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**TECHNICAL REPORT**

**BACKGROUND OF PLANKTON II:  
HARMFUL ALGAL BLOOMS (HABs) - POSSIBLE CAUSES**

By:

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## 1.0 Introduction

There is evidence to indicate that HABs have increased in occurrences (temporal and spatial) worldwide over the last decade. The blame inevitably is always put on eutrophication of coastal waters from human activities. Despite years of research in several countries, we are still far from understanding the exact causes for these blooms, and to expect that a common cause function in all blooms would be naive. It might be possible, however, to identify a set of most probable factors that promote HABs in a particular location and close monitoring of these factors may provide some degree of certainty to predict HAB occurrences in that location.

## 2.0 HAB cycle

Most HABs do share some common properties, which are listed below.

1. HABs are often detected after cell densities get high enough to discolor the water (red tides) or cause seafood toxicity.
2. The blooms are often patchy vertically and horizontally, the patches persisting for several days.
3. At its peak the blooms are almost monospecies.
4. Both the appearance and disappearance of the bloom are abrupt events.

In many ways, the development of these HABs resembles the development of microbial populations in batch cultures (Figure 1). The challenge for researchers is to explain the processes involved in each phase. Currently, some of the phases are better understood than others.

### 2.1 Seed population and bloom initiation

In temperate waters explanation of bloom initiation is relatively straightforward. Over-wintering cysts germinate once temperature and light regime becomes favorable. In northeastern United States, for example, areas *Alexandrium* cyst accumulation have been identified. In this case the occurrence of HABs can be predicted with some certainty.

In tropical waters the seeding of HABs is not so clear cut. Most tropical dinoflagellates, including the toxic *Pyrodinium bahamense*, do form resting cysts. More interestingly, we have found that *P. bahamense* cysts have an obligatory quiescence phase of ca. 90 days. It is still not known how this obligator phase relates to environmental cues, since light and temperature vary over a narrow range.

The importance of resting cysts in the initiation of blooms of *P. bahamense* is open to question. On the West Coast of Sabah we have not been able to identify areas where cysts accumulate. On the other hand it is common to find a few vegetative cells of *P. bahamense* in the water column year round in protected bays and lagoons. It is thus likely that blooms develop from these background populations. In Manila Bay (Philippines), however, there is evidence that *P. bahamense* blooms may originate from cyst beds in the Bataan and Cavite region (Figure 2). Clearly, the question of source population is an important issue that should be addressed, especially in tropical systems. This information is important in determining suitable locations for shellfish

aquaculture projects and the setting up of an efficient HAB monitoring network.

## 2.2. Bloom growth

For several reasons, this is the most important phase of a HAB development. Unfortunately, it is also a phase that is least understood. Vegetative cells that germinate from cysts, or present in the plankton, will not form blooms unless conditions are conducive. What are the factors that may determine whether or not blooms develop? These could be:

1. competitive advantage
2. release from nutrient limitation
3. favorable hydrographic conditions
4. release from predation

Since HAB events are generally not continuous events, it follows that favorable combinations of these control factors for the HAB species are also episodic in nature.

## 3.0 Nutrients and Species Competition

### Macronutrients

Nutrient discharges into coastal waters usually have an N:P ratio that differs from the receiving water. This could cause shifts in species composition. HABs are known from both P- and N-controlled environments, indicating that the apparent global increase HABs is not related to a general shift in supply ratios. In other words, there is no evidence at present that P-limitation leads to a higher frequency of HABs than N-limitation, or *vice versa*. Generally though N- and/or P-enrichment is normally not accompanied by Si-enrichment. Thus if the increase in HAB occurrence is related to a macronutrient enrichment-induced increase in phytoplankton biomass, non-diatoms will be favored, providing that light and temperature are not limiting growth.

Competition experiments with mixtures of dinoflagellates, haptophytes and diatoms generally indicate that dinoflagellates are poor competitors compared to haptophytes. If sufficient silicate is available, diatom will always outcompete non-diatom under nutrient- and/or light-limiting growth conditions. A model of the relative niche of major phytoplankton groups is shown in Figure 3. When light is limiting (center of diagram), diatoms are the fastest growing organisms. Under saturating light conditions (around the center of the circle), the nature of the growth-limiting nutrient will determine species composition.

Dominance of a particular phytoplankton species may also result from the dominant form of nutrients available at a particular time. This is particularly true of nitrogen, which can exist in a wide variety of forms. In coastal waters, for example, organic nitrogen may at times form the dominant fraction. Phytoplankton species vary in their capability to utilize these various forms of nitrogen and this could lead to dominance of a certain species. An example of how different phytoplankton species respond to various forms of nitrogen enrichment is shown in Figure 4.

## Micronutrients

Trace metals have been widely studied in relation to HABs. In growth experiments of *P. bahamense* it was found that the species has a high requirement for selenium (Figure 5). This is an element that is not naturally found in high concentration in marine environments. Most of the input originates from terrestrial sources. It could be delivered into coastal waters through land runoff. Also interesting the fact that *P. bahamense* could only utilize selenium as selenite or organic selenide. Thus not only supply is important, but also be speciation. Other trace elements considered to be important for the growth of HAB species are shown in Table 1. While experimental evidence for the influence of Fe, Se, Co, and Cu on the growth of harmful algal species exist, the exact role played by trace elements and chelators in the initiation and maintenance of most HABs remain unknown. The over-riding question is not if the harmful organism has a general requirement for a particular metal. The nutritional physiology of HAB species is often very similar to their non-toxic counterparts. Trace elements are essential for the growth of all phytoplankton. The question is whether HAB species have unique trace element requirements, physiology, and uptake or detoxication mechanisms that would allow them to dominate non-toxic species.

### **4.0 Hydrography and physiology**

Most observations on HAB events show that dinoflagellate blooms occur under stratified stable water column conditions. It is also an established fact that generally dinoflagellates do not like turbulence, as opposed to diatoms which thrive under such conditions. This fact was recognized many years ago by Margalef (Figure 6), although the scientist basis for this observation was not known until recently. It is now known from experimental evidence that strong shear inhibits DNA replication in dinoflagellates, especially if the shear is experienced during certain stages of the cell cycle. It is thus not surprising that in vertically well-mixed upwelling systems such as the Benguela current, Peru, and California, diatoms often dominate algal blooms. However, once turbulent energy decays, dinoflagellates can take advantage of the upwelled nutrients and form blooms. This, for example, is the case for *Dinophysis* and *Gymnodinium* blooms in the rias of Spain.

Turbulent events can also result in the abortion of blooms. On the west coast of Sabah, for example, we have often found that *P. bahamense* blooms abort (indicated by cell density) following strong winds. An even more pronounced example is bloom development in the northeastern United States. Here bloom events starts with germination of *Alexandrium* cysts from the mouth of the Kennebec River in Maine in early spring. These cells will then be transported by the plume of less dense water southwards, closely hugging the coastline. Since this plume of warmer water floats over the colder, denser water of the Atlantic, its movement can be easily tracked from SST images provided by NOAA satellites every four hours. Occasionally, winds from the south can blow for a period of time, enough to cause upwelling of cold Atlantic water. This will result in the destruction and dispersion of the plume offshore. In this case the blooms will die off. One thing that is still not clear in these events is whether the dinoflagellate cells actually die or whether they just get dispersed.

Life in a stratified water column presents its own problems to phytoplankters, especially if the nutrient is deep. It is likely that in a bloom patch the nutrient pool

will rapidly become exhausted and recycling would not be fast enough to sustain a dense population (can reach ca.  $10^6$  cells  $L^{-1}$ ). Laboratory experiments in plankton towers showed that dinoflagellates overcome this problem through vertical migration. Dinoflagellates are motile and vertical migration could be further enhanced by buoyancy regulation, for example through changes in lipid composition.

What is the physiological status of cells in these blob patches? Is the high density of cells in bloom due to enhanced in-situ growth rate or is it due to accumulation by physical forces? These are very important questions that need to be addressed. Most growth experiments in the laboratory indicate that the maximum growth rate achieved by the dinoflagellate were 0.3 - 0.5 divisions  $day^{-1}$ . At these rates, a reasonable inoculum density (ca. 100 cells  $mL^{-1}$ ) would actually be able to yield typical bloom densities within a period of 10 - 12 days, assuming minimum cell loss. It is thus possible to explain bloom formation just on the basis of passive accumulation, without invoking greatly enhanced growth rates. It would still be desirable though to be able to measure *in situ* growth rates in order to gain a better understanding of bloom formation. However this aspect is still very much limited by lack of suitable technology. It would also be desirable to know the nutritional status of these cells since this can have significant impact on cellular contents of toxins and photopigment.

## 5.0 Predation

In order for blooms to fully develop, population increase has to greatly exceed loss. Population loss can occur through physical dispersion, cell sinking, death, and predation. The persistence of a bloom patch is an indication that dispersal is not significant. Not much is known about sinking, although this could be significant during the later part of the bloom when resting cysts are formed. Ditto for natural cell death. Predation is an interesting aspect, not least because these cells are toxic. Experiments that have been carried out have been inconclusive. While some copepod species have been shown to avoid toxic cells, other predators such as the tintinnid *Favella* seem to feed on these toxic cells without adverse effects. The bottomline is that it is still not known how much impact predation, or the lack of it, has on HAB development.

## 6.0 Conclusion

HABs are, without doubt, becoming increasingly important in coastal waters worldwide. It is a very interesting phenomenon simply because most of the species involved are those that are not normally dominant in the plankton. Even over decades of research, still a lot is not known on the causative factors for these HABs. While increased nutrient loading undoubtedly increase the standing crop of phytoplankters in coastal waters, the relationship between eutrophication and HAB events is not always straightforward. In most cases we still do not know which species will benefit most from increased nutrient inputs. Treatment of effluents prior to discharge can reduce the amount of macronutrients that enter the sea, but at the same time this can also alter nutrient ratios. Experience in certain countries, for example in Japan and Scandinavia show that alteration of nutrient ratios can have quite unexpected outcomes on the phytoplankton assemblage. More studies, especially *in situ*, have to be conducted in order to better understand HAB events.

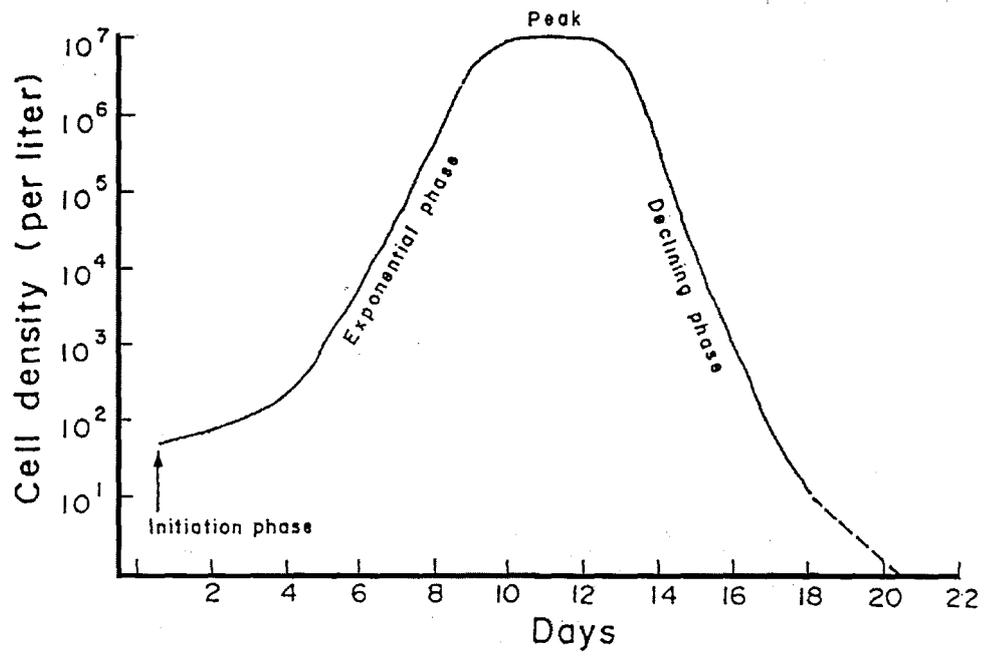


Figure 1: An idealized growth curve of a typical *Pyrodinium bahamense* var. *Compressum* red tide on the West Coast of Sabah.

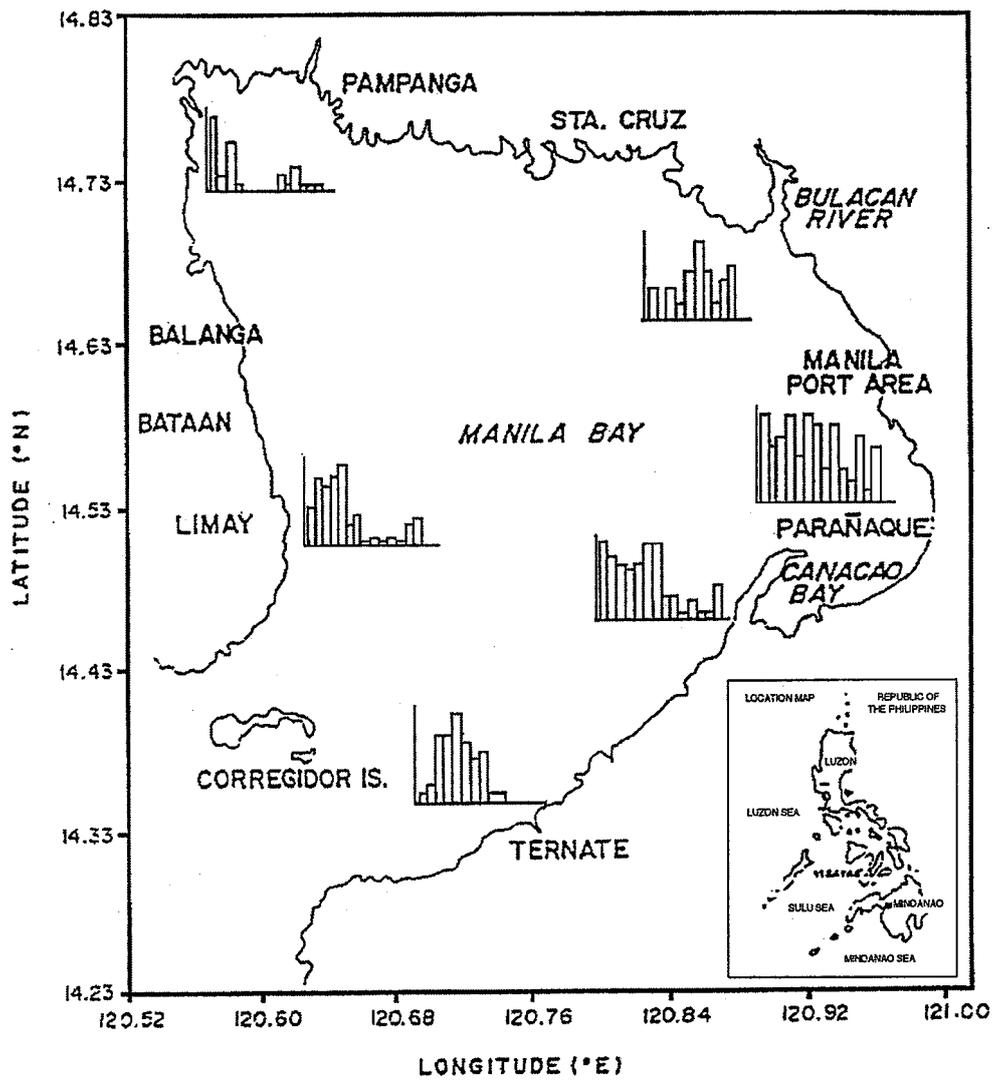


Figure 2: The distribution of *Pyrodinium bahamense* resting cysts in the surface sediment of Manila Bay from August 1993 to May 1994. Cyst densities (cysts  $\text{cm}^{-3}$  sediment) for each region were as follows: Bataan 0-450; Pampanga 0-70; Bulacan 0-40; Paranaque 0-60; Canaco 0-300; Ternate 0-80. (from Corrales and Crisostomo, 1996).

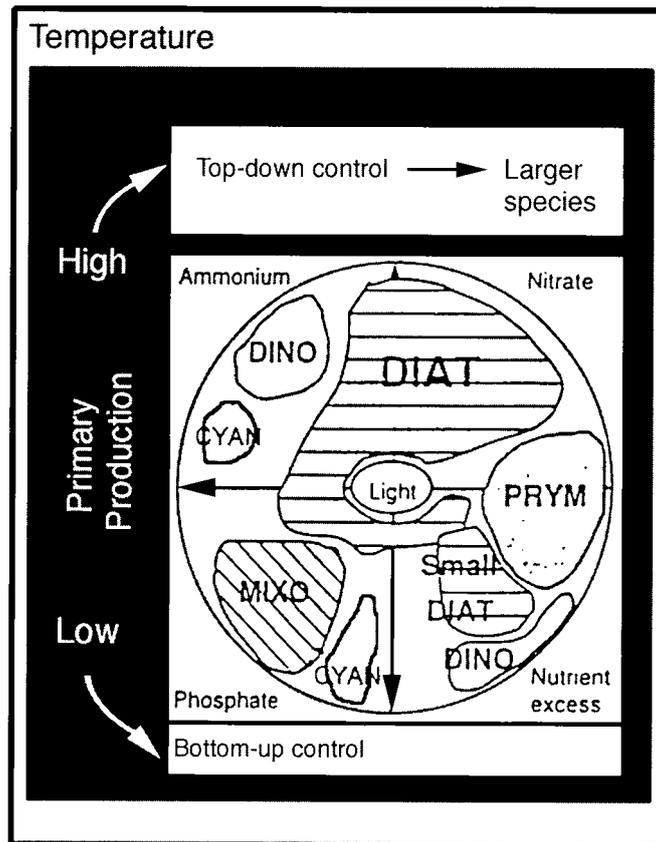


Figure 3: Ecological niche of dinoflagellates (DINO), diatoms (DIAT), haptophyceae (PRYM), cyanobacteria (CYAN) and mixotrophic algae (MIXO), according to their performance in competition experiments as described in Riegman *et al.* (1996). Centre of circle indicates light limiting conditions; border of circle indicates saturating irradiance levels and nutrient limitation or nutrient excess. Within the indicated taxonomical groups, primary production in the ecosystem may have, depending on its magnitude, its effect on species composition *via* top-down and corresponding size selective grazing.

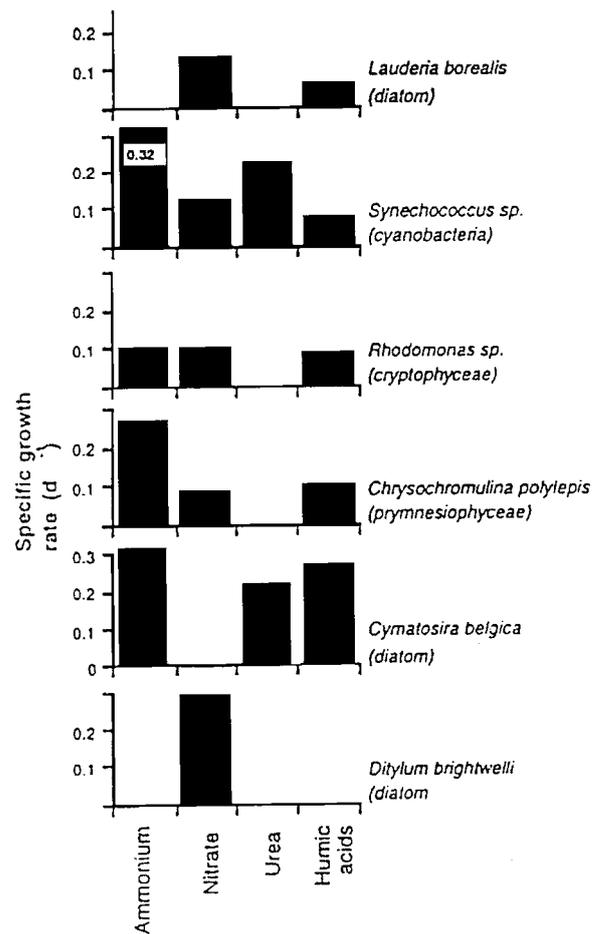


Figure 4: Response of different algae to different nitrogen sources under N-limiting growth conditions, in discontinuously diluted mixed algal cultures at a dilution rate  $0.1 \text{ d}^{-1}$ .

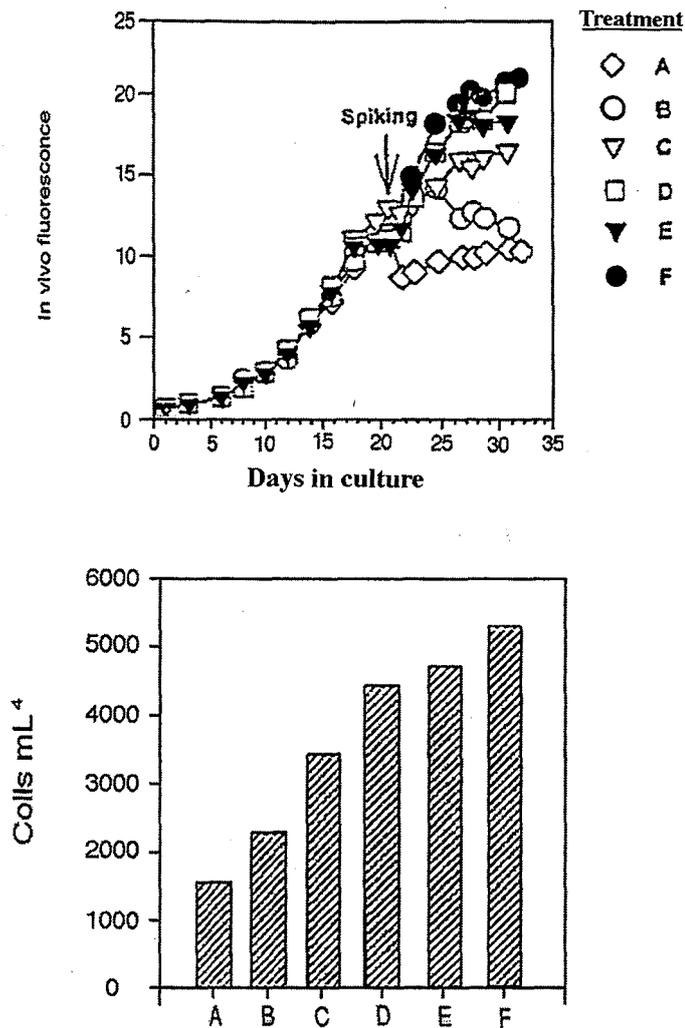


Figure 5: Effect of selenite supplement on *P. bahamense* var. *compressum* growth. Upper panel – *in vivo* fluorescence profiles of batch cultures spiked at early stationary phase with various nutrient combinations. Lower panel – highest yields obtained from the cultures. Spiking combinations were: A-no spiking; B-10 ml L<sup>-1</sup> modified ES medium nutrient mix; C-4 mL L<sup>-1</sup> soil extract; D-10<sup>-7</sup> M selenite; E-10<sup>-7</sup> M selenite, 200 mg bicarbonate, 10 mL L<sup>-1</sup> medium nutrients mix; F-10<sup>-7</sup> M selenite, 10 ml L<sup>-1</sup> medium nutrient mix. (From Usup, 1995).

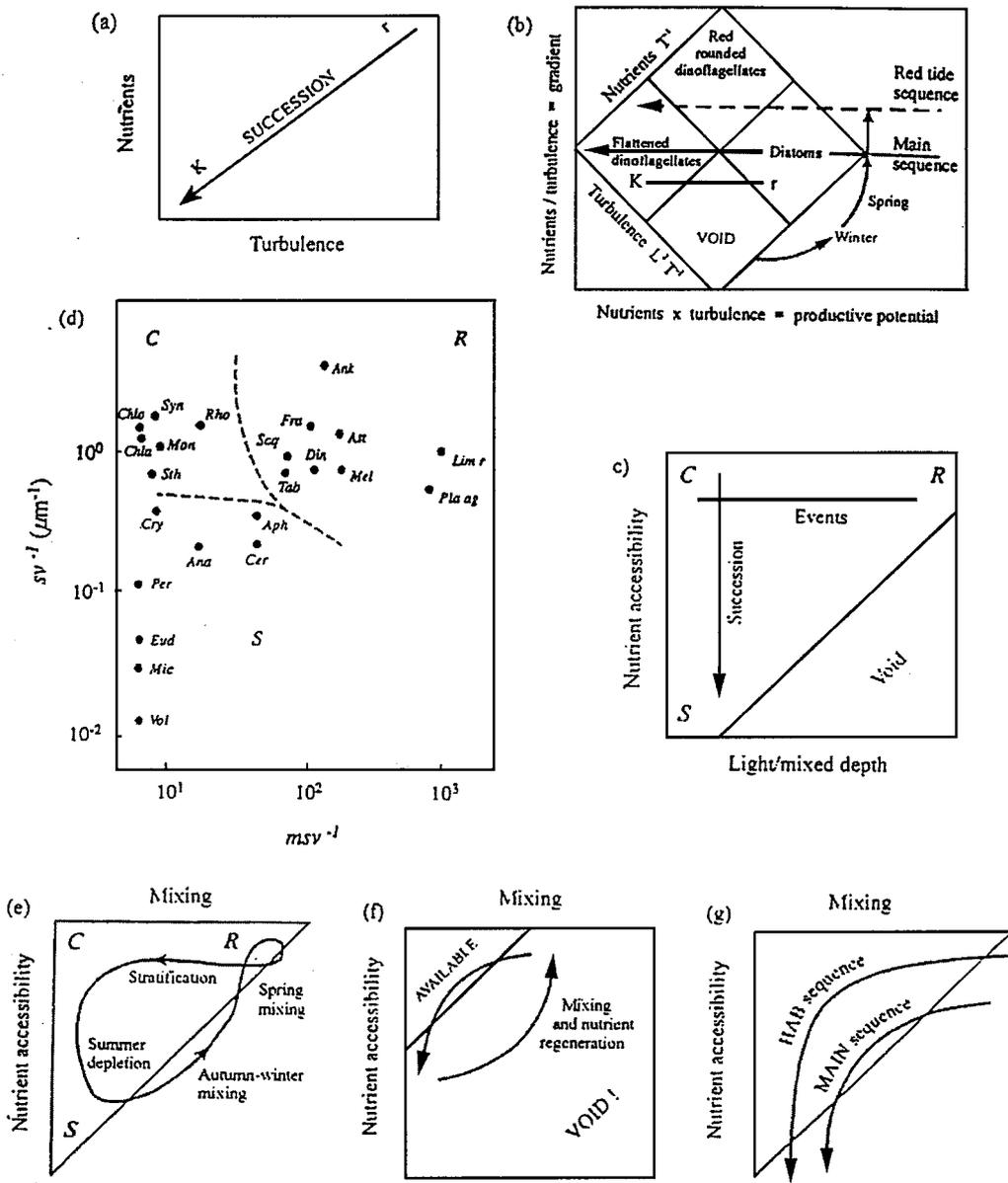


Figure 6: (a) Margalef's matrix summarising the sequence of phytoplankton (the main sequence) as a function of diminishing 'turbulence' and nutrient availability; (b) the Mandala of Margalef *et al.*, developed from (a) and including a 'red-tide', or HAB, sequence; (c) Reynolds' distinction of trajectories attributable to autogenic successional processes and allogenic forcing, and the selection of appropriately-adapted (C, S or R) species, which behaviour is influenced more by morphology than phylogeny; (e) ideal year-long trace of the selective trajectory imposed by (temperate) seasonal habitat variability; (f) diminished opportunities in the sea, which is mostly too nutrient- and light-deficient to support phytoplankton growth; (g) top left-hand corner of (f) showing how the main sequence (literally) skirts the edge but, with more nutrients available, trajectories pass more deeply to select more strongly for C-strategist algae.

Metal	Species	Reference
IRON	<i>Alexandrium tamarens</i>	Wells <i>et al.</i> 1991a
	<i>Aureococcus anophagefferens</i>	Gobler 1995
	<i>Chatonella antiqua</i>	Okaichi <i>et al.</i> 1989 Nakamura 1990
	<i>Gymnodinium breve</i>	Ingle and Martin 1971 Kim and Martin 1974
	<i>Gymnodinium sanguineum</i>	Doucette and Harrison 1990, 1991
	<i>Heterosigma sp.</i>	Yamochi 1983; 1989
COPPER	<i>Alexandrium tamarens</i>	Anderson and Morel 1978 Schenk 1984
	<i>Chatonella antiqua</i>	Nakamura <i>et al.</i> 1987 Nakamura 1990
	<i>Gymnodinium breve</i>	Martin and Olander 1971
	<i>Gymnodinium sanguineum</i>	Robinson and Brown 1991
	<i>Prorocentrum minimum</i>	Graneli <i>et al.</i> 1986
SELENIUM	<i>Aureococcus anophagefferens</i>	Cosper <i>et al.</i> 1993
	<i>Chattonella verruculosa</i>	Imai (this volume)
	<i>Chrysochromulina polylepis</i>	Dahl <i>et al.</i> 1989
	<i>Gymnodinium nagasakiense</i>	Koike <i>et al.</i> 1993 Ishimaru <i>et al.</i> 1989
	<i>Pyrodinium bahamense</i>	Usup and Corrales (this volume)
COBALT	<i>Chrysochromulina polylepis</i>	Graneli <i>et al.</i> 1993 Graneli and Risinger 1994

Table 1: Trace metals implicated in the growth of harmful algal species.