ACTIVITY	Year 1	Year 2	Year 3	Year 4	Year 5
	JFMAMJJASOND	JFMAMJJASOND	JFMAMJJASOND	JEMAMJJASOND	JFMAMJJASOND
Laboratory studies	XXXX XXXXX	XXXX XXXXX	х		
Field Sampling and experiments.	ХХХ	ХХ	XXX	XX	
Analysis	*****	XXXXXXXXX	XXXXXXXXX	XXXXXXXXX	
Data Reduction	XXXX	XXXX	XXXX	XXXX	
Writing			XXXXXXXXXXXXX	*****	XXXXXXXXXXXXX
Costs: Vessels Personnel Supplies, trave	60,00 200,0 el,etc. 15,00	0 25,00 00 200,00 0 15,00	0 65,0 0 220,0 0 20,0	00 25,0 00 220,0 00 20,0	00 00 75,000 00
	~\$300,00	0 ~\$300,00	0 ~\$300,0	00 ~\$300	,000 \$75,000

BLOOM DYNAMICS IN THE KATTEGAT /SKAGERRAK AREA

INTRODUCTION

During the last decades an increasing number of large scale phytoplankton blooms of both toxic and non-toxic species have been observed in the Kattegat/Skagerrak area. Before these outbreaks of harmful algal blooms, toxic mussels were almost unknown but became a serious problem during the eighties. A major project run during the first part of the eighties identified eutrophication as an important contributer to the increase in phytoplankton bloom events (Granéli et al. 1989 a). The distribution pattern of the blooms suggests that the features of the hydrodynamic processes in the frontal systems in the Kattegat/Skagerrak area (strong stratification, advection and entrainment) in combination with an increased anthropogenic load may be favouring the formation of large scale and harmful phytoplankton blooms. (Lindahl 1992). The anthropogenic load includes changes in land run off, such as increased metal and humic acid concentrations due to acidification.

The objective and reason for the studies proposed here is the need for society to understand phytoplankton bloom dynamics in order to implement countermeasures, so that negative effects are minimized. An increased understanding of the processes leading to phytoplankton blooms in general and harmful phytoplankton blooms in particular will also help to predict outbreaks.

SPECIES

The harmful phytoplankton events in the area include anoxia, caused mainly by Ceratium spp., DSP-toxicity, caused by Dinophysis spp. (Dahl and Yndestad 1985, Krogh et al. 1985), fish kills, caused by Gyrodinium aureolum (Tangen 1977, Dahl et al. 1982) and the naked form of Dichtyocha speculum (=? flagellate X = ? Heterosigma akashiwo), and mortality of a large number of marine organisms, caused by Chrysochromulina polylepis (Lindahl and Dahl 1990, Maestrini and Granéli 1991), and Prymnesium parvum (Granéli et al, 1992).(?)

Ceratium spp. reach their annual maximum during August-October. Dinophysis spp. occur most of the year and can form their annual maxima any time between June and October. G. aureolum starts to occur during July in the Skagerrak. Along the Swedish and Norwegian coast it usually forms blooms in September-October (Lindahl 1986). Dichtyocha speculum develops during spring into local blooms, affecting the gills of fish. Chrysochromulina polylepis develops its peak in May and Prymnesium parvum during the summer months. In the Kattegat/Skagerrak area a number of other toxic or potentially toxic species occur regularly. These include Alexandrium tamarense which has caused PSP-toxicity in mussels a number of times in the Skagerrak, and Nitzschia pungens and N. pseudodelicatissima, shown to be able to produce domoic acid which is responsible for ASP, although ASP outbreaks have not been observed in the Kattegat/Skagerrak area.

OCEANOGRAPHIC REGIME

The Kattegat area is dominated by a two layer estuarine flow driven by the low saline (15 PSU) water flow from the Baltic Sea. Deep water enters from the Skagerrak with salinities between 32-34 PSU. This water originates mainly from the Atlantic but also North Sea surface water enters the Kattegat. A strong halocline is located at about 15 meters depth, but varies due

to the fluctuating flow from the Baltic Sea and to local winds. Mixing across the halocline is dominated by upward wind entrainment.

The Swedish and Norwegian Skagerrak coast is characterized by a current entering from the North Sea north of a Jutland which is mixed with fresher Kattegat water along the Swedish coast. The entrainment in the coastal current is about four times larger in the Skagerrak than in the Kattegat, depending on the decreasing stratification along the current. The current changes due to prevailing weather conditions. Upwelling may also be important in the supply of nutrients in the initiation and mantainance of phytoplankton blooms. However, we believe that the strong stratification which stabilizes the water is the most important physical factor for the development of flagellate blooms in the area.



Figure 1. Map of the proposed study area, with the main water masses and currents indicated (From Lindahl, 1992).

The nutrient situation in the Kattegat/Skagerrak has been quite well studied (see Rydberg et al. 1990 for details). There are data of the seasonal variation of nutrients in these waters for more than 40 years. The data reveal that there has been an increase of both inorganic nitrogen and phosphorus in these waters during the last decades (Wulff et al. 1990). The same trend is also found for the water discharged in the coastal areas, where nitrogen and phosphorus have increased dramatically during the last decades.

THE SKAGERRAK





The ways macro- and micronutrients and organic compounds select and stimulate harmful algal species is nearly unknown. However, we do know that nitrogen is the most limiting nutrient for biomass formation of the total phytoplankton communities in these waters (Granéli et al. 1987, 1990). There are indications that some of the dinoflagellates are mixotrophic and that they might be using organic compounds as nutrient sources e.g. humic acids (Granéli et al. 1985, 1989). Humic acid has increased dramatically in the runoff over the last 15 years, due to acidification (Forsberg and Petersen, 1990).



Figure 3. Flow scheme of nitrogen circulation in the Kattegat. (From Rydberg et al. 1990).
During 1990 and 1991 a major ICES project, SKAGEX, was run in the Skagerrak/Kattegat area (Danielssen et al. 1991). The objective of the study was to define and quantify the water masses entering and leaving the area and to study physical, chemical and biological oceanographic processes within the area. The first part of the study in May-June 1990 included seven countries, seventeen research vessels and more than 200 scientists. The data now emerging from SKAGEX will make a sound base for the proposed project on phytoplankton bloom dynamics. x

ECOLOGICAL-OCEANOGRAPHIC PROBLEMS

This proposed multi-national and multi-disciplinary study will focus on the problem of eutrophication and hydrodynamic processes, resulting in large scale and often harmful phytoplankton blooms, comprising a multitude of species. We plan to concentrate the study on three groups of species which are likely to be present every year in the study area. Studies in this area and in other places of the world have given us some basic knowledge of the selected species.

Gyrodinium aureolum may form blooms which kill fish. It may also have negative effects on other marine organisms. Sedimentation of the bloom can contribute to anoxia. The problems are mainly occurring in the outer archipelago. The blooms are probably formed offshore and large subsurface populations of the species can often be found in central Skagerrak (Danielssen et al. 1991, Lindahl 1992). Gyrodinium aureolum has not been a problem in the Kattegat, probably due to the lower salinity there. The dynamics of G. aureolum show many similarities to the bloom dynamics of Gymnodinium breve in Caribbean waters. Parallel studies of the two organisms would thus be beneficial for the understanding and generalization of the processes governing the bloom outbreaks.

Dinophysis spp. cause Diarrhetic Shellfish Poisoning (DSP) by the production of dinophysitoxins and okadaic acid. In this area there are three species known or suspected to produce toxins; *D. acuminata*, *D. acuta* and *D. norvegica*. These toxins are accumulated in mussels which may become toxic despite very low *Dinophysis* densities in the water. At other times very high *Dinophysis* densities (>40 000 cells/l) do not result in toxicity of the mussels. For unknown reasons DSP-toxicity has only been recorded in the outer archipelago (Haamer et al. 1990).

Ceratium spp. develop large and patchy blooms nearly every year in the Kattegat and Skagerrak. There are at least five different species that can form the blooms: *C. furca*, *C. fusus*, *C. lineatum*, *C. longipes*, *C. tripos*. Sometimes they develop together into multi-species blooms. Other times one of them dominates the bloom. The sedimentation and subsequent decomposition of these blooms causes anoxia in areas very important for the fisheries of crayfish (*Nephrops norvegica*). Areas up to 4700 km² in the Kattegat have been affected in this way (Granéli et al. 1989).

The understanding of the role of eutrophication and hydrodynamic processes for the phytoplankton bloom dynamics will develop from a combination of in situ studies and experiments. To approach this understanding we have formulated a number of basic questions for each species or species group which need answers.

Gyrodinium aureolum

The scenario of G. aureolum bloom dynamics is shown in figure 4. From this model we have formulated the following questions:



- Where does the annual development of G. aureolum originate from?
- How is the subsurface population of G. aureolum initiated (advection or seeding)?
- Do the subsurface populations advect to the surface and develop into large scale blooms?
- Why are G. aureolum cells found in subsurface layers?
- What do they do down there? Is G. aureolum strictly autotrophic or is it mixotrophic? If mixotrophic, is it only by ingestion of dissolved organic substances or is phagotrophy involved?
- What is the productivity of G. aureolum in situ?
- Are G. aureolum -cells grazed?
- How do the blooms terminate? (sedimentation = anoxia as consequence?, cell lysis, grazing?).
- Which factors decide why and when the subsurface population will turn into a surface bloom? (light, temperature, nutrients, turbulence, etc?).

Dinophysis spp.

Most of the ecophysiological characteristics of *Dinophysis*-species are unknown (as e.g. grazing, mode of nutrition, growth rate, etc.): We know only that these species are not found in high numbers in these waters (between a few cells to max 45 $000 \cdot \Gamma^1$). Thus our studies will aim to answer the following questions:

- When do Dinophysis spp.-cells start to develop?
- Where in the water column is the early growth taking place? In the deep water? In thin layers?
- What are in situ growth rates for the different Dinophysis species?
- Do changes in N:P:Si ratios affect Dinophysis spp-growth and the selection of these species?
- Does grazing on *Dinophysis* and on other phytoplankton species structure the phytoplankton towards a selection of *Dinophysis* spp?
- Are Dinophysis spp real autotrophs or mixotrophs?
- If mixotrophs, do they utilize dissolved organic substances for their nutrition or are they phagotrophs?
- Are DSP concentrations found in mussels correlated with Dinophysis spp-cell numbers?
- Why, when DSP in mussels occurs late during the year (November), are the mussels toxic for several months. Is this due to cold water, or to the low concentration of phytoplankton cells in the water?



Figure 5. Conceptual model of Dinophysis-spp bloom dynamics.

Ceratium spp

The factors behind the initiation and mantainance of Ceratium blooms are unknown, as is *in* situ information on their growth rates. From experiments in mesocosms it seems that they are

not severely grazed by the dominant copepod in the area (Granéli et al. 1989, Olsson et al. 1992). The following questions have been formulated to make the basis for the study.

- When are the late summer-autumn blooms of Ceratium initiated? Already in the spring?
- What mechanisms select the species or group of species which form the blooms?
- Where in the water column is the early growth taking place? In the deep water? In thin layers?
- Why do Ceratium spp. blooms develop into such magnitude nowadays?
 - a. Increased growth rate?
 - b. Longer growth period?
 - c. Increased nutrient supply? Anthropogenic, climatic?
 - d. Changed grazing pressure?
- The blooms often have a very large, but very patchy distribution.
 - a. Does the bloom develop from one or several places?
 - b. Are the patches formed by physical concentration mechanisms or by biological factors?
- The blooms seem to occur mainly in the coastal current area. Which hydrodynamical processes contribute to this?
- Why and how does the bloom terminate? Nutrient depletion, infection (virus, fungi, bacteria)?
- What is the relationship between Ceratium blooms and anoxia in the Kattegat?



Figure 6. Conceptual model of Ceratium bloom dynamics.

TIME DURATION OF THE STUDY

The project is planned for four years, with three years for experimental operations and field studies running parallel and a final year for evaluation and publishing.

As the target algal species develop mainly from early June to the end of October, the field studies will run only during this period.

RESOURCES NEEDED

In the initial phase an important part of the study will be to localize the origin and spread of the seeding populations. This requires coverage of an extensive area which can be accomplished by cooperation with several institutes in the Skagerrak/Kattegat area, as well as the North Sea area, performing short cruises with frequent sampling. During bloom and post bloom conditions a few vessels capable of hosting many scientists with the needed competence from all over the world should operate for periods of one to three weeks.

The mesocosm approach has been proposed for studies of grazing and nutrients as important factors in structuring phytoplankton communities towards selection of undesirable species. This approach will be used since *in situ* grazing experiments are difficult and flask experiments have artificial effects. (For *Dinophysis* spp. however *in situ* technique have to be used, see below). The mesocosm experiments suggested are based on natural communities rather than being monospecific, so results can be obtained for different species.

For the grazing experiments the predator community is varied in the mesocosm in two ways (See Olsson et al. 1992 for details):

i) changing composition of predators.

ii) increasing numbers of predators.

For the nutrient experiments, different additions of nitrogen, phosphorus and silicon will be added to different containers containing water and the natural phytoplankton communities. N:P:Si will be added in different proportions in order to obtain different ratios between these elements.

In situ growth rates of Dinophysis sp. can be determined using the 14C- uptake technique. Determination of growth rate of several species of Dinophysis spp. and Ceratium spp. have been done by Granéli et al. (1992). This work will serve as the basis of a more elaborate experimental set-up. In the same experiment a strict autotroph and a heterotroph will also be isolated and their 14C uptake measured both during light and dark periods. This will enable us to determine whether the growth of Dinophysis is similar to that of autotrophs or heterotrophs.

SHIPS

<u>Prebloom conditions</u>: Sampling is performed by all research vessels operating routinely in the area during time periods of importance for bloom initiation and formation. Ferries can also be used. The existing monitoring programmes in the area could be expanded to cover the special objectives of this case study.

<u>Bloom or subsurface maxima conditions</u>: One large vessel should operate for one to three weeks. It has to be on standby until bloom conditions occur.

Bloom termination and fate: See above.





STAFF

It is not possible at this stage to decide on the size of the project. Several scientists from the surrounding countries, Denmark, Norway and Sweden will form a basis for the study and it is expected that scientists from other countries will also take part in the project.

INSTRUMENTS

Instruments needed to get adequate information during the field studies include: (here we have also included the mesocosm studies):

ADCP

CTD with particlecounter, fluorimeter and transmissometer. UNDULATING CTD, with particle counter, fluorimeter and transmissometer. FLOW CYTOMETER. SCINTILLATION COUNTER (on board). AUTOANALYZER TURNER FLUOROMETER INVERTED AND LIGHT MICROSCOPES INFLATABLE SWIMMING POOL + POLYETHYLENE CYLINDERS OF SEVERAL HUNDRED LITERS VOLUME (c 12 cylinders)

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Grazing experiments in detail Mesocosms:

The experiments will be performed on board ship in culture rooms (50 liter polyethene cylinders) or in the sea. The shore experiments can follow the system of Olsson et al.(in pres, 300 l polyethylene cylinders in an inflatable swimming pool), which is easy to handle.

To resume: the cylinders are filled with seawater filtered through a 100 μ m mesh size nylon net. Mesozooplankton collected on a 100 μ m mesh size net is added back to 4 of the cylinders at the same concentration found in situ (1 X zooplankton cylinders and to 4 other cylinders at 10 times the natural zooplankton concentration to increase the grazing pressure on the phytoplankton communities shortening the time of the experiment (10 X zooplankton cylinders) 4 cylinders will remain without "extra" zooplankton additions (control cylinders), which will give us the information on how the phytoplankton communities behave in the absence of the mesozooplankton (macrograzers). These latter cylinders will also give us information on how the microzooplankton (<100 μ m) is controlling the lower spectrum of the phytoplankton communities. To all cylinders nitrogen, phosphorus and silicon will be added in small amounts daily in order to minimize the addition of nutrients in the cylinders containing extra zooplankton amounts (and also to give us enough amount of phytoplankton biomass for good analyses). Samples will be taken daily during 8 to 12 days for chlorophyll, nutrient analyses, phytoplankton and microzooplankton identification and counting). The mesozooplankton will only be counted and identified at the end of the experiment, as it is necessary several liters of seawater to get reliable amount of zooplankton number.

MESOCOSM STUDIES

I. INTRODUCTION

The notion that a global increase in harmful algal blooms is occurring comes from the increasing number of regional reports on such occurrences. This global expansion hypothesis is probably, in part, an artifact of increased observational coverage, at least for certain regions, and includes events attibutable to natural variance, exclusive of *bona fide* increased regional occurrences accompanying cultural modification of the chemical habitat and food web structure. Thus, the validity and true magnitude of this hypothesized global expansion are unresolved; increased regional field studies, time-series observations and experimental evaluation of testable hypotheses are needed. The mechanisms regulating the selection of harmful algal species and their bloom events also need study, given the increasing threats to human health, to aquaculture and natural resources. Such evaluation is scientifically more tractable than efforts to confirm the global expansion hypothesis, although the results would be relevant to resolution of the latter. Such insight will also help in efforts to manage blooms of harmful species.

Harmful bloom events are attributable to a complex of causative species and environmental conditions regulating their outbreaks, rather than are bloom phenomena of one or two cosmopolitan species occurring at predictable time intervals, locations and environmental conditions. Regional surveys of such bloom episodes are needed to establish the multiple conditions under which blooms occur, the species involved, their bloom patterns, etc. Such descriptions will help to establish the commonalities generally characterizing harmful bloom events and the diverse habitat - and unique species-specific characteristics of regional bloom events. The dynamics of different species and functional groups, and different habitat factor combinations are involved in coastal current blooms, in blooms accompanying upwelling cycles, in nutrient enriched habitats, at aquaculture sites, and as periodic successional events. Quantification and modeling of these diverse bloom types require experimentation to establish rate constants of essential processes affecting population recruitment and losses, processes which determine bloom occurrences, duration, magnitude.

Available field evidence has established the regional, seasonal and interannual diversity in bloom occurrences, their duration, magnitude and causative species. However, lack of a theoretical framework, formulation and examination of testable hypotheses, and absence of direct experimentation to supplement descriptive field studies have compromised an understanding of the basic mechanisms regulating bloom events. We do not understand the mechanisms which select for bloom species and communities. We do not have even a basic, quantitative understanding and model of harmful algal blooms generally, let alone can satisfy the need for species-specific or habitat-specific models.

Regional studies have clearly established that nutrients and grazing are two factors generally significant in regulating harmful bloom dynamics, even though their relative importance varies with bloom types. A pilot study using experimental mesocosms should be undertaken to investigate the regulation of bloom events by nutrient concentration, supply ratios, and herbivorous grazers. The focus should be a first principles approach, rather than directed towards a specific region, toxin type or species, although the experimental species/community used should be of a bloom type.

II. PROBLEM FORMULATION

Community Structure and Growth

Natural phytoplankton communities are composed of populations of species whose individual population dynamics are under multifactorial regulation:

$${}^{dN}/_{dt} = KN - K_{a}N - K_{m}N - K_{s}N - K_{g}N$$
 (1)

where the population growth rate, d^{N}/dt , is determined by its growth (recruitment) term, KN, and the sum of its loss terms: advection (K_s), natural mortality (K_m), sinking (K_s) and grazing, (K_s). Thus, the abundance (N) of a given phytoplankton species at time, t, is given by:

$$N_{t} = N_{0}e^{(K-Ka-Km-Ks-Kg)t}$$
(2)

and indicates that changes in population abundance of a bloom species will result from changes in its growth coefficient (K) and in its loss terms, both singly and in various combinations. A population increase can occur at both low and high growth coefficients; low population abundance (N_0) does not mean that cellular growth rate is limited (Hecky and Kilham, 1988). The total community growth curve is therefore a result of the differing bloom patterns characterizing the species present. Some species will be in bloom, others in decline; still others will be in stationary phase and the seed stock for the evolving successional pattern. A major ecological problem within biological oceanography is the identification and quantification of the factors and their interactions regulating bloom events and cycles. There is no reason to believe that the factors which regulate harmful bloom events differ from those regulating the "normal" seasonal bloom patterns characterizing the region of interest.

Nutrients and Cellular Growth

The population growth equations (1) and (2) are affected by nutrients through their influence on the growth coefficient (KN), which is also a function of light, temperature, nutrient supply rate and their interactions. Nutrient-limited growth can be modeled using the Monod model:

$$\mu = \mu_{\rm m} \left[{\rm S/K_s} + {\rm S} \right] \tag{3}$$

where μ is the specific growth rate, μ_m is the maximum specific growth rate, S is the concentration of limiting nutrient and K, is the half-saturation coefficient for growth. The Monod equation relates growth rate to external nutrient concentrations (Hecky and Kilham, 1988; Kilham and Hecky, 1988; Sommer, 1989).

The Droop Model, which relates growth rates to internal nutrient levels, is preferably used for species which store nutrients intracellularly for subsequent use, a physiology not accommodated by the Monod model:

$$\mu = \mu_{\rm m} (1 - {\rm qo}/{\rm_q})$$

where q_0 and q are the minimal cell quota and intracellular concentration of limiting nutrients, respectively.

Studies applying Monod and Droop modeling concepts and related experimentation reveal individual bloom species follow one of three options in exploiting their variable nutrient environment, rather than have a uniform strategy (Sommer, 1989). Some species employ an *affinity strategy* with which they efficiently use low nutrient concentrations to achieve growth. *Growth strategy* species achieve high maximal growth rate, μ_m , through a rapid uptake of transient peak nutrient levels, whereas *storage strategy* species have much higher nutrient uptake rates than growth rates and build up intracellular storage pools.

Our present knowledge of which nutrient-limiting model and what nutrient uptake strategies characterize the different harmful algal bloom species is virtually non-existent. This gap obscures current understanding of how nutrients regulate harmful bloom population dynamics and requires experimentation.

Nutrients, Population Growth, and Species Selection

Numerous experimental studies have confirmed the tautology that if nutrients regulate cellular growth, then phytoplankton population dynamics are likewise affected. Thus, total population biomass yield is related to nutrient availability; multiple nutrient regulation of a single species does not occur, only one nutrient may be limiting at a given time or growth stage; in multiple species chemostats, multiple nutrient limitation of growth can occur; and nutrient supply ratios can be a strong determinant of species selection and community structure (Hecky and Kilham, 1988; Kilham and Hecky, 1988; Sommer, 1989). Given this complexity, specific experimentation on these processes is required, since these issues can not be resolved as experimental adjuncts in descriptive field studies on harmful bloom species.

Long-term studies and inter-regional comparisons indicate that increased nutrient levels, notably in N and P, indicative of a changing chemical environment have occurred in numerous inshore, coastal waters globally (Smayda, 1989, 1990). The expected increased phytoplankton biomass levels and production rates in response to this nutrient loading have been confirmed for many of these regions. An increased occurrence and blooms of novel, harmful, noxious or nuisance species have been a significant, parallel phenomenon in many of these chemically altered regions. A conspicuous shift in phytoplankton community structure to an increased importance of flagellates of diverse phylogenetic origin has likewise occurred. Smayda (1989, 1990) has hypothesized that the parallel increases in nutrient availability, changes in ratios of essential nutrients, their sources (agricultural, domestic, industrial), changes in species composition, and altered harmful bloom dynamics are significant factors regulating the apparently changing patterns and types of harmful bloom events occurring on a global, regional and local scale. Specifically, culturally induced changes in the ratios of availability of N:Si and P:Si are hypothesized to select for the predominance of harmful bloom species over the silicarequiring diatoms; that diatoms, initially favored in nutrient-enriched environments, outcompete non-diatom components until available silica concentrations are utilized. At that point, phytoflagellates and/or non-motile, non-diatomaceous taxa become favored, and utilize remaining elevated nitrogen and/or phosphorus levels, often forming harmful blooms during this predominance.

Experiments to evaluate this testable nutrient-ratio hypothesis are recommended, the results of which can be validated/nullified by comparison with existing field observations of community structure and harmful bloom events in nutrient-disturbed regions. Substantial physiological evidence from studies on freshwater phytoplankton species indicates that nutrient ratios, notably Si:N, Si:P, N:P, do indeed select for limnetic species occurrences and regulate their competition along nutrient resource and ratio gradients (Sommer, 1989). There is no reason to believe that the physiology and competition coefficients of marine species are not similarly influenced by nutrient ratios and resource gradients.

Grazing and Harmful Bloom Events

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Competition for nutrient resources and nutrient limitation of bloom events and species occurrences represent a bottom-up control mechanism of food web structure and maintenance. Grazing, a significant loss term in the population growth equations (1) and (2), in contrast, represent a top-down mechanism of food web structure and maintenance. Grazer-gradient experiments have established multiple consequences characterize grazer/phytoplankton interactions (Sterner, 1989; Smayda, 1992). Algal growth rates may increase or decrease with grazer population density and taxonomy; shifts in phytoplankton species composition and abundance accompany selective grazing pressure; allelopathic substances secreted by bloom species inhibit, or are lethal to grazers; nutrient excretion during grazing influences phytoplankton species competition. Theoretically, fluctuations in the relative importance of top-down vs. bottom-up control during harmful bloom events can be expected. Just as there is a lack of quantitative understanding of the diverse roles and mechanisms of nutrient regulation of harmful species selection and their bloom events, regulation of these events by grazers and their interactions with nutrients are likewise poorly understood and require experimentation.

III. PROPOSED STUDY

An experimental mesocosm study to evaluate the role of nutrients and grazers in regulating harmful bloom species selection and their blooms is recommended. Specific details of the experimental design, procedures, mesocosm scale, etc. will vary with the hypotheses being tested and the organismal complex under consideration.

The minimal objectives of this study should be to evaluate the role of nutrient ratios (N:P, N:Si, P:Si) in selecting bloom organisms, and the influence of nutrient concentration on bloom dynamics. These testable elements are subsets of the general hypothesis that nutrient enrichment initially stimulates non-toxic diatom blooms until silica becomes limiting, after which non-diatomaceous species will bloom, including harmful dinoflagellates, phytoflagellates or non-motile chrysophyceans.

The role of grazers on harmful species selection, their blooms and derivation of grazing constants for use in the population growth equations should also be evaluated. Herbivorous grazers representative of the micro-zooplankton, macro-zooplancton and benthic communities need assessment.

Initial efforts should be directed towards "proof-of-concept", e.g. to establish the suitability of the recommended mesocosm approach towards quantifying certain harmful bloom processes, the basis of which should be the recommended nutrient and grazer experiments.

Heterosigma akashiwo: SUITABILITY FOR EXPERIMENTATION

Heterosigma akashiwo increasingly has been implicated in red tide blooms, sometimes of prodigious magnitude, globally in nearshore waters: Canada, Japan, Korea, China, Taiwan, New Zealand, Spain, U.S. (Narragansett Bay, Puget Sound) and Chile. Taxonomic issues are largely resolved; frequent past confusion with Olisthodiscus luteus is not a problem, since the latter taxon is now recognized to be a benthic-dweller; H. akashiwo is planktonic, with an epibenthic stage in its life cycle.

Significant mortality of cultures has resulted from A. akashiwo blooms. In Japan, total economical loss over a 16-year period has been about 2 billion yen. In Puget Sound (U.S.) and Chile, extensive mortality and economic loss have accompanied its blooms. H. akashiwo, in fact, may be the leading, present cause of catastrophic fish kills in high-value fish aquaculture, replacing *Chattonella* spp. in this regard. H. skashiwo has been show experimentally to be antagonistic or lethal to diatoms, microzooplankton (tintinnids, rotifers), copepods, benthic larvae, and certain shellfish, among others. Thus, H. akashiwo is a broad spectrum antagonist or lethal to several trophic levels within the food web. The nature of its presumed toxin has not been established.

Numerous autecological studies have been carried out on *H. akashiwo*; it is easy to culture, and many geographical, clonal isolates are available. We have considerable knowledge as to its basic cellular properties with respecto to the effect of temperature and salinity on its growth rate, its phosphorus metabolism, photosynthetic properties, phototaxic properties, etc. In addition, a 25-year data set from Narragansett Bay, based on weekly quantitative measurement, provides insight into interannual variations in its bloom dynamics and associated environmental conditions. Numerous field studies have been carried out in Japan as well. Thus, an extensive autecological data base on cellular processes of *H. akashiwo* and its *in situ* occurrences is available to design and interpret large-scale experimental manipulation of this flagellate to assess its population dynamics. It is recommended as a candidate species for use in the mesocosm experiments leading to their evaluation and potential development as an experimental tool in quantifying harmful algal bloom processes and in model development.

MESOCOSM PANEL MEMBERS: G.C. Cadée, A. Cruzado, P. Gentien, T.J. Smayda, J.C. Smith.

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TOXIC FLAGELLATES BLOOM DYNAMICS IN THE ATLANTIC COAST OF THE IBERIAN PENINSULA

The Atlantic coast of the Iberian Peninsula offers an excellent opportunity for the simultaneous study of toxic algal blooms which can cause both PSP and DSP. The threat of both syndromes is annual in Portugal and Galicia, and an interdisciplinary programme aimed at increasing our knowledge of bloom dynamics in this region would contribute to worldwide knowledge of the phenomena involved.

I. GEOGRAPHICAL AREA

The shelf and inshore waters between Cabo Roca in Portugal (lat 38°45'N) and Cabo Finisterre in Spain (lat 42°50'N) has been identified as a region which presents common problems annually to the aquaculture and fishery industries and public health, as a result of the presence of two groups of toxic dinoflagellates responsible for PSP and DSP episodes every year. The southern (Portuguese) part of the region has a number of lagoons which are estuarine in character and with somewhat restricted exchange with the adjacent coastal waters, and the northern (Spanish) part is broken by a system of rias.

This region is very rich in shellfish resources. The Galician rías support the largest mussel cultivation industry in the world. Annual production of mussels (*Mytilus* galloprovincialis) is of the order of $150-200 \times 10^3$ t. There is little cultivation in Portuguese waters, but 70-80 x 10^3 t of wild stocks of clams, mussels, oysters and other bivalves are harvested. All these resources are jeopardized by recurrent harmful algal blooms of the species listed in II. The present management of this problem is based on toxicity monitoring followed by a ban on harvesting when toxicity thresholds are exceeded.

Areas which face the problems posed by G. catenatum blooms elsewhere are Tasmania and Japan. Regions where comparable DSP episodes associated with Dinophysis spp occur are the Chilean and Swedish fjords and Ireland. On the other hand, places like the Ebro Delta (Cataluña, Spain) and the eastern coast of U.S. have never suffered DSP episodes despite the occurrence of Dinophysis populations on their coasts.

II. SPECIES

The toxic species which cause problems in this region are Gymnodinium catenatum and Alexandrium lusitanicum+minutum both of which can give rise to PSP, and Dinophysis spp, especially D.acuminata and D.acuta, which cause DSP.

Heterosigma akashiwo and Prymnesium parvum responsible for fish kills in many parts of the world has caused extensive blooms in this area. A green flagellate of uncertain affinity (Gyrodinium sp), probably identical with a species said to be increasing in French Atlantic waters (Sournia et al, 1992), and the German Bight (Elbrächter, in press), has formed an extensive bloom in two occasions (Reguera et al, 1990; Jiménez et al, 1992). Gymnodinium breve, the well known Florida fish killer, has also been reported. Thus the diversity of species identified as problematical in this region is as high as anywhere in the world (Blanco et al 1985; Fraga and Sánchez, 1985; Sampayo, 1989; Reguera et al, 1991)

III. OCEANOGRAPHIC REGIME

The Iberian Peninsula lies at the northern limit of a typical eastern boundary current system, hence experiences upwelling under the influence of the Azores anticyclone when it becomes established, usually between April and October.

The upper ocean layer of this region is influenced by the Western North Atlantic Central Water which has two branches: the subtropical and the subpolar. The first seems to originate south of the Azores Islands and is advected NE, occupying the upper levels of the permanent thermocline and vanishing off the Galician coast; the subpolar branch seems to originate in the western part of the Bay of Biscay, influences all the subtropical northeast Atlantic Ocean and interacts with Mediterranean Water (Fiuza, 1984). The different origins of these two branches give them different chemical characteristics.

The subpolar branch off the Iberian Peninsula flows southward and is associated with a less saline superficial layer (50-60 m) detectable most of the year along the northern continental shelf. During summer, this layer extends to the shelf break and is usually limited by the seasonal thermocline (Jorge da Silva, 1987). Its southern limit is located in the region of the Nazaré canyon, which corresponds approximately with the northern boundary of the subtropical branch of the WNACW. Recent investigations revealed the presence of this layer during spring when thermal stratification is incipient. During winter, the southward flow seems to be blocked by the subtropical branch of WNACW which reaches the surface and occupies almost the entire shelf (Fiùza, 1984).

North of Nazaré canyon upwelled waters from 60 to 120 m depth, with subpolar branch characteristics, enters the Portuguese lagoons and Spanish rías during the upwelling season (spring to early autumn). The less saline waters which leave the rivers, lagoons and rías tend to flow southward under the influence of the northerly winds, and separate from the coast. During very sunny periods, between May and August, when the upwelling is moderate or intermittent, shallow subsurface thermoclines (and nutriclines) are established in the rías and adjacent shelf that enhance the development of various flagellate species (Figueiras and Ríos, in press).

A poleward surface slope current, stronger near the coast, is prominent during southwest winds, when upwelling relaxes and especially in winter, and turns eastwards into the Cantabrian Sea. This current is weaker during northerly winds, tends to break up into gyres, and moves offshore at Cabo Finisterre instead of turning east. During the upwelling season, this current lies farther from the coast. Broad aspects of the physical oceanography of the coastal area (Wooster et al, 1976; Frouin et al, 1990; Haynes and Barton, 1990) and within the rfas (Fraga, 1981; Blanton et al, 1984) are well described. South winds can reverse the circulation pattern in the rías, which then act as negative estuaries. Under these circumstances, convergence zones can appear in the outer parts of the rías that block the outward flow of the inner water (Fraga and Prego, 1989).

During summer, when the water column is stratified and therefore susceptible to nutrient depletion, regeneration processes in the lower part of the water column play an important role as an additional source of nutrients. This nutrient enriched water is upwelled into the euphotic zone contributing to the maintenance of phytoplankton populations.

Within the rías, nutrients are abundant year round; regeneration processes are a prominent feature of the lower part of the water column, and can be the main source of nutrients for the phytoplankton at certain times of the year when upwelling ceases (Prego, 1992).

IV. THE PROBLEM

1. Paralytic Shellfish Poisoning was first detected in Portugal in 1960 and in Galicia in 1976. The 1976 event affected people in five different European countries (Italy, France, Switzerland and Germany, as well as Spain - see Lüthy, 1979), and was only the second major outbreak in Europe this century (the first was in the United Kingdom in 1968). Plankton samples taken after the event revealed the presence of *Gymnodinium catenatum* (the first record for European waters), but the toxin profiles indicated that *Alexandrium* (the probable culprit in the U.K. and the Portuguese events (Silva, 1963 and 1980) may also have been involved. This episode led to the establishment of a monitoring programme in Galician coastal waters in 1977. PSP associated with *Gymnodinium catenatum* has been detected in 8 of the 15 years since, and in all but one since 1985 in the Spanish Rías, and in Portugal in all but one since 1986. Low levels of PSP toxins (around the quarantine limit) were recorded in 1987 and 1992 in the Rias Bajas associated with *A.lusitanicum+minutum* and these species were the agent of a major PSP outbreak in Ria de Ares (Rias Altas) in 1984 (Blanco et al, 1985).

2. Proliferations of *Dinophysis spp* in several pulses are a common feature in the Spanish Rias and the Portuguese lagoons from spring to mid-autumn. *D.acuminata* usually exhibits one peak in the spring and another one in late summer/autumn. Highest number of *D. acuta* are usually found from August to October. Due to the persistent occurrence of one *Dinophysis* species or the other from March to November, the presence of low levels of DSP toxins in bivalves (together with a more and more strict application of the toxin controls) has become a real nightmare for the mussel growers in Galicia and the bivalve collectors in Portugal.

3. There is no extensive fish-farming business on the Atlantic-Iberian coast. Therefore, the potential harmfulness of fish-killer blooms (*H.akashiwo*, the "green flagellate" and *Prymnesium parvum*) has not been realized. Nevertheless, the negative impact of these blooms on the recruitment of pectinids and other groups with very sensitive larvae should be evaluated.

V. PRESENT UNDERSTANDING AND GAPS IN OUR KNOWLEDGE

Annual phytoplankton succession patterns in this area are reasonably well known (Figueiras and Ríos, in press) as are the periods when problem species appear (Reguera et al, 1991). We also have a general understanding of the hydrographic conditions which accompany proliferations of these species. But we do not know where the inoculating populations come from, nor the dynamics of the critical periods of early growth. The relative importance of in situ growth and concentration due to physical processes (upwelling, tides, estuarine circulation) is not known either, but upwelling can reintroduce sinking cells into the photic zone (Varela et al, 1991). In addition, there is almost complete ignorance of the life cycles of some of these species, their sources of nutrition, and their relationships with other components of the food web.

In summary, we need to deepen our understanding of harmful algal bloom dynamics so as to be in a position to explain their appearance, and improve our predictive ability, minimizing their effects.

Dinophysis spp

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Isolated cells of *D. acuminata* can be found all the year round. Proliferations are concomitant with the upwelling season, usually exhibiting one peak in the spring and another peak in the autumn. Growth seems to take place especially when a shallow thermocline (5-10m) is established in May-June, and the cell maxima is in the thermocline, but eventual mixing of the water does not cause a collapse of the population. Later in the season, the cell maxima are found at the surface. *D. acuta* occurs in a narrower season (July-October), associated with moderate upwelling, very dry summers and a deeper thermocline (10-15m), and in contrast with *D. acuminata* seems to be quite stenothermal and stenohaline, and more neritic in character. The blooms of *D. acuta* seem also to be associated with lower salinity plumes of continental origin (Moita, in press). Many aspects of the biology of the species in this genus are unknown or poorly understood. Some of the questions currently under investigation in the Iberian region are:

- Are the morphotypes observed in the declining phases of *Dinophysis spp* blooms permanent cysts?

It has recently been found that some of these species have several different morphotypes, and are suspected of producing cysts (Reguera et al, 1990; Bardouil et al, 1991; Moita and Sampayo, in press; Mackenzie, 1992). The relations between these morphotypes need to be established so that the life cycles can be understood.

- Are the initial populations advected into the rías by upwelling ?

D. acuta is present in the shelf waters beyond the rías and coastal lagoons even in years when it is not found within the rías and lagoons. The cells may be associated with a zone of less saline water identified on the shelf, which may enter the rías and lagoons in response to the intensity of upwelling events.

- What physiological reasons cause the cells to occupy different layers of the water column at different times in the growing season?

They may, for example, occupy the thermocline during growth to avoid being dispersed by the estuarine circulation, and they may swarm in the surface layer at the end of the growth season in connection with sexual reproduction.

- Are there physiological reasons for daily vertical migration, or is it a tactic to maintain the cells at an optimal depth?

- When do the cells behave autotrophically and when do they become hetero- or mixotrophic? Does this influence toxin production of the cells? Would this explain the lack of toxicity of *Dinophysis spp* in other regions ?

Growth rates are not known, and the nutritional status of *Dinophysis* is uncertain, but *Dinophysis acuminata* cells have been observed at different times of the growing season swollen and full of digestive vacuoles (Reguera et al, in press). They must be mixotrophic. The relations between toxicity and growth remain to be established, and the role of vertical migration in their ecology is not known.

Gymnodinium catenatum and Alexandrium spp

These organisms are autotrophic, but much remains to be learned in the other four areas -life cycles, growth rates, toxicity and behavior- for all species. It is not known whether *G. catenatum* blooms are initiated by *in situ* cyst germination, or advection or both, and the oceanographic changes which lead to its interannual variations in abundance have not been established. There are toxic and non-toxic strains of *G. catenatum*, and the taxonomic relationship between them is not known.

The timing of *Alexandrium* blooms is thought to depend in part on an internal biological clock mechanism, and this may be true of the other problem species too.

Gymnodinium catenatum

This species has appeared in the Rías Bajas associated with PSP episodes each year from 1985 to 1990 (Fraga et al, 1988 and 1990). Its cyst has been described (Anderson et al, 1988). Laboratory experiments show that a high proportion of cysts germinate without the need for a dormancy period, and that temperatures above 16°C are necessary for a good germination rate (Bravo and Anderson, in prep). It is interesting to mention that this temperature is very rarely reached at the bottoms of the rías, except when upwelling relaxes. The major outbreaks of this species in Portugal and Galicia have taken place at the beginning of autumn, associated with southern winds, relaxation of upwelling and advection of coastal waters into the rías. Very moderate outbreaks have taken place sometimes in summer, in stratified conditions, and in these cases the cell maxima are in the nutricline.

In Portuguese coastal waters, the distribution of G. catenatum is well related with the intensity, duration and variability of upwelling (Moita, in press). The blooms are associated with a nitrate depleted coastal plume of slightly lower salinity than the oceanic water (Moita,

in press; Fraga et al, in press). Figueiras and Fraga (1990) suggest that this species, by virtue of its vertical migration capacity, exploits the nutrient rich layer under the thermocline.

- Is there a "red spot" area with a high concentration of cysts in the Aveiro - Oporto region, the germination of which would expain the very rapid proliferations of G. *catenatum* in the autumn, with a clear south to north propagation?

It is necessary to complete cyst mapping of the region (between ría de Arosa and the Portuguese coast).

- What is the origin of the inoculum populations? Massive "in situ" cyst germination when the bottom waters reach a suitable temperature (and maybe light and other factors we do not know) at the end of the upwelling season? Advection of already established shelf populations? Advection of populations from the poleward surface slope current when it moves nearer to the coast?

- What is the main nutritional source for G. catenatum?

We know that this species is autotrophic, but we do not know the relative importance of "new" nutrients from the upwelled water, or regenerated nutrients or even of organic compounds in its nutrition. The effects of trace elements (Fe, Se, etc.) on vegetative growth has not been studied either. The influence of the inputs of rivers Miño and Duero in stimulating the development of the blooms needs to be evaluated.

- Why did G.catenatum not bloom in 1991 when the south wind season arrived and the offshore warmer water invaded the rías?

May be the bottom temperature was not high enough to stimulate cyst germination, or that different patterns in the poleward current circulation took place (too close to the coast too suddenly).

- Why does G. catenatum not proliferate on the southern Portuguese coast and north of Cape Finisterre ?

Alexandrium spp

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Toxic blooms due to *Alexandrium* in this region are caused by the *A. minutum* group, and are limited to coastal areas with estuarine characteristics. They are associated with pronounced stratification and very low salinity values, of the order of 20-25ppt, and are restricted to the thermocline which coincides with a marked halocline. These blooms occur more frequently and with greater intensity in spring and summer in Obidos Lagoon in Portugal and in the Rías Altas of Galicia. The reasons these blooms are of less importance in the Rías Bajas are not known. Cysts of this group are found in the sediments along the entire Galician coast (Blanco et al, 1985), but their role in bloom dynamics in this region remains unknown.

VI. PROPOSED PILOT STUDY

1. Field Studies

A two-pronged field study is proposed, with mesoscale and microscale components.

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The mesoscale component will survey the region between Cabo Roca and Cabo Finisterre, with the following aims:

- To define the boundary conditions for the two areas (see below) selected for microscale studies,

- To explore the geographical ranges of the problem species, including their cyst stages in the sediments,

- To describe the hydrography of the region and its seasonal variability.

The microscale component will involve frequent surveys (monthly, fortnightly or weekly) of Ría de Aveiro in Portugal and Ría de Pontevedra in Galicia. The monitoring progammes already operating in the region will form an essential guide to the necessary frequency of these surveys in the two selected rías. Some ecophysiological field studies (vertical migration, incubations..) will require very intensive sampling at a definite stages during the blooms.

2. Laboratory studies.

G. catenatum studies on toxicity, growth, mortality, behaviour and grazing by copepods or mussels, will be run with cultured strains already available.

During blooms of *Dinophysis spp*, laboratory studies on toxicity and growth will be run. The trials to culture *Dinophysis* (Sampayo, in press) will continue.

Comparative studies will be run with other harmful species already in culture such as Alexandrium spp, Heterosigma akashiwo, and Prymnesium parvum.

3. Modelling

Some numerical modelling of the shelf region is already in progress (Rodríguez, pers.comm.) based on procedures developed by Backhaus (1985). There are also models of Ría de Arosa (Pascual, 1987) and Ría de Vigo (Prego and Fraga, 1992) available which can be developed for other rías. The boundary conditions for future model development will proceed from the field surveys.

Measurements needed

It may be possible in some cases to measure in situ growth rates by intensive sampling at favorable times. In the case of *Dinophysis acuminata* and *D.acuta*, which

separate after cell division, the daughter cells can easily be recognized before their lists recover the mature form. Estimates of division rate could therefore be obtained by counting dividing, recently divided and fully formed cells.

Chain length in Gymnodinium catenatum might provide a means of estimating in situ growth rate, but probably also responds to turbulence. It should be possible to establish the impact of turbulence on chain length in laboratory experiments, and hence obtain correction factors allowing chain length to be used to estimate growth rate. Field measurements of shear rates in G.catenatum patches might then allow this approach to be used under natural bloom conditions.

When a problem species is dominant, it may be possible to use the fractionated ¹⁴C technique.

The mixotrophy or heterotrophy of *Dinophysis* will be examined using the very sophisticated pigment analyses procedures by HPLC available in the Centro Experimental de Vilaxoán.

Resources needed

a) <u>Ships</u>

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- Mesoscale surveys: 2 large ships for 8-10 days to operate simultaneously 4 times a year.

- microscale surveys: small ships currently used in the Spanish and Portuguese institutions.

b) <u>People</u>

- Permanent staff (scientists and technicians from the institutions involved) plus some post graduate students. International participation would be most welcome.

c) Instruments

- Normally equipped oceanographic research vessel with CTD, ADCP, Niskin bottles.....

d) <u>Shore laboratory analyses</u>

- Existing facilities at INIP (Lisboa and Aveiro), IEO (Vigo), Xunta de Galicia Centres (Coruña), and Sanidad Exterior (Vigo).

- There are experts on the taxonomy of target species in both Spain and Portugal, and expertise on DSP and PSP toxin analyses (bioassays and HPLC) and pigment analyses (HPLC)

- Satellite and meteorological data, available at the Instituto de Investigaciones Marinas (Vigo), that has an on-line reception system.

- Sediment cores: Geology Department, University of Lisbon.

e) <u>Duration</u>

- Field and laboratory operations: 3 years.
- Analyses and Interpretation : 1 year

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Figure 1. Map showing the proposed study area.



Figure 2. Simplified scheme of the surface and subsurface circulation in summer and winter in Iberian area and Biscay.