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ASSESSMENT OF THE STATE OF EUTROPHICATION IN THE MEDITERRANEAN SEA

FIRST DRAFT

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1. INTRODUCTION

According to the Protocol for the Protection of the Mediterranean Sea against Pollution from Land-based Sources (LBS Protocol) the Contracting Parties shall take all appropriate measures to prevent, abate, combat and control pollution of the Mediterranean Sea Area caused by discharges from rivers, coastal establishments or outfalls, or emanating from any other land-based sources within their territories.

Article 6 of this Protocol stipulates that:

- The Parties shall strictly limit pollution from land-based sources in the Protocol Area by substances or sources listed in Annex II to this Protocol;

- To this end they shall elaborate and implement, jointly or individually, as appropriate, suitable programmes and measures;

- Discharges shall be strictly subject to the issue, by the competent national authorities, of an authorization taking due account of the provisions of annex III to the Protocol.

Annex II to the protocol and specifically item 6 refers to inorganic compounds of phosphorus and elemental phosphorus while item 10 includes substances which have, directly or indirectly, an adverse effect on the oxygen content of the marine environment, especially those which may cause eutrophication.

According to the proposal of the Meeting of Experts for the Technical Implementation of the LBS protocol (December, 1985) which was adopted by the Fifth Ordinary Meeting of the Contracting Parties to the Barcelona Convention (September, 1987), assessment documents should include inter alia chapters on:

- sources, point of entries and amounts of pollution for industrial, municipal and other discharges to the Mediterranean sea;
- levels and effects;
- present legal, administrative and technical measures at national and international level.

The preparation of the present document was entrusted to four experts who have worked extensively on the problem of eutrophication in the Northern Adriatic sea. These are:

- ***Professor R. A. Volleinweider, National Water Research Institute Burlington, Canada;***
- ***Dr. A. Rinaldi, Laboratory "M.N. Daphne", Region of Emilia-Romagna, Italy;***
- ***Professor R. Viviani of the University of Bologna; and***
- ***Professor E. Todini of the University of Bologna.***

The present document is a first draft and is presented to the Joint Meeting of the Scientific and Technical Committee and the Socio-Economic Committee (Athens, 3-8 April 1995) for their comments requesting delegations at the same time to submit, for its improvement, information relevant to their countries especially on existing legal provisions.

The document describes the present state of eutrophication in the Mediterranean sea by addressing the main themes covered by this phenomenon through an accurate analysis of the theoretical aspects as well as a by describing the circumstances, effects and remedies to be applied. It is divided into two parts: the first part which deals with the assessment of the situation and the second part which concentrates on remedial actions and control measures.

The causes and mechanisms affecting the phenomenon are described as well as aspects connected with the aquatic ecosystem and the complex interactions existing in the trophic chain/network. The main nutrients capable of triggering off the process, such as phosphorous and nitrogen, are also considered.

The anthropogenic and natural origins of the various nutrient sources are defined and an estimation of the inputs into the Mediterranean is attempted. This estimation considers all sources: rivers, waste waters from urban and industrial plants, agriculture, atmospheric precipitation and uncultivated land/forests. Earlier reported figures for the total quantity of nutrients in the basin are also up-dated. The extent of these phenomena observed and studied in the Mediterranean is also examined in detail. The phenomenon has been recorded all around the Mediterranean sea and this is apparent from the review of the publications from various countries. In many cases, the increased trophic level observed in coastal areas is creating problems of various kinds for the local inhabitants.

The document also examines the adverse effects on the marine ecosystem as well as on man's activities at sea such as fishing and tourism. The problem is mainly connected to the direct consequence of the presence of algae capable of synthesizing icthyotoxins and the result of the formation of hypoxic and anoxic layers in the bottom waters. The economic repercussions on the fishing and tourist trades depend to a great extent on the degree of deterioration of environmental conditions and the organoleptic alteration of the body of water along the coastline.

The effects on human health conditions are also considered through a detailed examination, not only of cases directly involving the Mediterranean but, given the importance of this subject, also of those affecting the entire planet. A list is given of the various types of toxins that are capable of causing serious diseases in man with information on where and how they are manifested.

After describing the theory behind the eutrophication process and its relevant characteristics the document deals in the second part with the methods of control and remedies which can be applied. It gives guidelines to be followed in monitoring programmes with particular emphasis on the principles and methods to be adopted. Remedies aimed at resolving or at least limiting the effects of the eutrophication process are also set out; amongst these are the following possibilities:

- elimination/reduction of nutrients at source;
- elimination/reduction of nutrients in effluent waters;
- elimination/reduction of quantities of nutrients discharged into the sea (coastal zones).

The chapter on existing national and international legal measures is not complete. It covers only France, Spain and Italy and the European Commission. It is hoped that delegations will provide information so that the revised version will contain the legal action taken by all countries bordering the Mediterranean sea aiming at the protection of water ways (fresh water and sea).

A. ASSESSMENT

2. DEFINITION AND DELINEATION OF THE PROBLEM

2.1 Introduction

The following few basic questions set the main frame pertinent to the problem regardless whether we deal with fresh or marine water eutrophication:

a) What is eutrophication?

b) Where, and under what circumstances is eutrophication a problem; therefore, why do we wish to control eutrophication?

c) How does eutrophication relate to other water pollution problems?

d) What scientific knowledge is required to understand the cause-effect relationships, at the basis of which the selection and the appropriateness of the choice(s) among the available control options can be evaluated to counteract eutrophication in any given situation?

e) What are the key factors that have to be considered in the development of integral management strategies to bring eutrophication under control, where and when control is desirable?

These are all apparently simple questions; yet, they span over a wide range of scientific and non-scientific topics, and over several levels of complexity. Accordingly, what will be said further below cannot be more than a very superficial glance at some main aspects of the problem. Yet, it is important to keep in mind the frame within which the problem has to be visualized.

2.2 Definition of eutrophication

Eutrophication in its most generic definition that applies to both fresh and marine waters, is the process of enrichment of waters with plant nutrients, primarily nitrogen and phosphorus, that stimulates aquatic primary production. Its most serious manifestations are algal blooms (red tides), algal scum, enhanced benthic algal growth, and at times a massive growth of submersed and floating macrophytes (Vollenweider, 1968; 1981). Sometimes, these manifestations are accompanied by, or alternate with cycles of visible bacteria blooms (Aubert, 1988) and fungal developments.[1]

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[1] This definition of the term underscores the nature of eutrophication as a process and a pollutant, dissociating it from its terminological associations. The occurrence of "eutrophic" waters and eutrophic systems can be entirely natural. "Eutrophied" waters have undergone a process of transformation that alters the structure and dynamics of the system - which in its original conditions can even be a "eutrophic" one. The main criterion here is that eutrophication typically leads to an alteration of biotic diversity. At the upper scale of the process diversity normally decreases; at the lower scale, diversity may increase.

Visibly, eutrophication and its side-effects cause discolouration of waters, reduced transparency, unsightliness and disturbance to bathers thus impairing recreation. Dense macrophyte and macro-algae agglomerations chop channels, lagoons and estuaries impairing fishery and navigation, and reducing flow and the holding capacity of freshwater reservoirs, etc.

When aging, the decaying organic material consumes, and in serious cases depletes the oxygen reserve of the water causing an array of secondary problems such as fish mortality, formation of corrosive and other undesirable substances such as CO_2, CH_4, H_2S, NH_3, organoleptic (taste and odour producing) substances, organic acids, toxins, etc.

Sticking algal material and high pH can cause dermatitis and conjunctivitis, and ingestion of algae can cause diarrhoea in sensitive individuals. The development of toxin producing algae in the marine environment, when accumulated in fish, particularly shellfish, is a threat to human health. In fresh waters, toxic blue-greens (cyanobacteria) are a cause of livestock mortality.

2.3 Anthropogenic versus natural eutrophication

Eutrophication as a water quality problem differs from those listed above mainly in the increased difficulty to distinguish the process of eutrophication caused by man from processes and phenomena that may occur also naturally. This difficulty has unavoidably led to some controversial positions within the scientific community about the reality and importance of eutrophication as a water quality problem. Lakes and enclosed marine systems may, or may not become naturally eutrophied by processes of aging. Certain aquatic systems lie in catchment systems, or exhibit morphometric and hydraulic conditions that favour elevated trophic conditions. Climatic variations that cause alterations in the vegetation and soil conditions of the respective catchment basins may lead to alterations in trophic conditions of bodies of water. In the marine environment changes in current and upwelling patterns due to climatic variations may also cause changes in the local and regional trophic state of waters. However, such variations may act in either direction, i.e. leading either to eutrophication, or to oligotrophication.

Still, it is to be noted that the time scale at which the natural processes operate, is substantially different from that of man induced processes. The time scale of natural processes is at least in the order of centuries, and mostly longer, while anthropogenic eutrophication occurs at time scales of decades and less. Even with this qualification, the distinction between natural and anthropogenic causes of eutrophication is not always clear cut.

2.4 Delineation of the problem of eutrophication versus other forms of pollution

Eutrophication is but one form of water quality problems. Other water quality problems result from pollution of waters with heavy metals, from inorganic, organic and microbiological pollutants in urban, agricultural and industrial sewage; from agricultural run-offs that also contain herbicides and pesticides, from industrial effluents and discharges and other losses such as oil and petroleum residues, radioactivity and heat in thermal effluents; from acid precipitation, and not least from simple litter (Waldichuk, 1987; Coté, 1989).

Pollution of this sort, though not being its main cause, may directly or indirectly enhance or counteract eutrophication. All the respective interactions and interconnections are not fully understood yet, but should not be ignored as a potential though minor cause of eutrophication.

2.5 Potential impact of eutrophication on human and ecosystem health

Among the problems of perhaps widest concern in the marine environment is the apparent spread and increase in frequency of algal blooms of toxic algae involved in causing paralytic and diarrhetic shellfish poisoning (PSP and DSP, respectively, produced by saxitoxin and other toxins in certain dinoflagellates and chrysophyceae), both already known for some time, and the appearance of new forms previously unknown or ignored such as amnesic shellfish poisoning (ASP) produced by domoic acid in diatoms (cf. e.g., reviews by Aubert, 1992; Viviani, 1992).

Many questions about sea-born toxicity in humans remain yet open. e.g., ciguatoxin is now recognized to be produced by a dinoflagellate species and transmitted along the food-chain to ciguatera producing fish. Yet, it is not clear whether eutrophication has any bearing on such phenomena. Still, the mechanisms involved in, and the conditions necessary for toxin formation perplex scientists; also the fact that in recent years less known algal species, as it seems, have become producer of toxic red tides (Okaichi et al., 1988), and/or that toxicity can result quite severe at relatively low algal concentrations. This has been born out with the *Chrysochromulina* incidents in the Skagerrak and Kategat in 1988/89 causing fish and bottom fauna kill (Rosenberg et al., 1988).

While this, and other cases suggest algal toxin to be involved in fish and bottom fauna mortality - though fish and bottom fauna kills are usually caused by anoxia -, it remains a matter of contention how far eutrophication, and the presence of algal toxins in particular, is responsible for the mass death of sea mammals. There is evidence, however, that the dolphin kill offshore the American East Coast in 1987/88 was linked with the outbreak of the brevetoxin producing algae *Ptychodiscus brevis*, and that the toxin was transmitted to dolphins along the food-chain.

Regardless of such uncertainties eutrophication that impairs shelf fishery, fish farming and aquaculture, has indeed become a serious concern worldwide (cf., e.g., "Red Tide Newsletter", vol 1 and 4).

Localised anoxia in shelf regions, estuaries, lagoons and fjords is usually the consequence of eutrophication, but can also be caused by discharges of untreated urban and industrial sewage rich in organic substances. On the other hand, the connection between eutrophication and the phenomenon of massive production of mucilage (marine snow), which in the summers of 1988 and 1989 covered a large part of the Northern Adriatic Sea (Cognetti, 1989), has still to be established.

2.6 Beneficial aspects of eutrophication

Although certain phenomena of red tides may be natural, it is to be noted that man-made eutrophication is not necessarily negative but with limits can even be beneficial. Indeed, without a certain level of primary production, fish production would remain low (Kerr and Ryder, 1992). Filter feeders, like shellfish that utilize phytoplankton directly, will benefit from modest algal blooms. Increases in zooplankton and bottom fauna due to increased food supply will be transmitted to higher trophic levels augmenting commercially valuable fish stocks. These beneficial aspects of moderate eutrophication are not in question here. What is in question is eutrophication becoming a nuisance, i.e., when its manifestations begin to affect activities like fishery and aquaculture, in particular of commercially valuable species, and/or begin to interfere with other water use such as recreation (bathing, water sports, tourism), and become a direct or indirect threat to human health.

It is to be noted that aspects regarding the balance and breaking point between beneficial and deleterious effects of eutrophication have not been well studied and documented yet. Also, there is a lack of what could be termed a kind of benefit/damage scale that would permit to evaluate in more objective terms the results of eutrophication.

2.7 Adriatic eutrophication in context of marine eutrophication worldwide

While the process of eutrophication in fresh waters has a history dating back to at least the last century, widespread phenomena of eutrophication in the marine environment are more recent, though both, fresh and marine eutrophication has been accelerated in the aftermath of growing urbanization, intensification of agriculture, and industrial development generally following World War II. Estuaries and Inland Seas and marine basins of but limited hydraulic exchange have been first affected; yet, as has been reported by many authors (Gray and Paasche, 1984; Rosenberg, 1985; Okaichi et al., 1987; IOC, 1988; Forsberg, 1991; Dederen, 1992; Vollenweider, 1992, a.o.), coastal marine eutrophication has increased in many parts of the world. Eutrophication in the Mediterranean (UNESCO, 1988) makes part of this worldwide evolution of the problem.

3. CAUSES AND MECHANISMS OF EUTROPHICATION

Causes and mechanisms of eutrophication have to be evaluated within the context of our scientific knowledge about the structural and dynamic properties of aquatic ecosystems, and the metabolic processes that govern them. Indeed, the process of eutrophication (as well as the reverse process of oligotrophication) represents merely a particular aspect of the aquatic ecosystem dynamics. To set the frame work about marine eutrophication, some of the relevant facts about aquatic ecosystems are summarized in the following sections. Appendix I deals with the methodological questions concerning the measurement of biomass in the aquatic environment.

3.1 Structure and compartments of aquatic ecosystems

Aquatic ecosystems do not differ essentially from terrestrial ones as to structure. The main difference between the two kinds of systems lies in the difficulty to allocate single aquatic ecosystems to major geographic complexes, such as biomes, which is practically impossible. This does not mean there is no geographic differentiation between aquatic ecosystems, but their distinctive properties refer to the compositional make-up of secondary producers, rather than to that of the primary producers. Many primary producer species, particularly phytoplankton, exist over large geographic areas, while the natural area of many secondary producers is confined.

Simplifying the actual structural complexity of aquatic ecosystems, their components belong, in principle, to one of the four interrelated main compartments:

a. Primary producers
b. Secondary producers
c. Mineralizer / Detritus
d. Nutrient pool

3.1.1 Primary producers

This compartment encompasses all those species called autotrophic that build their biomass from inorganic nutrients and utilize for the synthesis of organic matter either radiant energy or the energy of inorganic chemical reactions. Chlorophyll that acts as energy capturer is found in all photo-autotrophic ('green') plants; specific accessory pigments, such as xanthophyll, phaeophytine, rhodophytine, etc. occur in certain major plant divisions only.

Cyanophyta, or cyanobacteria, the common blue-green algae (now united with bacteria under the division of Procaryotes because of their primitive nuclei) are for the most photo-autotroph. Some species can utilize, like do other specialized chemo-autotrophic bacteria, chemical energy.

Some of the otherwise photo-autotrophic algae are facultative heterotrophic; i.e., they can (a) either uptake water dissolved organic compounds (e.g., sugars) and utilize the stored energy, or (b) ingest organic particles, including living cells, as do some dinoflagellate species.

Algae (micro- and macro-algae)[2] are the most important representatives of photo-autotrophic aquatic primary producers. Phytoplankton of both, fresh and marine waters is an assembly of freely buoyant micro-algae (size in the sub-millimetre range), the movement of which is largely controlled by horizontal and vertical water motions. Depending on shape and whether the phytoplankton species are unicellular or form colonies, their size ranges from about 1 to 500 (<1000) µm in diameter or length, and volumes from about 2 to 10^5 ($<10^6$ to 10^7) μ^3.

There are several thousands of algal species, but typically only a few hundreds are found with some frequency in phytoplankton. Of these, a few dozen make up the bulk of the biomass. Taxonomically, phytoplankton recruit from both, prokaryota and eukaryota; for classification, the prevailing pigment composition and primary and secondary synthesis products, which are specific for various classes and their subdivisions, are of key importance. Because knowledge about elemental composition, biochemical properties, and metabolic specificity down to the species level is of considerable significance in the present context taxonomic references will be unavoidable (cf. Chpt 5, 6 and 7). Algal species involved in eutrophication may come from almost any of the classes listed, but the more noted nuisance species recruit from either Cyanophyceae (Cyanobacteria), Chrysophyceae, Prymnesiophyceae, Bacilariophyceae (diatoms), Dinophyceae (dinoflagellates), and Chlorophyceae. Diatoms, green algae (chlorophyceae), and blue-greens (cyanobacteria) are dominant in fresh waters, while in marine waters diatoms, dinoflagellates and calcareous nanoplankton (e.g. *Emiliana huxleyii* mostly in tropical warm waters) prevail.

Microalgae are also important constituents of periphyton communities of littoral rocky zones, or attached on other algae and macrophytes, or living as submersed benthic community on mud. Their size range is more variable, however, and can exceed the mm range. Vis-a-vis phytoplankton, periphyton is less important in deep waters, but in more shallow waters periphyton can make up a substantial fraction of the primary producers.

With the exception of a few species (*Chara, Nitella*), macro-algae are lacking in fresh waters, but in the marine environment many green, red and brown algae species are important macro-components of littoral, sublittoral benthic, and free floating communities (e.g., Chlorophyceae: *Ulva*, *Cladophora*, *Caulerpa*, a.o.; Rhodophyceae: *Chondrus*, *Gigartina*, *Gracilaria*, *Polysiphonia*, *Delesseria*, a.o.,; Phaeophyceae: *Fucus*, *Sargassum*, *Cystosira*, *Laminaria*, *Macrocystis* , a.o.).

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[2] **Note:** Algae is not a taxonomic term

A significant number of macrophyte species that recruit from many families inhabit humid environments, i.e. wetlands such as swamps, marshes, reeds, which are vital habitats for water fowl, spawning grounds for anadromous fish, and other animals closely associated with the water milieu. Macrophyte communities are important primary producers in and around estuaries and lagoons, as well as in sublittoral seacoast beds that receive sufficient light to make photosynthesis possible. Among typical marine species that form extended submersed communities are: *Zostera*, *Posidonia*, *Cymodocea*.

3.1.2 Secondary producers

This compartment includes all those species called heterotrophic that need for their metabolism and survival organic substances acquired in the form of food through predation on other species. Secondary producers in the marine environment derive from all phyla and classes of the animal reign covering a very wide range of biotypes, habitat occupancy, food preference, etc.; fresh waters are less rich in this respect. This wide variety makes it difficult to characterize the category 'secondary producers' in a succinct way. Body size is among the more significant criteria (Peters, 1983). Characteristic size class spectra that encompass the whole range of biotypes present in aquatic communities reflect fundamental structural properties of aquatic systems generally (cf. below).

Plankton (that operationally includes both, zoo- and phytoplankton, and hence, is not a term referring to secondary producers only) has been typified as: (a) net plankton with two subcategories (a.1) mesoplankton (>200 µm; mostly zooplankton), (a.2) microplankton (200-64 µm; includes also most of the larger phytoplankton), (b) nanoplankton (<64 µm). This division that historically came about from the mesh size of nets commonly used, is somewhat arbitrary. A more recent classification, (Sieburth, 1979), which covers all biological categories, i.e. zooplankton, phytoplankton, bacteria regardless of trophic type, is based on a logarithmic scale and distinguishes between (a) mesoplankton (>200 µm), (b) microplankton (200-20 µm), (c) nanoplankton (20-2 µm) and (d) picoplankton (2-0.2 µm).

Actively swimming organisms of larger size (cm range and up) are generally categorized as nekton, but many of the smaller species, both phyto- and zooplankton, have the ability of active locomotion.

As regards food preference species are categorized as herbivores, carnivores, and omnivores. These categories are somewhat artificial either, as not all types of secondary producers can be allocated unequivocally to this scheme: e.g., detritus feeders, which fulfil an important role in both, aquatic and terrestrial ecosystems. 'Herbivorous' plankton feeders obtain their feed from living phytoplankton (some species seems to be able to be selective in their choice), but also from the large pool of organogenic seston. Also, it has been claimed that some species cover part of their energy need by osmotic uptake of dissolved organic substances.

Secondary producers may also be characterized by the mode of feed acquisition: (a) species showing 'raptorial feeding' (active or passive random encounter between predator and prey, most carnivorous species), (b) 'filter feeding' species concentrating their food by appropriate sieving mechanisms (e.g., certain cladocera and copepoda species like *Calanus*, mollusc, etc.), and (c) 'diffusion feeding' (accidental collision and sticking). Of particular importance also in terms of eutrophication are species of the second category, because the kind and size of the feed they can retain depends on the fine structure of their filter system.

Marine Bottom fauna is not less diverse in species composition, habitat and food requirements, and mode of food acquisition. Many crustaceae, echinodermata, certain gastropods, a.o. are voracious raptorial feeders. Coelenterata and bivalve molluscs are among the most important filter feeders in the marine environment. Worms are detritus feeders, etc.

Others: There are few herbivorous fish species; others are omnivorous, or species that change their food preference from young to adults. Forage fish like sardines, anchovies, herrings serve as food for secondary predators like cod, sea bass, which in turn feed on zooplankton, or on mollusc, worms, eggs, etc. Sharks, but also mammals like porpoise feed on intermediate size class fish, while e.g. Baleen whales filter mesoscale zooplankton.

Birds are the most important biological links between bodies of waters and their surroundings. Some birds species are herbivorous feeders on submersed macrophytes, while raptorial birds feed on fish.

3.1.3 Mineralizers

Mineralizers are mostly bacteria, but also fungi and protozoa that ingest and metabolize organic detritus. The products of the mineralization processes are dissolved organic intermediates and inorganic end products, such as phosphate ions, and ammonium, sulfide ions, etc., which in the presence of oxygen are microbiologically oxidized to nitrate and sulphate.

3.1.4 The nutrient pool

The nutrient pool consists not only of the water dissolved inorganic and organic compounds that are utilized by primary producers, but also of the nutrient stock stored in sediments, which under appropriate conditions may become mobilized. In deep, oxygen rich offshore waters, the importance of nutrient mobilization from sediments vis-a-vis the importance of the water dissolved nutrient stock is normally negligible; yet in more shallow waters, depending on geomorphological and hydrodynamic conditions, on overall productivity, and on the degree of oxygenation of waters near the water-sediment interphase, mobilization of the nutrient reserve bound in sediments may be significant in the overall local and regional nutrient budget.

3.1.5 Concatenation: Food-chain versus food-web

Summing up the previous paragraphs, the different components and compartments (or trophodynamic levels) of an ecosystem are thought of as linearly linked transfer chains of material and energy, i.e., food-chain

```
+ENERGY===>
               I
               I
 PRIMARY PRODUCERS ===> HERBIVORES ===> CARNIVORES-1 CARNIVORES-2
               ^                    I                     I                  I
               I                    I                     I                  I
     NUTRIENTS   <==== MINERALIZERS  (microbial loop) <====
               ^                    I                     I                  I

               I                    I                     I                  I

               I                    =====> -energy        =====>-energy     =====>energy
 (External Input)
```

Because of incomplete transfer from one compartment to the next, the total biomass (standing crop) in each subsequent compartment should be smaller than that of the preceding one. The superposition of the compartments forms, what is called, a biomass-pyramid. The biomass-pyramid of marine systems stretches over about 7 logarithmic size classes, i.e., from micrometers to decameters. This conceptual scheme is often the basis for food-chain simulation models.

While this conception is correct within very broad terms, it is incorrect in some details. First, the structural and functional relationships at the lower trophodynamic levels appears to be more intricate than depicted in the general scheme given above. The primary production level is surely the most important energy input compartment in both, terrestrial and aquatic self-sufficient ecosystems. Yet, the environment in which this compartment is embedded in pelagic systems, appears to be that of a functionally related network of autotrophic and heterotrophic activities. Excretion, recycling of organic and inorganic substances, resorption and phagotrophy is particularly intense at this level. Accordingly, the concept of a sequential relationship, as depicted in the linear model, has to be supplanted by a more diverse one that includes additional levels arranged as a net of relationships (for more details cf. e.g., Fenchel, 1988).

Second, at higher levels where the situation seems to be more clear, the unequivocal allocation of species to well defined categories is not straight forward either. Food preference of species varies with availability, and in some species can be very complex, depending also on age. Juvenile stages of some species may be herbivorous, while adults may be carnivorous. Symbiotic relationships between species belonging to different taxonomic groups are numerous in the marine environment ranging from loose associations to strictly bonded unions; to mention e.g., symbiosis between Zoantharia and zooxanthellae. Structurally, then, an ecosystem does not function as a linear sequence, i.e., as a food-chain but rather as a food-web.

Third, the concept of biomass pyramid of decreasing compartment size may apply to terrestrial ecosystem, but hardly describes aquatic systems correctly. For oligotrophic, more or less self-contained oceanic steady state systems it has been estimated that the compartmental biomass amounts of the logarithmically ordered size class spectra, ranging from bacteria to whales, are in the same order of magnitude, i.e., 0,1 to 0,01 g/m^3 (Sheldon et al., 1972; Kerr, 1974; Platt and Denman, 1977). Numbers of individuals/volume in each size class tend to decrease inversely proportional to body weight.

This model does not necessarily apply to more eutrophic systems. The highly dynamic nature of such systems, particularly those plankton dominated (high rotation at the primary input level where seasonal inputs of nutrients cause large population oscillations; phase displacement between compartments because of increasing mean live-span of the component species in sequential compartments; top-bottom down control, etc.) produces the most varied combinations of biomass present in the various compartments, both in time and space.

On the other hand, in order to maintain the system, the total energy input at the primary production level, integrated over time, must exceed the sequential energy transfers to successive compartments (energy cascade; Odum, 1971), regardless of the variations of the momentary compartmental biomass spectra. This condition may not hold, however, if the system receives a substantial fraction of allochthonous organic material (which either may be imported e.g., by advection from other areas, or by discharge from land) that can directly be utilized by species of higher trophodynamic levels.

3.2 Aquatic ecosystem metabolism

The metabolic processes that are at the basis of aquatic ecosystem dynamics are undoubtedly quite complex. Still, in terms of mass transformations, they are governed by relatively simple stoichiometric principles, on the basis of which these processes can be quantified and correlated. Central to these processes is the role of biomass that presupposes at least a cursory knowledge of its the chemical composition.

3.2.1 Elemental composition of bioseston (primarily phytoplankton, zooplankton, bacteria)

Depending on the point of view, the elemental composition of sestonic biomass can be listed under three or four main groups:

(1) C, N, H, O, P, S;
(2) Ca, Mg, K;
(2a) Na, Cl;
(3) Fe, Mn, Co, Zn, Mo;
(3a) Cu, B, V;
(4) Si; Al; others;

Group (1) comprises those elements that make up the bulk of the main cell constituents: proteins, carbohydrates, lipids, DNA, RNA, etc. Group (2) lists the so-called 'antagonists' that are required to various degree in cell metabolism. Group (3), commonly known as micro- or trace-elements, contains elements that are mainly connected with catalytic reactions involving enzymes. Group (4) are elements that normally do not seem to have any specific structural or metabolic function except silicon, which is a necessary component of diatom cell walls.

The percent ash content (i.e., the residue remaining after ignition at 400 C^{o}) of sestonic biomass varies considerably depending on species composition. The ash content of diatoms may be as high as 40% and higher, but the ash content of other phytoplankton species is normally less than 15-20%. "Ash free biomass" is roughly composed of 45-55% carbon, 8-10% nitrogen, 7-8% hydrogen, 30-35% oxygen, and 1-2% sulphur and phosphorus, while trace elements generally make up less than 1%.

The chemical composition of zooplankton is similar to that of phytoplankton, but because of a certain fraction of nitrogen being bound in chitin, the nitrogen fraction tends to be slightly higher. Conversely, organogenic (dead) detritus may be deficient in phosphorus.

On average, carbon, nitrogen, phosphorus and sulphur found in marine bioseston approximate an atomic proportion of 106 : 16 : 1 : 1 (41 : 7.2 : 1 : 1 by weight), the so-called Redfield Ratio. In combination with figures given above, this may be expressed as

$$C_{106}N_{16}H_{180}O_{44}(PO_4)SH,$$

which represents a kind of molar composition of biomass. Of course, as the percent elemental composition of specific samples varies depending on the species, and on the relative amounts of proteins, carbohydrates, lipids, and other compounds present in cell material (cf. Vollenweider, 1985), this formula has to be taken *cum grano salis*; still, as a reference it has proven to be quite useful (cf. below).

3.2.2 Metabolic processes

3.2.2.1 Primary production and biomass formation

Primary production is defined as the process by which organic matter is produced from dissolved inorganic substances. This process requires energy that either comes from radiation (light), or from inorganic chemical reactions. Considering the magnitude of utilization of light as energy source by organisms (plants containing chlorophyll), the process involving chemical reactions is of minor importance, and can be ignored in the context of the objectives of the present report. Chemodynamic autotrophic processes are the only ones to maintain deep sea hydrothermal-vent communities.

Photoautotrophic processes are secluded to occur in the euphotic zone, which extends to the depth at which light intensity is reduced to about 1% of the normal surface irradiation in the wave length range of between 400 and 700 nm. Light attenuation in water is an exponential function measured by the so-called attenuation coefficient, §. Accordingly, the depth of the euphotic zone, z_{eu}, equals 4.6/§. In the most transparent oceanic offshore waters z_{eu} may extend to more than 200 m. Normally, z_{eu} is much less, and in turbid inshore waters may be only a few meters, and even less. Light attenuation in water is higher, not only due to mineral turbidity and dissolved organic substances, such as humic substances, but also due to the presence of biota, particularly algae. The cross-sectional attenuation coefficient relative to chlorophyll present in planktonic algae is in the order of 0.01 to 0.02 m^2/mg Chl a. These relationships, in connection with the extent and frequency by which periods of density stratification alternate with cycles of deep water mixing, have a bearing on the degree single bodies of waters are affected by the process of eutrophication.

Photosynthesis, i.e. the formation of sugars and polysaccharides from CO_2 (carbon dioxide) and water, is the initial process in primary production. This reaction is expressed in formal stoichiometry as:

$$106\ HCO_3^- + 106\ H_2O \xrightarrow{(+\ energy)} \underset{(sugar)}{106\ C_6H_{12}O_6} + 106\ O_2 + 106\ OH^- \qquad \text{Eq.(1)}$$

Photosynthetic quotient PQ = mol O_2 / mol CO_2 = 106/106 = 1

Accordingly, the incorporation of 106 molecules of carbon dioxide is accompanied by an equal number of 106 oxygen molecules[3] liberated; i.e. the photosynthetic quotient PQ equals 1. This oxygen accumulates in the water, and if the process is very intense, as in eutrophic waters with algal blooms, waters become supersaturated in oxygen, and pH increases.

Phytoplankton biomass formation. In reality the reaction described in equation (1) is much more complex, and proceeds over various component reactions. Chlorophyll plays the role of light energy captor and transmitter, and phosphorus bonds (e.g, the ADP-ATP system acts as transient energy repository that in connection with the NADP-NADPH redox system fuels important reactions involved in cellular metabolism and the synthesis of proteins and other cell components). This explains the key role of phosphorus in eutrophication.

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[3] For consistency with subsequent arguments the molar figure 106 has been used; any integer, also 1, could be used for the purpose.

Despite the complexity of the reaction pathways involved in biomass formation, it is possible to reduce this process to a few simple summary equations. Unlike equation (1) that only refers to carbon, hydrogen and oxygen, these equations include at least three other essential elements, N, P, S. Their proportions relative to carbon are given according to the Redfield Ratio. Also, the equations are written to apply to marine rather than to fresh water. The following two equations differ depending on whether the source of nitrogen is nitrate (NO_3) or ammonium (NH_4).

a) <u>Nitrate as nitrogen source</u>:

$$106\ HCO_3^- + 16\ NO_3^- + HPO_4^{2-} + SO_4^{2-} + 100\ H_20 \ <==> \qquad \text{Eq.(2a)}$$

$$C_{106}N_{16}H_{180}O_{44}(PO_4)(SH) + 150\ O_2 + 126\ OH^-$$

PQ = 150/106 = 1.42

b) <u>Ammonium as nitrogen source</u>:

$$106\ HCO_{3-} + 16\ NH_4^+ + HPO_4^- + SO_4^{2-} + 52\ H_20 \ <==> \qquad \text{Eq.(2b)}$$

$$C_{106}N_{16}H_{180}O_{44}(PO_4)(SH) + 118\ O_2 + 94\ HO^-$$

PQ = 118/106 = 1.11

Both processes lead to pH increases; yet, with prevailing uptake of nitrate the pH increase would tend to be larger than with prevailing ammonium uptake. Further, eqs. (2a) and (2b) result in (apparent) PQ values that exceed unity[4] 1. Accordingly, by using appropriate techniques to measure the carbon input and the oxygen evolution simultaneously, one would have a way to evaluate whether nitrates or ammonium is the prevailing nitrogen source. In reality, the matter is less straight forward. Apparent PQ found in the marine environment may be as high as 1.8 and higher (Platt and Denman, 1977), which makes simple interpretation difficult.

3.2.2.2 <u>Mineralization of bioseston</u>

Mineralization of biomass that occurs after cell death in part by simple lysis, but mostly by microbial activity, is basically the reverse process of biomass formation. Here, the main distinction that has to be made rests on whether the process takes place under aerobic, or anaerobic conditions.

a) Aerobic mineralization of bioseston can take place already in the euphotic zone, but occurs mostly in deeper waters after sedimentation of biogenic detritus. As indicated with double arrows eqs.(2), can be read in reverse. Under natural conditions, total

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[4] **Note:** There is a more subtle difference between eq.(1) and (2), however. Because chemical energy is required for biomass formation that is accompanied by a certain metabolic heat loss, the amount of energy captured and stored corresponding exactly to eq.(1) would not be sufficient to satisfy the total energy requirements of eq.(2a) and (2b).

mineralization occurs essentially in two steps: first according to eq.(2b) in which ammonium is the end product; ammonium is then oxidized to nitrate over two follow-up reactions (cf. below).

If mineralization occurs mainly in the euphotic zone, one can speak of recycling, meaning that the mineral components released are newly available for re-incorporation into new biomass.

b) Anaerobic mineralization, instead, occurs under anoxic conditions, or at very low oxygen concentrations, mostly in deep waters. In this case, there exists no simple inversion corresponding to eqs.(2). The end products of mineralization under severe anoxic conditions are but only in part identical with the original mineral components, which may result in vital nutrient losses to the system.

Among the various possibilities there are two variants of particular interest to the theme, the first one when nitrates are still available, the second when nitrates are absent. Nitrates, though being weaker oxidants than oxygen, are next in the redox-chain. One of the possible reaction patterns is described with eq.(3a).

a) Nitrates available:

$$C_{106}N_{16}H_{181}O_{44}(PO_4)(SH) + 93\ NO_3^- \implies \qquad \text{Eq.(3a)}$$

$$106\ HCO_3^- + 16\ NH_4^+ + HPO_4^{2-} + SH^- + 46.5\ [N_2]_{gas} + 5H_20$$

b) Nitrates absent, sulphates available:

$$C_{106}N_{16}H_{180}O_{44}(PO_4)(SH) + 58\ SO_4^{2-} \implies \qquad \text{Eq.(3b)}$$

$$106\ HCO_3^- + 16\ NH_4^+ + HPO_4^{2-} + 59\ [SH_2]_{gas} + 24\ OH^-$$

The meaning of eqs.(3a) and (3b) are that nitrogen is likely lost in the mineralization process as nitrogen gas, and if nitrates are used up, sulphates are reduced to hydrogen sulphide.

c) Under slightly less reducing conditions the following reaction may also take place

$$C_{106}N_{16}H_{180}O_{44}(PO_4)(SH) + 66\ H_2O + 34\ OH^- \implies \qquad \text{Eq.(3c)}$$

$$16\ NH_4^+ + HPO_4^{2-} + 48\ HCO_3^- + 58\ [CH_4]_{gas} + [SH_2]_{gas}$$

Accordingly, carbon would be lost in the form of methane, yet only as long as hydrogen sulphide does not inactivate the microbiologically mediated process.

The processes described by eq.(3a) take place primarily in boundary layers between oxic and anoxic waters, and those described by eq.(3b) and eq.(3c) near and in anaerobic sediments. Depending on the particular circumstances under which they occur, these processes may also take place in parallel. Also, other metabolic pathways may be followed, such as processes of fermentation involving as intermediary products alcoholic compounds and/or organic acids.

Despite of their cursory nature the equations presented can be used to estimate, at least approximately, the quantities that are involved in biomass formation and mineralization. For example, with a %-content of carbon in biomass of about 50%, mineralization of 1 g of ash free particulate organic matter, according to eq.(2b), would require about 1.5 g of oxygen (end product of nitrogen = NH_4^+); complete oxidation 1.9 g O_2/g., or 3 and 3.8 g O_2 /g C, respectively; etc. Strong experimental deviation from these figures would indicate some particular situation, e.g., incomplete mineralization.

3.2.2.3 Generalization

The processes described above can be considered summarily as oxidation-reduction reactions. The sequence with which the single reactions take place is regulated by their relative equilibrium position along the redox scale. Yet, some of the possible reactions are biologically catalyzed. With regard to the energies involved, reduction processes require energy, oxidation processes produce energy that can be utilized in further reactions.

Carbon. The first part of the generalized reaction of carbon species,

$$CO_2 \Longleftrightarrow H_2CO_3 \Longleftrightarrow CH_4$$

is regulated solely by chemical equilibria and pH, whereas in the second one biomass is converted to methane by bacteria.

Nitrogen. The sequence is complicated by intermediate nitrogen species (NH_2OH, NO, N_2O), while the pathway to N_2, in principle, leads over NO^{2-}.

$$\begin{array}{ccccc} NO_3^- & \Longleftrightarrow & NO_2^- & \Longleftrightarrow & NH_4^+ \\ & & | & & \\ & & N_2 & & \end{array}$$

Denitrification of NO_3^- to N_2, that is coupled with the respiration of organic substances (mediated by bacteria, such as *Pseudomonas*, *Bacillus*, *Micrococcus*, a.o.), can be formulated as follows:

$$4\ NO_3^- + 5\ (HCOH)_x \Longrightarrow 2\ N_2 + 5\ CO_2 + 3\ H_2O + 4\ OH^-$$

and,

$$4\ NO_2^- + 3\ (HCOH)_x \Longrightarrow 2\ N_2 + 3\ CO_2 + H_2O + 4\ OH^-$$

Oxidation of NH_4^+ in the presence of oxygen and organic substances may be incomplete and also lead to N_2 i.e.,

$$4\ NH_4^+ + 6\ O_2 + 3\ (HCOH)_x \Longrightarrow 2\ N_2 + 3\ CO_2 + 4\ H^+ + 9\ H_2O$$

Denitrification is probably of lesser importance in marine offshore waters, but can make up an important loss of biologically available nitrogen to primary producers in hypoxic and anoxic eutrophic coastal waters and lagoons.

Complete oxidation of ammonium to nitrates occurs in two steps, the first yielding nitrites (mediated by *Nitrosomonas*),

$$NH_4^+ + 3/2\ O_2 ==> NO_2^- + 2\ H^+ + H_2O$$

Nitrites are then oxidized to nitrates (mediated by *Nitrobacter*),

$$NO_2^- + 1/2\ O_2 ==> NO_3^-$$

Nitrogen fixation, i.e. the incorporation of N_2 into biomass directly, is carried out by only a few species, such as *Azotobacter*, and certain cyanobacteria. Nitrogen fixing cyanobacteria are most important in fresh water eutrophication, but nitrogen fixing species occur also in the marine environment.

Figure 1 shows the principal features of the nitrogen cycle of the reactions described above.

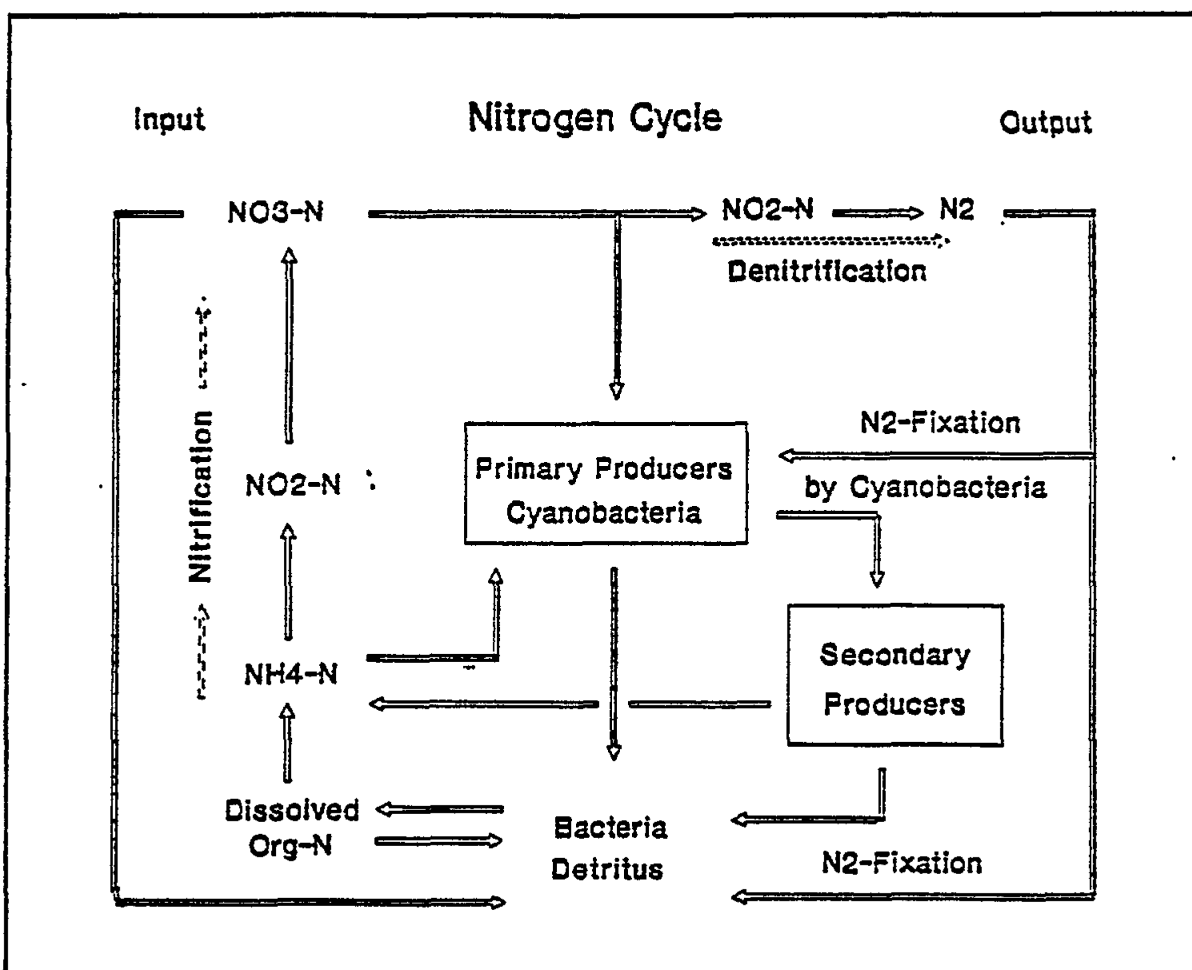

Fig. 1 The main features of the nitrogen cycle

<u>Sulphur</u>. Besides nitrate ion, sulphate ion is another strong oxidant. In the process, sulphate is reduced according to

$$SO_4^{2-} \Longleftarrow\!\!\!= [S] \Longleftarrow\!\!\!= S_2^-$$

or,

$$SO_4^{2-} + 2\,H_2O \Longleftarrow\!\!\!= SH_2 + 2\,OH^- + (2\,O_2)$$

Sulphide ion reacts easily with bivalent iron to form FeS_2. The presence of hydrogen sulphide always indicates highly reducing conditions. Hydrogen sulphide is not produced as long as nitrate is available. Sulphide ion is rapidly oxidized in the presence of oxygen, but likely not by nitrate reduction-denitrification.

Iron. The redox reaction of trivalent-bivalent iron, i.e.

$$Fe^{3+} \Longleftarrow\!\!\!= Fe^{2+}$$

plays an important role in regulating PO_4 availability. Trivalent iron oxide-hydroxide complexes that are formed under oxic conditions adsorb multiple amounts of PO_4 ions. The oxidized iron flocs that are deposited on sediments form a barrier layer, which prevents the return flow of phosphorus to supernatant waters. Under reducing conditions this barrier is broken down, and phosphorus, as well as that fraction of iron that does not eventually become bound as iron sulphide, can move freely and return to the water phase. This process is known as phosphorus release from sediments. Its magnitude varies, but under anoxic conditions it can be as high as 10 mg P/m^2.day.

3.2.2.4 Kinetics aspects

Among the kinetic aspects mention should be made of the rules that govern nutrient uptake and growth kinetics in phytoplankton. Nutrient uptake is regulated by Michaelis-Menthen kinetics, according to which the uptake rate, μ, is proportional to the concentration at low nutrient concentrations, while rates reach an upper limit, μ_{max}, with increasing concentrations. Mathematically, this is expressed as

$$\mu = \mu_{max}\ M/(k_M + M),$$

M being the concentration of the nutrient in question (nitrogen, phosphorus, silica, etc.), and k_M the half-saturation constant.

Growth is regulated in a similar fashion. At concentrations of $M << k_M$, growth is limited by the nutrient in question; conversely, if $M >> k_M$, growth is not limited by the said nutrient. However, under natural conditions, growth, and accordingly yield, will simultaneously depend on several nutrients, as well as on other factors (light, temperature), that act in concert. The combination of all the factors, and the species specific genetically determined maximum growth rate, regulate the potential and actual growth of phytoplankton. Accordingly, some species may be able to produce algal blooms within a few days, while the growth of other species always remains modest.

Michaelis-Menthen kinetics and corresponding growth relationships are important concepts used in mathematical simulation models. Pertinent treatments of this subject are to be found e.g., in Chapra and Reckhow (1983), Strasskraba and Gauch (1983), Jørgensen (1988), a.o.

3.3 Relevance to eutrophication

Eutrophication stimulates primary production, i.e. aquatic plant life (algae and macrophytes) as defined in the previous chapter. The processes that lead to visible discolouration of waters and algal blooms, etc., are complex, and have to be understood in the context of marine processes, i.e., the interactions between abiotic and biotic factors that control them.

3.3.1 Conceptual and analytical difficulties

In most circumstances biological production is controlled simultaneously by several factors that vary in a spatial-temporal setting. The continuing variation of physical factors such as light, temperature, water motion, stratification and mixing, etc., in combination with variations in nutrient availability create a rich resource spectrum for algal growth (Harris, 1986). Also, the conditions that control phytoplankton growth at the micro-scale level may differ from those that determine the overall production capacity of the system at the macro-scale (Gray and Paasche, 1984). The sense of this becomes immediately intelligible if one examines the patchiness of algal blooms: they normally occur in non-continuous spatial-temporal packages distributed over areas of various extension and temporal duration. Accordingly, inferences made about causes that promote algal blooms become contingent on such circumstances, as well as on the spatial-temporal scale of interest (Harris, 1986).

The hierarchical aggregation of levels of complexity presents not indifferent methodological difficulties to the investigator who for practical reasons has to define what factor determines the system at the macro-level. It must be born in mind that measures aimed at controlling eutrophication apply to this level, regardless of what mechanisms might be operative at the micro-level. These difficulties can only be overcome by appropriately designed long term studies that embrace both, consistent monitoring and experimentation at various scales of temporal and spatial resolution. Short term studies are indicative, at best, worst, they may not only be useless but become entirely misleading.

3.3.2 General conditions for algal growth

Nutrient poor, i.e., oligotrophic waters cannot sustain massive algal blooms and macro-algae agglomerations for any length of time. Therefore, the question is not whether nutrients are involved in causing eutrophication, but which ones are mostly responsible for the phenomenon. Nitrogen and phosphorus species have been recognized for a long time to limit production in most circumstances. Still, certain micro-elements involved in enzymatic reactions such as Fe, Mn, Co, Mo, a.o., may be at times in short supply. Interestingly, some dinoflagellate species that are of concern in red tides seem to require selenium. Many algal species also require vitamins (biotin, thiamin, B_{12}) either singly, or in various combinations (Provasoli, 1963). Variation in the availability of micro-elements and vitamins, and perhaps other organic compounds such as chemical mediators (Aubert, 1990) have a likely bearing on species selection.

For predicting algal blooms and species composition realistically it is important to understand the interactive role of these factors in production dynamics thoroughly. At present we are still far from this goal. On the other hand, the number of algal species that most often produce marine algal blooms, is relatively limited (cf. Table 17 and Fig. 13). Typical spring blooms are mostly due to diatoms, while dinoflagellate blooms occur normally in summer and fall. The prevailing factors for this differentiation are in part physical (low temperature, high turbulent energy, deep mixing, etc., that favour diatoms; high temperature, water stability that favour dinoflagellates), in part nutritional. The exact role of these factors in causing certain species to bloom, and not others, is difficult to define, however. High nutrient concentration and/or high nutrient supply (e.g., nitrogen and phosphorus; cf. below) are undoubtedly "necessary conditions" for algal blooms, but for themselves are not "sufficient conditions" for specific algal blooms. Also species reproductive strategies, the interactions within the biotic network and along the food-chain (Mann, 1969; Fenchel, 1988), the presence and dynamics of complexing agencies, the role of nutrient turnover rates and microbiological interactions (Seki and Iwami, 1984), etc., determine the dynamics at the primary production level.

3.3.3 The limiting factor concept, and the concept of target factors

The complexity portrayed in previous paragraphs is of but little use for practical purposes, unless restated. As already said, the manifest production is governed at any time by numerous factors simultaneously (acute limiting conditions or factors); yet, the overall productivity of waters under otherwise comparable conditions is largely determined by those factors that limit production over a substantial length of time during the main growth period (chronic limiting conditions or factors). Though not in all circumstances, chronic limitation is most often due to limited availability (systems internal) and supply (from outside the system) of key nutrients. Both, acute and chronic limitation, are governed by some generalized form of the Michaelis-Menthen relationship (cf. above).

To evaluate which element, or group of elements, are most likely to act as limiting factor, the amounts and proportions of all critical elements present in biomass must be ranked against their concentration and proportions in the water environment. With this, one can eliminate immediately hydrogen and oxygen, and the elements of group 2 (section 3.2.1) as potentially limiting. This leaves carbon, nitrogen, phosphorus and sulphur (group 1). Carbon in the form of free CO_2, bicarbonates and carbonates, and sulphur in the form of sulphate are normally in excess relative to nitrogen and phosphorus in both, fresh and marine waters, while trace elements may or may not be. This makes nitrogen and phosphorus primary candidates for chronic nutrient limitation.

Nitrogen and phosphorus are singled out as target substances not only because of their overriding role in regulating aquatic productivity, but also because these factors are the only ones that are amenable to control at source. Because control options for either nitrogen or phosphorus reduction at source differ in regard to technologies and respective strategies that are of dissimilar administrative and legislative economic consequences, it is further important to know whether nitrogen or phosphorus is the prevailing limiting factor in any given situation.

On a worldwide oceanic scale the prevailing limiting conditions vary considerably. For open oceanographic conditions, nitrogen rather than phosphorus is generally assumed to be the production limiting factor. Nitrogen can also be limiting in coastal waters as evidenced by experimental studies near a South California sewage outfall (Eppley, 1971), along the North American east coast (Yentch and Vaccaro, 1958; Ryther and Dunstan, 1971), and along the Swedish West coast (Rydberg, 1982). Still, in other circumstances nitrogen and phosphorus availability may be fairly balanced (Skagshaug and Olsen, 1986). In contrast to oceans, phosphorus limitation seems to be the norm in fresh waters (OECD, 1982), though exceptions to this rule are known.

3.3.4 Oceanic versus inshore processes

While eutrophication has been found to potentially affect fresh water lakes of practically any size and depth, it is hardly justifiable to consider oceans as a whole to become eutrophic within a few years or even decades. Nevertheless, there are signs and actual trends of increasing productivity of larger marine areas, particularly of enclosed seas (e.g. the Mediterranean, the Baltic, the Black Sea, the Seto Inland Sea) and relatively shallow marine areas (e.g. the North Sea). On a secular scale, however, the deep-mixing, apparently self-contained oceanic gyres may also be affected by the increased supply of nitrogen and phosphorus from land-locked sources and eolian deposition (GESAMP, 1991).

Marine eutrophication is mainly an inshore problem that affects lagoons, harbours, estuaries and coastal areas adjacent to river mouths of highly populated river basins and/or that receive sewage from coastal cities. This creates particular situations that differ in their production characteristics from those of offshore waters. Production in offshore waters is largely maintained by internal recycling and advection/ diffusion of nutrients from deep water layers (primarily nitrate nitrogen: "regenerated" versus "new" production, cf. e.g. Platt and Denman, 1977). Upwelling supplies nutrients from deep waters also inshore, but this mechanism that rests on geographic, morphometric and hydrodynamic circumstances is confined to certain oceanic areas and regions. Accordingly, offshore waters are naturally low productive, upwelling regions show naturally higher productivity. Ecosystem characteristics in both are relatively stable, though productivity in upwelling regions is more variable.

The main consequence of anthropogenic eutrophication is ecosystem destabilization that results in large fluctuations of the affected systems. The amplitude of these fluctuations depend on numerous local factors, such as geomorphological, hydrodynamic and meteo-climatic features, variability of the hydrologic regime of rivers, ecosystem characteristics, chemical characteristics of, and steadiness or variability of sewage discharges, and other anthropogenic influences.

High, but variable nutrient supply, results in the proliferation of opportunistic phytoplankton and other algal species of high reproductive capacity, yet of low tolerance to nutrient deficiency. Eutrophication leads often to changes in phytoplankton species that either cannot be filtered out by filter-feeding species, or block their filter system, because of size and shape. The development of algal species that exhibit either bulky morphological features, or have unfavourable biochemical properties (e.g., toxins produced by some phytoplankton species that is toxic to secondary producers), may therefore impede the normal food transfer from the primary production to higher trophic levels causing a disruption of the normal food-web relationships. Food-web disruption can be a kind of feed-back mechanism involved in the development of unusual algal blooms.

A classic case is the proliferation and excessive growth of *Phaeocysts pouchetii* in the North Sea (Lancelot et al., 1987). Once the colonies of this species reach a certain size, they are no longer sufficiently grazed by zooplankton; the decaying biomass then accumulates as foam along the French, Belgian, Dutch and German coast.

Also, the extent to which the metabolic processes described previously (cf. 3.2.4) take place, depend on the same conditions mentioned above. Local morphometry, depth, magnitude and duration of density stratification are major factors. Shelf regions in particular may develop conditions of hypoxia and anoxia in the follow of intense algal blooms that lead to fish and bottom fauna kills, and in the long run to alteration of the biocoenotic structure of the local ecosystems.

An additional point of interest is the fact that eutrophication is often caused by sewage discharge. At least, the fraction of organic material supplied either by direct or by indirect sewage discharges relative to the total nutrient supply can be substantial. Accordingly, part of the secondary production could be sustained directly by sewage. However, in most cases, the organic material is rapidly mineralized using oxygen, or other oxidants. Therefore, distinction should be made between oxygen consumption due to autochthonous production and oxygen consumption due to allochthonous organic material. In practice, the discrimination is not easy, and perhaps even not necessary. The main point here is the implication that untreated, or insufficiently treated sewage is not only of sanitary concern, but is also one of the causes that contributes to local situations of hypoxia and anoxia.

3.3.5 Loading criteria

Nutrient supply from external sources is the driving factor of aquatic eutrophication. From a practical point of view of management the question arises, therefore, whether it is possible to establish a quantitative relationship between nutrient supply and the degree of eutrophication. To this end, Vollenweider (1968) introduced the concept of loading tolerance, which was further developed in several papers by the same author with particular reference to phosphorus. This concept defines the general relationship between nutrient supply and the trophic reaction of an aquatic system, with emphasis placed on levels of nutrient supply that determine the transition between oligotrophic and eutrophic conditions in any given situation.

Vollenweider's concept has been quantified for lake systems using a few systems parameters, such as mean depth, hydraulic load and water residence time. In this specific form, the model conception is not immediately transferable to marine systems, yet the concept as such remains of general applicability regardless of the specificity of bodies of water. Chapra and Reckhow in their treatise on modelling (Chapra and Reckhow, 1983) consider also the principles that apply to coastal areas. Lateral coastal currents and inshore-offshore exchange are of particular relevance for the build-up, dissipation of nutrients and maintenance of nutrient gradients along shores. Giovanardi et al. (1992a; 1992b), instead, considered the situation south of the river Po, using a simple dispersion model with salinity as a tracer, and redefined the OECD trophic classification scheme for that area. The combination of both would provide a basis to define acceptable loadings, which, however has not been done yet.

A variety of this approach, more along the above conception has been pursued by Wallin and Håkanson (cf. Wallin, 1991). In addition to parameters considered by Vollenweider, the authors introduced also a coastal form factor, Vd, and a statistically defined sensitivity array, S, which are used to construct a kind of loading tolerance diagram for coastal marine areas corresponding to that developed by Vollenweider for lakes.

Clearly, more complex models and alternative approaches have been pursued in the appropriate scientific literature, all with the implied aim to define in some way nutrient load tolerance conditions of aquatic systems. Although substantial progress has been made thus far, non of these various approaches will be fully applicable to all marine situations; each situation, and accordingly the method taken to characterize the particular circumstances that determine the local or regional marine trophic conditions, must be evaluated in its proper context.

3.3.6 Nutrient limitation in the Mediterranean

Of particular interest in the context of the present report is the question of nutrient limitation in the Mediterranean Sea. Studies of this sort are not plentiful, however. Experimental studies in the coastal waters of Emilia-Romagna have shown that phosphorus rather than nitrogen limits production (Chiaudani et al., 1980; Marchetti, 1985; Mingazzini et al., 1992), as has also been found for the brackish waters of the Baltic (Fonselius, 1978). On the other hand, it must also be noted that marine waters adjacent to major river outfalls are not nutrient limited at all after periods of high water discharge and/or discharges of nutrient rich waters (e.g., during early spring and fall periods), and that situations of this sort give raise to substantial algal blooms (Vollenweider et al., 1992). Periods of nutrient limitation occur mostly during summer, and production may be either phosphorus or nitrogen controlled, when concentrations often fall to low values and N/P ratios decrease from values >25 to values <10, as has been found in coastal waters south of the River Po (cf. Annual Reports, Emilia-Romagna Region, Series 1978 to 1992).

Regionally and seasonally, instead, production may either be phosphorus or nitrogen limited, or both, as has been reported by Becacos-Kontos (1977) for the Aegean Sea. From the statistical analysis of Saronikos Gulf and Island of Rhodes inshore and offshore waters by Ignatiades et al. (1992), one may conclude that, on average eutrophic inshore Gulf waters are rather nitrogen limited, and nitrogen limitation decreasing toward offshore waters, while both, oligotrophic inshore and offshore pelagic water around the Island of Rhodes are phosphorus limited.

Krom et al. (1991) conclude from their study of the NO_3^-, PO_4^{3-} and Chl a distribution in a quasi-stationary warm-core eddy south of Cyprus and further analyses of waters in the southern Levantine basin that the Eastern Mediterranean is phosphorus rather than nitrogen limited. Other workers came to similar conclusions for the Western Mediterranean, although this basin may be slightly nitrogen limited (Owens et al., 1989, ctd. in Krom et al.). The authors suggest also an interesting hypothesis for the low phosphorus content of the basin, relating it to phosphorus sorption on iron-rich Saharean dust fall-outs.

As will be pointed out in Chpt. 4, the nutrient load of both, nitrogen and phosphorus to the Mediterranean basin as a whole is still low in comparison to other closed seas. Eutrophication is largely a problem of coastal and adjacent waters, where, however, it can be very serious. For details cf. Chpt. 5.

4. NUTRIENT SOURCES, SOURCE TYPES, AND LOAD ASSESSMENT

4.1 General

Sources considered here refer primarily to those containing phosphorus and nitrogen because of the overriding importance of these elements in eutrophication (cf. Chpt. 3.). However, considerations pertaining to eutrophication cannot easily be separated from taking a wider view about pollution as a whole. Certain forms of pollution enhance the process of eutrophication (e.g. industrial discharges containing e.g., trace elements such as iron, manganese, molybdenum etc., or sources that emit organic growth factors, such as vitamins). Others may contain toxic factors, or even biologically important substances but at concentrations that may be toxic to biota. The complex interaction between these various possibilities is not well understood at present and requires further study.

In the following sections a number of principles are discussed that have major bearings on eutrophication per se, ignoring largely the points raised in the preamble. Later in the chapter an attempt is made to evaluate the nitrogen and phosphorus load to the Mediterranean Sea.

4.2 Land use and source identification

The type and importance of pollution sources, and in particular nutrient sources, are intimately associated with the use of land and its transformation by men. Under pristine conditions, the geochemical dynamics, which also include the flow of nitrogen and phosphorus, are regulated by natural processes that depend on the specific geological, orographic, sedimentological, geochemical, climatic and biotic environment in which these processes take place. Thermic and hydrological conditions are of primary importance. However, historically all these factors have been seriously affected or changed by man to various degree. A majority of present-day landscapes, even of some where man's influence is not immediately apparent (e.g., the Mediterranean macchia), are to be classified under the category of anthropogenically transformed landscapes, in which natural expanses remain as disjunct pockets only.

A breakdown into land use categories, and their implied relationship to pollution and nutrient sources, is given in Figures 2 and 3. This breakdown has been kept as complete as possible as a reminder, although in reality every situation differs from each other, and accordingly must be evaluated and assessed separately.

Besides the still natural landscapes, which are listed for completeness but will not be treated further, the main anthropogenically transformed land use categories of interest in the context of eutrophication are:

- Urban Settlements and Communications Areas
- Farm Lands
- Industrial Areas

Urban Development. With the rapid expansion of population centres that began in the last century in developed countries, and now involves also developing countries, new technologies and facilities have been introduced. Of greatest hygienic and environmental consequences was the almost general introduction in developed countries of water supply and water disposal systems, i.e., sewers that often were discharged directly to rivers and/or lakes and other stagnant waters. With the improvement of centralized treatment facilities and secondary treatment during this century, some of the worst pollution effects resulting from the discharge of untreated sewage to waterways have been alleviated. Still, secondary treated urban sewage contains substantial amounts of phosphorus and nitrogen, which became the main cause of eutrophication at the turn of the century. With the introduction of household and industrial detergents containing polyphosphates after World War II the problem was further aggravated. Following the adoption of tertiary sewage treatment and due to a substantial reduction of polyphosphates in household detergents in some countries further development of the problem has been halted or slowed down in recent decades. Still, secondary and in particular tertiary treatment of urban sewage is not general, specially in countries bordering the Mediterranean. Accordingly, urban sewage remains a major source responsible for the continuing eutrophication of fresh and marine waters.

Further to direct discharges of insufficiently treated sewage, run-offs from urban streets and highways, and connected areas continue to cause concern, in particular in situations of combined sewer systems that receive both urban sewage and urban run-offs. This causes difficulties of effective treatment in conditions of storm-run-offs because of excessive sewage dilution that reduces and endangers the correct biodynamic functioning of treatment plants.

Farming. The relative role of various farming practices regarding their effects on eutrophication, instead, depends largely on whether farming is extensive or intensive. Extensive farming may have a low impact on eutrophication, even if animal stock raising is involved. As long as the uptake capacity of soils remains high, extensive farmlands may act

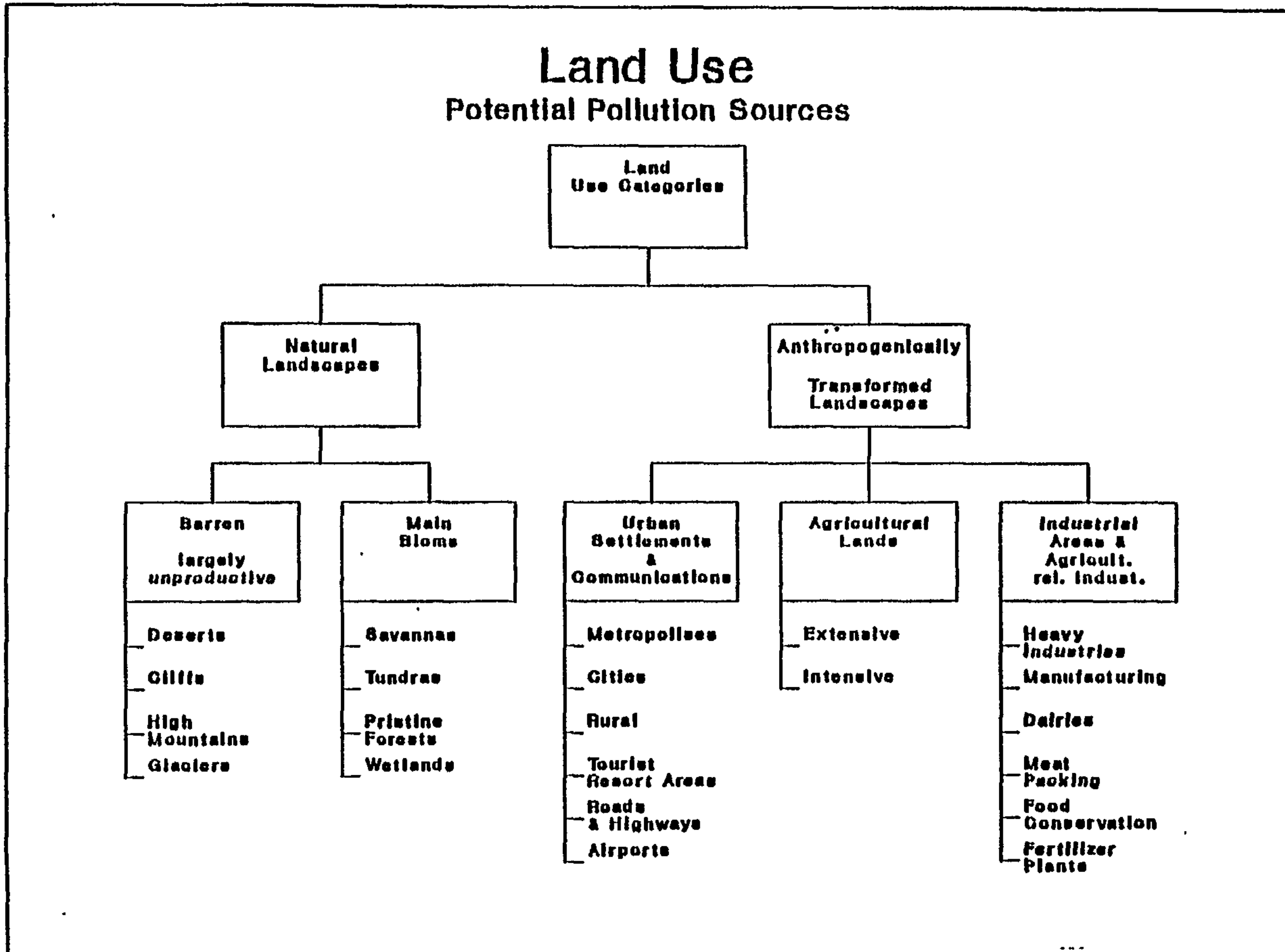

Fig. 2 Land use: Potential pollution sources

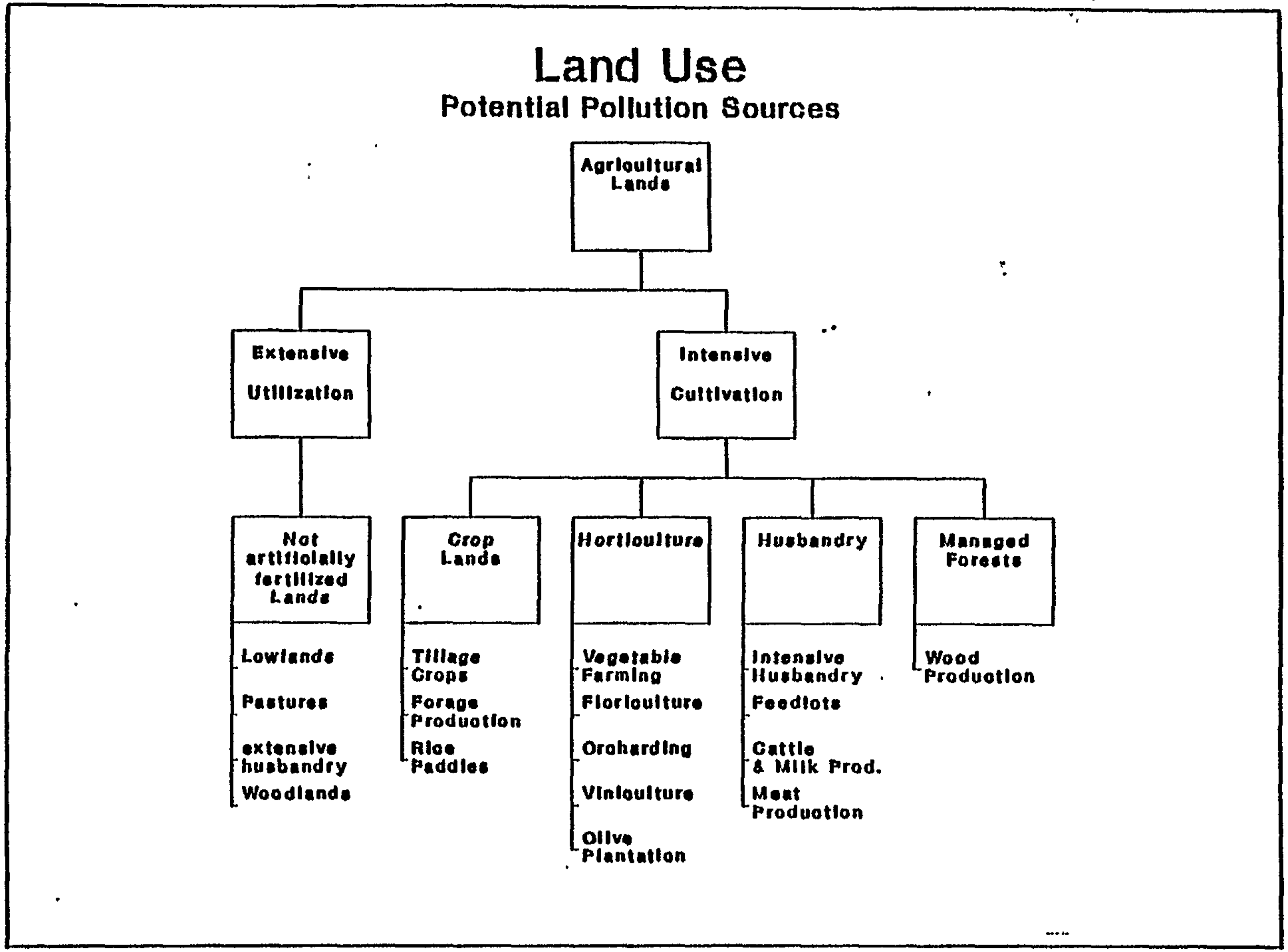

Fig. 3 Land use: Potential pollution sources

as partial sinks for phosphorus and nitrogen. However, while this is true for low density areas, the situation in high density areas may be different. Accordingly, conditions have to be assessed on a case basis.

Intensive farming, that encompasses both high crop production and high density animal husbandry, can be a major contributor to nutrients causing eutrophication due to either the use of large quantities of synthetic fertilizers, and the production of high amounts of solid and liquid manure by farm animals. The net effect of manure on eutrophication is augmented by direct discharges from feed-lots and from stable washing, and practices of manure application to soils of reduced uptake capacity, e.g., sandy, loamy or frozen soils. With regard to synthetic fertilizers, their net effect on eutrophication depends on a number of factors, foremost on the chemical nature and formulation of fertilizer used, on the amounts used, the mode, frequency and period of application, and the general soil conditions, as well as on the kind of crop produced.

Industrial Activities. Among the industries that are of major concern as regards eutrophication are those, which are closely related to agricultural activities such as dairies and dairy products; food transformation and conservation industries such as canneries, sugar mills, breweries, distilleries, slaughter houses, etc., while other industries are often major sources of gross pollution, but their bearing on eutrophication per se remains secondary. Industrial BOD production is generally a good indicator for the relative prominence of industries in this sense, but there are exceptions like fertilizer industries. The contributing role of petroleum and petrochemical industries to eutrophication, on the other hand, is not well understood, but there are indications that petroleum products discharged to waterways may stimulate eutrophication, though not be its major cause.

4.3 Principles of source characterization and assessment

For the characterization of individual sources, several general source attributes have to be taken into consideration. The most important are listed in Figure 4. Others will be discussed further below.

4.3.1 Source type

Source types will be discussed in some detail further below. The major distinction to be made that has bearing on source characterization is between point and diffused sources. Point sources are generally well definable both qualitatively and quantitatively, while the respective characterization of diffused sources is often difficult.

Frequency, Location, Variability: Within the two categories the weight of various single sources depends on frequency, distribution patterns, i.e. location in the basin, and regularity or irregularity of emission. Sources distributed sparsely throughout a drainage basin may be of lesser importance than sources concentrated in a few critical points; also, sources situated far up in a drainage basin may bear lesser weight than sources of comparable source strength located near sensitive receiving waters. Average emission of some sources (e.g., municipal sewer discharges; certain industrial sources) may remain relatively steady over longer periods of time, though the actual discharge pattern over shorter periods of time (24 hour cycle; 7-day period) may vary considerably. In tourist resort areas, instead, municipal discharges vary also seasonally, depending on the magnitude of tourist fluctuations. Diffused sources may also vary seasonally, as e.g. wash-offs from agricultural fields during spring melting of manure applied on snow and frozen ground; air born wash-outs of nitrogen and phosphorus depend on precipitation patterns, etc.

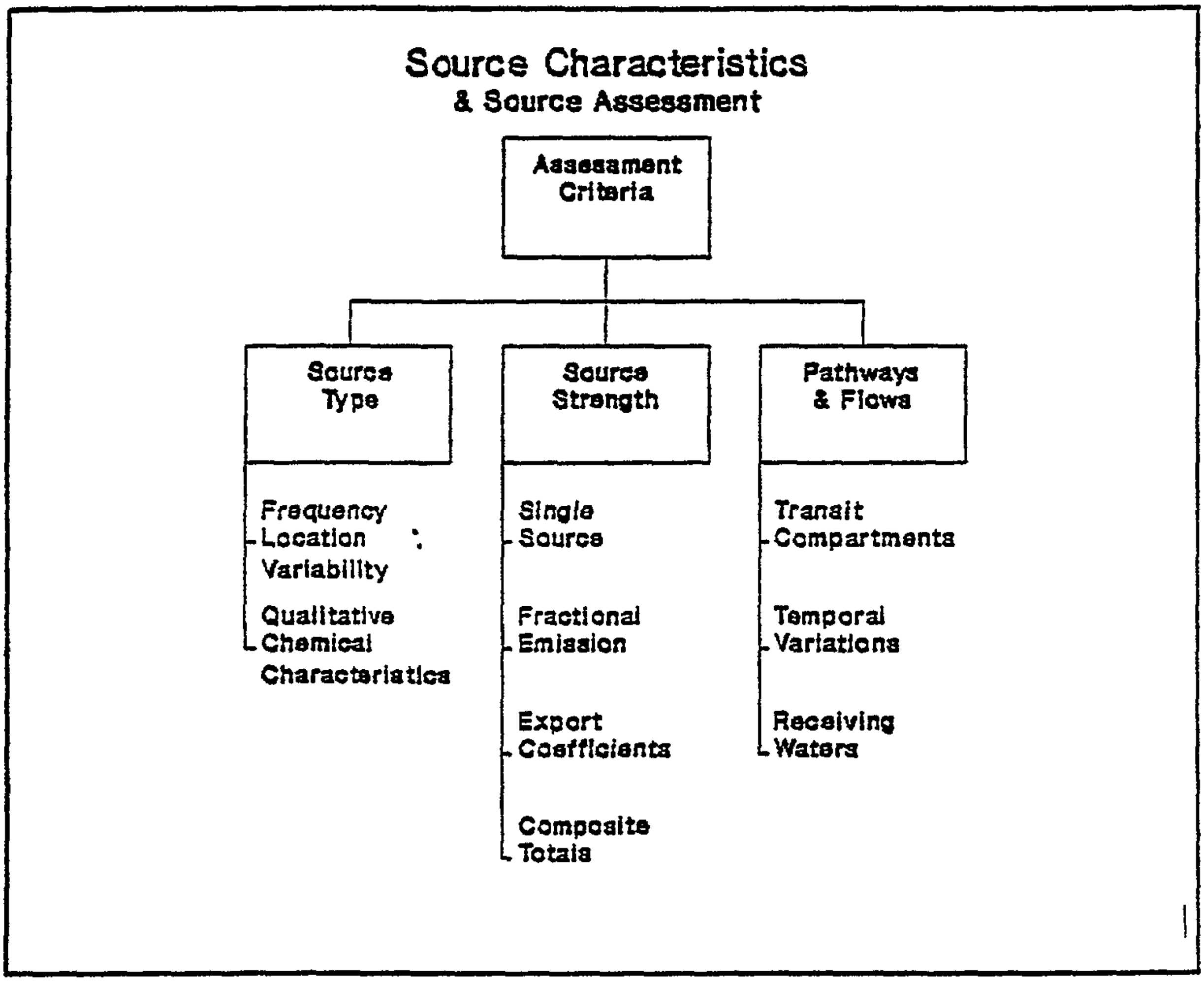

Fig. 4 Source characteristics and source assessment

Qualitative chemical characteristics: Not only the total amounts of phosphorus and nitrogen emitted from the various sources, but also the chemical forms, and their relative composition in effluents are of importance. These factors vary substantially from source to source. Basically, distinction must be made between inorganic and organic forms of nitrogen and phosphorus, but this distinction is not sufficient. Depending on the nature of the source, either element may be present as molecular or colloidal dissolved compounds in a variety of forms, or as inorganic or organic particulates. Also, some fraction of phosphorus and nitrogen may be adsorptive bound on mineral and/or organic particles.

This large variety creates certain analytical difficulties, since it is not generally known to what extent the various components have bearing on eutrophication. Accordingly, it remains a matter of judgement how detailed a chemical analysis should be, and how far it is necessary to account for various components. A typical case is phosphorus present as hydroxy-apatite in the erosional turbidity load of rivers; erosional hydroxy-apatite in certain river basins can make out the prevailing fraction of the total phosphorus load. Regarding organic components, their relative importance depends on the degree to which such compounds are readily hydrolysed and/or metabolized microbiologically, or whether they are chemically and/or biologically inert, i.e., refractory.

Considering the practical approach to the above question, however, a synopsis of necessary analyses for both nitrogen and phosphorus is given in Figure 5. This programme, which is operationally defined, is normally quite adequate for the purpose of routine assessment and surveillance, and in practice entails the determination of some components, usually nitrate, nitrite, ammonia and ortho-phosphates, and filtered and unfiltered totals of nitrogen and phosphorus, while the organic and particulate fractions are estimated by difference. Often the hydrolysis steps are omitted.

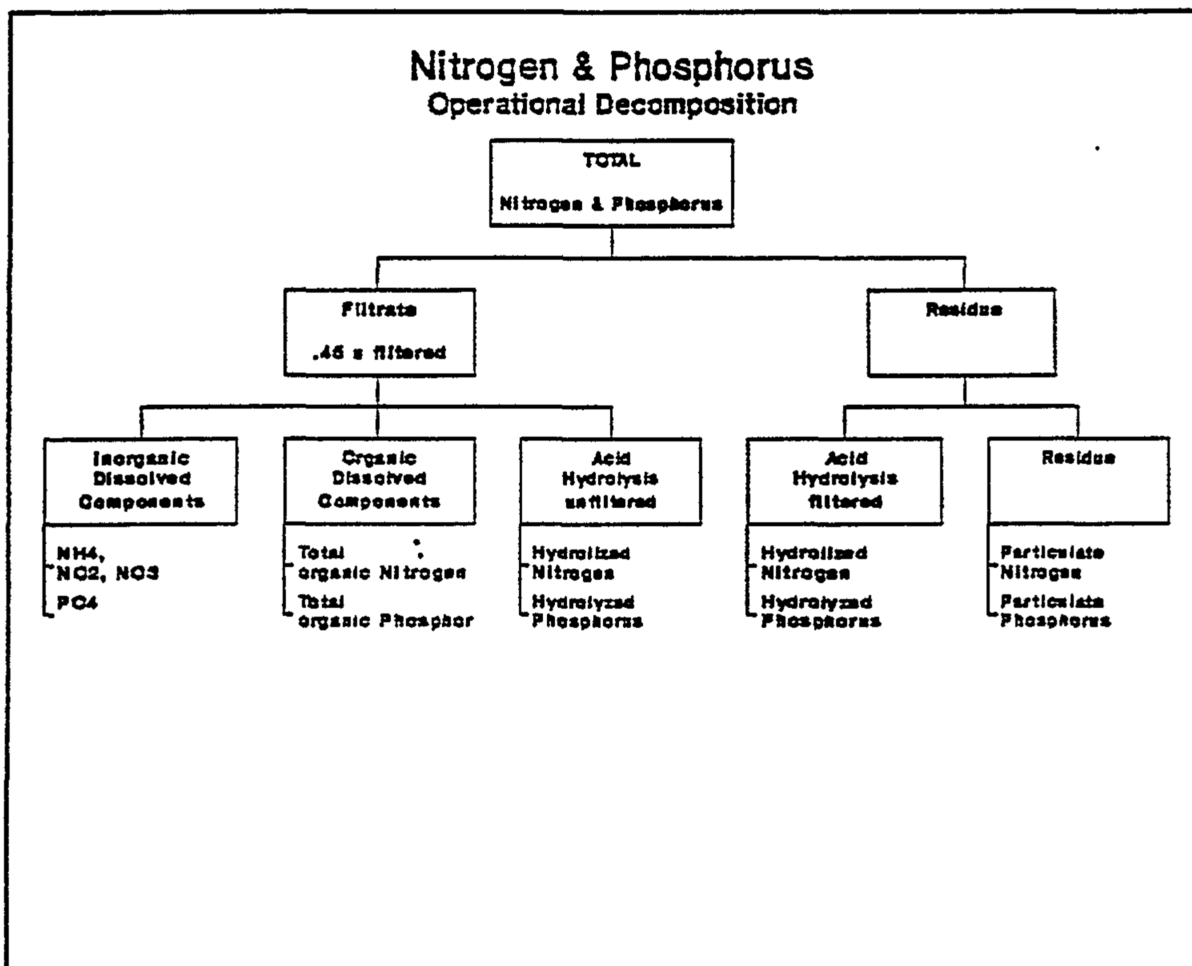

Fig. 5 Nitrogen and phosphorus operational decomposition

4.3.2 Source strength

Source strength refers to the total amount of phosphorus and nitrogen potentially available per unit of time from any specific source (e.g., kg/day). This value should not be confounded with source emission. E.g., the amount of phosphorus and nitrogen in faeces produced per a 250 kg cow/day, determined under controlled rearing conditions, is rather constant, but the amount of phosphorus and nitrogen discharged to waterways from a herd of cows may vary according to forage conditions, age composition, rearing environment, zootechnical practice, etc. In a similar fashion, the unit amount of a nitrogen fertilizer applied to agricultural fields represent the unit source strength, but the amount lost from different fields depends on a variety of factors, such as the exact chemical nature and composition of the fertilizer used, soil properties, slop and drainage conditions, crop produced, etc. Accordingly, in order to assess the relative importance of all nitrogen and phosphorus sources, each single source has to be evaluated separately, and its fractional emission determined. The composite total is then obtained by summation. For reference cf. e.g., Vollenweider (1968), Porter (1975).

In practice, a complete source assessment for each single case and basin is a tedious task, and certain shortcuts may be unavoidable. Assessment of point sources at the outlet point (normally discharge pipes) is generally straight forward on the condition that temporal variations in source discharges are duly taken into account. Quantities may be expressed as e.g., kg/day, t/year, etc., but in some cases it may desirable to refer quantities also to certain units, such as e.g., kg/inhabitant/year. Diffused sources cannot be measured directly, but may be assessed indirectly. This is possible, if basin conditions are reasonably well known, and experience gained from pilot studies or appropriately selected sub-basins can justifiably be transferred to and/or extended to the basin in question as a whole. Unit source emission of diffused sources is often expressed in terms of export coefficients, e.g., as kg/ha.year. Export coefficients are estimated experimentally in appropriately selected sub-basins, and careful analysis of the exported nutrient load at a calibrated river station.

Table 1 provides some ideas about the magnitude and variation of such export coefficients as obtained in some 40 agricultural basins in Europe and North America. Values very much larger than the means resulted from highly fertilized fields.

Table 1

Export Coefficients for Phosphorus and Nitrogen from Agricultural Areas, kg/ha.year

	AVG	STD	Nr	GeoMean		±1 STD		±2 STD
Phosphorus:								
USA/Canada	0.26	0.29	15	0.15	0.43	0.05		
Europe	0.45	0.58	11	0.21	0.79	0.06		
All	0.34	0.45	26	0.17	0.57	0.05	1.89	0.02
						Max/Min	2.14	0.01
Nitrogen:								
USA/Canada	23.32	34.59	17	10.17	34.64	2.98		
Europe	13.11	11.35	25	6.84	30.21	1.55		
All	17.39	24.52	42	8.08	32.62	2.00	131.8	0.50
						Max/Min	120	0.10

Table 2 instead summarizes data of integral basin export coefficients, expressed in t/km^2/year, for some river basins of the Mediterranean. Export coefficients of this sort account for the composite emission from all sources, including diffused and point sources, and possible loss factors (cf. below). The data show that while the mean integral basin export coefficients for nitrogen are comparable in magnitude to those obtained from agricultural fields, those for phosphorus are roughly half an order of magnitude larger. Export values of this sort are comparable with those found in North America. The average loss of nitrogen from more than 900 watersheds in the U.S.A. is reported to be 954 kg/km^2.year (Omerik, 1977); according to Reckhow et al. (1980), phosphorus losses amount up to 300 kg/km^2.year in areas of intensive agriculture, between 40 to 170 kg in medium intensive areas, while in other areas the losses can be as low as 10 kg/km^2.year. The implications will be discussed further below.

4.3.3 Pathways and flows

This introduces still another aspect of composite source assessment that takes into account the flow pattern and flow size through individual paths and compartments of phosphorus and nitrogen throughout the basin as a whole. The term compartments is used in this context in two senses (a) as the summation of single sources having essentially similar characteristics (e.g., all household sewers which make up the compartment 'household discharges'; the sum of discharges from animal husbandry of a certain kind, eg., hogs, etc.), (b) pools of transformation inserted in particular paths (e.g., sewage treatment plants; food transformation industries; etc.). The common denominator of all the various compartments is the fact that they have one or several distinct inputs and outputs (flows) that can be measured or reasonably estimated. As a whole, flows and compartments that belong to a basin make out a system of interwoven input-output channels, whereby certain compartments may act as either source or sinks for phosphorus and nitrogen. Typical sinks are soils in which a substantial fraction of the inflowing phosphorus and nitrogen is retained by processes of physico-chemical binding and/or biological transformation such as uptake in crops, or regarding nitrogen, by processes of denitrification.

Pathways and size of flows may show temporal variations depending on input-output variations and modifications. From the point of view of eutrophication, the most important aspect considers the amount, location and timing of that fraction of the total load that ends up in the receiving waters (rivers, lakes, sea shores, etc.).

In order to draw up a complete balance sheet, total imports and exports of nitrogen and phosphorus in and out of a catchment system need to be assessed; however, because of the many difficulties to obtain the necessary raw data, this is rarely done. A specific example will be presented later.

4.4 Practice of source assessment and source inventory

While what has been said above summarizes the general principles that need be considered in dealing with nutrient source assessment, the practice of drawing up a complete nutrient load inventory for the sake of load management is normally guided by a scheme along the following points:

A) According to origin
   a. natural
   b. human settlements

Table 2

Export Coefficients for Italian River Basins and the Rhône and Ebro Basins

River	Length km	Drainage sqkm	Discharge ckm/y	Nitrogen 1000 t/y ($N-NO_3$) Total-N	Phosphorus 100 t/y ($P-PO_4$) Total-P	Exp.Coef Nitrogen t/sqkm/y	Exp.Coef Phosphorus t/sqkm/y
Po	652	69,974	46.4	(66.7)	(10.9)	0.953	0.156
				81.7	10.7	1.168	0.153
				110.0	13.0	1.572	0.186
minimum					7.5		0.107
maximum					19.5		0.279
Adige	410	12,200	7	(6.6)	(0.4)	0.541	0.033
				7.7	1.3	0.631	0.107
				13.0	1.2	1.066	0.098
Tiber	405	17,169	7.2	(9.9)	(3.5)	0.577	0.204
				20.2	3.2	1.177	0.186
Arno	241	8,247	2.05	(2.5)	(1.0)	0.303	0.121
				4.6	1.8	0.558	0.218
16 basins south Po		12,571		30	2.2	2.386	0.172
		Average			Total	1.230	0.167
		±STD				0.677	0.059
		Average			Mineral	0.594	0.129
		±STD				0.269	0.072
Rhône	812	99,000	67.9	(54.4)	(12.0)	0.549	0.121
Ebro	930	(110,000)	12.4	(ca 34)	(ca 9?)	(<0.34)	(<0.1)

Sources: Pettine et al., 1985; Provini et al., 1992
Marchetti and Verner, 1992
Kempe, 1985
World Resources 1988-89
Note: Pettini's Total-N = Total inorganic N

Other Rivers	Export estimates:						
	km	sqkm 1)	ckm/y	$N-NO_3$	$P-PO_4$	t/sqkm/y $N-NO_3$	t/sqkm/y $P-PO_4$
Seine	776	(100,100)	8.7	36.7	1.6	0.367	0.016
Loire	1010	120,500	33.7	78.5	3.5	0.651	0.029
Garonne	650	(83,850)	19.2	28.7	1.7	0.342	0.020
Rhône	812	99,000	67.9	54.4	12.0	0.549	0.121
France					Avg->	0.477	0.047
Rhine 1	1326	175,400	69.7	200.0	17.4	1.140	0.099
Rhine 2	1326	175,400	79.0	250.0	25.0	1.425	0.143
Rhine Hellman		175,400	68.6	Total P->	50.6		0.288
Weser	502	(64,760)	8.5	38.0	5.4	0.587	0.083
					Avg->	1.051	0.108
Weser+Ems+Elbe		(262,280)		Total P->	38		0.145
W. Germany		248,678		Total P->	111		0.446

Comments: (Surface estimated from river length)
1 Rhine 1963-78
2 Rhine 1978
Sources: Kempe, 1985; Bernhardt, 1978/ World Resources, 1988-89

c. animal husbandry
d. fertilizers
e. industrial byproducts

B) According to type
f. inorganic (e.g., detergent polyphosphates)
g. organic

C) According to loss or discharge mode
h. surface run-off, leaching and erosion
i. sewage
j. industrial discharge
k. aeolian

D) According to general categories
l. point sources
m. diffused sources

E) According to controllability
n. from a theoretical point of view
o. from aneconomic - strategic point of view

These and the various aspects discussed in previous paragraphs are arranged in Figure 6 from a practical point of view, and may serve as a basis for organizing the data to be collected. In this scheme, emphasis is given to controllability. As already mentioned, point sources are generally easier to control than diffused sources, but the proportion of nutrients originating from point versus diffused sources normally differs according to whether phosphorus or nitrogen is in question. Experience accumulated over recent decades shows that in most situations of man induced eutrophication 50% and more of phosphorus originates from point sources, while the reverse holds for nitrogen. Clearly, there are exceptions to this rule. Approximate percent ranges of source contributions that cover a wide variety of actual situations are given in Table 3.

Nitrogen and Phosphorus from the domestic environment, urban settlements and industries. The per capita defecation coefficients for nitrogen and phosphorus of a population having a mixed balanced diet have been estimated to be 12 and 1.5 g/day, or 4.4 and 0.54 kg/year (cf. Bucksteeg, 1966). These figures may vary slightly depending on diet conditions, but as a whole, they are rather representative for a broad segment of the human population. The polyphosphate contribution from household detergents, instead, has changed dramatically over the last three decades. While the per capita contribution of phosphorus originating from laundry detergents in the 60s reached values two to three times the human metabolic value, values of present conditions are generally much lower, due to reformulation of laundry detergents, in part due to legislative measures introduced by countries, in part due industrial reassessment of their formulations. Still, polyphosphate contents in laundry detergents vary largely from country to country. The content in industrial detergents, on the other hand, remains generally still high.

Nitrogen and phosphorus contributions from urban runoff, from streets and highways, and industrial contributions, are difficult to assess because of the insufficiency of appropriate studies. Rough estimates assume a 10% quota of population contributions.

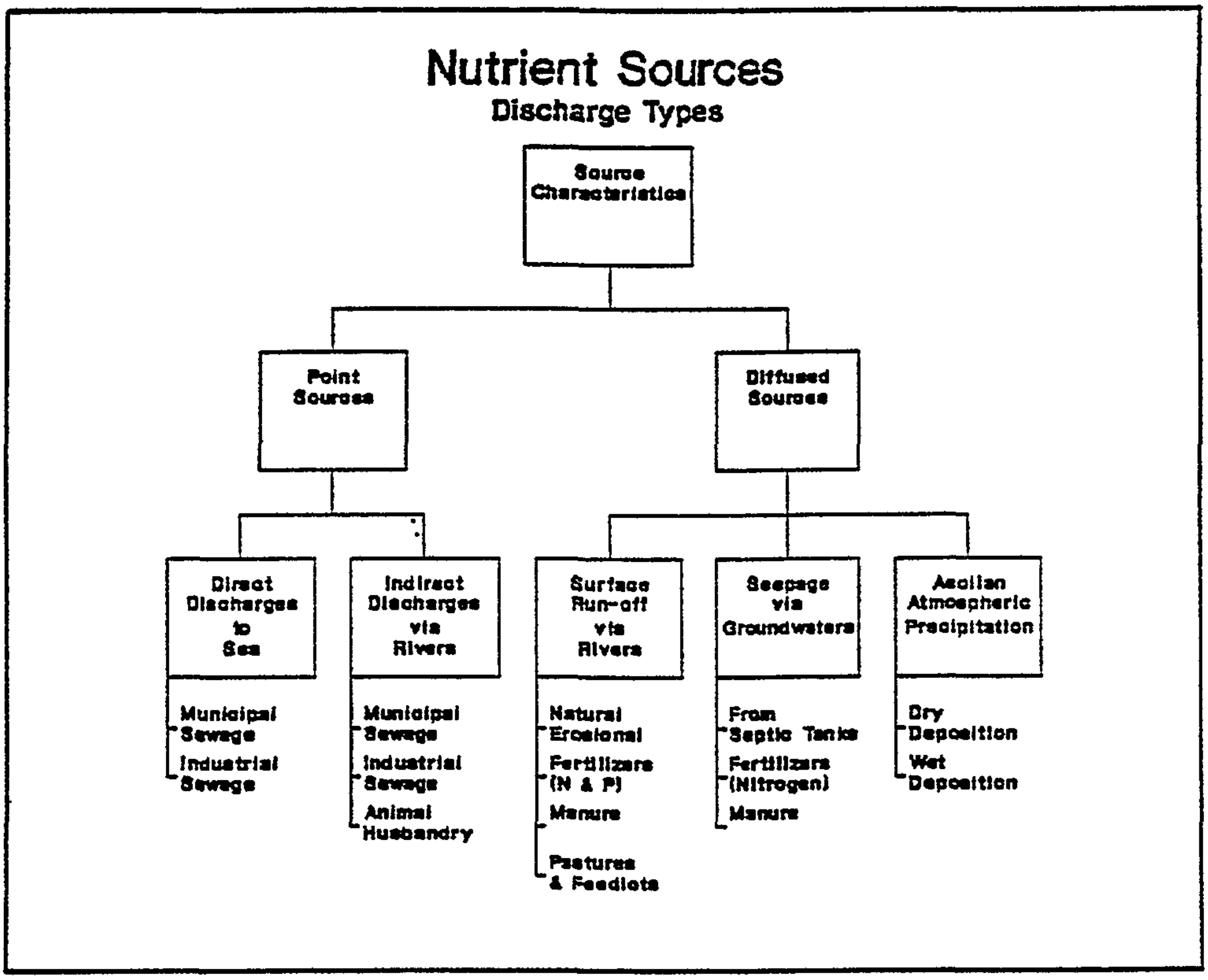

Fig. 6 Nutrient sources. Discharge types

Chemical Fertilizers. Estimates of fertilizers applied to croplands vary considerably between crop types, and from region to region, and between countries, as does animal husbandry. Total fertilizer application to crop lands ranges at present from 0 to a country mean of some 800 kg/ha (cf. Table 8). While quotas tend to stabilize in countries that used higher amounts for some time, these quotas increase still in countries of generally lower fertilizer use. Regarding loss fractions, some general export figures have been given above.

Loss of phosphorus and nitrogen to surface and ground waters occurs via surface washout, surface erosion and percolation. The distinction between these paths is important, but it is generally correct to conclude that the loss of phosphorus from chemical fertilizers is substantially below that of nitrogen, and that therefore nitrogen from agricultural applications can be a major contributor to eutrophication, in particular during the spring period when nutrient concentrations in sea waters are high, and usually blooms of diatoms occur.

Table 3

Origin of Nitrogen and Phosphorus Loads from
Anthropogenic Sources

	% Nitrogen	% Phosphorus
Urban Sewage:	20 - 40	40 - 60 - (80)
Agro Industries: Crop production and Fertilizer use, Animal husbandry, Food transformation	40 - 60 - (80)	20 - 40
Others: Industries, Urban runoff, Atmospheric Precipitation	10 - 20	<10 - 20
Natural:	<10 - 20	<5 - 10

Phosphorus is mostly lost by surface washout and erosion; percolation losses to ground waters is minimal because of the generally high phosphate (the main form of phosphorus in fertilizers) binding capacity of agricultural soils. Regarding nitrogen, instead, it is important to consider the form in which nitrogen is applied to soils. While the chemical binding of ammonia and urea in soils is rather complicated, nitrates have a high mobility, and substantial fractions may leach into ground waters. Nitrate accumulation in ground and well waters in the lower Po Valley is a predicament that, besides being a factor in eutrophication, raises questions about the safety of drinking water. Still, the problem has not risen to an acute crisis yet.

Animal husbandry. Animal husbandry is likely a major agricultural contributor of nitrogen and phosphorus in many environments (Porter, 1975). Manure is a source of ammonia and urea, and in particular, of mobile phosphorus in situations of intense animal husbandry, when liquid manure is more or less directly washed into surface waters. Citing from Vollenweider's 1968 review, the quantities of nitrogen and phosphorus produced by livestock, expressed in terms of live weight, vary considerably from one species to another. Cows and pigs produce about 150 kg N/1000 kg live weight per year, goats and sheep about 120 to 130 kg, and poultry about 85 kg. Pigs produce the most phosphorus, with nearly 45 kg/1000 kg live weight per year, followed by hens with about 30 kg, and sheep and goats with 20 kg (Miller, 1955). Slightly different values but of the same order of magnitude are reported by ASAE (1979).

In European countries with intensive animal husbandry, the arising of nitrogen and phosphorus from livestock relative to the human population varies by a factor from 6 to 16 with a mean of 8 for nitrogen, and from 5 to 26 with a mean of 11 for phosphorus. Accordingly, the total source strength of nitrogen and phosphorus is roughly 10 times that of the human population. These high amounts, contained in manure, fortunately are mostly spread on land. A certain fraction of these will be washed into surface and ground waters. Depending on local conditions the percent loss factor is normally in the order of 10 to 25 % for nitrogen, and 1 to 5% for phosphorus. According to the studies by Chiaudani et al. (1978)

and Marchetti (1987) the relative contribution of nitrogen from animal husbandry in Italy would be around 5-6% from all sources, and that of phosphorus around 14% making animal husbandry third in importance. Countries practicing extensive stock raising (e.g., sheep and goats), as is the case in various semi-arid Mediterranean countries, would probably arrive at lower loss fractions.

Aeolian Deposition. This argument will not be treated here, but reference to marine deposition rates will be made in a later context (cf. 4.5(d)).

A model situation. To bring the pieces together a model has been conceived for an average landscape with population density of about 150 inhabitants/sqkm and a corresponding distribution of land use activities (crop production and fertilizer use, animal husbandry, etc.) that is typical for such a situation. Polyphosphates in detergents have been assumed to be 50% of the per capita phosphorus value. For the prevailing conditions of peak contents of polyphosphates in detergents during the 1960 to about 1975 period, this value is on the low side, but is now more in line with recent detergent formulations and the usage of polyphosphate free detergents.

Modifications that make the model more realistic consider also a fractional retention of nitrogen and phosphorus in treatment plants or due to land disposal from septic tanks by a quota of 25%, but further reductions that are achieved now by tertiary treatment facilities are ignored. As to losses from diffused sources a minimum quota of 10% and 1% for nitrogen and phosphorus, and a corresponding maxima quota of 25% and 5%, respectively, is assumed. Accordingly, the model output gives not a fixed value but the range within which one can expect the real values to lay. As Table 4 shows the approximate amounts exported from 1 sqkm would range under average conditions from between 1000 to 3000 kg/year for nitrogen, and from between 130 to 250 kg/year for phosphorus. The breakdown of these export quotas shows also that the relative contribution of nitrogen from point sources is generally less than 50-60% of the total, while the reverse would apply for phosphorus.

Application to Mediterranean Conditions. How far are these estimates confirmed by actually measured values, and how far can it be applied to Mediterranean countries? Italy is the only Mediterranean country that has adapted and perfected the approach to loading estimates originally developed by Vollenweider (1968). Therefore, the following assessment that may serve as a guide relies heavily on the Italian experience.

Originally, the model was designed to meet the conditions typical for Middle Europe, and for this purpose the model prediction is in excellent agreement with actual measurements. For three watersheds in Germany (Mohnethal, Briggethal, Wahnbachthal) with population density ranging from 120 to about 200/km^2, nitrogen exports have been reported from 1500 to 2500 kg/km^2, and phosphorus exports from 70 to 290 kg/km^2. With slight adaptation of the model to the Emilia-Romagna conditions, the nitrogen and phosphorus export from the 4 Provinces of Bologna, Ferrara, Ravenna and Forlì (11,104 sqkm; 2.27 Mill. inh., 204/km^2, 1975) was estimated to range from 18,000 to 31,000 t N/y, and from 1,900 to 2,800 t P/y around 1975 (Vollenweider, 1977). These figures are corroborated by data of the recent more detailed studies on the 16 basins of that region by Marchetti and Verna (1992), who estimated the nitrogen and phosphorus exports from an area of 12,571 km^2 to 30,000 and 2,200 t/y, or 2386 and 172 kg/km^2, respectively (cf. Table 2).

Table 4

Estimated Nitrogen and Phosphorus Exports from a Representative European Model Area

Model Assumptions: 150 inh/sqkm
20% urban & wastelands
20% forests
30% arable (fertilizers applied: 80 kg N & 30 kg P/ha)
30% grassland, pasture & animal husbandry
(livestock/human excrements: N=8:1; P=11:1

Main Sources:	Nitrogen		Phosphorus	
	kg/sqkm			
A) Sewage & Urban Runoff				
- from human origin	660	660	80	80
- detergents	-	-	40	40
- highways	66	66	8	8
- industrial	66	66	8	8
Sub-Total A)	792	792	136	136
B) Land Runoff	a)	b)	c)	d)
- from forests	50	250	5	25
- from arable lands *	120	600	9	45
- from pastures & grasslands **	265	1320	9	45
Sub-Total B)	435	2170	23	115
Total A) + B)	1227	2962	159	251
% A) of Total	65%	27%	86%	54%
Total A)** + B)	1062	2797	129	221
% A) of Total	59%	22%	82%	48%

* Assumed export coefficients on land applications:
a) = 5%, b) = 25%, c) = 1%, d) = 5% of totals of fertilizers & manure applied on arable, pastures & grasslands

** Assuming another 25% of domestic nutrients in treatment plants and/or deposition on land

This confirms the applicability of the model in principle, contingent to its correct tuning to the particular situation investigated. In the case above, the model gave the correct range because of several concurrent factors, to mention the approximate population density of 200 inh/km^2, the rate of fertilizers applied (100-150 kg N/ha.year; 20-30 kg P/ha.year; Rossi et al., 1992), and the kind and level of animal husbandry similar to that assumed in the model.

Actual export values referring to Po basins as a whole instead (cf. Table 2) are rather on the low side of the model predictions, particularly regarding nitrogen. This may have several reasons: climatic, orographic and soil conditions, land use activities, crops produced, etc., and not least relative basin size. While the mean area of the 16 basins south of the Po studied by Marchetti and Verna (1992) is around 800 sqkm, the Po Valley has an extension of some 70,000 sqkm of which a large proportion (ca. 50-60%) makes out the Po plane. On the West and North side, the Po plane is flanked by the Alps, on the South by the Apennines, both with steep valleys. Between Turin (km 122) and the closure of the basin at Gaiba (km 572), the mean slope of the river is about 0.5 m/km. Below this point the river is impounded and does not receive any further tributary till it reaches the sea about 80 km downstream. The transport way of the Southeast rivers, which originate in the Apennines and discharge directly into the sea, relative to that of the river Po is relatively short. This differences in orographic and hydrographic conditions has bearing on a number of factors: velocity of river flow, determining intensity of erosion and sediment erosion-accretion patterns, total erosional load, residence time of river waters between source and outflow, etc. Residence time of water in the river bed may affect denitrification, and this, besides intensive agriculture, may be one of the reasons for higher nitrogen export coefficients of the lower basins as compared to that of the River Po as a whole. Conversely, phosphorus export is not affected by gaseous losses to the atmosphere, but phosphorus may accumulate in river sediments. A substantial fraction of the total phosphorus load in rivers is bound on river suspensions (up to 98%) (Santiago, 1991; Thomas et al., 1991; Santiago et al., 1992; Barbanti et al., 1992), and these in turn are subject to sedimentation during periods of low water load and re-suspension and washout during periods of high water load.

As regards the behaviour of single diffused sources, the situation is rather complicated. Rossi et al. (1991; 1992) have studied the nitrogen and phosphorus contribution from chemical fertilizers used in well drained agricultural areas selecting 5 pilot fields situated in the lower Po plane, which rarely receive manure from animal husbandry. Nitrogen release to drainage waters shows a characteristic time pattern: nitrogen concentrations in the underground drainage water are high (40 to about 90 g N/m^3) during winter-spring to about June depending on the year, and then dropping to very low values during summer and fall; phosphate concentrations vary irregularly, but modest concentrations remaining below 200 mg P/m^3 with means ranging from about 50 to 100 mg/m^3. The total loss per ha and year was estimated to range from 22 to 83 kg/ha for nitrogen, and 0.018 to 0.153 kg/ha for phosphorus. With an application of 100-150 kg/ha of nitrogen and 20-30 kg/ha of phosphorus, between 20 and 55% of nitrogen is lost, while the fractional loss of phosphorus would amount only to about 0.1%. This is substantially below the range assumed in the model discussed above. The high phosphorus binding on soils, and accordingly the low release rates, is possibly a consequence of the silty nature of soils in that region, as has also been concluded by Vighi et al. (1991), who found phosphorus release rates from lowland watersheds near the Adriatic Sea comparable to those of Rossi et al. (0.03 to 0.21 kg/ha.year). Conversely, the same authors report erosional losses from mountain watersheds as high as 0.6 kg/ha.year. Also Marchetti (personal communication) studying phosphorus release in Lombardy fields found much higher phosphorus loss rates.

Multiple regression analysis of data pertaining to sub-basins of the River Po (data collected by Marchetti in the 1970s, and further analyzed by Vollenweider) provides a further perspective to the problem of estimating actual exports (cf. Table 5 and 5a). In this analysis the only load generating compartments that have been considered are basin size and population. The resulting multiple correlation coefficients are significant for both, nitrogen and phosphorus. About 68% of the variability of the nitrogen data and about 75% of the variability of the phosphorus data is explained by the two compartments alone. Yet, there is a substantial difference in significance of the respective regression coefficients, regardless of whether the analysis includes a constant or not. In the case of nitrogen, both coefficients are significant at the $P=<0.05$ level ($P.05;14 = 2.145$), while in the case of phosphorus only the coefficient pertaining to population is significant, but this at a significance level of $P<<0.01$.

Table 5

River Po: Nitrogen and Phosphorus Export by Sub-Basins. (Data: Marchetti 19...)

No. Basin	Size sqkm	Population 1,000,000	Nitrogen kg/day	Phosphorus kg/day	N calc.	P calc.
0 Upper Po	4,885	0.465	28,189	2,196	14,988	437
1 Dora/Stura	2,523	1.639	5,197	487	15,837	1,541
2 Orca/Dora B	6,532	0.343	4,582	364	18,428	322
3 Sesia	3,072	0.452	9,384	887	19,349	425
4 Tanaro/Scriv	9,498	1.038	49,000	1,026	29,916	976
5 Agona-Ticino	9,020	1.084	23,804	944	28,979	1,019
6 Staffora	1,120	0.123	601	101	3,531	116
7 Lambro	3,858	4.605	40,858	4,869	36,365	4,329
8 Trebbia	1,396	0.040	1,277	46	3,746	38
9 Adda	7,636	1.668	16,363	1,324	28,876	1,568
10 Nure	1,069	0.062	677	28	3,050	58
11 Arda-Parma	4,457	0.578	12,053	684	14,565	543
12 Crostolo	574	0.208	1,817	183	2,649	196
13 Oglio	6,693	1.165	29,129	1,498	23,591	1,095
14 Mincio	3,083	0.348	3,146	270	9,775	327
15 Secchia	2,367	0.366	9,800	1,100	8,077	344
16 Panaro	2,191	0.472	8,072	1,054	8,247	444
Sum	69,974	14.656	243,948	17,061	260,967	13,777
			N/P:	14.3		

Multiple Regressions:		Nitrogen:			
Regression Output:			Regression Output:		
Constant		-3423.30	Constant		0
Std Err of Y Est		8842.04	Std Err of Y Est		8766.6
R Squared		0.6878	R Squared		0.6711
No. of Observations		17	No. of Observations		17
Degrees of Freedom		14	Degrees of Freedom		15
	Basin:	Popul.:		Basin:	Popul.:
X Coefficient(s)	3.0194	6199.60	X Coefficient(s)	2.5172	5787.7
Std Err of Coef.	0.8117	2106.03	Std Err of Coef.	0.5612	2033.8
t-value	3.720	2.944	t-value	4.486	2.846
		Phosphorus:			
Regression Output:			Regression Output:		
Constant		191.68	Constant		0
Std Err of Y Est		613.20	Std Err of Y Est		602.60
R Squared		0.7542	R Squared		0.7456
No. of Observations		17	No. of Observations		17
Degrees of Freedom		14	Degrees of Freedom		15
	Basin:	Popul.:		Basin:	Popul.:
X Coefficient(s)	0.0053	916.53	X Coefficient(s)	0.0334	939.59
Std Err of Coef.	0.0563	146.06	Std Err of Coef.	0.0386	139.80
t-value	0.0939	6.275	t-value	0.866	6.721
	not sign!			not sign!	

Table 5a

Summary Assessment of Po-Basin Export

Nitrogen: Basin: Population:	 0.9188 t/sqkm.year = 919 kg/sqkm.year 2113 t/million = 2.113 kg/inh.year
Phosphorus: Basin: Population:	 0.0122 t/sqkm.year = 12.2 kg/sqkm.year 343 t/million = 0.343 kg/inh.year
Basin export of phosphorus adjusted from regression underestimate: 17,061 (13,777) = 3284 kg/day or 3284*365 kg/year = 17.1 kg/sqkm.year Total average basin P-export coefficient: 39.3 kg/sqkm.year	

From this, one would conclude that nitrogen export results from both, the domestic and the land use environment, while the phosphorus export would result only from the domestic environment. Converting the coefficients to annual values gives:

	Population kg/inh.year	Land kg/km^2.year
Phosphorus:	0.335-343	1.9-12.2
Nitrogen:	2.11-2.26	919-1102

Comparison between the measured and predicted export quota for phosphorus shows that (a) there is some internal compensation, (b) the predicted quota are underestimates for at least three basins, most noticeably for the Secchia and Panaro that show notoriously higher phosphorus export coefficients similar to those found by Marchetti et al. for the lower basins that discharge directly into the Adriatic Sea (cf. above). On the other hand, it is to be noted that the very uncertain regression coefficients for phosphorus from land are in the order of magnitude of Rossi's and Vighi's et al. findings. If one assumes that the difference between the predicted and measured totals (about 3000 kg P) is due to model uncertainties, but essentially due to export from land, then the phosphorus export coefficients given above would have to be increased by some 15 to 20 kg/km^2. year, which would bring the total export coefficients from land closer to the estimates given in Table 1. What ever is the correct answer to this question, it can be assumed that the loss of phosphorus from agriculture per se (fertilizers) is easily overestimated, although the above values are clearly on the low side. High actual phosphorus export rates from land, on the other hand, are likely due to either increased erosional losses, and/or to animal husbandry.

The land export coefficients for nitrogen estimated by the multiple analysis, instead, are in accord with export coefficients given in Table 2. Regarding the population contribution of both, nitrogen and phosphorus, the analysis would validate the assumption of an about 50% basin retention.

In conclusion, the procedures for estimating nutrient exports from basins as discussed above are largely supported by the Italian experience. Still, it remains questionable how far the respective findings and methodologies can be transferred to other Mediterranean countries. There are several factors that differ substantially from country to country: population density and its regional variations, land use distribution and agronomic utilization such as prevailing agricultural crops, fertilizer use on crop land, and animal husbandry, the latter being rather extensive in many Mediterranean regions. It would therefore be inappropriate to extend the above model approach to single Mediterranean countries or the Mediterranean as a whole without substantial modifications. As there are essential difficulties to do this properly, several alternative methods are used in the following to determine the range and limits within which the actual figures of the nitrogen and phosphorus load from land-based sources to the Mediterranean may lie.

4.5 Load assessment for the Mediterranean as a whole

The assessment of the nutrient load for the Mediterranean as a whole encounters major difficulties due to the lack of comparable data for the whole area. Therefore, a step by step approach is taken using substantially simplified methodologies. This entails (1) a general pollution and sensitivity index, (2) a review of the Italian attempt to asses its total load, and (3) a generalized methodology for estimating the probable range within which the total nitrogen and phosphorus load to the Mediterranean is to be expected.

a) Potential pollution and sensitivity index

Prior to embarking on the difficult question, how much nitrogen and phosphorus is discharged into the Mediterranean a simplified overview approach is taken that pinpoints areas of major concern, and that will be supplemented by concrete data about eutrophication incidents in Chpt. 5.

For this purpose a simple index that has bearing on potential regional and local consequences of population on nutrient loads and eutrophication is introduced. This index is defined as the number of inhabitants per km of shoreline and, accordingly, represents a coarse measure of coastal nutrient load density. Using population and shore length figures as listed in Table 6, this index is calculated for all Mediterranean countries and would range from 400 for Yugoslavia to 17,400 for Egypt.

Interpretation of these figures is not straight forward, however. Depending on how the figures on shoreline length and the number of inhabitants that weigh directly on the sea have been estimated in the first place, the index can be open to considerable uncertainty, as within countries there is large variation in the distribution of population, and hence, variation in inhabitant/shoreline density. As example the Italian figure of 5,300 taken at face value, would be entirely misleading, particularly as regards the Adriatic Sea. Of the 57 million inhabitants of the 20 administrative Regions about 24 million residing in the 7 Northern Regions: Piemonte, Valle d'Aosta, Lombardia, Emilia-Romagna, Trentino Alto Adige, Veneto, Friuli Venezia Giulia, weigh on the North Adriatic coast of roughly only 400 km; this gives an inhabitant/shoreline density of about 60,000, the highest in the whole Mediterranean. Accordingly, the inhabitant/shoreline density for the rest of Italy would reduce to about 4.5.

Similarly in Greece e.g., the corresponding density of the Gulfs of Saronikos and Thermaikos, and else where would result substantially higher than the national average, and probably the same is true for coastal stretches of countries like Turkey, Tunisia etc. On the other hand, the high density for Egypt, Israel and Lebanon adjacent to a largely oligotrophic marine environment indicates an actual (in Egypt e.g., the Alexandria shores and harbours are highly polluted) or the potential for, high local eutrophication.

Table 6

Mediterranean Basin Population by Countries
Population-Shorline Density Index
Metabolic Nitrogen and Phosphorus generated by Population

	Total[1] Population 1985 *1000	in Medit Basin 1985 *1000	%	Length of Coastline km	Density Index inh/km	Generated N t/y	Load[2] P t/y
Albania	3,050	3,050	100%	418	7.3	13,420	1,647
France	54,621	11,790	22%	1,703	6.9	51,876	6,367
Greece	9,878	8,862	90%	15,000	0.6	38,993	4,785
Italy[3]	57,300	57,300	100%	7,953	7.2	252,120	30,942
Malta	383	383	100%	137	2.8	1,685	207
Monaco	27	27	100%	4	6.8	119	15
Spain	38,542	13,860	36%	2,580	5.4	60,984	7,484
Yugoslavia[4]	23,153	2,582	11%	6,116	0.4	11,361	1,394
Sub-Total	**186,954**	**97,854**	**52%**			**430,558**	**52,841**
Algeria	21,718	11,500	53%	1,200	9.6	50,600	6,210
Egypt	46,909	16,511	35%	950	17.4	72,648	8,916
Libya	3,605	2,284	63%	1,770	1.3	10,050	1,233
Morocco	21,941	3,390	15%	512	6.6	14,916	1,831
Tunisia	7,081	4,965	70%	1,300	3.8	21,846	2,681
Sub-Total	**101,254**	**38,650**	**38%**			**170,060**	**20,871**
Cyprus	669	669	100%	782	0.9	2,944	361
Israel	4,252	2,886	68%	190	15.2	12,698	1,558
Lebanon	2,668	2,668	100%	225	11.9	11,739	1,441
Syria	10,505	1,155	11%	183	6.3	5,082	624
Turkey	49,289	10,000	20%	5,191	1.9	44,000	5,400
Sub-Total	**67,383**	**17,378**	**26%**			**76,463**	**9,384**
Total	355,591	153,882	43%			677,081	83,096

1 Source: MAP-UNEP, 1989; % adjusted for Italy and France
2 Assuming 4.4 kg N & 0.54 kg P excreted per capita/year
3 There is more detailed information available for Italy;
Density/km in the Northern Adriatic Sea 8-10 times national average!
4 Read: former Yugoslavia

Although the inhabitant/shoreline density index has its shortcomings, a more differentiated mapping of this sort around the whole Mediterranean would nevertheless be quite valuable, particularly for regions where other information is lacking, it is scanty or difficult to obtain otherwise. As population statistics are among the easiest available in most countries, compilation of potential coastal pollution and sensitivity indices would be relatively easy to accomplish by countries.

Mapping of this sort could be made more meaningful if adjusted for levels of sewage treatment installed and pollution abatement measures taken generally. These, and progressive completion of water quality assessment of coastal waters could serve for a long-term evaluation of likely pollution trends. Of course, the primary goal must remain the complete inventory and assessment of all sources that contribute to coastal eutrophication, and its abatement.

b) Estimation of Nitrogen and Phosphorus discharged to the Mediterranean Sea

i) The Italian Pilot Studies. Chiaudani et al. (1978) in their extended investigation on eutrophication related subjects have compiled extended statistics on population, industries, fertilizers used in agriculture, and animal husbandry in Italy, and calculated source strength and emission of phosphorus for each category, region by region, and by hydrographic basins. The authors conclude that the total phosphorus emission from all sources would amount to 59,000 t/year (1974-76 data), of which 42% alone are generated by the four Northern Adriatic Sea bound Regions: Piemonte, Lombardia, Emilia-Romagna, Veneto.

Further, the estimated totals per category and percent distribution of phosphorus emission by category is reported as following:

			1000 t/year	%
a)	Domestic	Detergents	19.60	
		Metabolic	16.24	60.65
b)	Agriculture		10.52	17.80
c)	Livestock	Cattle	3.88	
		Hogs	2.50	
		Sheep and Goats	0.69	
		Horses	0.32	
		Poultry	0.49	
		Total livestock	**7.88**	13.35
d)	Industries		3.58	6.06
e)	Uncultivated Lands		1.27	2.14
		Total	**59.09**	

In principle, this distribution is still valid except for household detergents, the use of which has been restricted by law to 2.5% since 1986, and to 1% as of March 1988. Marchetti (1987) has re-evaluated the earlier estimates on the basis of new data taking into account the reduction of polyphosphates in detergents and assuming a retention coefficient of 50% of phosphorus from the domestic environment. In addition, he has extended the estimates to include nitrogen assuming that 3% of the phosphorus fertilizers and 20% of the nitrogen fertilizers is exported to waterways. As regards phosphorus and nitrogen from animal husbandry, he assumes an export coefficient of 5% of the quantities produced by category. Accordingly, the new estimates read as following:

			1000 t/year	%
	Phosphorus			
a)	Domestic	Detergents	6.21	
		Metabolic	16.39	
		Total	**22.60**	46.36
b)	Agriculture	Cultivated lands	14.42	29.58

c)	Livestock	Cattle	3.15	
		Hogs	1.71	
		Sheep and Goats	0.36	
		Horses	0.22	
		Poultry	1.45	
		Total Livestock	**6.88**	14.11
d)	Industries		3.60	7.39
e)	Uncultivated Lands		1.25	2.56
		Total	48.75 t/year	
			1000 t/year	%
	Nitrogen:			
a)	Domestic	Metabolic	127.13	19.31
b)	Agriculture	Cultivated lands	420.00	63.80
c)	Livestock	Cattle	23.29	
		Hogs	5.09	
		Sheep and Goats	2.21	
		Horses	1.55	
		Poultry	4.08	
		Total Livestock	**36.21**	5.50
d)	Industries		50.00	7.59
e)	Uncultivated Lands		25.00	3.80
		Total	658.340 t/year	

Accordingly, the total phosphorus load would amount to approximately 49,000, and that of nitrogen to about 660,000 t/year. The latter is about 20% higher than Provini's et al. estimate of 540,000 t/y (Provini et al., 1979).

Comparison between these estimates shows the difficulty in estimating total loads. Although the main difference regarding phosphorus is due to the assumed reduction in polyphosphate and retention in treatment plants and septic tank disposal, the respective estimates are of the same order of magnitude, despite the variation in the single component data. The difference as regards nitrogen results mostly from different estimates of nitrogen loss from land, instead.

The study further shows that of the total Italian load about 50-55% of the generated phosphorus from all sources, domestic environment, agriculture and animal husbandry, respectively, flow into the North basin of the Adriatic Sea across less than 5% of Italy's total coastline. Because of proportionality between phosphorus and nitrogen in rivers, it can be assumed that similar proportions hold for the total nitrogen load, although the relative contribution from component sources are at variance.

Whatever the uncertainty of the absolute loading values may be, the Italian study, in as far as it carefully considers the distribution of loads according to source category and major hydrographic basins, can serve as a model to be followed by other Mediterranean countries. Data of this sort provide the basis on which to develop management strategies. Yet, besides the knowledge of such gross facts, knowledge of the basin internal pathways and flux pattern of nitrogen and phosphorus is also of importance. This concept has been pioneered by Bernhardt and his Commission (Bernhardt, 1978) for phosphorus in Germany. A simplified version of Bernhardt's methodology has been elaborated by Vollenweider (1992) for, and adapted to the prevailing conditions of the Emilia-Romagna Region as an example. Data needed are: imports and exports through all external systems boundaries, definition of compartments within systems boundaries, size of, and fluxes between compartments. In addition to data used from Chiaudani et al. others have been taken from the most varied statistics available. The pieces are brought together in Figure 7 showing the major features of the internal basin structure, and the prevailing flow and exchange patterns. The figure illustrates both, the complexity, and also how incomplete our knowledge about the various compartments and flows still remains. Regarding the input output balance, of the roughly 35,000 t of phosphorus that enter the Region in the form of fertilizers and polyphosphates, some 16,000 to 18,000 t leave the Region in the form of farm products (wheat, produce, fruit, meat and bones, dairy products etc.) and others, while only some 10 to 15 % end up in the sea.

Implicitly, this balance sheet accounting contains a warning that managing fertilizers for the sake of phosphorus load reduction to the sea has its limitations, which not only depend on technological constrains but also on implicit constraints that derive from the potential impact, which fertilizer reduction may have on regional economic activities. In other words, fertilizer imports cannot be below those of equivalent product exports. Simple source data listings would hide this problem.

On the other hand, the conclusion regarding nitrogen, may be different, because it seems that nitrogen fertilizers are used in excess of the amounts required to maintain crops at the actual level of production. Unfortunately, no corresponding analysis has been made yet.

ii) Basin load to the Mediterranean. No comprehensive estimate of the total nutrient load to the Mediterranean Sea as a whole seems to have been made yet by any author. This is a lacuna difficult to fill because of the lack of exhaustive source and reliable input data for all countries bordering the Mediterranean Sea. As already discussed, Italy is an exception. Therefore, any estimate that is made from partial information must remain tentative.

Partial budget estimates have been made by Béthoux (1979; 1981; 1986), who discusses the exchange balance between the Mediterranean and the Atlantic, and estimates possible contribution of nitrogen fixation to the nitrogen budget. River inputs have been estimated in a UNEP study (UNEP, 1984). Estimates about atmospheric input have been made by Martin et al. (1989). Uncertainty remains, however, about the magnitude of retention in the basin of nitrogen and phosphorus by processes of sedimentation and accumulation in sediments. A wealth of information is available for the Adriatic Sea, and therefore it would be possible to draw up reasonable balance sheets for this basin. Partial nutrient budgets have been proposed by Vukadin (1992), but some of his figures are questionable. Bombace (1985; 1992) gives figures for total fish catch, which further could be elaborated in terms of nitrogen and phosphorus removed from the sea by fisheries. For the Mediterranean as a whole the extended FAO Fisheries Statistics are available.

The following attempts to estimate the total inputs of nitrogen and phosphorus from land-locked sources are based on the resident population of the Mediterranean basin, the

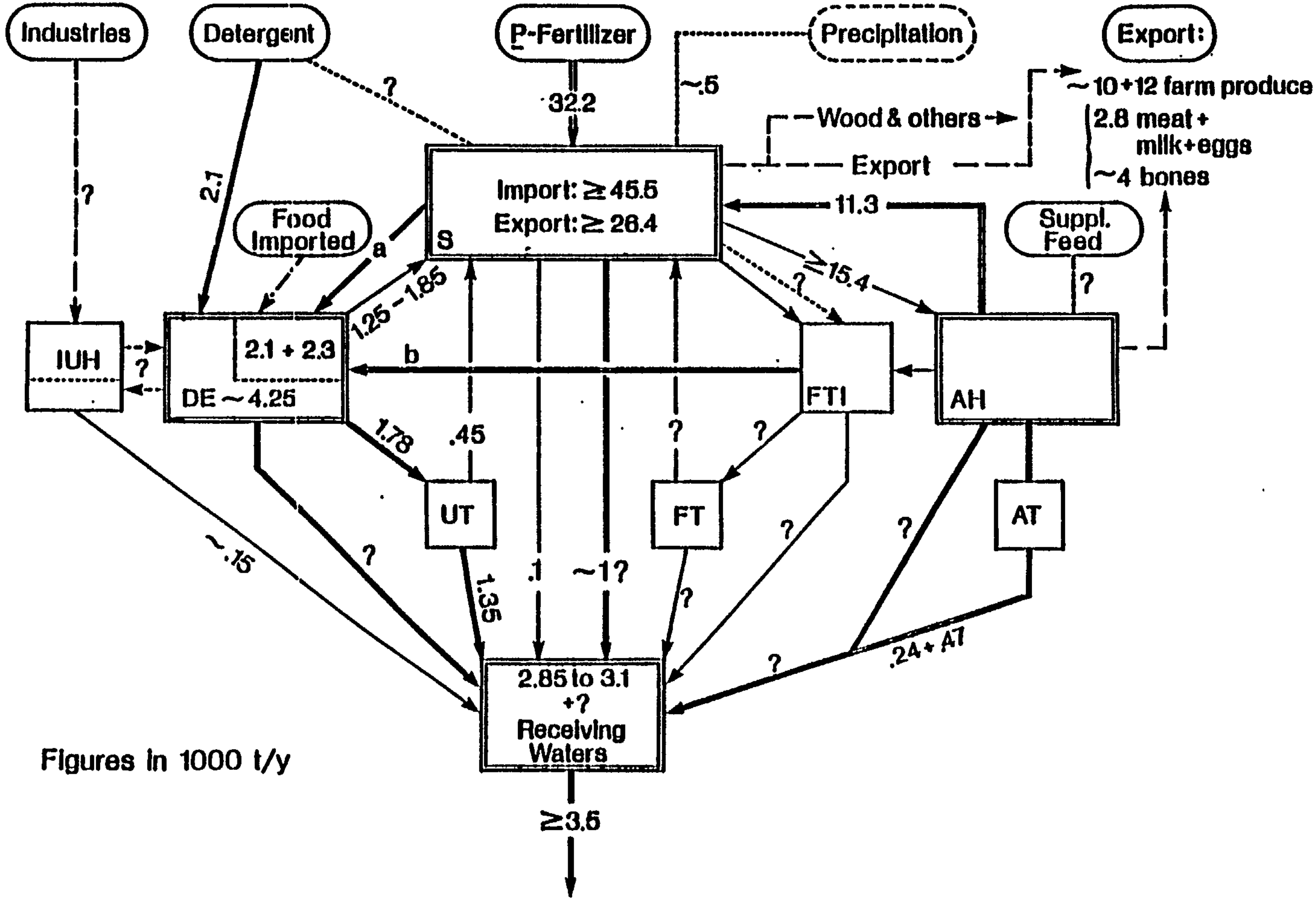

Fig. 7 Phosphorus imports

"active"[5] size of this basin, and known river inputs. Conceptually, the amounts of nutrients that flow into a body of water are a function of natural losses augmented by anthropogenic inputs that in some way are proportional to human activity, which latter in turn is proportional to population density. Accordingly, the total loads cannot exceed a fixed upper boundary; hence, the question arises how to estimate this boundary, as well as how to evaluate within some degree of confidence the probable range of the likely real load.

1) Upper Limits. The 1985 population of the 18 countries bordering directly the Mediterranean (i.e. excluding all Black Sea bound countries, and the Upper Nile African countries) totals 355.6 millions, and the total surface of these countries amounts to 8.5 million sqkm (cf. Table 7). Accordingly, the mean population density would be 39/sqkm.

However, even for approximate estimates of nutrient loads these values are unusable in this form. Actual drainage basin size and resident population would be a better starting point. UNEP (1989) provides figures for the resident Mediterranean population, country by country, but no figures for the respective actual basin surfaces. Correcting the MAP-UNEP data given for France and Italy, the population directly Mediterranean bound would sum up to 153.9 millions, or 43% of the population of the 18 countries. Further, according to UNEP (1984) of the 154 millions about 40 to 45% would reside in coastal areas (UNEP, 1976 data report 44 millions).

Using the % population figures to approximate the corresponding basin size, a first correction reduces the basin estimate to 3.9 million sqkm. Still, in considering the approximate land needed to provide the food and other resources to maintain a self sustaining population of 150 Millions, the figure of 3.9 Million sqkm seems too high for the "active" basin size to use as reference for estimating the nitrogen and phosphorus load from land that may reach the sea. Taking also the extent of unproductive areas, country by country, into account, the figure for the Mediterranean bound "active" basin would reduce to between 1 to 1.5 million sqkm and increase the mean density to about 125 inh./sqkm. These values are somewhat lower than the UNEP (1987) value of 1.8 million sqkm for the Mediterranean region, and slightly larger than the estimated catchment area of 0.85 million sqkm (excluding the River Nile basin) estimated for 69 rivers with a total discharge of some 8840 m^3/sec draining into the Mediterranean.

Using the regression model valid for the Po (cf. Table 5a), the expected nitrogen load from land-based sources could be in the order of 1.5 million t/y, while the phosphorus load would amount to some 0.1 million t/y (Table 8). Yet, this latter figure appears to be on the low side. Using instead the model figures of Table 4, the estimated nitrogen export for a 1 million sqkm area might range from 1.2 to 3 million t/year, and that of phosphorus from 0.16 to 0.25 million t/y; taking 1.5 million sqkm area as reference, the maximum loads would be in the order of 4.5 and 0.38 million tonnes, respectively, which likely represent upper boundary values.

The difficulty lies now in the attempt to narrowing down these figures to values that are consistent with the known nutritional and dynamic conditions of the Mediterranean.

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[5] The term "active" basin is used here as the size of the area that includes population settlements plus all more or less intensively managed land areas to sustain the needs of the population. Accordingly, barren areas, or areas utilized that do not essentially contribute to nitrogen and phosphorus exports are not considered.

Table 7

Mediterranean Basin Population by Countries[1]
Estimation of "Effective" Basin Size and Population Density

	Basin Population *1000	%	Country Surface sqkm	Adjusted Surface sqkm	Density inh/sqkm	Unprod %	"Effective Basin" sqkm
Albania	3,050	100%	28,748	28,748	106	21	22,711
France[2]	11,790	22%	543,965	119,672	99	16	100,525
Greece	8,862	90%	131,990	118,414	75	10	106,573
Italy[2]	57,300	100%	301,262	301,262	190	19	244,022
Malta	383	100%	316	316	1,212	59	130
Monaco	27	100%	2	2	13,500	0	2
Spain	13,860	36%	504,750	181,512	76	7	168,806
Yugoslavia	2,582	11%	255,804	28,527	91	8	26,245
Sub-Total	**97,854**	**41%**	**1,766,837**	**778,453**	**126**		**669,013**
Algeria	11,500	53%	2,381,741	1,261,167	9	82	227,010
Egypt	16,511	35%	1,001,449	352,489	47	97	10,575
Libya	2,284	63%	1,759,540	1,114,782	2	91	100,330
Morocco	3,390	15%	458,730	70,876	48	23	54,575
Tunisia	4,965	70%	163,610	114,719	43	46	61,948
Sub-Total	**38,650**	38%	**5,765,070**	**2,914,033**	**13**		**454,438**
Cyprus	669	100%	9,251	9,251	72	69	2,868
Israel	2,886	68%	20,255	13,748	210	34	9,074
Lebanon	2,668	100%	10,400	10,400	257	62	3,952
Syria	1,155	11%	185,000	20,340	57	21	16,069
Turkey	10,000	20%	779,452	158,139	63	37	99,628
Sub-Total	**17,378**	**26%**	**1,004,358**	**211,878**	**82**		**131,590**
Total	153,882	43%	8,536,265	3,904,365	39		1,255,041

1 Source: MAP-UNEP, 1989
2 France adjusted to 22% and Italy to 100%

2) Estimation of nitrogen and phosphorus loads from of source strength estimates. Given the incomplete information about the various existing sources, estimation of this sort cannot be done without substantial simplification and generalization. In essence it involves making maximum use of those data that have most bearing on generation of nitrogen and phosphorus, i.e., population and agro-industrial activity. An index for the latter, which reflects the degree of modern agricultural development, is the amount of fertilizers used/ha agricultural land, whereby the precise specification of the kind of crop and crop land in question and the composition of fertilizers used must remain open.

Point Sources: domestic and related sources. Figures about the metabolic production of nitrogen and phosphorus by the resident population are among the most reliable for estimating total source strength. Estimates about other sources without extended measurement programmes, on the other hand, are affected by large uncertainty margins. Nitrogen and phosphorus produced by a population of 154 million amount to some 680 000 and 83,000 t/year, respectively (cf. Table 6). Adding another 10% to 20% for unaccounted domestic, industrial and other direct sewage sources, and to phosphorus another 50% to 100 % to account for polyphosphates in detergents, the respective totals increase to between 750,000 and 820,000 t for nitrogen, and to between 145,000 to 185,000 t for phosphorus. Of these one can expect that a some 50% to 60% are discharged into waterways and into the Mediterranean Sea. Accordingly, the maximum loadings from the sources mentioned would rang from between 390,000 to 470,000 t for nitrogen, and 80,000 to 100,000 t for phosphorus.

Table 8

Load Estimate applied to the Mediterranean Basin as a whole
using the coefficients of Table 5a

Effective Basin: Resid. Population:	1.25E+06 1.54E+08	sqkm inh	(cf. Table 7)		
Basin Coeff. (kg/sqkm.y): Popul. Coeff. (kg/inh.y):		Nitrogen 920 2.2	Phosphorus 40 0.35		
Load Estimates:	N kg/y	P kg/y	N/P	N % Contribution	P
Basin Population	1.15E+09 3.39E+08	5.00E+07 5.39E+07	23.0 6.3	77% 23%	48% 52%
Total	1.49E+09	1.04E+08	14.3		

Diffused Sources: Contrary to domestic and related sources, estimates about diffused sources are uncertain, and for the most tentative. Also, the distinction between point and diffused sources is not always easy.

Fertilizers: Fig. 8 and Table 9 report the most recent information on land distribution and fertilizer application in countries bordering the Mediterranean Sea, and these are confronted with data from selected countries North of the Alps. The data listed refer to total commercial fertilizer, and hence, are not directly comparable to the model data used; still, they reflect the correct proportions. Except for France, average fertilizer application in all the Northern Mediterranean countries is substantially below that of Transalpine countries, and that of countries bordering the Mediterranean South and East is generally very low except for Egypt and Israel. It is extremely difficult, however, to estimate with any degree of confidence the amounts that are exported from this source to the Mediterranean Sea. Rather than to give any figure, this question is left open, yet, the quota of fertilizer application is used in a subsequent model estimate.

Animal husbandry: Variations between countries of animal husbandry relative to population are also substantial (cf. Table 10), and regional differentiation becomes even more apparent if data are ordered according to high and low agronomic activity sectors (Table 11). Arab and non-Arab countries differ clearly with regard to pig rearing; cattle raising is generally high in Northern Mediterranean countries and in Turkey (except Greece), while sheep and goat raising is high except in Malta, Israel, Egypt and Italy.

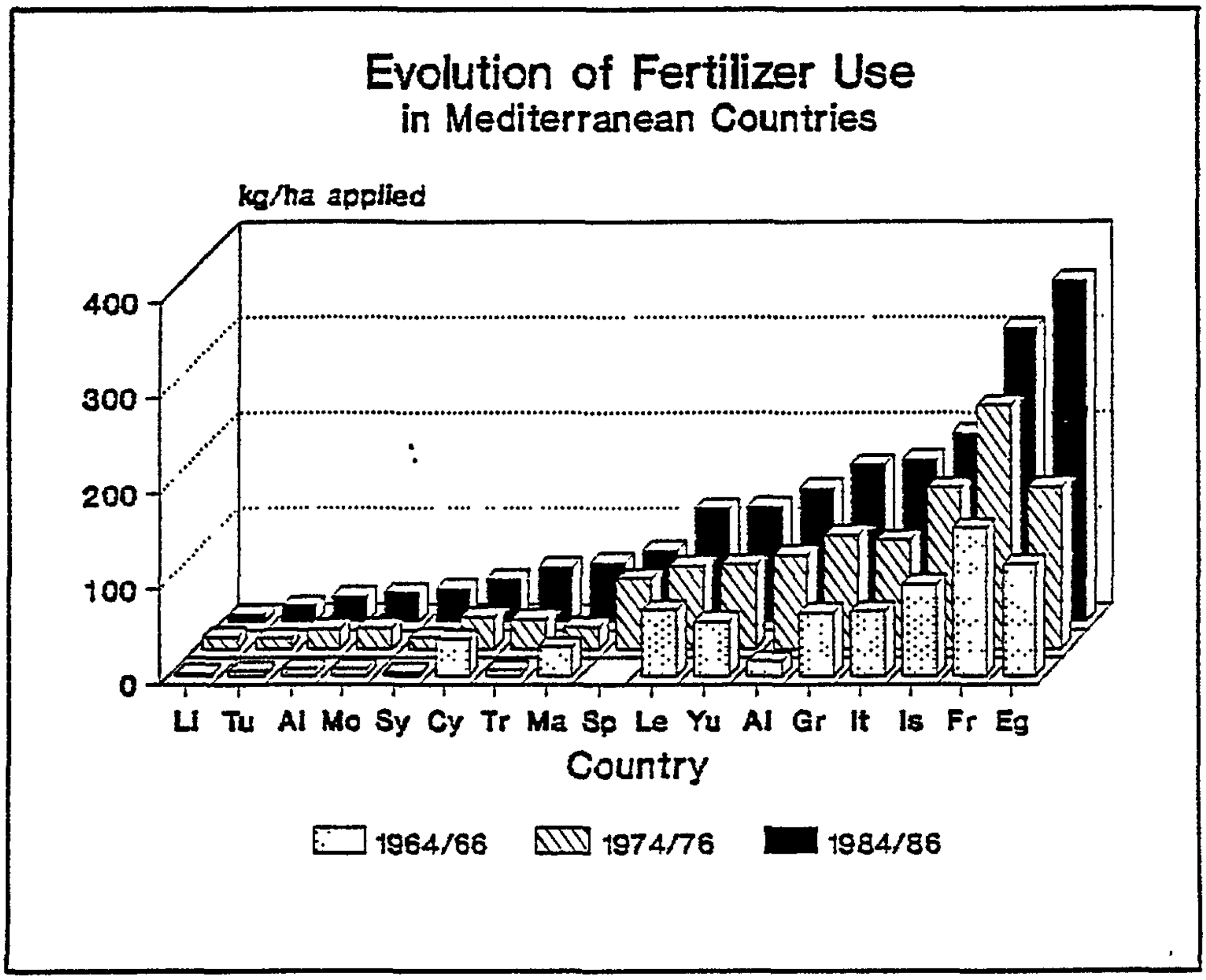

Fig. 8 Evolution of fertilizer use in Mediterranean Countries

Assuming that the Mediterranean basin resident animal population is proportional to the resident human population (which, of course is a coarse simplification) the magnitude of the source strength of the compartment 'animals' in term of phosphorus and nitrogen generated can be reasonably estimated from the population figures in Table 6, the number of animals per inhabitant, and appropriately selected generation coefficients per category. To make estimates comparable to the Italian estimates, the same coefficients, which are essentially those proposed by Vollenweider (1968), have been used. The respective estimates are summarized in Table 12a and 12b, respectively. Accordingly, the total nitrogen generated would be around 2.35 million t/year, and phosphorus around 0.4 million t/year. The export fraction for nitrogen is likely between 5 to 25%, and that of phosphorus between 1 to 5%. This would give a range for nitrogen between 0.12 and 0.6 million t, and for phosphorus between 0.004 and 0.02 million t/year. Interestingly to note that for both nitrogen and phosphorus generation, cattle raising makes out some 50% of these figures, although there are substantial variations from country to country.

Table 9

Land Use and Trend in Fertilizer Use on Croplands[1]

	Cropland Pastures	% Land Use Perm			Fertilizers applied kg/ha			Fert. Factor
		Meadows	Forests	Others	1964/66	74/76	84/86	
Mediterranean Countries:								
Albania	26	15	38	21	15	99	140	1.23
France	35	22	27	16	155	255	308	2.70
Greece	30	40	20	10	66	119	165	1.45
Italy	42	17	22	19	68	114	170	1.49
Malta	41	0	0	59	30	23	62	0.54
Monaco								
Spain	41	20	31	7		74	75	0.66
Yugoslavia	33	25	37	8	57	89	121	1.06
					Weighted Avg		168	1.48
Algeria	3	23	2	82	7	21	27	0.24
Egypt	3	0	0	97	117	170	357	3.13
Libya	1	8	0	91	2	13	8	0.07
Morocco	19	47	12	23	7	22	31	0.27
Tunisia	31	20	4	46	5	11	17	0.15
Cyprus	17	1	13	69	38	33	45	0.40
Israel	21	40	5	34	96	169	198	1.74
Lebanon	29	1	8	62	69	87	119	1.04
Syria	31	45	3	21	3	12	35	0.31
Turkey	36	12	26	37	6	31	58	0.51
					Weighted Avg		77	0.68
Black Sea Countries:								
Bulgaria	37	18	35	9	82	145	232	2.04
Iran	9	27	11	53	3	21	69	0.61
Romania	46	19	28	7	25	104	153	1.34
USSR	10	17	42	31	27	71	102	0.90
					Weighted Avg		169	1.49
Selected European Countries:								
Belgium	25	22	21	33	466	535	536	4.71
Denmark	62	5	12	21	183	234	257	2.26
Germany	31	19	30	21	367	430	423	3.71
Netherlands	26	33	9	32	582	756	787	6.91
Poland	49	13	29	9	84	237	231	2.03
Sweden	7	1	64	27	121	171	154	1.35
Switzerland	10	40	26	23	324	374	432	3.79
					Weighted Avg		379	3.33
Avg Europe	30	19	27	24	Avg F.factor ->			1.00

[1] Sources: UNEP Environmental Data Report 1989/90
World Resource Institute Report 1988/89

Table 10

Livestock Population in Mediterranean Countries

Animals/1000 Inhabitants [1]						
	Cattle	Sheep & Goats	Chickens thousands	Pigs	Horses Mules Asses	Buffalos & Camels
Albania	199	630	1639	69	38	1
France	424	219	3424	203	6	0
Greece	77	1491	3037	111	38	0
Italy	158	184	1937	159	7	2
Malta	37	26	2611	235	5	0
Monaco						
Spain	131	526	1349	292	14	0
Yugoslavia	224	334	3110	372	21	2
Sub-Average	**178**	**487**	**2444**	**206**	**18**	**1**
Algeria	70	823	1013	0	33	6
Egypt	59	109	1087	1	40	58
Libya	55	1776	7490	0	28	50
Morocco	113	748	1504	0	63	3
Tunisia	89	943	2260	1	48	25
Sub-Average	**77**	**880**	**2671**	**0**	**43**	**28**
Cyprus	61	1286	5979	327	73	0
Israel	74	90	6115	28	3	2
Lebanon	18	219	3748	7	6	0
Syria	71	1250	1333	0	26	1
Turkey	339	1166	1217	0	42	13
Sub-Average	**113**	**802**	**3678**	**73**	**30**	**3**
Gross-Avg.:	**129**	**695**	**2874**	**106**	**29**	**10**

[1] Data elaborated from:
UNEP Environmental Data Report 1989/90
World Resource Institute Report 1988/89

Accordingly, the following minimum estimates would result:

	Nitrogen : t/year	Phosphorus : t/year
Domestic and related (60%) :	470,000	100,000
Animals :	120,000 to 600,000	4,000 to 20,000
Total	590,000 to 1,070,000	104,000 to 120,000

To these figures one would have to add a base export from land and unaccounted sources, which, however, would unlikely exceed an additional 25 to 35%. According to Vighi et al. (1987), the European average of diffused agricultural sources (fertilizers + background) amounts to 28% for phosphorus. Thus, the upper actual nitrogen export figures would fluctuate around some 1,450,000 t/year, and that of phosphorus around 175,000 t/year.

A generalized Model. Essentially the same order of magnitude is obtained from a modified model approach. In this, the assumption is made that the generated total load is a function proportional to the human population and its agro-industrial potential. To this end it is assumed that the load contribution from agricultural activities (livestock and crop farming, etc.) is correlated with population density and the level of agricultural development, and the index for this latter in turn would be the amount of fertilizers used per unit of cultivated land. This avoids giving to high weight to areas of prevalently extensive agricultural utilization (e.g., sheep and goats raising), emphasizing instead intensive animal husbandry and crop farming. Indeed, there is loose inverse proportionality between sheep and goats/inh. and fertilizer use/ha (cf. Table 11).

To this end the following model assumptions have been made:

i) estimation of nitrogen and phosphorus generation from population is reasonably accurate;

ii) of the total domestic and industrial load generated, a certain minimum fraction is retained and recycled in the basin;

iii) the percent estimates of population generated loads vary within certain limits of the total basin load generated;

iv) it is reasonable to assume the percent contribution of population lies within the range of 20 to 60% for nitrogen, and within 40 to 80% for phosphorus (cf. above).

v) the loss of nitrogen and phosphorus from land use (including both crop production and livestock raising) is a function of the level of agricultural development, which is assumed to be proportional to the intensity of fertilizer used.

Under i) the population figures in Table 6 are used assuming 4.4 and 0.54 kg per capita/year metabolic production of nitrogen and phosphorus, respectively. Under ii) the further assumption is made that the total nitrogen generated by domestic, industrial and related sources is 1.2 times and that of phosphorus 2.3 times the metabolic value (which includes polyphosphates in detergents[6] and from other sources, and that of both, nitrogen and phosphorus, 50% is retained in the basin either in treatment plants, septic tanks, or otherwise. With this simplified assumption the population values can directly be used for further calculation. iii), iv) and i) are then combined for calculating a table using different assumptions for the basic percentage to attribute to exports from the basin according to the following simplified formula:

Total export = M * ex * {1 + [basic %/(100 - basic %)] * F.f.},

where M = nitrogen or phosphorus generated by population;
ex = export factor: 0.6 for nitrogen, 1.15 for phosphorus;
basic % = assumption for all diffused load regardless of country;
F.f. = fertilizer use factor by country as calculated in Table 9.

[6] For some countries that introduced legislation to limit the polyphosphate content in detergents, like Italy, the figure 2.3 may be somewhat in excess of the present conditions.

Table 11

High-Low Density Agronomic Activity Sectors by Countries

	high		low
1) Fertilizer Use:	kg/ha	kg/ha	
Egypt	357	Spain	75
France	308	Malta	62
Israel	198	Turkey	58
Italy	170	Cyprus	45
Greece	165	Syria	35
Albania	140	Morocco	31
Yugoslavia	121	Algeria	27
Lebanon	119	Tunisia	17
		Libya	8
Avg+/-STD 114+/-98 kg/ha			
2) Cattle Raising:		cattle/1000 inhabitants	
France	424	Morocco	113
Turkey	339	Tunisia	89
Yugoslavia	224	Greece	77
Albania	199	Israel	74
Italy	158	Syria	71
Spain	131	Algeria	70
		Cyrpus	61
		Egypt	59
		Libya	55
		Malta	37
		Lebanon	18
Avg+/-STD 129+/-107 inhabitants			
3) Sheep & Goats:		Sheep & goats/1000 inhabitants	
Libya	1776	Albania	630
Greece	1491	Spain	526
Cyprus	1286	Yugoslavia	334
Syria	1250	Lebanon	219
Turkey	1166	France	219
Tunisia	943	Italy	184
Algeria	823	Egypt	109
Morocco	748	Israel	90
		Malta	26
Avg+/-STD 695+/-531 sheep & goats/1000 inhabitants			

The corresponding estimates are tabulated in Table 13a and 13b. Accordingly, the upper limit for total nitrogen load would be about 2.7 million t/y, the lower limit about 0.8 million t/y. The corresponding values for phosphorus, instead, would be 0.3 and 0.13 million t/y, respectively. Of these, about 65% would come from European countries situated in the North-Northwest, 25% from African countries in the South, and 10% from the other countries in the East-Northeast.

Table 12a

Livestock Population in Mediterranean Countries[1]
estimated for the Mediterranean bound basins

	Animals (thousands)						
	Cattle	Sheep & Goats	Chickens	Pigs	Horses	Buffalos & Camels	Sum:
Phosphorus generated in t/year							
Albania	4,492	1,536	850	798	1,009	17	8,702
France	37,011	2,062	6,862	9,073	648	0	55,655
Greece	5,026	10,569	4,575	3,723	2,896	8	26,976
Italy	66,918	8,434	18,870	34,702	3,515	896	133,335
Malta	104	8	170	342	17	0	641
Monaco	0	0	0	0	0	0	0
Spain	13,391	5,835	3,179	15,377	1,702	0	39,484
Yugoslavia	4,286	690	1,365	3,649	463	39	10,492
Sub-total	**131,227**	**29,134**	**35,871**	**67,663**	**10,250**	**960**	**275,105**
Algeria	5,948	7,571	1,980	10	3,312	590	19,412
Egypt	7,199	1,445	3,052	72	5,745	8,381	25,894
Libya	938	3,245	2,908	0	557	992	8,640
Morocco	2,833	2,029	867	5	1,863	77	7,673
Tunisia	3,279	3,745	1,907	11	2,092	1,092	12,127
Sub-total	**20,198**	**18,036**	**10,714**	**98**	**13,569**	**11,132**	**73,746**
Cyprus	303	688	680	832	426	0	2,930
Israel	1,577	208	3,000	310	65	59	5,219
Lebanon	355	467	1,700	76	139	0	2,738
Syria	605	1,155	262	0	258	9	2,288
Turkey	25,073	9,329	2,069	9	3,691	1,093	41,264
Sub-total	**27,913**	**11,847**	**7,711**	**1,227**	**4,580**	**1,160**	**54,437**
Total	**179,337**	**59,017**	**54,296**	**68,988**	**28,398**	**13,252**	**403,289**
[1] Data elaborated from: UNEP Environmental Data Report 1989/90 World Resource Institute Report 1988/89 Coefficients used:				SUM:			403,289 t/year
kg/ind.ye	7.4	0.8	0.17	3.8	8.7	8.7	

How far are these estimates reasonable? Clearly, given the simplifications, the level of accuracy of these estimates is probably not too high for any country listed, but the range of the totals within which the real loads lay is acceptable. Taking Italy as a test country, which is not only best known, but also the major relative contributor to the Mediterranean load, her total nitrogen export should range between 0.3 to 1 million t/year, and her phosphorus export between 0.027 and 0.058 million tones. Marchetti (1987) estimated the generated total nitrogen load to 0.66 million tones (of which 27% originating from domestic sewage and industry), and the generated total phosphorus load to 0.048 million tones (of which 54% domestic and industrial). This corroborates the range of our estimates. Accordingly, one may conclude that the basic diffused load lay between 60 to 80% for nitrogen, and between 20 to 40% for phosphorus. Regarding other countries, comparison can be made only for phosphorus with estimates made by Vighi et al. (1987) for a few European countries. Adjusting these values for the residence population Vighi's et al. estimates would give 3,600 t for Albania, 20,300 t for France, 13,500 t for Greece, 61,000 t for Italy, 22,500 t for Spain, and 4,100 t for Yugoslavia. In terms of diffused source contribution these figures would compare with the 40 to 50% assumption of the present estimates.

Extending these conclusions to the Mediterranean basin as a whole, then the most likely actual total nitrogen load from land-based sources would lie within the range of 1.5 to 2.5 million tones, and that of phosphorus between 0.15 to 0.25 million tones. These figures compare well with known N/P ratios from rivers (river N/P ranging from about 5 to 15, cf. Table 2 and Fig. 4).

Table 12b

Livestock Population in Mediterranean Countries[1]
estimated for the Mediterranean bound basins

	Animals (thousands)						
	Cattle	Sheep & Goats	Chickens	Pigs	Horses	Buffalos & Camels	Sum:
Nitrogen generated in t/year							
Albania	33,264	9,408	2,400	2,373	7,192	124	54,761
France	274,081	12,628	19,375	26,979	4,617	0	337,680
Greece	37,217	64,736	12,919	11,070	20,636	56	146,634
Italy	495,556	51,661	53,280	103,192	25,048	6,386	735,123
Malta	767	49	480	1,017	124	0	2,437
Monaco	0	0	0	0	0	0	0
Spain	99,163	35,738	8,976	45,727	12,129	0	201,734
Yugoslavia	31,742	4,227	3,854	10,850	3,298	277	54,248
Sub-total	**971,790**	**178,447**	**101,284**	**201,208**	**73,044**	**6,842**	**1,532,615**
Algeria	44,048	46,374	5,592	30	23,605	4,202	123,851
Egypt	53,313	8,849	8,616	215	40,939	59,729	171,662
Libya	6,944	19,878	8,211	0	3,967	7,071	46,071
Morocco	20,981	12,427	2,447	14	13,277	546	49,693
Tunisia	24,284	22,940	5,385	32	14,911	7,782	75,334
Sub-total	**149,571**	**110,469**	**30,251**	**290**	**96,700**	**79,329**	**466,610**
Cyprus	2,247	4,214	1,920	2,475	3,038	0	13,894
Israel	11,679	1,274	8,471	920	463	421	23,228
Lebanon	2,630	2,862	4,800	226	992	0	11,510
Syria	4,477	7,072	739	0	1,841	61	14,189
Turkey	185,672	57,140	5,843	28	26,303	7,786	282,772
Sub-total	**206,705**	**72,561**	**21,773**	**3,649**	**32,636**	**8,268**	**345,592**
Total	**1,328,066**	**361,477**	**153,308**	**205,147**	**202,380**	**94,440**	**2,344,818**
[1] Data elaborated from: UNEP Environmental Data Report 1989/90 World Resource Institute Report 1988/89 Coefficients used:				SUM:			2,344,818 t/year
kg/ind.ye	54.8	4.9	0.48	11.3	62	62	

Table 13a

Estimated Total Nitrogen Load to Mediterranean Sea under 3 assumptions of % diffused source load corrected for amount fertilizer used /ha by countries

basic % assumption> Population generated			Total estimated N-Load			estimated actual % point source [1]		
			80%	60%	40%			
	t/y	F.f.	t/y	t/y	t/y			
Albania	13,420	1.23	47,647	22,900	14,651	17%	35%	55%
France	51,876	2.70	367,855	157,399	87,247	8%	20%	36%
Greece	38,993	1.45	158,987	74,242	45,994	15%	32%	51%
Italy	252,120	1.49	1,054,547	490,000	301,818	14%	31%	50%
Malta	1,685	0.54	3,213	1,837	1,378	31%	55%	73%
Monaco	119	0.00	71	71	71	100%	100%	100%
Spain	60,984	0.66	132,982	72,737	52,656	28%	50%	69%
Yugoslavia	11,361	1.06	35,787	17,680	11,645	19%	39%	59%
Sub-total	**430,558**		**1,801,090**	**836,868**	**515,461**	14%	31%	50%
	64%		67%	66%	66%			
Algeria	50,600	0.24	59,152	41,157	35,159	51%	74%	86%
Egypt	72,648	3.13	590,174	248,559	134,687	7%	18%	32%
Libya	10,050	0.07	7,724	6,665	6,312	78%	90%	96%
Morocco	14,916	0.27	18,695	12,604	10,574	48%	71%	85%
Tunisia	21,846	0.15	20,934	16,043	14,412	63%	82%	91%
Sub-total	**170,060**		**696,680**	**325,027**	**201,143**	15%	31%	51%
	25%		26%	26%	26%			
Cyprus	2,944	0.40	4,558	2,813	2,231	39%	63%	79%
Israel	12,698	1.74	60,607	27,490	16,450	13%	28%	46%
Lebanon	11,739	1.04	36,484	18,084	11,950	19%	39%	59%
Syria	5,082	0.31	6,798	4,455	3,674	45%	68%	83%
Turkey	44,000	0.51	80,183	46,569	35,364	33%	57%	75%
Sub-total	**76,463**		**188,630**	**99,410**	**69,670**	24%	46%	66%
	11%		7%	8%	9%			
Total t/	**677,081**		**2,686,400**	**1,261,305**	**786,274**	**15%**	**32%**	**52%**
Total Load Estimate: = 0.6*Pop-N* {1 + [basic %/(100 - basic %)]* F.factor}								

[1] Correspondingly: Actual % diffused load = 100 - % point source load

c) Estimates from river discharges

Good experimental river discharge estimates are difficult to achieve even under favourable conditions. Meteo-climatic conditions play a major role: in dry years river loads may be substantially below average, while in wet years major fractions of the total load may be washed out during a few peak hydraulic discharge periods. Thus, in their study of the River Po, Provini et al. (1992) found the phosphorus load in dry years to be as low as 7,000 to 10,000 t/y, while for the same river the authors estimated values as high as 18,000 to 20,000 t/y in wet years. Therefore, load estimates calculated from one or two year data only, compounded by unreliable measurements of the hydraulic load and infrequent sampling, may be entirely misleading.

Table 13b

Estimated Total Phosphorus Load to Mediterranean Sea under 3 assumptions
of % diffused source load of total correction for amount
fertilizer used /ha by countries

basic % assumption> Population generated			Total estimated P-Load			estimated actual % point source [1]		
			60%	40%	20%			
	t/y	F.f.	t/y	t/y	t/y			
Albania	1,647	1.23	5,387	3,446	2,476	35%	55%	76%
France	6,367	2.70	37,025	20,523	12,272	20%	36%	60%
Greece	4,785	1.45	17,464	10,819	7,497	32%	51%	73%
Italy	30,942	1.49	115,261	70,996	48,863	31%	50%	73%
Malta	207	0.54	432	324	270	55%	73%	88%
Monaco	15	0.00	17	17	17	100%	100%	100%
Spain	7,484	0.66	17,110	12,386	10,024	50%	69%	86%
Yugoslavia	1,394	1.06	4,159	2,739	2,029	39%	59%	79%
Sub-total	**52,841**		**196,854**	**121,250**	**83,448**	31%	50%	73%
	64%		66%	66%	65%			
Algeria	6,210	0.24	9,681	8,270	7,565	74%	86%	94%
Egypt	8,916	3.13	58,468	31,682	18,289	18%	32%	56%
Libya	1,233	0.07	1,568	1,485	1,443	90%	96%	98%
Morocco	1,831	0.27	2,965	2,487	2,248	71%	85%	94%
Tunisia	2,681	0.15	3,774	3,390	3,198	82%	91%	96%
Sub-total	**20,871**		**76,455**	**47,314**	**32,744**	31%	51%	73%
	25%		26%	26%	25%			
Cyprus	361	0.40	662	525	456	63%	79%	91%
Israel	1,558	1.74	6,466	3,870	2,571	28%	46%	70%
Lebanon	1,441	1.04	4,254	2,811	2,090	39%	59%	79%
Syria	624	0.31	1,048	864	772	68%	83%	93%
Turkey	5,400	0.51	10,954	8,319	7,001	57%	75%	89%
Sub-total	**9,384**		**23,384**	**16,388**	**12,890**	46%	66%	84%
	11%		8%	9%	10%			
Total t/	**83,096**		**296,693**	**184,953**	**129,083**	**32%**	**52%**	**74%**
					Avg:	**50%**	**66%**	**82%**
Total Load Estimate: = 1.15*Pop-P* {1 + [basic %/(100 - basic %)]* F.factor}								

[1] Correspondingly: Actual % diffused load = 100 - % point source load

Unfortunately, the Mediterranean river load estimates are insufficient to make valid comparisons. Accordingly, stretching the existing information beyond its range of validity is unavoidable. UNEP (1984) lists water discharge rates and catchment basin size for 69 rivers representing about 50 to 60% of the Mediterranean bound basin. To use these figures for nutrient load estimates it is assumed that the export coefficients for nitrogen and phosphorus calculated for the 4 Italian rivers plus the Rhône are true for the whole Mediterranean. The respective river nitrogen and phosphorus load estimates are listed according to the 10 Mediterranean basins defined by UNEP (cf. Table 14).

While the individual figures appear to be reasonably correct for rivers of the Northern arc, they are very uncertain for rivers of other regions. Difficult to asses are figures from countries that drain only partially into the Mediterranean and/or having large badly drained unproductive areas (e.g., wadies). Therefore, the single estimates may not be too precise, but the respective totals, excluding the Nile, would amount to about 1 million t/y for nitrogen, and to 0.14 million t/y for phosphorus.

Further the effect of large irrigation systems, as installed in several Mediterranean countries, on nutrient export is difficult to assess without actual measurements. Thus, it is impossible to say what the effects on nutrient discharges to the Mediterranean of the Asswan High Dam construction, the closure of one Nile arm, and the changes in agricultural practice of Egypt have been. The highly developed millennium old irrigation system that drains mostly into the Northern delta lakes acts largely as sinks for phosphorus and nitrogen. On the other hand, the Egyptian use of fertilizers has dramatically stepped up over recent decades (cf. Table 9). Elster and Vollenweider (1961) and Vollenweider and Samaan (1972), studying L. Mariout, L. Edku and the Nousha Hydrodrome found this latter to be still oligo-mesotrophic by 1957-59, while Mariout receiving untreated city waters from part of Alexandria was already hypertrophic. On the other hand, the trophic conditions of Nousha Hydrodrome, which receives land drainage waters, have meanwhile strongly deteriorated. Thus it is possible that though the average water discharge of the Nile has decreased substantially, concentrations have increased, but probably not to the point of balancing the former nutrient load.

Regarding total loads, a source of uncertainty are direct discharges into marine waters from coastal cities and municipalities that are not draining into major rivers. On the other hand, the estimated totals of nutrients from river discharges are reasonably in agreement with the above model estimates, which, taken at face value, would largely confirm the figures obtained with different methodologies.

<u>The UNEP (1984) estimates for 1976</u>. Based on very scanty data, a UNEP expert group that met twice in 1976, estimated a nitrogen load from resident population and rivers discharging to the Mediterranean of 800,000 to 1,200,000 t/y, and correspondingly a phosphorus load of 260,000 to 460,000 t/y. While the fractional load estimates of the coastal population (44 millions) + industry and agriculture (ca. 200,000 t/ of nitrogen; 57,000 t/y of phosphorus) are reasonably comparable to the way of our estimates, the indirect load estimates that would result from the river drained hinterland are likely to be on the low side for nitrogen (600,000 to 1,000,000 t), but clearly in excess for phosphorus (200,000 to 400,000 t) compared to our estimates. The respective average river N/P load would be around 2.5 to 3, which in no way is supported by direct estimates and measurements. Accordingly, also the exchange load estimates between the Mediterranean and the Atlantic through the Strait of Gibraltar made by Béthoux (cf. below), are questionable.

d) Aeolian depositions

Beside nutrient supply to marine waters by rivers and direct discharges, aeolian deposition of nitrogen, phosphorus and other inorganic and organic trace species transported by air currents from land locked sources has increased over recent decades over the

Table 14

Regions and Rivers of the Mediterranean

Region Name	State	Rivers	Discharge m^3/sec	Surface 10^3 sqkm	Load Estimates* Nitrogen 10^3 t/y	Phosphorus 10^3 t/y
1 Alboran	Spain	1				
	Morocco	5				
	Total	6	120.2	56.42	121.71	16.55
2 Northwest	Spain	9				
	France	8				
	Italy	1				
	Total	18	2725.7	229.25	297.60	40.47
3 Southwest	Spain	1				
	Algeria	5				
	Italy	1				
	Total	7	107	79.4	99.25	13.50
4 Tyrrhenian	Italy	3				
	Tunisia	1				
	Total	4	388	46.9	58.63	7.97
5 Adriatic	Italy	11				
	Yugoslavia	4				
	Albania	3				
	Total	18	3365.2	145.75	182.19	24.78
6 Ionian	Greece	1				
	Italy	1				
	Total	2	180	1.4	29.51	4.01
7 Central		0				
8 Aegean	Greece	6				
	Turkey	1				
	Total	7	1020	80.65	169.47	23.05
9 Northeast	Turkey	3				
	Cyprus	1				
	Total	4	431.02	41.33	51.66	7.03
10 Southeast	Israel	2				
	Egypt	1				
	Total	3	501.02	2961.2	1.50	0.20
	Totals		**8838.14**	**3642.3**	**1011.52**	**137.57**

Discharge and Basin Data from UNEP, 1984
Note: a few lacking surface areas have been interpolated
* Export Coeff. used: Nitrogen 1.25 t/sqkm.y; Phosphorus 0.17t/sqkm.y
Load Estimates of Region 10 do not include River Nile Loads

otherwise natural yet low supply of many inorganic and organic compounds. Aeolian deposition occurs essentially either as dry deposition of particulates resulting largely from erosion and wind transport (e.g., fertilizers whirled up), or as wet washouts by precipitation. According to GESAMP (1989) wet aeolian nitrogen deposition makes out about 60 to 70 % of total oceanic deposition globally. Also, of the total about 60% is in the form of oxidized nitrogen species.

Flux rates vary considerably from region to region, but are highest in the Northern Hemisphere. Mean North Atlantic fluxes rates have been estimated to 0.24 g/m^2.year, but over the North Sea and the Baltic, flux rates of 0.6 to 1 g/m^2.year are more typical, likely due to the high amounts of fertilizers applied in surrounding countries (cf. also Vollenweider, 1968). On the other hand, the value of 4.1 g/m^2.year given for the Western Mediterranean (Martin et al., 1989), appears not to be acceptably high for the Mediterranean as a whole. Aeolian nitrogen supply in the Northwestern Mediterranean would then equal that of rivers and direct discharges (350 versus 400 thousand t/year; l.c.). Taking instead a more conservative value of 0.1 - 0.2 g/m^2 for the whole Mediterranean, which is more in line with (though still higher) Béthoux's estimate of 0.075 g/m^2.y measured near Nice (Béthoux, 1986); then the aeolian nitrogen input could be in the order of 250,000 to 500,000 t/year. As with river inputs, most of it would likely be deposited along the Northern arc. Disregarding these uncertainties, aeolian nitrogen deposition to the Mediterranean Sea is probably making a substantial contribution to the total nitrogen load.

Estimates about aeolian phosphorus fluxes are more scanty globally. Inland fluxes of some 10 to 50 mg/m^2.year have been found, and it may be reasonable to estimate the aeolian load to the Mediterranean to range between 25,000 to 50,000 t/y. Nevertheless, it is not warranted at this time to consider such figures as final.

e) Black Sea and Atlantic Sea exchange load.

The mean Black Sea net water exchange is in the order of 6,000 m^3/sec (12,600-6,100 m^3/sec), and that of the Atlantic through the Strait of Gibraltar amounts to some 70,000 m^3/sec (1,750,000-1,680,000 m^3/sec), both toward the Mediterranean (Svedrup, 1943). Recent estimates of the Atlantic exchange made by Béthoux (1979) are slightly lower but not essentially different (1680,000 and 1600,000, or 53 and 50.5*10^{12} m^3.year, respectively).

Transfer through, and exchange of pollutants between the Black Sea via the Marmara and the Aegean Sea have been studied by Aubert et al. (1990). The authors note that dissolved nitrogen and phosphorus increase from the Bosporus to the Dardanelles, but give no figures. The N and P production from Istanbul alone amounts to at least some 14,000 and 2,500 t/year, respectively. Of these one can assume that at least 50% is retained in the Marmara Sea. Accordingly, the load increment from this source to the Mediterranean as a whole remains modest (about 1-2%), but the relative load to the Northern Aegean Sea will be much higher.

The Atlantic exchange, instead, is a major factor determining the nutrient balance of the Mediterranean. Béthoux (1979; 1986), evaluating available information, derives values for the nitrogen and phosphorus exchange through the Strait (Table 15). Regarding deep water outflow totals he assumes that the bulk of nitrogen and phosphorus is inorganic using average concentrations of 6 mmol/m^3 for $N(NO_3)$ and 0.28 mmol/m^3 for inorganic phosphorus multiplied by the respective water load. Regarding inflow estimates for total nitrogen from the Atlantic to the Mediterranean the author considers three assumptions for DON in excess of

Table 15

Nitrogen Budget for the Mediterrranean
calculated from J.P. Béthoux; 1979 & 1986)

Assumption -->	1	2	3	Phosphorus t/year
	t/year			
Atlantic Inflow:				
- inorganic	742,000	742,000	742,000	
- organic	0	742,000	1,484,000	
Total	**742,000**	**1,484,000**	**2,226,000**	77,500*
Medit. Outflow:	4,242,000	4,242,000	4,242,000	
N-deficit to satisfy	3,500,000	2,758,000	2,016,000	
Terrestr. Discharges:				
- inorganic (UNEP)	1,036,000	1,036,000	1,036,000	
- organic estimated	546,000	546,000	546,000	
Total	**1,582,000**	**1,582,000**	**1,582,000**	359,600
Rainfall:	182,000	182,000	182,000	
Estimated N2-fixation:	1,736,000	994,000	252,000	*

* Balance values

measured inorganic nitrogen, and theorises from the respectively estimated balance deficits about the possible magnitude of nitrogen fixation by cyanobacteria (*Trichodesmium*), macrophytes (*Posidonium*), and their epiphytes growing in the Mediterranean. Inorganic phosphorus load, on the other hand, was simply derived as balance deficit using the UNEP river load data. The respective estimations are summarised in Table 16.

Béthoux's considerations are interesting but raise some serious questions. Regarding nitrogen, figures appear to be in tune with our estimates about river load and aeolian depositions, while the phosphorus budget is hardly sustainable by our figures. Our load estimates are about half of the UNEP figures. Besides, Béthoux budget estimates remain partial in any case neglecting retention by sedimentation of both, nitrogen and phosphorus, and nitrogen losses by denitrification. However, it might be that nitrogen retention and denitrification may approximately be balanced by the aeolian contribution. If our figures are correct as to the order of magnitude, aeolian nitrogen load could make out some 15 to 25% of the total.

A similar equalisation cannot be postulated for phosphorus as long as the aeolian contribution remains unknown. Also, it does not seem that as a whole the discrepancies could be explained by concentration increases due to increased nutrient discharges from land-based sources, as Mediterranean deep water concentrations remained practically constant over the last two decades (cf. data reported by Coste, 1969; McGill, 1969; Krom, 1991). It is more likely that a) the UNEP phosphorus load estimates have been overestimated, a conclusion that derives from an unacceptably low N/P average ratio (6.4 by atoms = 2.9 by weight), which is in contrast to our data; b) the return flow balance of 77,500 t/y is an underestimate. Taking our upper load estimate from land-based sources of 250,000 t/y, then

<u>Table 16</u>

Areal Nitrogen and Phosphorus Load in g/m^2.year;
Selected Examples*

	Nitrogen	Phosphorus **
Baltic proper (210,000 km^2):	4.3	0.3 (0.1-0.2)
North Sea (500,000 km^2):		
Atlantic exchagne load + atmospheric contribution	4.2	0.9
River + direct discharges	1.9	0.2
Inner Oslo Fjord (200 km^2):	17.0	3.0
Laholm Bay (300 km^2):	24.0	1.4
Japan:		
Tokyo Bay (1,400 km^2)	89.2	10.7 (0.5-1.0)
Ise Bay (170 km^2)	43.4	5.8 (0.5-1.0)
Seto Sea (22,000 km^2)	8.2	0.8 (0.15-0.3)
Adriatic Sea: Emilia-Romagna Coast (2,000 km^2)	70.0	7.8 (0.4-0.8)
(ortho-phosphate P)		3.6
Mediterranean Sea (2,505,000 km^2) land-based + aolian input	1.5$0.5	0.15$0.05 (#0.1)

* Data elaborated from IOC, 1988; Moller-Andersen, 1986

** Values in parentheses indicate loading tolerance range that would apply to lakes of corresponding morphometry and hydrology

the required deficit to account for would amount to 187,000 t/y. Assuming an aeolian load of 25,000 to 50,000 t/y (10% of the estimated aeolian nitrogen load), plus some 75,000 t/y of inorganic phosphorus (Béthoux), plus 75,000 t/y in organic particulate and dissolved form (modifying assumption 2 of Béthoux's table, using P/N (at)=1/16); then the sum would cover the estimated deficit. Accordingly, the total phosphorus return flow through the strait would amount to some 150,000 t/y.

Algal blooms along the Almaria-Oran front are common. Therefore, it is not only conceivable but likely that part of the required nutrient return flow to the Alboran Sea originates from the Guadalquivir basin, and that nutrient rich coastal waters from that region is mixed with nutrient poor atlantic water, or sucked along the coast. However, to verify this assumption, more detailed studies around the strait areas are required. If proven, then programmes of sanitation of the Mediterranean should also extend to areas outside areas directly discharging to the Mediterranean. Of course, this reasoning would also apply to the Black Sea-Marmara exchange.

4.6 Concluding remarks

In summary, and regardless of the many uncertainties that make integral estimates difficult, the load estimates to the Mediterranean made under b) are plausibly in the right order of magnitude. If so, this would re-dimension some of the cursory figures reported elsewhere. Vudkadin (1992) estimates the river input of nitrogen and phosphorus into the Adriatic Sea alone to 250,000 t and 82,000 t (N/P = 3). The same figures are cited by Attenborough (1987) in a popular book, and gives also input values of nitrogen and phosphorus into the Northern arc of the Western basin between Genoa and Valencia as 340,000 t N and 115,000 t P (N/P = 3). While the nitrogen input may be in the right order of magnitude, the phosphorus loads appear to be grossly overestimated, even if one doubles the input estimates under (b) for which there is no justification to do so.[7]

Another perspective resulting from these estimates concerns the question whether the Mediterranean as a whole is endangered by eutrophication. The answer follows from estimating the average areal load due to anthropogenic nutrient inputs. Accepting the figures under b), though neglecting the Atlantic and Black Sea interchanges, the mean areal nitrogen load to the Mediterranean as a whole would be $1.5 \ \$ \ 0.5\ g/m^2$.year, and that of phosphorus $0.15 \ \$ \ 0.05\ g/m^2$.year.

Comparing these figures to those known from other marine areas (cf. Table 16) show that the Mediterranean figures are still very low. Accordingly, it is safe to conclude that the main body of the Mediterranean as a whole is not yet seriously threatened by eutrophication over the next decades. The problem instead is local and regional, limited largely to specific coastal and adjacent offshore areas, where it still can be quite serious, as will be substantiated by the review of the specific eutrophication incidences around the Mediterranean in the following Chapter 5 of this report.

However, these local and regional problems must in no case be underestimated as to their potential socio-economic and sanitary impact on tourism, aquaculture, fisheries, and other water uses (cf. Chpt. 6 and 7). Without the necessary cure and precaution taken, and in view of the projected population increase (cf. Chpt. 5) and its related activities, these problems will become aggravated over the next 25 years.

However, it follows also that though coastal eutrophication in the Mediterranean is a widespread problem, it can be brought under control if the political will exists to take the necessary measures.

5. EXTENT OF EUTROPHICATION AND ALGAL BLOOMS IN THE MEDITERRANEAN

5.1 General description of the Mediterranean

The Mediterranean is an intercontinental sea. On the north, it laps the coasts of southern Europe, on the south North Africa and on the east Asia Minor. The Italian peninsula and Sicily divide the Mediterranean into two basins, the west and the east, communicating through the channel of Sicily. The length of the main east-west axis is 3,860 km.; although

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[7] We suspect that such phosphorus values have been calculated as ortho-phosphate, and not as P. This is a common error. Appropriate correction would bring down the loads to acceptable figures.

the average width is not over 700 km, maximum width is approx. 1,800 km. The average depth of the Mediterranean has been estimated to be 1,502 meters. The greatest depth is found at the Matapan trench, located in the eastern basin, at 4,632 meters. In the western basin, maximum depth is southwest of the Island of Ponza (Tyrrhenian Sea), at 3,731 meters, cf. Table 17. Excluding the Black Sea, the area of the Mediterranean is 2,556,000 square kilometers. Compared to other oceans, the Atlantic for example, which has a surface of 85,620,000 square kilometers, the extension of the Mediterranean Sea basin ranges among those of modest dimensions.

Given the latitude at which the Mediterranean is located, the temperature of the surface waters varies significantly with seasons. Temperature variations and rang ranges are more marked on the northern side of the basin, less on the southern and eastern parts. The difference in temperature in the northwest Adriatic is significant; a variation of 24EC can be measured between winter lows (+4EC) and summer highs (+28EC).

Due to strong evaporation and to limited fluvial input, the average salinity is generally much higher than in the other oceans. Around Gibraltar, the salinity is near 36 per thousand; as one moves eastward, the average salinity increases, reaching a level of 39.5 in the eastern basin. The high level of evaporation not compensated by fluvial or meteorological inputs leads to a positive exchange balance with mostly surface inflow of waters from the Atlantic. As far as a total estimate of inflow (Atlantic and Black Sea inputs, fluvial input, precipitation), and outflow (evaporation, Mediterranean outputs to the Atlantic and Black Sea) is concerned, see Table 17. Accordingly, the hydrological balance is principally maintained by the inflow of masses of oceanic water entering from the Strait of Gibraltar.

Due to this inflow and the earth's rotation, an anti-clockwise current is generated which, on the south side of the basin, moves from west to east, and in the opposite direction on the north side. Due to the basin conformation, there are secondary eddies in the Tyrrhenian, the Aegean and the Adriatic, moving in an anti-clockwise direction as well. However, in the Alboran Sea modified atlantic waters move mostly clockwise, and waters are slit to form the Almeria-ran jet (cf. below). In the Adriatic the abstraction of Mediterranean waters is activated mainly by density currents generated by fluvial inputs (Po, etc.) into the northwestern part of the basin.

Eutrophication

Satellite images of the Mediterranean able to show the variations in chlorophyll in surface waters, reveal that the highest levels of autotrophic biomass correspond to the areas close to river deltas or those off large urban agglomerations. Conversely, the open sea waters of the Mediterranean are generally close to oligotrophy or even ultraoligotrophy (Béthoux, 1981; Cruzado et al., 1988; Krom et al., 1988; Innamorati et al., 1992) except for cases generally caused by the upwelling of deep waters rich in nutrients.

The manifestations of eutrophication due to the fertilizing substances produced by man are not the same in all cases and there is no linear relationship between cause and effect. Above all, the degree of dilution by the receptor body of water, the hydrodynamics of the coastal systems, and seasonality are important parameters which may favour or prevent the formation of algal blooms and their secondary manifestations (hypoxia/anoxia of the waters near the seabed, bottom fauna kills and general deterioration of organoleptic qualities).

Table 17

Hydrographic data of the Mediterranean sea

A) Morphometry	Surface sqkm	Depth Max m	Mean m	Volume ckm
Mediterranean:	2,505,000	5,020	1,450	3,632,000
Basins:				
Alboran basin		1,470	<700	
Western basin		3,068		
Golf of Lyon			<97	
Ligurian Sea		2,560		
Tyrrhenian Sea	275,000	3,550		
Adriatic Sea	132,000	1,260		
North Adriatic (Golf of Venice)			<50	
Middle Adriatic			<200	
Ioanina Sea	50,000	5,020		
Aegean Sea	196,000	2,530	<800	
Eastern Basin		3,432		
B) Water Budget (cf. Svedrup 1943, p.179)		1000 m^3/sec	cm/y	Res.Time
Gains:				
Inflow from the Atlantic Ocean		1750.0		
Inflow from the Black Sea		12.6		
Precipitation		31.6	38	
Runoff		7.3		
	Total	1801.5		
Losses:				
Outflow to the Atlantic Ocean		1680.0		
Outflow to the Black Sea		6.1		
Evaporation		115.4	145	
	Total	1801.5		ca 75 years

Figure 9 summarizes the most important sites of reported phenomena of eutrophication in coastal and lagoon areas. Virtually all the countries around the Mediterranean offer more or less obvious cases; although the northern shores are generally the most affected, the problem of eutrophication is also causing serious problems in the south, and there is ground for fear that these problems may assume much vaster proportions than at present. If forecasts are correct, the population of the North African states will rise from today's 109,300,000 inhabitants to 208,000,000 in the year 2,025 (Agnelli Foundation, 1991). Considering further the development stage of production technologies in these countries and the virtually total absence of environmental policies, eutrophication problems will scale up in the future.

As to the scientific information available about Mediterranean eutrophication, coverage provides a fairly satisfactory picture of the present situation, although the density of information acquired on specific problems varies depending in part on the existence of qualified research institutes and the availability of funds. Thus, the absence of data on the

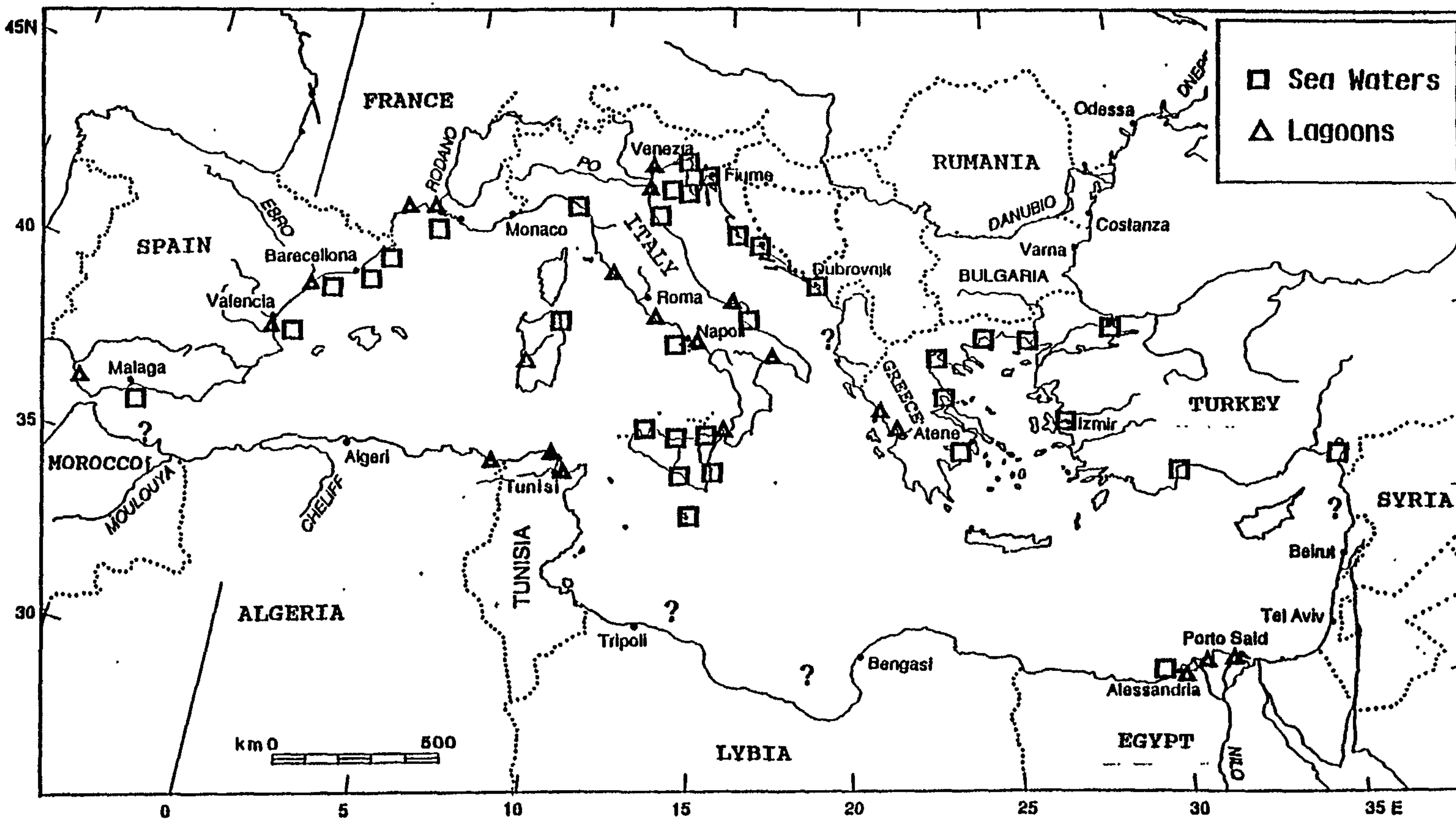


Fig. 9 Mediterranean areas where eutrophication phenomena were reported

coastal waters of Albania, Syria, Libya and Morocco makes it impossible to define the trophic status of their transition waters.

5.2 Review of recorded incidents of eutrophication by regions and countries

5.2.1 Spain: Western Mediterranean

Alboran Sea. Diatom blooms occur periodically along the southern shores of Spain from the Straits of Gibraltar to Almeria as a result of upwelling generated along the path of the Atlantic current entering through the Straits of Gibraltar. Moving along the Andalusian coast and offshore into the southern Alboran Sea at its eastern boundary, this current determines the so-called Almeria-Oran Front which showed a permanent bloom recently described by Tintoré et al. (1989) and Martinez et al. (1990). The same conclusions were reached by Minas et al. (1983), who emphasize that the high productivity of the basin to the North-West of the Alboran Sea is strongly related to upwelling associated with the anticyclonic vortex generated by the flows of Atlantic waters entering through the Straits of Gibraltar.

Localized eutrophication is reported in the Bay of Malaga, where Jmenez et al. (1986) describe the presence of winter blooms. Cortes et al. (1985) also reported high values of chlorophyll-a in the area off the city of Malaga in 1992; the authors indicate the effluents from Malaga and the input of the Guadalhorce river as the main causal factors. Significant quantities of dinoflagellates which produce PSP group toxins were also reported by Bravo et al. (1990) along the Andalusia coastal area between Malaga and the Bay of Algeciras; in this case the species was *Gymnodinium catenatum* which, in January-February 1989, was present with concentrations in excess of 3,000 cells/l.

East coast and Baleares. Describing the distribution of nutrients in the Gulf of Sant Jordi (Terragona), which is under the influence of the nutrient inputs of the Ebro river, Deya (1981) emphasizes the fact that the development of the autotrophic biomass is phosphorus controlled. At Barcelona, Margalef (1968) reports a case of "red tide" produced by *Chattonella subsalsa*. At the port of San Carlos de la Rapita, geographically to the South of the Ebro delta, in May 1989 Delgado et al. (1990) observed a large bloom (28,000,000 cells/l) of *Alexandrium minutum* (a dinoflagellate listed amongst the species capable of synthesizing group PSP toxins). Other reports come from the Cullera zone, where diatoms (*Thalassiosira* and *Chaetoceros* species) have generated blooms with values of 7.200,000 cells/l during the winter months (Del Rio et al., 1986). Balle Curellas (1965) also reports cases of abnormal proliferation of diatoms off the Balearic Islands. In view of the location these blooms should probably be attributed to normal cycles of upwelling of deep waters rich in nutrients.

Lagoons, bays, estuaries. In areas such as lagoons and estuaries where primary productivity is generally high, increased supply of eutrophying substances of human origin has been raising the trophic level to worrying levels over recent years. In areas of reduced water exchange this condition generates significant levels of dystrophy and states of hypoxia/anoxia (Lopez and Arte, 1971). Cases have been reported by Miracle et al. (1988) in the Albufera of Valencia; this lagoon (area 26 km^2; mean depth of 1.1 m) receives an influx of nutrients equivalent to 2.077 tons of nitrate+nitrite nitrogen, 1.908 of ammoniacal nitrogen, and 619 tons of phosphorus every year (Vicente et al., 1990). Other cases are reported for the lagoons of Encanizada, Platjola and Olles in the Ebro delta by Comin (1986), and for the Lagoon of Cadice in the Guadalquivir delta to the West of the Strait of Gibraltar. Some of these areas are of particular interest to naturalists because of the birds that winter and breed there. Amongst such zones, Cruzado (1990) mentions the Alguamoles of the Ampurda, the lagoons along the coast of Valencia, the Menor Sea and the Guadalquivir estuary system.

Even if the connection with eutrophication is doubtful, blooms of planktonic and nectonic organisms consisting of Salpae, jelly-fish and *Noctiluca miliaris* have been observed in various areas of the Southern coast of Spain. Early in 1986, a microalgae bloom which originated in the Bay of Rosas affected 120 km of coastline (Cruzado, 1989).

5.2.2 France: North-western Mediterranean

General situation regarding eutrophication along French coasts. Areas affected along the Atlantic French coast are mainly subject to excessive proliferation of macroalgae (Ulvaceae, particularly in the lagoons and in some Breton bays) and to phytoplankton blooms in the areas adjoining the estuaries of the major rivers. During the last twenty years these phenomena have gradually increased, in direct correlation with the increased presence of eutrophying substances of human origin. Naturally, the areas most affected are those with the longest water residence times, and those which receive water from river basins with large populations and/or from urban agglomerations along the coast (Menesguen, 1990).

The excessive proliferation of macroalgae has created fairly significant environmental dystrophies (summer anoxia in the lagoons and bays, production of volatile substances such as hydrogen sulphide and large accumulations of phyto-biomass) generating serious economic problems for the local authorities, which have had to remove the masses of Ulvaceae from the beaches (Piriou, 1986). Along the coasts of Brittany from 1985 to 1988 the total cost of collecting these biomasses rose from 1.6 to 2.5 million francs (CEVA, 1989).

Also, abnormal proliferations of microalgae seem to be more significant and widespread in the northern and Atlantic coasts than on the Mediterranean coast. During 1975-1988 Belin et al. (1989) recorded 62 cases on France's northern coast, 125 on the Atlantic and 26 on the Mediterranean (Fig. 10). In general the organisms which have led to "coloured water" are dinoflagellates of the species *Noctiluca scintillans*, *Prorocentrum minimum*, *Gonyaulax sp.*, *Gyrondinium aureolum* and *Gymnodinium sp.*. From an overall analysis of the events which have occurred in the North Sea, it appears that in many cases flagellates have acquired dominance over diatoms during the last 20 years (Radach and Berg, 1986). The algal blooms observed during the period 1975-1988 showed a constant increase until 1984, after which there was a reduction in events that in numerical terms settled around values comparable to those of 1982-83.

Eutrophication in the Mediterranean. From the geographical point of view, the French Mediterranean area can be divided into the following sub-areas:

- Western zone between the spanish border and the Rhône delta (Gulf of Lions). With many lagoons, it has a hinterland without particularly large-scale industrial activity and a few large towns that discharge their effluents straight into the sea. Nutrients affecting this area originate above all from the Rhône basin (95,000 km^2) which brings to the sea some 5,000,000 tons of suspended solids (Leveau and Coste, 1987), 76,000 tons of inorganic nitrogen and 8,400 tons of phosphorus per year (Coste et al., 1985). The coastal zones and marine waters mostly affected by the inputs from the River Rhône lie between Marseilles and Cape Creus extending over an area of about 11,000 km^2. The plume generally tends to move westwards, gradually diluting, since it is deflected by the oligotrophic waters of the Liguro-Provençal Current (Sournia et al., 1990). The general rise in the level of eutrophy causes widespread diatom blooms in winter and spring, and blooms of dinoflagellates in summer when the weather and sea conditions are favourable (low hydrodynamism, static waters and high temperature), (Perez et al., 1986).

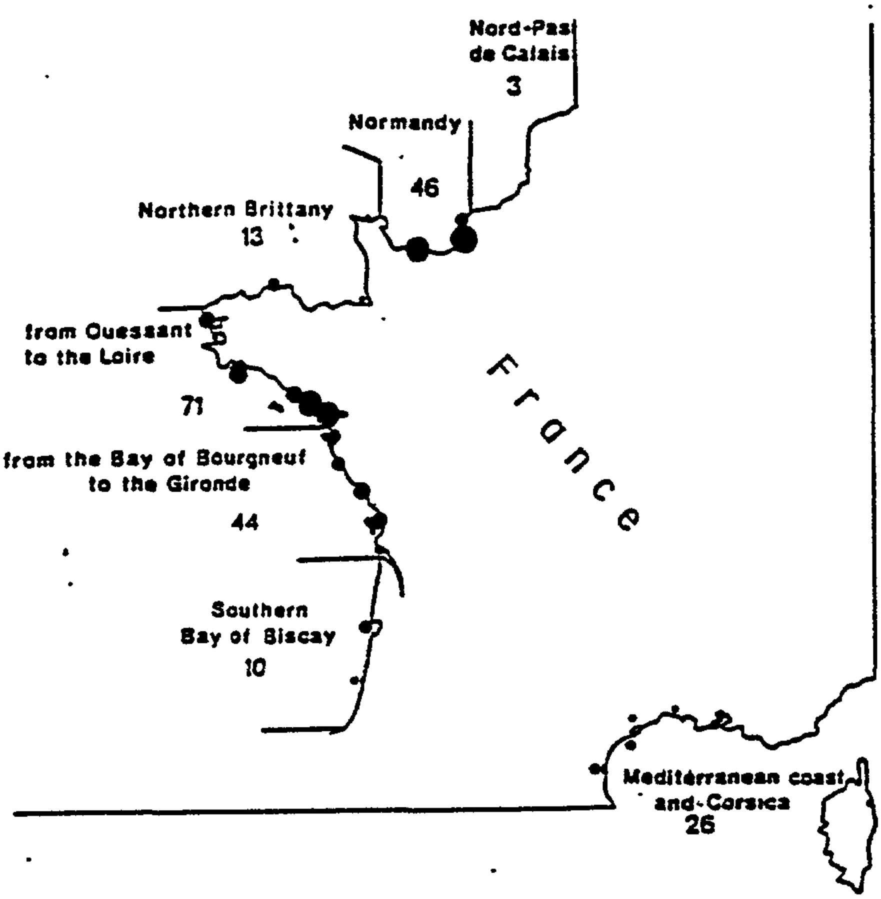


Fig. 10 Cumulative occurrences of discoloured waters along the French coast from 1975 to 1988 (after Belin et al., 1989)

Some of the coastal lagoons in the Languedoc-Rossignol Region are also affected by periodic events of eutrophication (Ménesguen, 1990). Lieutaud et al. (1991) attribute the gradual trophic deterioration of eight lagoons along the shoreline of Montpellier (from the Or to the Ingril lagoons) to the increased input of nutrients of urban and agricultural origin.

- Eastern zone between the Rhône delta and the Italian border. This zone receives the effluents from large towns and cities (Marseilles, Toulons, Cannes and Nice) and from important industrial areas (Region of Fos). There are generally no eutrophication phenomena in the open sea waters because of the westward drift of oligotrophic waters carried by the Liguro-Provinçal Current (Soumia et al., 1990). Events of algal blooms are mainly limited to ports and coastal waters near the points of urban and industrial effluent discharges. The same applies to the Berre and Vaine Lagoons, where Kim and Travers (1986) report high nutrient values and the development of massive algal blooms. Bellan (1972) discusses the gradual deterioration of the Berre and Vaine lagoon due to heavy pressure from the human population, which has caused profound modifications in the animal and vegetable communities typical of these ecosystems. Vitello and Keller (1991) reach the same conclusions from their studies on the meiobentos of the Berre lagoon.

- Corsica. This island, with few lagoons (Diana and Urbino) and with a fairly high level of hydrodynamism along its coasts, is only marginally affected by the urban effluents from the cities of Bastia and Ajaccio, and by the small amounts of industrial effluents discharged. Eutrophication phenomena are of little significance and very occasional off Corsica.

As to nuisance species it appears that the numbers of toxic flagellates present in French seas have increased over time. Until 1982 only *Gyrondinium aureolum* was present, while the *Dinophysis* species appeared in 1983 gradually becoming dominant. *Alexandrium minutum* was reported for the first time in 1988 (Belin et al., 1989). *Dinophysis* is present off the South coast of France, where it is thought to have made its first appearance in 1987 (Leveau et al., 1989; Lassus et al., 1991).

Routine analyses performed in areas affected by the presence of toxic flagellates have highlighted three important facts:

a) their presence cannot be correlated with and does not depend on the inputs of nutrients resulting from human activities;

b) in the areas where these organisms are present there is no alteration at the level of grazing due to inputs of pollutants (Leveau et al., 1989);

c) their presence even at low concentrations (a few individuals/litre) and in absence of real blooms may be unsafe to human health.

It must be noted that anoxia in French coastal waters has never reached the levels of the Skagerrat (Rosenberg, 1985) or the northern Adriatic (Rinaldi et al., 1993). Shortages of oxygen in the waters near the sea-bed after algal blooms were reported in the Bay of Vilaine in July 1984 (Marceron, 1987), but there were no serious consequences.

5.2.3 The Coasts of Italy

General. Manifestations of eutrophication occurring in the Italian seas depend on the hydrology, hydrodynamics and morphology of the areas concerned. In the Ligurian Sea, the Tyrrhenian Sea and the southern Adriatic, the phenomena are episodic and generally not widespread, with secondary effects (hypoxia/anoxia in the bottom waters) being of but little significance. Eutrophication causing conditions arise to a large extent from the effects of effluent discharges from urban agglomerations and only in a few cases from inputs of rivers. If we exclude a number of lagoon areas (Orbetello, for example) the negligible damages caused by eutrophication occurring on Italy's western coasts is due, in large part, to the hydrodynamic and morphological characteristics of these seas: the rapid processes of exchange with open sea waters, the resulting dilution factors and the low water residence times all attenuate the phenomena.

This is not the case throughout the northern Adriatic Sea. Apart from receiving huge quantities of fertilizing substances, this part of the Adriatic is very shallow (only 50 meters on the line from Rimini to Pula) and has physical and hydrodynamic characteristics which tend to segregate the nearshore and offshore systems for long periods; this is particularly noticeable in the area to the South of the Po delta, where, in the summer, water residence times may reach 40-50 days. Eutrophication under such conditions can be serious.

Italian West coast seas and Islands

Ligurian Sea. In general eutrophic conditions are not found in the Ligurian Sea. Near Genoa, waters show low trophic levels with orthophosphate values between 0.02-0.31 mg-at/m^3, nitrite-nitrogen levels between 0.5-41.8 mg-at/m^3, and 1.2-4.1 mg/m^3 of chlorophyll-a (Genovese, 1979). Carli et al. (1992) also found similar conditions in two coastal stations close to Genoa (Genoa Sturla and Genoa Quinto), and in the generally oligotrophic Bay of Riva Trigoso east of Genoa (Carli et al. 1994). Variations over time in the phytoplankton populations off Chiavari (eastern Liguria) also shows low autotrophic biomass (chlorophyll-a mean 0.2 mg/m^3; maximum of 5.4 near the coast), (Cattaneo, 1982).

The generally oligotrophic characteristics of the Ligurian offshore waters are confirmed by Innamorati et al. (1985; 1986a) who found phytoplankton concentrations rarely to exceed 200,000 ind/litre (200,329 cells/l with *Cryptomonoas spp.* dominant in 1980 and 278,063 cells/l with *Amphidinium curvatum* dominant in 1979). Similar conditions extend into the waters of the Tuscan archipelago and of the northern Tuscan Tyrrhenian Sea; phytoplankton populations normally show low concentrations (mean value in July 1983 11,400 cells/l.), (Innamorati et al., 1986b, 1992; Lazzara et al., 1989).

Off the La Spezia Harbour, Zurlini (1991) conducted a study on the nutrient loads adjoining the port area, and defined their trophic state on the basis of the criteria recommended by the OECD (1982). Overall, from the various sources the roads of La Spezia receive 86.1 tons/year of phosphorus and 506.2 of nitrogen, giving the system mesotrophic characteristics with a tendency towards eutrophy in the most internal areas directly affected by the urban inputs. Cattini et al. (1992) reach the same conclusions, highlighting the fact that the highest values of eutrophy are found near the port area of La Spezia, where a maximum chlorophyll-a value of 15.4 mg/m^3 was found.

The situation close to the mouth area of the River Arno is interesting. Although in general high nutrient concentrations are found (annual averages of 9.5 mg-at/m^3 of nitric+nitrous nitrogen and 1.02 of orthophosphate) in this area, phytoplankton biomass values are low (annual average 180 cells/l and 4 mg/m^3 of chlorophyll-a). The apparent inability of algae to assimilate the nutrients available seems to be due to inhibitory toxic substances (synthetic detergents and surfactants) discharged from industrial plants located in the hydrographic basin of the Arno (Innamorati et al., 1989).

Tyrrhenean Sea. One area repeatedly affected by high trophic conditions and significant dystrophic processes is the Lagoon of Orbetello (Grosseto - southern Tuscany). The large amounts of nutrients of urban origin which have been discharged into the lagoon for 15 years promote periodic algae blooms which, particularly during the summer and autumn, cause frequent crisis of anoxic. Still, once normal conditions have been restored the ecosystem generally recovers rapidly (Lenzi and Salvatori, 1986). The resulting damage to the fish population of this lagoon ecosystem have led to short-term measures including the collection of the macroalgal biomass and the forced exchange of waters (Lenzi, 1992).

Dystrophies caused by eutrophication phenomena are not reported in the coastal discharge area of the River Tiber (Pettine et al., 1983). In spite of the high trophic levels normally found at the two mouths of the river, as a result of the very active hydrodynamics of the site, the natural conditions of oligotrophy of the Tyrrhenian Sea and/or the presence of toxic substances capable of inhibiting the growth of autotrophic biomass, no microalgal blooms are reported (Zoppini et al., 1989).

After the conspicuous increase in the human population of the adjacent areas, which started in the '30s, the coastal Lake of Sabaudia (7 km^2 in area, in southern Latium) gradually passed into a state of eutrophy; sewage inputs from the town of Sabaudia (5,000 residents + 15,000 tourists during the summer season) and from a pig farm of 1,500 head led to recurrent anoxic crises. The most serious one occurred in July 1979, when all the fish stocks were destroyed (Perdicaro, 1980). From 1980 the treatment of the sewage and the closure of the pig-farm led to a gradual improvement of the basin (Perdicaro, 1985).

Other reports of eutrophic environments come from the Lagoons of Miseno, Fusaro and Patria (Naples), where the discharge of untreated sewage and industrial effluents have seriously reduced water quality. For the Fusaro lagoon, Carrada et al. (1988) also reports a bloom of *Gymnodinium catenatum* (dinoflagellate capable of synthesizing PSP group toxins) with concentrations of 6,000,000 cells/l.

Gulf of Naples. Two subsystems can be recognized in the Gulf of Naples: one is of "open sea water" with oligotrophic characteristics essentially determined by waters originating offshore; the other, nearshore system presents pronounced eutrophication phenomena triggered by the inputs along the coast (Genovese, 1979). However, there are significant variations in the coastal area; because of the poor circulation of the waters particularly in the summer months, plumes of dark waters caused by sewage outfalls may often be identified. Carrada et al. (1979) recorded high algal biomass values to the East of Naples in the areas with the highest nutrient input during the summer of 1977. Zingone et al. (1985) report a bloom caused by *Chaetoceros simplex* in the summer of 1983 that reached 120,000,000 cells/l in the areas most affected by the effluents from the city of Naples. Ribera D'Alcalà et al. (1989) encountered algal blooms in the same area in May 1987 which, although not widespread, reached peak concentrations of chlorophyll-a of 176 mg/m^3. Modigh et al. (1985) emphasize the inverse correlation between salinity and chlorophyll-a values that further underlines the role of nutrient-rich waters and biostimulants from the sewage system.

Carrada et al. (1982) found in the Gulf of Salerno in November 1981 the coast-offshore gradients of the autotrophic biomass to be low with concentration between 0.1 - 0.8 mg/m^3 of chlorophyll-a.

No further episodes of eutrophication are reported from other areas of the Tyrrhenian, including southern Campania and the Calabrian coast which seems to be unaffected by eutrophication (De Domenico, 1979).

Sardinia

In general, the coastal waters and lagoons of Sardinia seem to be little affected by eutrophication. The few reports concern the Gulf of Olbia, the Gulf of Cagliari and the Santa Giusta Lagoon (Oristano).

For the Gulf of Olbia Sechi et al. (1987) report a bloom of *Gymnodinium sp.* which occurred in September 1985. This event caused kills of fish and molluscs as a result of prolonged anoxia of the bottom waters. The trigger causes are attributed above all to the discharge of sewage from the town of Olbia. States of anoxia leading to fish kills have also occurred in the Lagoon of Santa Giusta (central-western Sardinia); in a study conducted during 1990-91 by Delogu et al. (1992) the phenomenon is associated to the abnormal growth of microalgae (Ulvaceae) and diatoms caused by high eutrophying inputs of urban origin.

In the Gulf of Cagliari, Loi et al. (1981) found high nutrient concentrations in the coastal area most affected by urban and industrial effluents. For the same gulf, Genovese (1979) also reports high primary productivity values (446 mg C/m^2/day) in the presence of *Rhizosolenia firma*.

Sicily

Northern shores. Frequent blooms of Chlamydomonadaceae are reported (De Domenico, 1979) for in the Bay of Milazzo, but their development is probably not correlated with the inputs of nutrients. In the adjoining Gulf of Patti (Messina), in July 1969 Gamgemi (1973) detected blooms of the same microalgae after heavy rains.

Also in the province of Messina, cases of eutrophication were reported in the brine Lakes of Ganzirri (Magazzu' et al., 1991) and Faro (Magazzu', 1982) that lay in basins of high human activity. Large numbers of fish died in the Lake of Ganzirri in 1990 as a result of anoxia. *Dinophysis sacculus* (a toxic species capable of synthesizing DSP group toxins) reaching 40,000 cells/l have been reported in other brine lakes (Olivieri and Tindari) in the same area.

More or less frequent algal blooms occur in coastal waters of the Gulf of Palermo summer (Genchi et al., 1982; 1983). Due to discharges of inadequately treated sewage high values of nutrients and chlorophyll-a were found above all in the area off and to the East of the port of Palermo (for the area near the port: 25 mg-at/m^3 of ammoniacal nitrogen, 158 of nitric nitrogen, 360 of orthophosphate and 30 mg/m^3 of chlorophyll-a in March 1981). Coastal waters are also affected by a high bacterial pollution.

The Gulf of Castellammare also shows recurrent cases of eutrophication caused by the excessive inputs of nutrients from sewage and industrial effluents (Calvo and Genchi, 1989); total input of 321 tons/year of phosphorus and 1,471 of nitrogen, 195 and 685 of these respectively of industrial origin have been estimated.

Riggio et al. (1992) report an interesting succession of biological indicators in the same area. As a consequence of increased eutrophication the bivalve *Mytilaster minimum*, which favours oligotrophic-eutrophic environments, was partially supplanted by *Mytilus galloprovincialis* that grows better in eutrophic-hypertrophic conditions.

Southern shores. A corresponding case is reported for the Gulf of Gela. Increased trophic conditions induced by urban and industrial waste had detrimental effects on the fishing industry. Modification in the texture of the sediments, which had become sandy-muddy, favoured limivorous species of little commercial interest; thus, high productivity is actually contrasted by a low value of the fish caught (Arculeo et al., 1990).

Eastern shores. Giacobbe and Maimone (1991) report widespread presence of significant amounts of *Dinophysis spp.* in the coastal waters off Syracuse with concentrations reaching 2,000 cells/l. De Domenico (1979) reports eutrophic conditions off Augusta, where recurrent blooms occur above all in the western section. In January 1977 very high nutrient concentrations were measured in the north-western and central part of the roads, with 1.6 mg-at/m^3 of orthophosphate and 20.0 mg-at/m^3 of nitric nitrogen.

Ionian Sea. In the Gulf of Taranto, microalgae blooms occur during the summer months only in the bay known as the "Mare Piccolo". One report of this is given by Magazzu' (1982) who describes a bloom which occurred in 1973.

Southern and Central Adriatic Sea

Pulia, Gargano, Abruzzi, Marche. Conditions of oligotrophy normally predominate along the easter coasts of Puglia. Marano and Rizzi (1985) report fairly high concentrations of phytoplankton organisms only for the areas corresponding to Porto Nuovo, Bari, and the Gulf of Manfredonia.

The coastal Lake of Varano (Foggia) is more seriously affected by high trophic levels; this lagoon is periodically subject to eutrophic events with fairly serious dystrophic consequences. There is a risk of widespread putrefaction during the summer months unless increased exchange with sea-water is guaranteed (De Angelis, 1964). In a study carried out from May 1985 to April 1986, Tolomio et al. (1990) highlight the presence of high concentrations of nutrients and autotrophic biomass.

There are few reports from the southern and central Adriatic about events of eutrophication; in fact, this area is generally considered oligotrophic. South of the Conero promontory (Ancona) waters three miles off shore are between mesotrophy and oligotrophy; small areas of eutrophy only occur along the coast near the mouths of rivers and urban agglomerations (Artegiani et al., 1979).

Going north reports about increased trophic levels and microalgae blooms become more numerous. Off the Conero promontory, Artigiani et al. (1985) record a large-scale bloom of *Gymnodinium sp.* monitored during October 1984. This bloom, indeed the largest in distribution and intensity during the last twenty years, started at the end of July 1984, and actually affected a large part of the north-western Adriatic. Very high biomass values were found adjacent to the river Po delta, and in the zones close to larger towns; close to the Po delta chlorophyll-a values in excess of 600 mg/m^3 were measured (Emilia-Romagna Region, 1985; cf. Fig. 11). Although its intensity and distribution waxed and waned, the bloom died out in December of the same year. Blooms caused by this microalga have special features which differ from those of other dinoflagellates, since this organism is able to withstand steep thermal and salt gradients without showing signs of stress. The start of the bloom (July) coincided with water temperatures around 24EC, while by December when the bloom disappeared, the temperature had dropped to 8-9EC. Unaffected by salinity it was equally abundant both in low-salinity (20-24 in the area near the Po delta) and high-salinity areas (35-37 off Ancona). None of these episodes caused anoxia of the bottom waters, probably because this microalga is not thecated, so it floats instead of sedimenting when dead; vast areas of sea covered in floating yellowish patches were visible particularly in October, when the bloom started to die off.

The situation in the coastal waters of the Marche, North of the Conero (Ancona) is oligotrophic, although it may be slightly affected by variations due to the inputs of the river Po (Scaccini Cicatelli et al., 1972; Scaccini Cicatelli, 1974). Modest increases in trophic levels are also noted near the mouths of local rivers and close to sewage inputs from the towns along the coast. Scaccini Cicatelli (1967) was one of the fist researchers to draw attention to the state of phosphorus-limitation in the coastal waters of the north-western Adriatic; the data used refer to two cruises in August 1965 and March 1966 in the area between the Po delta and the Conero promontory. Poli Molinas and Olmo (1968; 1969) and Olmo and Poli Molinas (1970), Scaccini Cicatelli and Falcioni (1972) and Tegaccia and Tegaccia (1985) all came to the same conclusions; the data obtained in a limited area, off the mouths of the Metauro and the Arzilla, demonstrate that in all three years there was always an excess of soluble nitrogen salts and a lack of orthophosphate. Soluble nitrogen (nitric, nitrous and ammoniacal) and orthophosphate discharged into the sea by the Metauro in 1969 amounted to 238 tons for the nitrogen and 6.5 tons for the phosphorus, respectively.

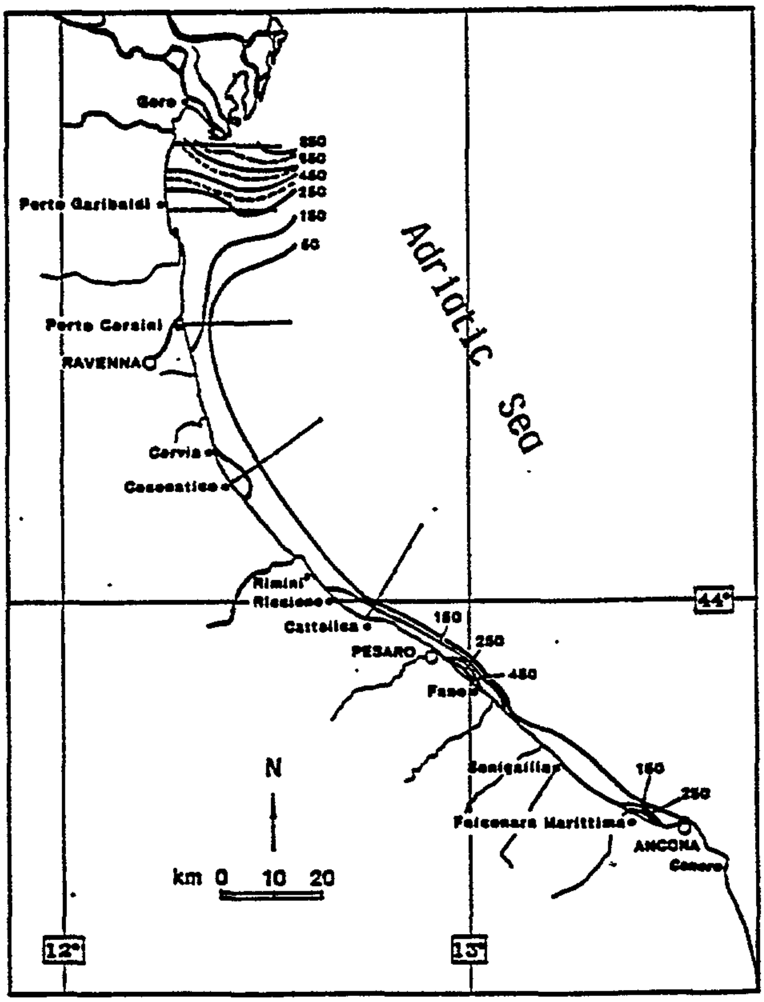


Fig. 11 Distribution of chlorophyll concentrations (mg/m^3) in the coastal waters of Emilia-Romagna and Marche 1984; (after Annual Report, Regione Emilia-Romagna 1985)

In 1969 the area also experienced the first widespread bottom fauna kill; the zone involved reached from the Po delta to the northern part of Marche's coast. Piccinetti and Manfrin (1969) attribute the causes to an abnormal proliferation in May of dinoflagellates (*Peridinium depressum* dominated). Other cases of anoxia along the Marche's coasts, caused more by the passive transport of anoxic waters from the North than by abnormal proliferations of microalgae, are referred to by Penna et al. (1986), who record the beaching of benthic organisms along the coast of the Pesaro area in June 1986.

Northwestern Adriatic

Moving northward, we come to the area most directly affected by the inputs of the river Po.

Emilia-Romagna coasts. Phenomena of eutrophication with distribution and persistence much greater than in any other case that have occurred in other parts of the Mediterranean, have occurred and continue to occur in the coastal waters of Emilia-Romagna to the South of the Po delta (Rinaldi and Montanari, 1988). The first cases reported date back to 1969 (cf. above). These were followed by a relatively long period in which the phenomenon no longer occurred, until it returned in 1975 (Mancini et al., 1986). In September of that year an immense bloom of flagellates caused widespread anoxia in the bottom waters, accompanied by bottom fauna kills and huge beachings of bottom fish (7,000 tons in the Municipality of Cesenatico alone).

Similar events succeeded each other with a certain regularity in the summer of almost all the following years (Fig. 12). The blooms which occur in this area are normally caused by Diatoms and Dinoflagellates (Emilia Romagna Region, 1981-1991 and Vollenweider et al., 1992). The former, although they may cause blooms at any time of the year, they tend to dominate during winter and spring, while flagellate blooms occur especially in the summer and autumn (see Fig. 13 and Table 18).

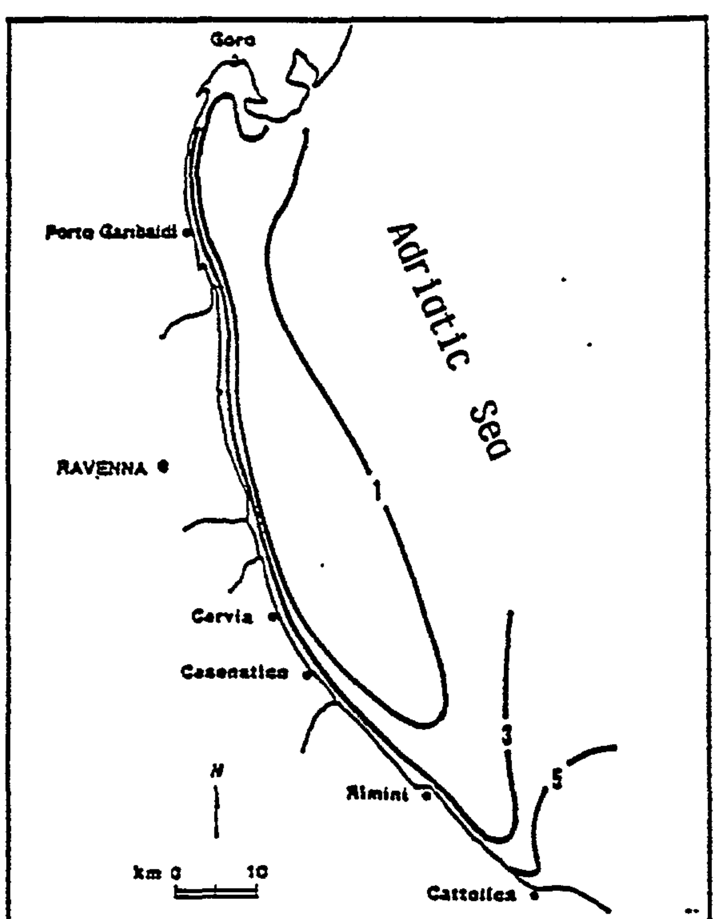


Fig. 12 Distribution of hypolimnetic oxygen concentrations (mg/l) along the Emilia-Romagna coast in August 1982; (after Annual Report, Region Emilia-Romagna 1983)

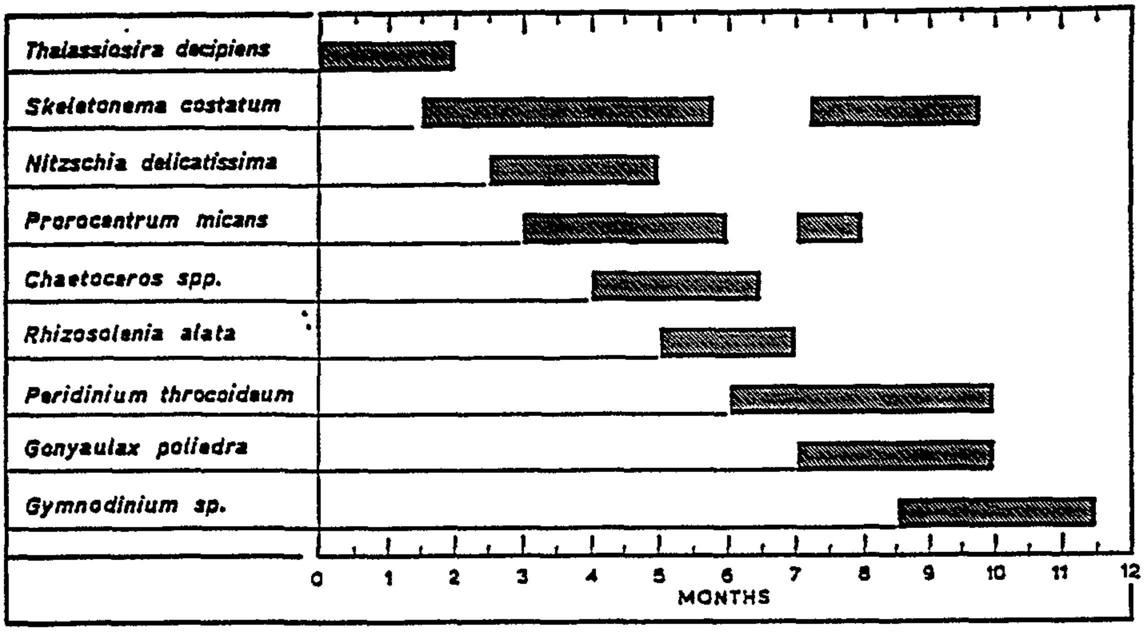


Fig. 13 Prevailing seasonal occurrence of some of the most common algal bloom-forming phytoplankton species

Table 18

Mean red-tide episodes due to dinoflagellates in the Northern Adriatic 1976-1985; (after Mancini et al., 1986)

SPECIES	YEARS 76	77	78	79	80	81	82	83	84	85	Max. cell/l x 10^3
Gymnodinium sp.	*	*	*	*	*	*		*	*	*	230,000
Gonyaulax polyedra		*	*	*			*		*	*	35,000
Protogonyaulax tamarensis							*			*	11,800
Prorocentrum micans	*	*							*	*	3,800
Prorocentrum minimum					*					*	29,000
Prorocentrum triestinum										*	20,000
Glenodinium foliaceum				*							5,500
Glenodinium lenticula					*	*	*				33,000
Glenodinium quadridens					*			*			15,000
Noctiluca miliaris			*		*			*			1,200
Katodinium rotundatum					*	*		*	*		224,000
Scrippsiella trochoidea							*			*	13,300

The effects of recurrent anoxia of bottom waters caused profound modifications in the benthic ecosystem; there were considerable reductions in the original populations of the least mobile bottom organisms (molluscs, crustaceans and polychaetes) most sensitive to oxygen defficiency. Repetition of these dystrophies has led to the disappearance of about fifteen species of molluscs and three of crustaceans. For many other species, a general thinning-out of the existing stocks has been noted (Montanari et al., 1984; Rinaldi et al., 1993).

Further, the recurrent phenomena of eutrophication, the repeated states of anoxia and the general deterioration of water quality in the north-western Adriatic have had, and continue to have negative repercussions on the economy of the region, like tourism and fisheries. The abnormal colour of the waters due to the high concentrations of suspended phytoplankton biomass, the resulting poor transparency and the unpleasant smells caused by the processes of putrefaction all combine to make the coastal waters extremely "unattractive" to bathers. With regard to fishing, and mollusc farming in particular, considerable damage has been done by the dinoflagellate of the genus *Dinophysis*, which produces DSP group toxins. The occurrence of these flagellates, which have become more plentiful during the last decade, have led to temporary and prolonged bans on the harvesting and sale of mussels (*Mytilus galloprovincialis*) farmed in the coastal and lagoon areas of Emilia-Romagna (Viviani et al., 1990; Boni et al., 1992). Further, *Alexandrium tamarensis*, a dinoflagellate capable of producing PSP group toxins, has been observed in the waters of the northern Adriatic (Fortuna et al., 1985; Mancini et al., 1986; Honsel et al., 1992); yet, no pathologies in the resident populations which can be blamed on PSP intoxication have ever been encountered.

Lagoons. Eutrophication has caused and continues to cause serious problems in the lagoon areas around Ravenna (Pialassa della Baiona). Another lagoon, the Sacca di Goro (Po delta) has been the subject of numerous studies (Franzoi et al., 1986; Rinaldi et al., 1988; Emilia-Romagna Region 1989; Bencivelli and Castaldi, 1991; Barbanti et al. 1992). These transition basins are affected both by phytoplankton blooms and by the invasive presence of microalgae (Ulvaceae in particular). During the summer, the abundance of vegetable biomass leads to persistent states of anoxia with serious damage to the lagoon ecosystem and the activities related to fish breeding. The cause of deterioration of these lagoons is not only excessive input of nutrients (especially from the Po and from the surrounding agricultural areas), but deterioration is aggravated by the effects that result from construction of new installations (ports, quays and breakwaters) which in many cases reduce the water exchange between the lagoon and the sea, and thus increase water residence times.

Northern Adriatic (Veneto, Friuli-Venezia Giulia):

Gulf of Venice. The deterioration of the 550 km^2 sized Lagoon of Venice has even more serious implications because of the city's important social and cultural activities. Cossu et al. (1984) have estimated that the potential nutrient inputs from domestic effluents into the Lido basin (about one third of the entire lagoon area) alone amount to 635 and 577 tons/year of nitrogen and phosphorus, respectively, 2,015 tons/year of nitrogen and 150 of phosphorus from industry, and 1,332 and 422 from the agricultured sector. More recent figures are given by Cossu et al. (1992) for the whole lagoon of Venezia that account for existing sewage treatment facilities and discharge modi for the whole population, including seasonal tourist presence. These are at variance however, and would amount to 2188 t (potential) and 1208 t (real) of nitrogen, and 445 (potential) and 275 t (real) of phosphorus, respectively.

Inputs from the sediments, from the atmosphere and from the sea (through the tides) are difficult to estimate, but would probably double these quantities. The consequences are frequent algal blooms and mostly abnormal proliferations of Ulvaceae. During the summer, the size of the macroalgal biomass, assessed at a maximum of 15 kg/m^2 (Venice City Council, 1991) cause anoxic states with high production of hydrogen sulphide. Apart from its toxicity for aquatic fauna, this latter compound is released into the atmosphere, creating serious problems for the local population (Bernstein, 1991). With monthly samplings during 1985-1989 at a number of stations around the lagoon of Venice, Alberotanza et al. (1992) demonstrate that although the phosphorus concentrations show a downward trend, the lagoon system is still in a state of extreme environmental stress as a result of persisting hypertrophy.

The other areas of the northern Adriatic between the Po delta and Trieste generally had lower trophic levels than the adjacent area. Due to the hydrodynamics of this basin, Po waters tend mainly to be carried south by southward currents. Further, the area North of the Po delta receives lower input of eutrophying substances and water residence times of local tributaries are shorter because of the absence of clear low salinity fronts.

Montresor et al. (1981) found diatoms prevailing during the spring-summer period of 1978; in particular, for some stations off the southern Veneto coast, they report concentrations of 5,000,000 cells/l of *Skeletonema costatum*. Data obtained from three cruises in the northern Adriatic during 1979 (Socal et al., 1981) show that very high concentrations of diatoms (*Rhizosolenia* in the summer and *Chaetoceros* and *Leptocylindrus* in the autumn) overlap spatially with the plume of the Po river.

Microalgal blooms occurring in the coastal band in the period 1984-1990 were generally caused by diatoms. Likewise as in other areas in the northern Adriatic, blooms of *Skeletonema* and *Chaetoceros* occurred with values which, in the period 1984-1987, occasionally reached 36,000,000 cells/l (Veneto Region, 1991). The same study also demonstrates that the distributions of nutrients are particularly significant in the areas influenced by the rivers, especially the Livenza, Sile, Brenta and Adige.

Gulf of Trieste. Although sometimes fairly intense, the microalgal blooms reported in waters off the Friuli coast as far as Trieste are normally limited in diffusion. As early as 1973, Bussani (1974) observed an intense bloom of *Peridinium ovum* in the waters near Trieste. Significant increases starting from 1977 in microalgal blooms caused by dinoflagellates are reported by Fonda Umani (1985); blooms of *Noctiluca miliaris*, *Gonyaulax poliedra*, *Prorocentrum lima* and *Scrippsiella trochoidea* have occurred particularly in the areas of the Gulf of Trieste with a small water exchange. Cassinari et al. (1979) report a bloom of *Noctiluca miliaris* with peaks of 4,800,000 cells/l in June 1977. A bloom of *Scrippsiella trochoidea* with 7,000,000 cells/l is reported by Fonda Umani and Honsel (1983) in May 1983 in a limited zone of the port of Trieste. The same dinoflagellate generated a red tide in September 1987 (Cabrini et al., 1990).

While cases of anoxia in the strictly coastal band are reported only for the Bay of Muggia (Trieste) (Orel, 1990), more widespread and persistent anoxic crises are recorded in the deep waters off the Gulf of Trieste and the coasts of Istria. Unlike events on the western shores of the northern Adriatic, these cases are generally not preceded by algal blooms; therefore, the most likely cause is hydrodynamic stasis during the summer, accompanied by marked persistent stratification of the waters (Faganelli et al., 1985; Ghirardelli and Fonda Umani, 1989). As a consequence, bottom fauna kills have been observed both in the Gulf of Trieste and in the eastern area of Istria in 1974, 1983, 1988

(Stachowitsch, 1991) and more recently in 1989, 1990 (Hrs Brenko et al., 1992) and 1991 (Aleffi et al., 1992). The benthic ecosystem in areas of anoxia have shown a general reduction in numbers of species and of individuals.

5.2.4 Slovenia, Croatia, Montenegro. Eastern Adriatic

Justic et al. (1987) emphasize that oxygen supersaturation in the surface waters and oxygen depletion in bottom waters in the northern Adriatic occur in all seasons except winter. They attribute this to the increase in phosphorus, since this element is able to govern the development of the phytoplankton biomass (Justic, 1987; 1991a). The ecological consequences of states of hypoxia presented by Justic (1991b) agree with those of other authors (Stachowitsch 1991; Rinaldi et al. 1993). Drastic reduction in the density of populations of *Turritella communis* after repeated hypoxic crises is significant here.

It further appears that water transparency in the northern Adriatic has also been significantly decreasing over time with a gradual increase in suspended organic particles (phytoplankton biomass and debris). Compared to data obtained in the period 1911-1913 with those form 1972-1982 (Justic, 1988) transparency in the western part of the northern Adriatic has fallen by 1/3. Domijan and Smircic (1992) reach the same conclusions: decrease of transparency measured in the entire basin in different periods (1956-1971 compared with 1972-1990) was estimated at 1/3 in the northern Adriatic and at about 1/5 in the central and southern Adriatic.

The orography of the eastern Adriatic basin and its coasts (the former Yugoslavia) is generally mountainous that prevents the transfer to the sea of waters from large internal hydrographic basins. Therefore, and in contrast with the north-western nearshore waters, eutrophication phenomena which occur in the coastal waters of the easter Adriatic are normally of a local nature. In almost all cases, the zones affected by the phenomena are the ports and bays, whose trophic state is influenced by sewage outfalls and industrial effluents.

Bay of Pula. Because of the inadequacy of the sewage treatment system, during the last 20 years this area has gradually deteriorated (Maretic et al., 1977). Recurrent dinoflagellate blooms may reach chlorophyll-a concentrations in excess of 120 mg/m^3. *Prorocentrum micans* generally causes blooms during the spring, while in the summer *Gonyaulax poliedra* blooms occur; other blooms are periodically caused by *Noctiluca miliaris* and by the genus *Gymnodinium* (Degobbis, 1990).

Bay of Rijeka. Although oligotrophic throughout most of its area, the bay is affected by high trophic levels in the northern part due to the input of the Rjecina river, sewage outfalls and industrial effluents (Degobbis, 1990).

Dalmatian coast. Recurrent eutrophic episodes are also reported from the estuary of the Krka river, including the Bay of Sibenik. The western part of the bay is particularly affected because of the nutrient inputs from the city of Sibenik (population 30,000) and industrial areas. Legovic et al. (1991a; 1991b) report a bloom of *Gonyaulax poliedra* in the centre of the bay in October 1988. This event, associated with inputs of nutrients, heavy rainfall and a marked halocline, caused anoxia in the bottom waters with widespread bottom fauna kills.

A research project lasting 14 years aimed at identifying the physical-chemical and biological characteristics of a vast area off the main cities of Dalmatia (Zadar, Sibenik, Split, Ploce and Dubrovnik), by Pucher-Petkovic and Marasovic (1992) show blooms in the Bay of Sibenik normally caused by dinoflagellates. They also report large increases in nitrogen (particularly nitrates) during the last few years.

Bay of Kastela. There are many reports of blooms in the bay on which the city of Split stands. Marasovic (1985; 1987; 1990), Marasovic et al. (1991) and Gacic et al. (1988) highlight that recurrent algal blooms are coincident with the growing input of fertilizing substances. Primary productivity has risen from 120 gC m^2/year in 1963 to 250 gC m^2/year in 1984 (Pucher-Petkovic et al. 1988; Pucher-Petkovic and Marasovic 1988). The summer microalgal blooms recorded in the Bay of Kastela during the last twenty years have been mostly of the dinoflagellate *Gonyaulax poliedra*. The 1980, 1985, 1987, 1989 and 1990 blooms were followed by anoxia of bottom waters that caused kills of bottom fauna, and alterations in the organoleptic characteristics of waters (Marasovic, 1990).

Eutrophication of the Bay of Kastela stems above all from the rapid urbanization of the adjacent coastal area from 1960 to 1989 with population increase from 60,000 to 250,000. Nitrogen and phosphorus load has been estimated at 593.6 and 101.3 tons/year, respectively. The main sources of input are the urban sector (40.5% of nitrogen and 45.5% of phosphorus) and rivers (34.5% of nitrogen and 21.0% of phosphorus) followed in decreasing order of contribution by runoffs, industrial effluents and the atmosphere (Baric et al., 1991).

Other observations come from the area of the Port of Dubrovnik and the Bay of Kotor. In the first case data of a research project carried out during 1988-1989 by Caric et al. (1992) show that quantities of phytoplankton biomass, albeit still modest (max 6.15 mg/m^3 of chlorophyll-a), have increased above the natural trophic level of the area. In the second case the excessive inputs of nutrients of urban and industrial origin since the 1970's combined with the slow exchange of waters due to the morphology of the bay have provided favourable conditions for microalgal blooms to occur. In July 1975 there was an intense dinoflagellate bloom with 28,000,000 cells/l of *Prorocentrum scutellum* and 13,000,000 of *Prorocentrum micans* (Giovanardi and Bent, 1990).

General characterization of the trophic conditions in the Adriatic. Chiaudani et al., (1982) and Emilia-Romagna Region (1990) have underlined that hypertrophic-eutrophic states are generally limited to the areas off and close to the Po delta. Further to the North or South, conditions settle at mesotrophic-oligotrophic levels with areas of eutrophy found only in coastal areas influenced by the inputs of the minor rivers and effluents from towns. Data obtained from weekly monitoring in the area from Trieste to Pesaro in summer 1992 (Adriatic Authority, 1992) clearly showed that trophic parameters are highest in the areas affected by the inputs of the Po.

While conditions in offshore waters in the central and southern Adriatic Sea are oligotrophic, Fonda Umani et al. (1992), in their definition of the trophic characteristics of the Adriatic, emphasize that high phytoplankton biomass and recurrent red tides are found in general in the coastal areas of the northern basin. On the basis of the research carried out by various researchers during the last twenty years the trophic conditions of the Adriatic sea can be summed up as follows (see Fig. 14):

- a coastal area in the north-western Adriatic and in some sites on the coasts of Croatia and Montenegro which is affected by recurrent microalgal blooms and with trophic levels is to be classified as eutrophic;

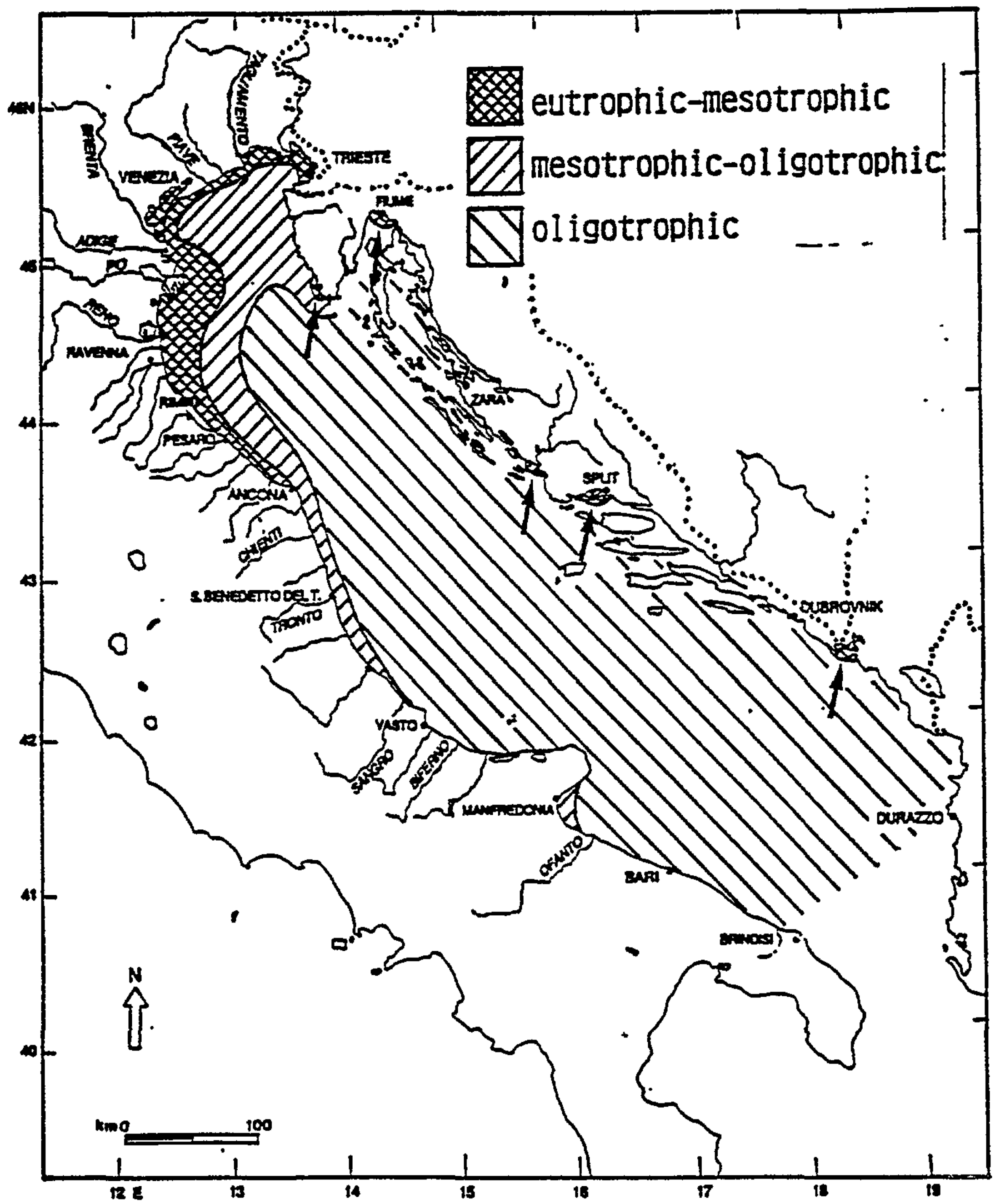


Fig. 14 Areal mapping of trophic conditions of the Adriatic sea

- the open sea waters of the north-western basin with mesotrophic-oligotrophic characteristics;
- the majority of the central-southern basin with trophic levels classifiable as oligotrophic.

Of course, the actual trophic conditions of these specified areas, particularly those along coasts, may vary depending on the quality/quantity of the inputs of human and land origins, and the time of the year. Franco (1981; 1984) and Franco et al. (1982) emphasize that the dynamics of the phytoplankton populations are governed by the seasonal sequence in the hydrodynamic and physical characteristics of the mass of water in the basin. In their hypothesis, the marked stratification of the water column in the summer determines a heterogeneous vertical distribution of phytoplankton, with the highest biomass values in river input diluted surface layers. Vertical instability during late autumn and winter and a marked frontal system along the western coast separating the diluted waters from the rest of the basin generate a distribution of phytoplankton biomass where horizontal heterogeneity predominates.

The causes of the serious deterioration that has occurred in the northern area for over twenty years are attributed to the a nutrient input in amounts that exceed the basin's natural assimilation capacity. The Po, carrying some 100,000 tons/year of inorganic nitrogen and some 6,000 tons/year of inorganic phosphorus, contributes the most of the total nutrient load of the northern Adriatic basin (Marchetti, 1990). The next largest of the rivers flowing into the northern Adriatic, the Adige, contributes 14,002 tons/year of total nitrogen and 1,202 tons/year of total phosphorus, although its mean nutrient concentrations are lower than those monitored in the Po (Provini et al., 1980). The total nitrogen and total phosphorus discharged into the northern Adriatic from Italy alone amounts to some 270,000 and 24,000 tons/year, respectively; cf. Table 19 (data produced by Marchetti; 1987 Emilia-Romagna Region, 1991). To these must be added the inputs from Istria estimated at 7.0 and 0.4 tons/year of total nitrogen and total phosphorus, respectively (Smodlaka and Degobbis, 1987).

5.2.5 Mucilage aggregates in the Adriatic and Tyrrhenian Seas

Adriatic Sea. After a year of apparent absence of visible agglomerations of mucilage in the surface waters, though present in deeper strata (5 to 10 m) in 1990, the phenomenon reappeared in 1991, first toward the end of May in Yugoslav waters, from where it spread to the northern and northeastern part of the Adriatic basin (see Fig. 15).

Mucilaginous material observed with underwater telecameras in regular weekly surveys presented itself initially as loose "marine snow" and ribbon like aggregates with highest concentrations in the thermocline. Later, after conditions of continuing calm weather and high insolation, batches of mucilage of various spatial extension and duration in time, surfaced over the whole northern Adriatic Sea. The phenomenon was most pronounced during the warmer hours of the day, but locally the appearance of mucilaginous batches was influenced by prevailing winds and currents.

In comparison to the situation found in 1988 and 1989, the 1991 mucilage events were of less severity, however, but of considerable longer persistency over time, i.e. from June 5 to the end of August. While strong sea-storms and surface mixing, as well as strong north-south currents were absent for the whole period, frequent light winds from land (Libeccio) and modest mixing of waters prevented the buildup of more serious mucilage accrual in coastal areas. On the other hand, further offshore and in deeper waters and near the sediments, considerable amounts of mucilage continued to be present, creating - besides difficulties to fisheries - a situation of hypoxia and modest bottom fauna kills.

Table 19

Load of nitrogen and phosphorus by Region and main sources

NITROGEN (tons/year)			
Regions	Total	Regional contribution	
		Po	Other rivers
Piemonte	42,772	42,772	0
Valle d'Aosta	946	946	0
Lombardia	90,774	88,883	1,891
Trentino Alto Adige	7,699	660	7,039
Veneto	62,353	4,474	57,879
Friuli Venezia Giulia	17,288	0	17,288
Liguria	930	930	0
Emilia-Romagna	48,945	24,385	24,560
Grand Total	271,707	163,050	108,657
PHOSPHORUS (tons/year)			
Regions	Total	Regional contribution	
		Po	Other rivers
Piemonte	3,759	3,759	0
Valle d'Aosta	93	93	0
Lombardia	9,175	9,033	143
Trentino Alto Adige	671	66	605
Veneto	4,621	226	4,395
Friuli Venezia Giulia	1,125	0	1,125
Liguria	131	131	0
Emilia-Romagna	4,406	2,233	2,172
Grand Total	23,981	15,541	8,440
% Distribution by source (cf. 1986 - Marchetti, 1987)			
	P(t)	N(t)	
Humans	33.6	19.3	
Agriculture	29.5	63.8	
Husbandry	14.1	5.5	
Detergents	12.7		
Industry	7.4	7.6	
Uncultivated soils	2.7	3.8	

Chemical and microscopical analyses confirmed that the prevailing components of the mucilaginous material are mucous polysaccharide agglomerates in which inorganic and organic particulate matter such as mineral fragments, organic detritus, zooplankton (mostly dead), etc. are occluded. As regards the phytoplankton component, which is normally dominated by *Nitzschia delicatissima*, the analyse have shown concentrations of this latter species to be as high as 150 - 200 million cells per dm^3 mucilaginous material. Other diatom species, *Navicula* and *Pleurosigma* were often present but in minor quantities, whereas dinoflagellates (*Gonyaulax fragilis*, *Gymnodinium sp.*, a.o.) were found only rarely, and then only in degradation state.

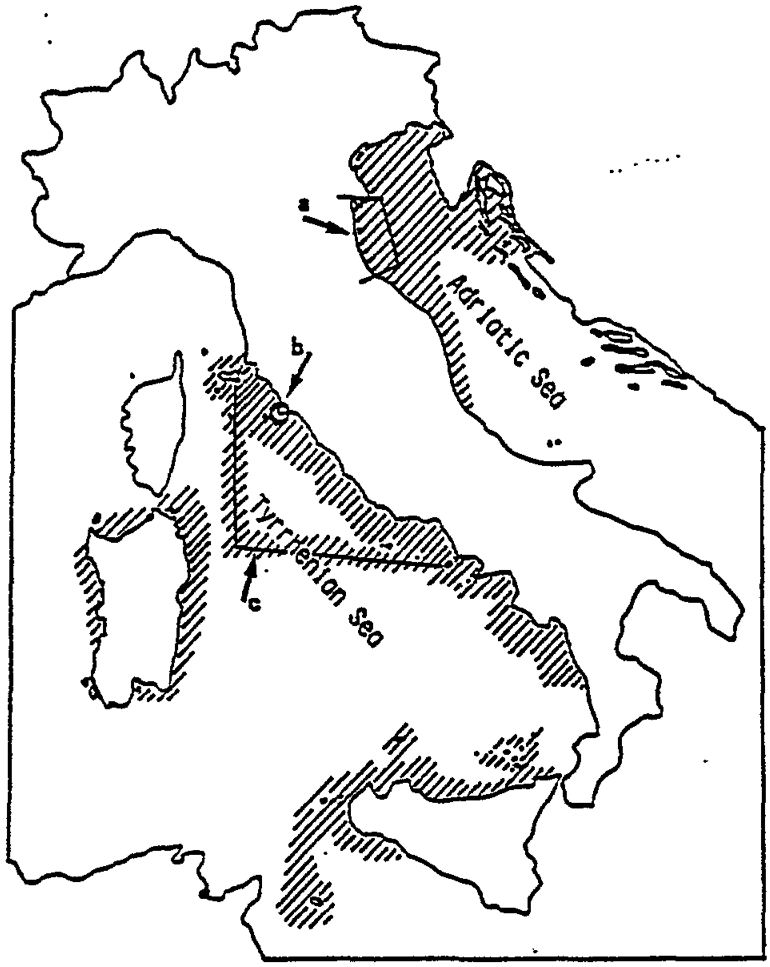


Fig. 15 Massive occurrences of mucilage aggregates 1988-1989 along the Italian coasts. a) and b) indicate zones of periodic observations; c) observations made during cruises of the oceanographic vessel "Minerva"

Tyrrhenian Sea. While mucilage phenomena in the Adriatic Sea have been known to occur and described by scientists since at least the last century, researchers from the Universities of Pisa and Florence, and associates of the "Daphne II" Laboratory of Emilia-Romagna have detected conspicuous quantities of mucilaginous filaments in deeper coastal waters of the Tyrrhenian Sea in July 1991 for the first time (see Fig. 15). The most severe conditions were found along the coasts of Tuscany and the adjacent archipelago. Similar problems have also been reported from the coasts of Lazio, Campania, and around Sardinia (cf. map), and by the University of Palermo for the northwestern part of Sicily.

This material (seemingly unobserved for the area or perhaps ignored, but probably as a phenomenon noted by local fishermen for decades under the term "heavy Waters") was found to float in dense ribbon like suspensions till it becomes entangled on rough surfaces (e.g. rocks) and organisms (Gorgonians, Bryozoa, Sponges, Posidonia, etc.) covering such communities with a spider's web-like tendon. It is at present unclear how seriously the benthic biota is affected and damaged by this phenomenon. erious suffocation of bottom fauna under such conditions has however been observed in the northeastern Adriatic sea.

5.2.6 Greece: Eastern Ionian and Aegean Seas

The trophic state of the open sea waters of the Eastern Ionian and Aegean Seas can be defined as oligotrophic (Friligos 1986a; Boussoulengas and Catsiki, 1989). However, in various areas with poor exchange such as the gulfs, lagoons and estuaries eutrophic conditions can be found, generally caused by sewage outfalls and agricultural and industrial effluents.

Moving from West to East, cases of eutrophication have been observed in the following areas:

Gulf of Amvrakikos. This narrow-mouthed bay on the Ionian Sea coast is an important breeding area for fish and invertebrates of valuable species. Recently this area has shown signs of deterioration because of the excessive inputs of eutrophying substances, generally from sewage outfalls and agricultural effluents (Pagou, 1990). High levels of nutrients (silicates and nitrates in particular) are carried into the north of the lagoon by the Arachthos river (Friligos and Balopoulos, 1988).

Lagoon of Messolonghi (Gulf of Patraikos). Data obtained during 1983-84, Friligos (1986b) demonstrate that conditions of anoxia frequently occur in the northernmost part of the lagoon during the summer season. The nutrient concentrations are generally high, with the highest values in the area close to the town outfalls.

The Gulf of Saronikos and its bays. Algal blooms that occur in the Gulf of Saronikos are generally widespread, which makes it one of the zones hardest hit by eutrophication phenomena in the whole of Greece. The causes for this deterioration are attributed mainly to the sewage outfalls from Athens and Piraeus (a population of 3,000,000 producing about 7 m^3/sec. of effluents) and the industrial effluents discharged into the nearby Bay of Elefsis. In this bay, identified as the most eutrophic area in the whole Gulf of Saronikos, widespread persistent cases of anoxia occur during the summer months (Catsiki, 1991; Nakopoulou et al., 1992). These events tend to favour the release of nutrients from the sediments, which in turn trigger increases in the phytoplankton biomass (Friligos and Barbetseas, 1986). Pagou (1990) reports 10,000,000 cells/l of *Gymnodinium breve* in November 1977 and 29,000,000 of *Scrippsiella trochoidea* in May of the same year. At Alimos Beach (eastern Gulf of Saronikos), in 1987 *Gymnodinium sp.* reached concentrations between 12,000,000 and 27,000,000 cells/l; the same genus was present with 27,000,000 cells/l in the waters off Psitallia Island near the sewage outfall. In the Gulf of Vouliagmeni (outer Gulf of Saronikos) 58,600,000 cells/l of *Pyramimonas sp.* were observed in July 1988. In the waters of Hellenikon (eastern Gulf of Saronikos)in March and April Pagou (1990) reports concentrations of over 1,000,000 cells/l of diatoms (*Thalassiosira sp.*, associated with *Leptocylindrus danicus* and *Nitzschia delicatissima*), and of *Noctiluca scintillas*. A maximum chlorophyll-a value of 27.0 mg/m^3 was measured at a station close to the Athens sewage outfall in Keratsini Bay in 1980-81 by Scoullos et al. (1983).

Gulf of Pagassitikos. High trophic levels are found above all in the northern area of the Bay of Volos. The nutrients come mainly from the town of Volos and industrial facilities. Friligos and Gotsis-Skretas (1988) record a case of red tide in the most polluted port area in July 1987, caused by flagellates of the species *Gymodinium catenatum* (11,150,000 cells/l) and *Cachonina niei*; diatoms were dominant in the less polluted open sea waters.

Bay of Thessaloniki (north-west of the Gulf of Thermaikos). A eutrophic situation similar to that in the Bay of Elefsis and the Gulf of Amvrakikos (Balopoulos and Friligos, 1986; Samanidou et al., 1986). Huge amounts of nutrients are carried into this Gulf by industrial effluents and the sewage outfalls of the city of Thessaloniki (population 1,300,000) and the waters of four rivers. The Axios river is held responsible for transferring large quantities of nitrates of agricultural origin into the Gulf. In this zone, the increase in the phytoplankton biomass is directly correlated with the increases in nitrates and phosphates and with the reduction in salinity values. The blooms are generally caused by diatoms: *Nitzschia closterium*, *Cerataulina bergonii*, *Leptocylindrus minimus*, *Chaetoceros socialis* and *Thalassiosira sp.* are the dominant species in the majority of cases (Gotsis-Skretas and Friligos, 1988).

Gulf of Kavala. Although the trophic levels are generally lower than in the other areas mentioned, it appears that a bloom of the dinoflagellate *Gonyaulax polyedra* with 10,000,000 cells/l that occurred in August 1986 in the north-western part of the Gulf was caused by the wrecking of a ship transporting phosphate fertilizers (Panagiotides et al., 1989).

Estuary of the Vistonis River. Yannakopoulou (1992) reports an intense bloom in August 1984 with maximum chlorophyll-a values of 86.0 mg/m^3, consisting mainly of cyanobacteria.

Gulf of Alexandropoulis (to the North-East of the Aegean): A trophic situation similar to Kavala. Mainly because of the emissions of the Evros river, in 1981-1982 concentrations of silicates and nitrates were three times and six times respectively higher than normal (Pagou, 1990).

5.2.7 Malta: Central Mediterranean

A three year monitoring program (1989-1991) was carried out in Malta in order to define the trophic state of two intensely urbanized port areas with heavy commercial traffic, the Grand Harbour and the Marsamxett Harbour. Axiak et al. (1992) found that the nutrient and chlorophyll levels were comparable to those measured in other areas of the Mediterranean where clear signs of eutrophication already exist. Increased levels of primary productivity has reduced the transparency of the waters, particularly in the innermost areas with the longest water residence times, showing a mean of 2.6 m. The same areas have the highest mean values of nutrients (26.4 and 1.6 mg-at/m^3 for nitrates and orthophosphate, respectively) and chlorophyll-a. Nutrient inputs come above all from sewage outfalls (population of 73,000 in the capital Valletta and its suburbs), although the contribution of shipping and tourism should also be mentioned.

5.2.8 Turkey: North-eastern Mediterranean

Open marine waters of Turkey generally present conditions of oligotrophy; only in limited costal areas affected by the inputs of rivers, sewage outfalls or industrial effluents conditions of eutrophy are reported.

The Sea of Marmara is an exception because of both its size, hydrology and hydrodynamics. A semi-enclosed sea, with an area of 11,500 km^2 and a volume of 3,378 km^3, its dominant low-density surface current tends to flow from East to West, while the bottom current carries high-density Mediterranean water from the Dardanelles towards the Bosphorus. During an oceanographic cruise in the Sea of Marmara from 30 June to 8 July 1990, Aubert et al. (1990) measured particularly high orthophosphate and inorganic nitrogen values near the Bosphorus. The cause is believed to be the sewage outfall from the city of Istanbul (population 2,000,000). To the West, the orthophosphate values gradually decreased. The sites with the most abundant phytoplankton biomass were the areas immediately to the West of the Bosphorus and near the Dardanelles. The phytoplankton populations consisted mainly of dinoflagellates (dominant species of *Gymnodinium* with maximum concentration 540,000 cells/l) and Coccolithophorideae (mainly *Coccolithus pelagicus* and *Emiliania huxleyi* with densities between 10,000 and 1,900,000 cells/l), while diatoms were not plentiful.

In contrast, (Uysal et al., 1988) report that diatoms generally dominated during a long period (September 1985 - January 1987) in the eastern part of the Sea of Marmara (close to the Bosphorus) and their abundance was closely correlated with the nutrient inputs. Conditions of anoxia in the bottom waters accompanied by widespread bottom fauna kills have been reported in the easternmost part of the Sea of Marmara (Basturk et al., 1990). Referring to a case in August 1989, the authors attribute the event to a combination of strong haloclines and marked stability in the basin during the summer, large inputs of nutrients and organic particulates from the city of Istanbul that caused high primary production.

The Bay of Gemlik (south-eastern part of the Sea of Marmara) shows clear signs of eutrophy as a result of nutrient-rich emissions of agricultural origin from the area near Lake of Iznik (Aral, 1992).

Western coasts. Yaramaz and Tuncer (1986) studied the nutrient levels at ten coastal stations between the towns of Canakkale (North-eastern Aegean) and Bodrum (South-eastern Aegean) on the western coast of Turkey. Highest values were found in the station close to the Bay of Izmir, with means of 16.6, 1.0 and 2.5 mg-at/m^3 for ammoniacal nitrogen, nitric nitrogen and orthophosphate, respectively. Nutrient concentrations in the innermost parts of the Bay of Izmir, referred to by many authors as seriously degraded, show particularly high values, especially compared to those obtained in unpolluted neighbouring bays. Koray and Buyukisik (1988) report a bloom of the dinoflagellate *Alexandrium minutum* (a microalga producing group PSP toxins) that occurred in May 1983 in a zone particularly affected by large amounts of nutrients. The phenomenon was followed by anoxia in the bottom waters and bottom fauna kills.

The recurrent algal blooms in this area are due to algae which produce PSP-group toxins (Koray, 1990; Koray et al., 1992); amongst these *Alexandrium minutum* with peaks of 10,000,000 cells/l (dominant in March, April, May and June), *Gonyaulax poliedra* with 50,000 cells/l (in April, May and June) and *Gonyaulax spinifera* with 20,000 cells/l (particularly in May and June). Such algal blooms cause cases of anoxia in the bottom waters.

Using a multiple regression model Koray (1988) demonstrates that increases in diatoms in the Bay of Izmir are correlated in 70 % of cases with high nutrient values; the 30% failure rate is attributed to the grazing action of the zooplanktons or inhibitor effects deriving from pollutants. Koray and Buyukisik (1992) reach the same conclusions that increases in autotrophic biomass are generally directly correlated with increases in phosphorus, nitrogen, light and temperature.

Comparing the polluted Bay of Izmir with the unpolluted Bay of Gulbahce, Buyukisik (1988) states that while in the innermost part of the Bay of Izmir the main trophic indicators are high (5.3 mg/m^3 of chlorophyll-a, 10.1 mg-at/m^3 of total inorganic nitrogen, 1.4 of orthophosphate and 6.7 of silicates; 1984-85), the mean values in the Bay of Gulbahce are much lower (0.85 mg-at/m^3, 2.3 mg-at/m^3, 0.1 mg-at/m^3 and 5.7 mg-at/m^3, respectively), representing conditions of oligotrophy, normal for the area.

Southern coasts. Basturk et al. (1988) and Yilmaz et al. (1990) report oligotrophic conditions in the open sea waters, with increases in trophic level at some coastal sites. Particular mention is made of the Bay of Antalya and the Bay of Iskenderun, where significant increases in the trophic conditions may occur both through periods of upwelling and because of emissions from the land. In the Bay of Iskenderun in particular, the authors record nutrient concentrations of 0.1-0.5, 0.5-12.0, and 0.1-11.0 mg-at/m^3 of orthophosphate, nitric+nitrous nitrogen and reactive silica, relatively.

5.2.9 Lebanon: Eastern Mediterranean

Except for some eutrophic port areas where occasional blooms may occur, no eutrophic conditions are reported in the coastal waters of Lebanon. In general, these waters are oligotrophic, with limited seasonal fluctuations in autotrophic biomass featuring increases in spring and reductions in the hot season. The dinoflagellates present in this area belong mainly to the phytoplankton typical of the temperate Mediterranean areas. The presence of toxic species such as *Gonyaulax poliedra*, *Alexandrium minutum* and *Dinophysis spp.* is also recorded. However, Lakkis (1991) points out that these species have never created health problems since they are present in very low concentrations.

Abboub-Abi Saab (1990) and Abboub-Abi Saab and Kassab (1992) also report low phytoplankton concentrations and confirm the general trend for a reduction in the microalgal biomass during the passage from inshore to offshore waters.

5.2.10 Israel: Eastern Mediterranean

The open waters of the south-eastern Mediterranean are particularly low in nutrients. Krom et al. (1988) in an oceanographic cruise during August-September 1987 in the Levantine basin south of Cyprus found generally oligotrophic conditions. Down to depths of more than 500 m below the surface they measured quantities of 5.5-6.3 mg-at/m^3 of nitrates and 10-12 mg-at/m^3 of silicates. These values are very low, particularly if compared with those for other oceans at the same depth: 40 mg-at/m^3 of nitrates in the Pacific and Indian Oceans, 20 mg-at/m^3 of nitrates in the Atlantic, 130 mg-at/m^3 of silicates in the Pacific and in the Indian Ocean and 40-100 mg-at/m^3 of silicates in the Atlantic.

Correspondingly, autotrophic biomass values in the coastal waters also tend in general to be very low. In a research program (carried out in the period March 1983-February 1984) on the seasonal distribution of populations of diatoms and dinoflagellates in two stations near Haifa (one coastal and one offshore), Schneller et al. (1985) record phytoplankton biomass values, expressed as chlorophyll-a, between 0.19 and 0.63 mg/m^3 in coastal waters and 0.08-0.38 mg/m^3 in offshore stations.

5.2.11 Egypt: South-eastern Mediterranean

Cases of acute eutrophication in the Egyptian waters are reported above all from the ports and coastal waters off Alexandria, and from the lagoons in the Nile delta. Overall, eutrophication and the worsening of water quality (abnormal water colours, anoxia in bottom waters and production of hydrogen sulphide) in Egyptian coastal and brackish waters are caused by the combination of: (a) large inputs of fertilizing substances from urban, agricultural and industrial sources; (b) the long water residence times in the lagoons, partly due to physical barriers; (c) salinity stratification of the waters; (d) generally high water temperatures.

Coastal waters and ports. Along the coast, high trophic levels have been observed in the area off Alexandria and in a number of sites close to the outflows of the main arms of the Nile (Rosetta and Damietta). The city of Alexandria discharges some 183,000,000 m^3/year of sewage (Aboul Kassim et al., 1992) and industrial waste into the nearby Bay of El-Mex (El-Sherif, 1990). These waters are transported by the geostrophic currents eastward off and along shore causing not only a seriously trophic state but also a general deterioration in the coastal ecosystem. However, dilution prevents the most catastrophic manifestations of eutrophication along the most important Egyptian bathing beach areas.

Eutrophication is more serious in the two rather closed port areas, the East Harbour and the West Harbour. Conditions in these harbours have recently been extensively studied by members of the Alexandria University (Aboul-Kassim et al., 1992; Emara et al., 1992; Saad and Hemeda, 1992a, 1992b; Zaghloul and Halim, 1992).

Western Harbour (7.54 km^2). Beside Port Said east of the delta, this is the most important Egyptian harbour. Heavy shipping traffic and the discharge of effluent from the urban sector create recurrent algal blooms associated with anoxic conditions in the bottom waters (IOC/UNESCO, 1988; Saad and Hemeda, 1992). Zaghoul (1992) states that this area is markedly eutrophic; conditions of low transparency values and high concentrations of nutrients and chlorophyll-a occur frequently.

Eastern Harbour (2.53 km^2). Heavy algal blooms in this area are caused as a result of the excessive input of nutrients from the city of Alexandria, combined with the marked vertical stability of the basin. In June 1985 Dowidar and Aboul-Kassim (1986) estimated mean chlorophyll-a at 23 mg/m^3. Zaghloul and Halim (1992) record red tides caused by the toxic dinoflagellate *Alexandrium minutum*, and anoxia in the bottom waters. Dowidar et al. (1990) estimated the total amount of dissolved inorganic phosphorus discharged into the Eastern Harbour at 1,094 kg/year.

Lagoons in the Nile delta. With the exception of L. Burullus these extended lagoons of mean depth around 1 m (Mariout, Edku, Burullus, Menzalah) and their fisheries have been extensively studied in a combined FAO/UNESCO programme in the late 1950' prior to the construction of the High Dam (Elster and Vollenweider, 1961). The lagoons are in part enclosed by reed belts (*Phragmites*), and in part covered by macrophytes (*Potamogeton* species; floating *Eichhornia*). They are important habitats for commercially valued fish. All lagoons receive the drainage waters from the extended irrigation systems that provide water to the highly developed agricultural areas of the Nile delta. These waters are then discharged into the Mediterranean. The interplay between nutrient rich fresh water supply from the South and seawater intrusion via the connections to the sea in the North creates strong salinity gradients, both horizontally and vertically.

Since the '60s the inputs from the Nile have undergone drastic modification as a result of the High Dam construction. While the reduction in the quantities of solids carried down has affected the morphology of the delta area, the lower flow-rates have not only profoundly modified the hydrology and biology of the South-eastern Mediterranean, but also the lagoon areas. Further to the increase in fertilizer use and the rise in the human population of the delta area that has brought about an increase of nutrients, and a significant increase in primary productivity in the coastal sea-waters (IOC/UNESCO, 1988).

Lake Mariout. Mariout is not directly connected to the sea as the other lagoons; its waters are discharged to the sea via the Mex pumping station west of Alexandria. Since the study of Elster & Vollenweider, the lagoon area has been reduced due to land reclamation, and in part by the construction of fish farms. The so-called Nusha Hydrodrome (5 km^2) is an artificially impounded section of Lake Mariout. Originally, L. Mariout had a basin of quite different biological properties, one part mostly covered by *Potamogeton pectinatus*, while another part that received discharges from the city of Alexandria, was plankton dominated and of extremely high productivity. Primary production measured ranged consistently over 1-2 g C/m^2.day, and reached peak values over 5 g/m^2.day with *Spirulina jenneri* dominating. Also, vertical stratification over only 1 m depth during calm periods could be very high with oxygen saturation in excess of 200% below surface, and severe reducing conditions over the bottom.

This in essence is still the situation of to-day, which is further aggravated by the huge inputs of untreated sewage from the city of Alexandria and the effluents from industrial and agricultural sources (Saad, 1973). Deterioration is so bad that the intense production and release to the air of hydrogen sulphide makes breathing at times difficult. Of the cases of eutrophication studied by Elster & Vollenweider, this is likely among the worst situation world-wide.

The Lagoon of Burullus (420 km^2, average depth 1.25 m) shows the highest concentrations of nutrients in its westernmost part since this is the area which receives 75% of the inputs from drainage of the surrounding areas. It also has the longest water residence times, since exchanges with the open sea take place mainly in the eastern part (Abdel-Moati et al., 1988). Estimates made by Abdel-Moati et al. (1990) during 1987-8 show that the Burullus lagoon receives nutrients equivalent to 558 tons/year of phosphorus and 2,318 of nitrogen. The chlorophyll values are generally not particularly high (mean value 6.6 mg/m^3), although there are recurrent peaks between 10 and 15 mg/m^3.

The Lagoon of Menzalah (ca. 2000 km^2 between the Nile delta and the Suez Canal) is in the same condition; due to the run-off from agricultural areas it reaches high trophic levels and recurrent algal blooms occur (Halim, 1989). Mean chlorophyll-a of Manzalah are reported at 21 mg/m^3.

As to nutrient limitation, Elster and Vollenweider (1961) found often N/P ratios low, indicating prevailing nitrogen limitation. Increased use of artificial fertilizers over recent decades, is therefore a possible main factor in promoting eutrophication.

5.2.12 Tunisia: Southern Mediterranean

Cases of eutrophication are reported above all in the Lakes of Tunis and Ichkeul.

Lake of Tunis. A lagoon of 48.6 km^2 with an average depth of 1 m. The only exchange with the sea are the navigable canal (opening 500 m) and two other small channels (opening 40 m), one close to the town of Kherredine. The salinity varies between 25 in the winter and spring, and 45 in the summer. There is also a considerable variation in temperature in the course of the year, ranging from 10EC in the winter to about 30EC in July-August. Cut in two by a raised causeway running alongside the navigable canal, the lagoon has a southern part and a northern part. The lagoon receives the only partially treated sewage of the city of Tunis (population of 1,000,000), which flows mainly into the northern part, while the southern section receives urban and industrial inputs (Aubert and Aubert, 1986).

The high trophic state of the basin favours invasive proliferations of macroalgae and frequent, intense algal blooms. It has been estimated that during summer and autumn the macroalgal biomass consisting above all of algae of the *Ulva* may reach 1,479 g/m^2 with a total mass assessed at 43,658 tons (UNESCO, 1984).

Belkhir et al. (1987) underline the presence of high nutrient values. The concentrations observed at 15 stations in the period 11-15 July 1985 were between 1,852 and 11,064 mg/m^3 for soluble inorganic nitrogen, between 44 and 1,958 mg/m^3 for orthophosphate and between 8,700 and 15,400 mg/m^3 for silica. The presence of large amounts of phytoplankton biomass consisted both of diatoms and of dinoflagellates (*Gymnodinium sp.* and *Prorocentrum micans*); anoxia and hydrogen sulphide caused widespread fish kills.

Lagoon of Ichkeul. The lagoon of 95 km^2 and average depth of 1.2 m, located to the North of Tunisia is described by Ben Rejeb and Lemoalle (1986). Phytoplankton biomass is particularly abundant during the winter months, because of the hydrology and hydrodynamics of the basin. During winter and spring the lagoon receives run-off rich in fertilizing substances from the surrounding areas, while in the summer there is inflow of nutrient poor marine waters. Chlorophyll and salinity show an inverse correlation; low salinity values in the winter correspond to high chlorophyll values, and vice-versa.

5.2.13 Algeria: Southern Mediterranean

Cases of eutrophication have been reported in a number of lagoons and in the port areas with heavy commercial traffic. Coastal sea waters generally show conditions of oligotrophy. During the "Mediprod V" oceanographic survey of 27 May - 27 June 1986 the highest chlorophyll-a values (0.6 mg/m^3 max.) were observed near the coast, and were associated with the upwelling of deep waters (IFREMER, 1990).

The Lake of El-Mellah. Situated a few kilometres from the Tunisian border this brackish lagoon of 680 hectares with a maximum depth of 5 m is affected by recurrent conditions of dystrophy and in summer of anoxia in the bottom waters (De Casabianca-Chassany et al., 1986; De Casabianca-Chassany et al., 1988). More recently, Samson- Kechacha and Touahria (1992) have observed the presence of potentially toxic species such as *Dinophysis acuminata* and others of the genus *Gonyaulax* and *Gymnodinium*. Conditions of environmental stress are mostly manifested in the most confined part of the lagoon, which receives direct input from rivers.

6 EFFECTS ON MARINE LIFE, RESOURCES AND AMENITIES

6.1 Phytoplankton blooms noxious for marine invertebrates and vertebrates

Massive and widespread fish and invertebrate mortality and damage to the marine eco-systems associated with algal blooms and/or green, red and brown tides are phenomena which occur in all the seas of the world, yet, in spite of this, the mechanisms of the deaths of the marine animals have not been sufficiently studied. Recently, the problem has represented a notable scientific interest, both in Japan and Europe because of the health and economic damage caused.

The effects of phytoplankton blooms on animals are direct and indirect, primary or secondary. The primary or direct effects are those caused by the blocking of the branchial apparatus of the phytoplanktonic biomass, the effect on the branchial cells of specific ichthyotoxins, and on the cellular metabolism of biotoxins adsorbed via the digestive system (Taylor, 1990). The indirect or secondary effects, however, are due to lack of O_2 and to production of H_2S and NH_3, which can reach levels really toxic for fish.

Harmful algal blooms which create problems for fishery and aquaculture in the coastal area in the world and in the Mediterranean sea are composed by organisms of these algae classes: Dinophyceae, Prymnesiophyceae, Raphidophyceae, Dictyochophyceae (= Silicoflagellates).

6.1.1 Dinophyceae

The toxic blooms of dinoflagellates fall into three categories (Steidinger, 1983): (a) blooms that kill fish but few invertebrates (*Gymnodinium breve* Davis, the Florida red tide organism, is an example); (b) blooms that kill primarily invertebrates (several species of *Gonyaulax* are of this type); (c) blooms that kill few marine organisms but the toxins are concentrated within the siphons or digestive glands of filter-feeding bivalve molluscs (clams, mussels, oysters, scallops, etc.) causing paralytic shellfish poisoning (PSP).

It has been noted for some time that the mortality of fish during the blooms of dinoflagellates of the species *Ptychodiscus breve* (= *Gymnodinium breve*) and *Alexandrium* (= *Gonyaulax*), responsible for NSP and PSP in man, is due to neuromuscular lesions caused by the same biotoxins absorbed via the digestive system (Ray, 1971; Steidinger et al., 1973) (see chapter 7).

The information presently available indicates the possible link between dinoflagellates toxins (saxitoxins and brevetoxins) and recent mass mortalities of marine mammals (humpback whale and bottlenose dolphins) along the eastern coast of the United States (Anderson and White, 1989).

Genus GYMNODINIUM Stein

In Florida, the mortality in fish is due to *Gymnodinium breve* which was identified in 1948 as the etiological agent and is considered to be the sole agent responsible for all the outbreaks described since 1844. As regards benthic fish, the toxins may have a complex effect on the neuromotor system, but this is not true for various invertebrates for which the anoxic condition is probably the only cause of death (Steidinger et al., 1973). Ray and Aldrich (1965), Spikes et al. (1968), Martin and Chatterjee (1969) found that the lipid extract of *G. breve* produces toxins that have effects on fish, chicks and mice. The abundance and annual periodicity pattern of the unannoured dinoflagellate *G. breve* (Davis) was studied also in the Mediterranean sea in an eutrophic environment (Saronikos Gulf, Aegean Sea) during 1977-1983 and 1987 (Pagou and Ignatiades, 1990). In the Aegean Sea, fish kills are to date not reported.

Gymnodinium catenatum has already been found in the Mediterranean sea: in a Tyrrhenian coastal lagoon (Carrada et al., 1988) and on the Spanish Mediterranean coast (Bravo et al., 1990). Only *G. catenatum* cells of Spanish coast produce PSP, but ichthyotoxic effects are not reported (see chapter 7).

In the Adriatic sea, along the coast of Emilia-Romagna, *Gymnodinium sp.*, at first identified as *G. corii*, caused a green tide in 1976 and another in 1977 (Viviani, 1977; 1981), which was named "green soup" by the press (Goldoni, 1977). This phenomenon was repeated in November of 1984, and covered the sea from the Marche to Veneto (Centro Studi Ricerche Risorse Biologiche Marine Cesenatico, 1984; Regione Emilia-Romagna, 1984). In research carried out in 1976-77 on potential ichthyotoxicity it was possible to demonstrate that the phenomenon of fish death was due to an anoxic condition (Viviani et al., 1985). In 1988, the same species of *Gymnodinium* indicated as *Gymnodinium sp.*, caused a similar tide which lasted for three months without harmful effect on invertebrates and fish (Centro Studi Ricerche Risorse Biologiche Marine Cesenatico, 1988; Regione Emilia-Romagna, 1988).

In 1982-83 and 1985, green tides (3,000,000 cells/litre) were observed in the Bay of Vilaine and Marennes (Lassus, 1984).

Genus ALEXANDRIUM Halim (= GONYAULAX)

Alexandrium monilata (= *Gonyaulax monilata*), a common dinoflagellate in the Gulf of Mexico, produces a substance toxic for fish (Gates and Wilson, 1960; Aldrich et al., 1967), but it does not affect the chick, mouse or other warm-blooded animals (Ray, 1971). The oysters of the Gulf of Mexico do not filter water when exposed to *A. monilata*. Clemons et al. (1980) have tried to purify the toxin, following the toxicity against German cockroaches and haemolytic activity. In their experiment, the toxicity has been found in the water-soluble fraction of a molecular range over 100,000.

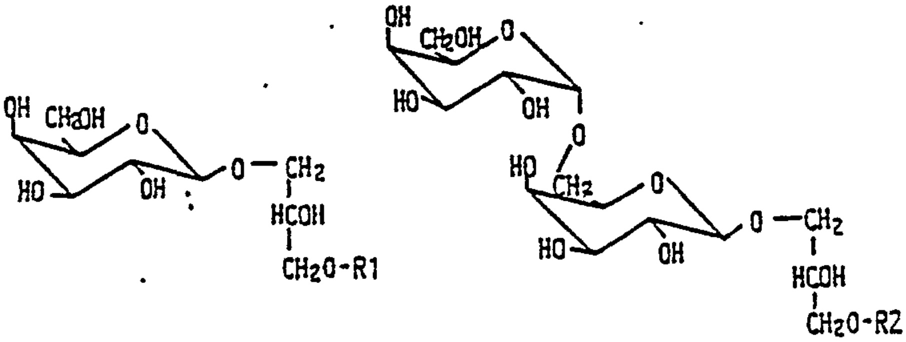
Alexandrium tamarensis from the English coast has caused death in aquatic animals, whereas that from the Atlantic coast of Canada has never produced such effects (Ray, 1971).

In Mediterranean, in the eastern lagoon of Alexandria bay outbreaks of red tides caused by *Alexandrium minutum* are recurrent summer phenomena since first observed in 1958 (Halim, 1960). Fish-kills occur, caused by clogging of the gills, but toxins are not reported (Halim, 1989).

Gonyaulax polyedra indicated as ichthyotoxic (Ballantine and Abbott, 1957; Sradie and Bliss, 1962; Reish, 1963), and responsible for young oyster mortality (Paulmier, 1977), caused in the Adriatic sea (Emilia-Romagna coast and Spalato Bay) "easy fishing" and mortality of fish and molluscs. This has been attributed to O_2 deficiency (Viviani, 1977; Marasovic and Vukadin, 1982).

Genus GYRODINIUM

In the blooms of *Gyrodinium aureolum*, closely connected to *Gymnodinium nagasakiense* of the Pacific (which in Japan causes fish kill), mortality of fish and salmon farms on the Irish, Welsh and Scandinavian coasts, and of invertebrates after 1966, have been described (Tangen, 1977; Ottway et al., 1979; Widdows et al., 1979; Roberts et al., 1983). At the end of the 1980s it has been highlighted that the ichthyotoxic effect, at the level of branchial functions of the above-mentioned dinoflagellate is due to polyunsaturated fatty acids and monoacyl-digalactosyl glycerol, which resembled haemolysin-2 of *Amphidinium carteri* (Fig. 16) (Yasumoto et al., 1990).



hemolysins-1 and 2 of A. carteri: R1= acyl($C_{18:4\omega3}$), R2= acyl($C_{18:4\omega3}$)
hemolysins of C. polylepis: R2= acyl($C_{18:5}$, $C_{20:5}$)
hemolysins of G. aureolum: R2= acyl($C_{20:5}$)

Fig. 16 Structures of hemolysin - 1 and hemolysin - 2 from *A. carteri* and proposed structures of hemolysin *C. polylepis* and *G. aureolum*

Gyrodinium spirale is a relatively large cell (40-200 μm) without photosynthetic activity, capable of catching live prey, widespread in many seas, including the European Atlantic coast and also the Mediterranean coast of France. In the Thau lagoon (brackish water) in 1985 this dinoflagellate caused the wiping out of 600 tons of mussels and 10 tons of oysters; however, there were no biotoxins present, and neither were biointoxication cases in man reported (Tournier and Guillon, 1985). *Gyrodinium spp.* represent, during the summer, the most abundant species of the dinoflagellate fraction in the Gulf of Trieste (Cabrini et al., 1989).

6.1.2 Prymnesiophyceae

Genus PRYMNESIUM

Another toxin of great interest is that produced by a yellow-brown alga *Prymnesium parvum*. This alga grows in areas of brackish water and sea water and produces a toxin that is lethal for fish and gill-breathing animals in some European countries and Israel (Shilo and Aschner, 1953; Shilo and Rosenberger, 1960; Shilo, 1967). Israeli research workers have used various biological methods to show that the toxin has a multiple action and produces three different effects: ichthyotoxic, haemolytic, and cytotoxic. The toxin inhibits the transfer of oxygen through the gill membranes and is one of the major problems of commercial carp farming in Israel (Shilo, 1967).

Initially, there is a reversible specific damage to gill tissues, consisting of the loss of their selective permeability, followed by a second stage leading to mortality, as a response of sensitized fish to non specific toxicants present in the environment in concentrations subletal to normal fish (Shilo, 1967).

The studies on the purification and analysis of *Prymnesium* preparations have shown that the active principle (prymnesin) is a glycolipid of high molecular weight (23,000 ± 1,800). The skeleton of its structure is a polysaccharide containing about 100 hexose molecules composed of glucose, mannose and galactose in a 2:1:1 ratio. In this polysaccharide 26 hydroxyl groups are esterified by four long chain fatty acids.

The various biological activities connected with prymnesin probably result from interactions with the cell membrane. It is presumed that prymnesin is attached to biological membranes and the resulting rearrangement of the membrane makes it permeable and loose.

Ulitzur and Shilo (1970) using a different separation technique, obtained a second toxin called "toxin B" which had 15 amino acids and a number of unidentified fatty acids. In contrast to prymnesin, toxin B resembled a proteolipid. It had six haemolytic factors.

Genus CHRYSOCHROMULINA Lackey

Also, the extensive flowering of the *Chrysochromulina polylepis* in 1988, along the coasts of Denmark, Sweden and Norway, which reached a maximum concentration of 50-100 million cells/litre, and which caused massive invertebrate and fish mortality both in the natural state and in farming, has opened up new health and economic problems for the European seas (Undertal et al., 1989). It must be noted that blooms of the *C. polylepis* are considered today to be an example of marked nutrient increase on coastal areas and therefore one of the characteristic parameters of anthropogenic eutrophication. The ichthyotoxins produced by this prymnesiomonadine is a glycolipid: monoacyl-digalactosylglycerol, whose fatty acid is a C 18:5 or 20:5 (1-acyl-3- digalactosylglycerol) (Yasumoto et al., 1990).

6.1.3 Raphidophyceae (Chloromonadophyceae)

Genus CHATTONELLA

Some chloromonadines also produce ichthyotoxins. *Chattonella antiqua* and *C. marina* are responsible for damage along the Japanese coast. It has been considered that their ichthyotoxic action can be due to the polyunsaturated fatty acids (Lassus, 1988).

The chloromonadines too, constitute for the European seas, and in particular the Mediterranean potentially ichthyotoxic phytoplanktons. As far as *Chattonella subsalsa* is concerned, in the 1960s this species was responsible for a fish kill along the Mediterranean coasts of France and Spain and for a bloom of 2,000,000 cells, in the Port of Algiers in 1956 (Hollande and Enjumet, 1957) and in the Port of Barcelona in 1968 (Margalef, 1968), and similarly in the Bay of Villefranche-sur-mer in 1961 (Tregouboff, 1962).

6.1.4 Dictyochophyceae (= Silicoflagellates)

Genus DICTYOCHA

The number of species of silicoflagellates in existence which are toxic to fish is probably only three. These organisms were abundant in the secondary era. The first cases of red tide produced by these silicoflagellates were described in Japan in 1955. After 1983, numerous cases of silicoflagellate red tides were described in Europe, not only at Kiel (Jochem, 1987), in the Kattegat (Aertebjerg and Borum, 1984), in Ireland (Doyle et al., 1984; Gowen, 1984) but also in the Mediterranean in the north-east of the Adriatic (Gulf of Trieste) (Fanuko, 1989).

The damage is attributable either to O_2 deficiency or to irritation of branchial apparatus by silicous structures, because a toxic substance has not been demonstrated to be present.

In 1983 it was discovered that *Dictyocha speculum* occurs also in the naked stage (characteristic of cell proliferation) and this stage is thought to be responsible for a fish kill in south western Denmark.

6.1.5 General tests on the toxicity of phytoplankton

To investigate the presence of biotoxins acting on marine animals when discoloured water red tide or mucilaginous aggregates occurs, various laboratory tests can be performed.

(a) Qualitative and quantitative determination of the phytoplankton

The qualitative and quantitative determination of the phytoplankton can be performed using Millipore filters (Margalef, 1969) and Utermöhl's method (1958). In the qualitative analysis of the phytoplankton particular attention must be paid to the species of the classes that produce ichthyotoxins: *Dinophyceae*, *Primnesiophyceae*, *Raphidophiceae*, *Dictyochophyceae*.

(b) Acute toxicity tests on fish using samples of water and phytoplankton extracts

(i) Tests on affected fish from areas of water or sea discoloured by phytoplankton blooms

Affected fish can be brought to the laboratory from their natural environment (brackish or sea water) in the water in which they were caught and then transferred and divided into equal numbers in suitable 10 litre continually aerated tanks containing: a) the discoloured water under test, and b) artificial control water. If all the fish kept under the two conditions recover and continue to live for 96 hrs it can be concluded that the disease or mortality in the natural environment was due to simple O_2 deficiency. If only the fish in tank a) die even with significant oxygenation, then biotoxins can be present. If some fish die in both tank a) and tank b) (control), it means that irreversible lesions (and branchial clogging) had occurred. In this type of screening it can also be useful to make preliminary tests for NH_3 and H_2S in the water, as their presence accompanies conditions of anoxia.

(ii) Effect of the acute toxicity of discoloured water on healthy fish in the laboratory

Because of its euryhaline characteristics, *Mugil cephalus* can be very useful in examining sea water samples of varying salinity. In this case also, the times of any deaths in the fish in the "discoloured water" tanks are observed. The absence of mortality over a 96 hrs period suggests that no water soluble biotoxins are present, whereas a high mortality suggests their presence. With a high mortality rate the type of phytoplankton must be considered, and investigations are started on the extraction or isolation of the biotoxins.

(c) Acute toxicity tests on mice using samples of water, phytoplankton and mussels extracts

(i) Direct extraction of biotoxins from water and phytoplankton

The biotoxins can be water or fat soluble. The water soluble toxins can be in solution as well as in the phytoplankton cells; the fat soluble toxins are found only in the cells or in particulate matter. At first the concentration of the phytoplankton can be done by filtration using Millipore filters or centrifugation. In the case of the fat soluble toxins from *G. breve*, direct extraction with ethyl ether (McFarren et al., 1965; Cummins et al., 1968) from 2-16 litres of sea water makes it possible to demonstrate the presence of the biotoxins by evaporating the ether extract and injecting the lipid fraction intraperitoneally into mice of 19-23 g (Cummins et al., 1968). For a more general assay of the toxicity in mice, it is possible to use either filter extracts or the residue after centrifugation. However, to carry out more detailed studies on the chemical nature of the toxin, it is necessary to use an appropriate plankton net to collect sufficient quantities of phytoplankton for application of the techniques for extracting water-soluble (AOAC, 1970) and fat soluble toxins (McFarren et al., 1965; Scheuer et al., 1967; Bagnis et al., 1974).

(ii) Concentration of the biotoxins by *Mytilus galloprovincialis* or *M. edulis*.

Another very useful method for demonstrating or excluding the presence of biotoxins of the PSP and NSP type in discoloured sea water is to filter the sea water in 100 litre tanks containing healthy mussels over a period of several days until the water becomes transparent or clean, and, if necessary, this can be repeated for a week (Viviani, 1977). The biotoxins of the water soluble (AOAC, 1970) and fat soluble type (McFarren et al., 1965) are extracted from the mussels and assayed in mice.

(d) Haemolysis test of extracts obtained from the algal waters or mussels on mouse blood cells

Bioassay using fish usually requires relative large amounts of samples, as the test materials should be dissolved in relatively large volumes of water to keep test fish. In addition dose-survival time responses of fish are often fluctuant. In order to surmount difficulty and use a rapid and sensitive bioassay method a haemolytic test for screening ichthyotoxins this has been applied. In fact many ichthyotoxins such as those of *Prymnesium parvum* (Shilo, 1967), *Amphidinium carteri* (Yasumoto et al., 1987), *Chrysochromulina polylepis* (Yasumoto et al., 1990; Edvardsen et al., 1990), *Gyrodinium aureolum* (Yasumoto et al., 1990) and maitotoxin of *Gambierdiscus toxicus* (Nakajima et al., 1981) are potent haemolysins. Also mussels accumulate the haemolysins. Mussels exposed to a *C. polylepis* bloom displayed a higher haemolytic activity than non contaminated mussels (Yasumoto et al., 1990). The discoloured water, red tide or mucilaginous aggregates could be screened for the presence of bioactive compounds using haemolytic tests. To this end the extracts obtained from the marine waters or mussels dissolved in chloroform were purified. After purification, haemolytic tests were carried out using mouse blood.

6.1.6 Studies on ichthyotoxic components of phytoplancton in the Mediterranean sea

Acute toxicity tests on fish

Using acute toxicity tests on *Mugil cephalus* with samples of marine water and phytoplankton extracts it has been possible to demonstrate the absence of biotoxins with acute ichthyotoxic effects during the "green tide" produced by *Gymnodinium corii* along the coast of Emilia-Romagna during 1976 (Viviani, 1978). Water with blooms of *Prorocentrum micans* (Viviani, 1977) and of *Gonyaulax polyedra* (Viviani et al., 1985) has also been demonstrated to be non-toxic.

Haemolysis test

The first studies on haemolytic and cytotoxic effect of ichthyotoxins and the analysis of toxic components are those of Israeli research workers (Shilo, 1967). It has been shown that the relationship between the different toxic activities can vary with different growth conditions of the *Prymnesium parvum* and that some of the haemolytic activity can be selectively removed from the phytoflagellate preparations by absorption on erythrocytes. Particularly interesting is the differential inactivation of the various toxic activities by alkalis and light. Although alkali rapidly inactivates the haemolysin which acts at 35EC, a haemolysin active at 8EC is only mildly affected (Shilo, 1967).

Polyunsaturated C 18 and C 20 fatty acids analysis

In the Mediterranean sea, instances of fish kill due to glycolipids and unsaturated fatty acids of phytoplancton origin have not yet been described. Previous research did however show that in the Adriatic there are phytoplankton which synthesize the fatty acid C 18:4 which, according to present knowledge has ichthyotoxic and haemolytic properties. Indeed, in the stomach contents of *Clupea sprattus* the presence of this fatty acid was demonstrated in the field of research on the relationship between fatty acids contained in diatoms and dinoflagellates, and their presence in the tissues of plankton-feeding fish in the Adriatic sea (Viviani et al., 1968).

When discoloured water, red tide or mucilaginous aggregates occur in a health and environmental monitoring programme for the presence of ichthyotoxic substances it is necessary to analyze by gas-chromatographic methods fatty acids of total lipids not only in marine waters, but also in mussels and in the stomach contents and tissues of plankton-feeding fish.

Significant levels of the polyunsaturated fatty acids C 18:4, C 18:5, C 20:5 could be the first screen before haemolysis tests, chromatographic and mass spectral data.

6.2 Damage to communities and the ecosystem by harmful algal blooms

The dystrophic effects which occur in the Mediterranean after eutrophication are generally caused by oxygen deficiencies in the bottom waters. A detailed description of the cases reported is given in chapter 5, but it is worth drawing attention to the North Adriatic, where the regular occurrence of widespread, persistent oxygen shortages has led to profound modifications in the benthic communities and ecosystem.

These modifications have been highlighted by research carried out in the Gulf of Trieste (Stachnowitsch, 1984) and in the North-West Adriatic off the coast of Emilia-Romagna (Crema et al., 1991; Rinaldi et al., 1993).

Laboratory research into the effects of oxygen deficiency has been performed using bivalves typical of the Northern Adriatic (De Zwaan et al., 1992).

6.2.1 Effect of oxygen deficiency on the benthic communities in the Gulf of Trieste

The detailed in-situ observations in the Gulf of Trieste in 1983 (Stachowitsch, 1984) were able to detect individual behaviour patterns and the sequence of mortality of benthic species due to oxygen deficiency, and will be used as a basic framework for comparing data of benthic community deterioration in the seas.

The animal groups chosen include sponges, polychaetes, anemones, bivalves, crustaceans, echinoderms and fish.

Sponges

A characteristic feature in the Gulf of Trieste was a mucus layer covering sponges and other sessile macroepifauna organisms. All sponges had died within the first two days after the onset of community deterioration. Sponges (35% of total biomass in the community investigated) are a major component of the multi-species clumps typical of the deeper Gulf.

Polychaetes

The large polychaetes *Eunice aphroditois* and *Dasybrancus caducus* emerged on the second and third days after the first signs of community stress: most individuals were dead on day 4.

Anemones

Both in the Gulf of Trieste and in the Limfjorden, anemones were the most resistant to oxygen depletion. *Calliactis parasitica*, normally found attached to hermit crab shells in the gulf, detached on days 3 and 4 and could be observed lying on the sediment with tentacles extended. After one week, however, virtually all species, including the large *Cerianthus sp.*, had died.

Bivalves

In the Gulf of Trieste the first signs of stress in bivalves were pointed out in *Cardium sp.* accompanied by extension of the siphons. Similar behaviour was recorded in Limfjorden, where the siphons of *Cardium edule* and *Syndosmya alba* stretched a few cm above the sediment, those of *Mya arenaria* 10-20 cm above the black mud (Jorgensen, 1980). This was followed by the critical condition of these species (the siphons of *Mya* extending 20-30 cm above the bottom). These bivalves were considered to be able to survive for an additional week in this state. However, substantial early losses of *Mytilus edulis* were reported in dense beds on the soft mud due to extremely high respiration even under normal conditions, coupled with H_2S sensitivity).

Crustaceans

In addition to the early mortality of small shrimp and the crabs *Pilumnus spinifer* and *Pisidia longicornis* associated with sponges, a number of larger forms were affected both in the Gulf and in the other areas under consideration here.

The first signs of unusual conditions were various reports of lobster (*Homarus americanus*) leaving their shelters and gathering on the highest parts of shipwrecks, a phenomenon paralleled by thousands of dead lobsters outside their dens and aggregated on the highest parts of outcrops in the North Adriatic Sea (Stefanon and Boldrin, 1982).

Living, maribund and dead *Squilla mantis* were on the sediment during the day and several individuals were observed swimming freely several metres above the bottom (Stachowitsch, 1984).

Echinoderms

Echinoderms, a major component of the macroepibenthos in the Gulf of Trieste, showed a variety of behavioural modifications before death in the 2-3 days after the first signs of stress.

On the second day, the sea start *Astropecten aurantiacus* was observed on mounds with greatly extended discs. This stress reaction characterized by a gas-filled stomach was also observed in Emilia-Romagna Region of Cesenatico, Italy (Rinaldi et al., 1993), and was followed on the third day by overturning individuals. *Amphiura chisjei,* the echinoderm species last to succumb, was aggregated in large numbers on the tops of mounds. All individuals of the epibenthic holothurian *Holothuria tubulosa* were found eviscerated on the first day, with death taking place on days 2 and 3. On day 1, the burrowing form *Thyone fusus* emerged from the sediment.

Fish

In the Gulf of Trieste, small benthic fish were also affected on the first day. Great numbers of dead goblids *Gobius jozo* as well as juvenile trachinidae and small flatfish were found dead on the sediment surface.

In the 1977 mortality event, these fish were concentrated at the leading edge of the affected area, resulting in increased catches of fish normally not caught together (Stefanon and Boldrin, 1982).

6.2.2 Effect of the oxygen deficiency on the benthic ecosystem of the North-West Adriatic

It is difficult to quantify the effects of the oxygen deficiencies on the stocks of bottom-dwelling fish because of the intensity of the fishing activities to which they are subjected. However, *Hippocampus antiquorum* and *Trachinus draco* are amongst the fish species which have disappeared or become much rarer, on which fishing practices have had only a negligible effect.

The most significant observations were made on molluscs and crustaceans.

The following is a list of the species of molluscs and crustaceans which have gradually become rarer (marked RA) or have actually disappeared (marked DS), since 1975. The observations refer to the area of sea off the coast of Emilia-Romagna, up to 20 km off shore (Rinaldi et al., 1993).

Molluscs

Grastropods

Aporrhais pes-pelecani (L.)	RA

Turritalla communis Risso	RA
Spheronassa mutabilis (L.)	RA
Truncularioppsis trunculus (L.)	DS
Murex brandaris (L.)	DS
Naticarius millepunctatus (Lamarck)	RA
Naticarius hebraeus (Martyn)	DS

Scaphopods

Dentalium inaequicostatum (Dautz.)	RA

Ophistobrancs

Acteon tornatilis (L.)	DS
Philine aperta (L.)	RA

Bivalves

Chlamys glabra (L.)	DS
Acanthocardia aculeata (L.)	RA
Acanthocardia paucicostata (G.B. Sowerby)	DS
Mactra corallina (L.)	RA
Spisula subtruncata (Da Costa)	RA
Ensis siligus minor (Chenu)	RA
Solen marginatus (Pennant)	RA
Angulus tenuis (Da Costa)	RA
Tellina fabuloides (Monterosato)	DS
Tellina nitida Poli	RA
Teeinella distorta (Poli)	RA
Donax smistriatus Poli	DS
Donax trunculus L.	DS
Abra alba (Wood)	RA
Pharus legumen (L.)	RA
Chamelea gallina (L.)	RA
Dosinia lupinus (L.)	DS
Venerupis aurea (Ghelin)	RA
Mysia undata (Pennant)	DS
Barnea candida (L.)	DS
Thracia papyracea (Poli)	DS

Crustaceans

Dorippe lanata (L.)	DS
Corvates cassivelaunus (Penn.)	DS

The effects on cetaceans and seabirds were different.

The drastic reduction in the occurrence of dolphins (*Tursiops truncatus*) off the coast of the North-West Adriatic is probably due to the variations in organoleptic characteristics (colour, odour and taste) and the loss of transparency caused by eutrophy. Once common even in the waters close to the shore, nowadays they can only be encountered (although sometimes in large numbers) over 10-20 miles from the coast. It seems that rather than a drop in numbers of these creatures we are witnessing a tendency for them to keep away because of a worsening in the condition of the waters, which before the '60s were certainly in a much better state than now.

In contrast, it seems that the seabirds (gulls in particular) seem to be benefiting from the situation: when the benthic fish come to the surface gasping for oxygen before dying, flocks of gulls (*Larus ridibundus* and *Larus argentatus*) gather in the area. It is well known that the numbers of these species are growing because of their ability to adapt in an opportunist way to various forms of human activity (fishing, agriculture and landfills), causing damage to more "delicate" species, particularly during the breeding and migration seasons. The way in which they interfere with and even prey on the eggs and nestlings of species such as oyster catchers (*Haematopus ostralegus*), avocets (*Recurvivostra avosetta*) etc. which nest in areas adjoining or overlapping with those of the herring gull (*L. argentatus*) is in fact well known.

6.2.3 Immature macrozoobenthic community along the coast of the Emilia- Romagna region

The macrozoobenthic community in the northern Adriatic Sea, south of the Po river, along the coast of the Emilia-Romagna region, was sampled in 1985 (Crema et al., 1991). The sampling site was in the centre of a highly eutrophied area with greatly increased intensity and frequency of dystrophic events over recent decades. The sampled community differs from all those described in the same area in a period (1934-1936) (Vatova, 1949) previous to the current degree of eutrophication. Large abundances of species indicative of unstable bottoms, such as the bivalve *Corbula gibba* and the polychaete *Lumbrineris latreilli* were recorded.

The recent biocenosis is characterized by a large abundance of *Corbula gibba*, a species typical of the transition zone between detrital and muddy bottoms. Its dominance is of particular interest. In fact, *Corbula gibba* is known to be a pioneer species in the recolonization of defaunated bottoms (Bonvicini Pagliai et al., 1985; Curini Galletti, 1987; Crema, 1989), and is prominent in subnormal zones in areas polluted or enriched by organic material (Ghirardelli and Pignatti, 1968; Pearson and Rosenberg, 1978; Bourcier et al., 1979; Russo, 1982).

This bivalve was also dominant, in association with *Lumbrineris latreilli,* in the Gulf of Fos, subjected to extensive dredging, and it has been included in a stock of species typical of unstable bottoms (Selen-Picard, 1981).

Moreover, the community structural features indicate a state of immaturity, such as in early successional stage communities. The increased frequency of acute dystrophic events and consequent shortening of the time between consecutive disturbances is proposed as the cause of the modification of biocenosis, and its current structure and composition in the Northern Adriatic Sea, and in eutrophicated coastal areas of the Mediterranean.

6.2.4 Research in the laboratory and in the field on the resistance of bivalves to oxygen deficiency

De Zwaan et al. (1992) carried out laboratory survival tests to determine the resistance of benthic organisms to oxygen deficiency. In sea water where oxygen levels were reduced by bubbling through nitrogen, he performed tests on a number of bivalves endemic to the North-West Adriatic such as: *Chamelea gallina*, *Tapes philippinarum*, *Mytilus* *galloprovincialis* and *Schapharca inaequivalvis*. The 50% survival times of the individuals used demonstrated that the most resistant is *S. inaequivalvis* with 19 days, followed in decreasing order by *M. galloprovincialis* with about 16 days, *T. philippinarum* with 12 and finally *C. gallina* with about 6 days.

Observations in the field have shown that the mortality times of *C. gallina* and *S. inaequivalvis* are generally shorter than the times calculated in the laboratory (of the order of 40-50%).

The reasons probably lie in the combined effects of a number of factors, since as well as the oxygen deficiency there is the simultaneous action of substances of a toxic action such as hydrogen sulphide and ammonium, which are generally found in high concentrations in such situations.

6.3 Direct economic effects

6.3.1 Effects on fisheries and coastal fish-farming in regions and subregions of the Mediterranean

The Mediterranean has always been considered an oligotrophic sea; only limited coastal areas and a number of secondary basins (such as the North-West Adriatic) are affected by high trophic levels because of the discharge of nutrients deriving from human activities.

The effects of eutrophication on the stocks of the species most highly prized by the fishing industry are extremely difficult to quantify, mainly fishing has contributed to the reduction in fish stocks recorded in the Mediterranean during the last twenty years.

Since in the Mediterranean there have so far been no reports of microalgae capable of synthesizing fish toxins, and that the cases of benthic fauna kills have always been caused by oxygen deficiencies in the bottom waters, a number of comments may be made:

- It seems that eutrophication has had no negative repercussions on offshore fish. On the contrary, their numbers should tend to increase because of the positive effects on primary productivity and on the trophic system in general. This reinforces the hypothesis that the reduction in stocks of offshore fish has been caused above all by overfishing showing no regard for breeding cycles. An example is provided by the North Adriatic, where during the last ten years there has been a considerable reduction in the numbers of sardines (*Clupea pilchardus*) and anchovies (*Engraulis encrasicholus*). As an example, Fig. 17 shows the trends for this Clupeiforms caught by fishermen from Cesenatico (Italy - North-West Adriatic). Although the data are of local reference, they are actually representative of the entire North-West area of this area.

- The situation with regard to the benthic organisms (invertebrates and vertebrates) which are fished is different. In the North-West Adriatic there have been many reports of kills of bivalves of commercial interest (*Venus gallina* and *Mytilus galloprovincialis* in particular) as a result of oxygen deficiencies induced by algal blooms. Negative effects have also been reported on *Sepia officinalis*, particularly in cases where the opening of the eggs has coincided with periods of oxygen shortage. The same occurs to the bottom fish (*Solea vulgaris*, for example), whose larval/immature stages tend to move into the coastal waters

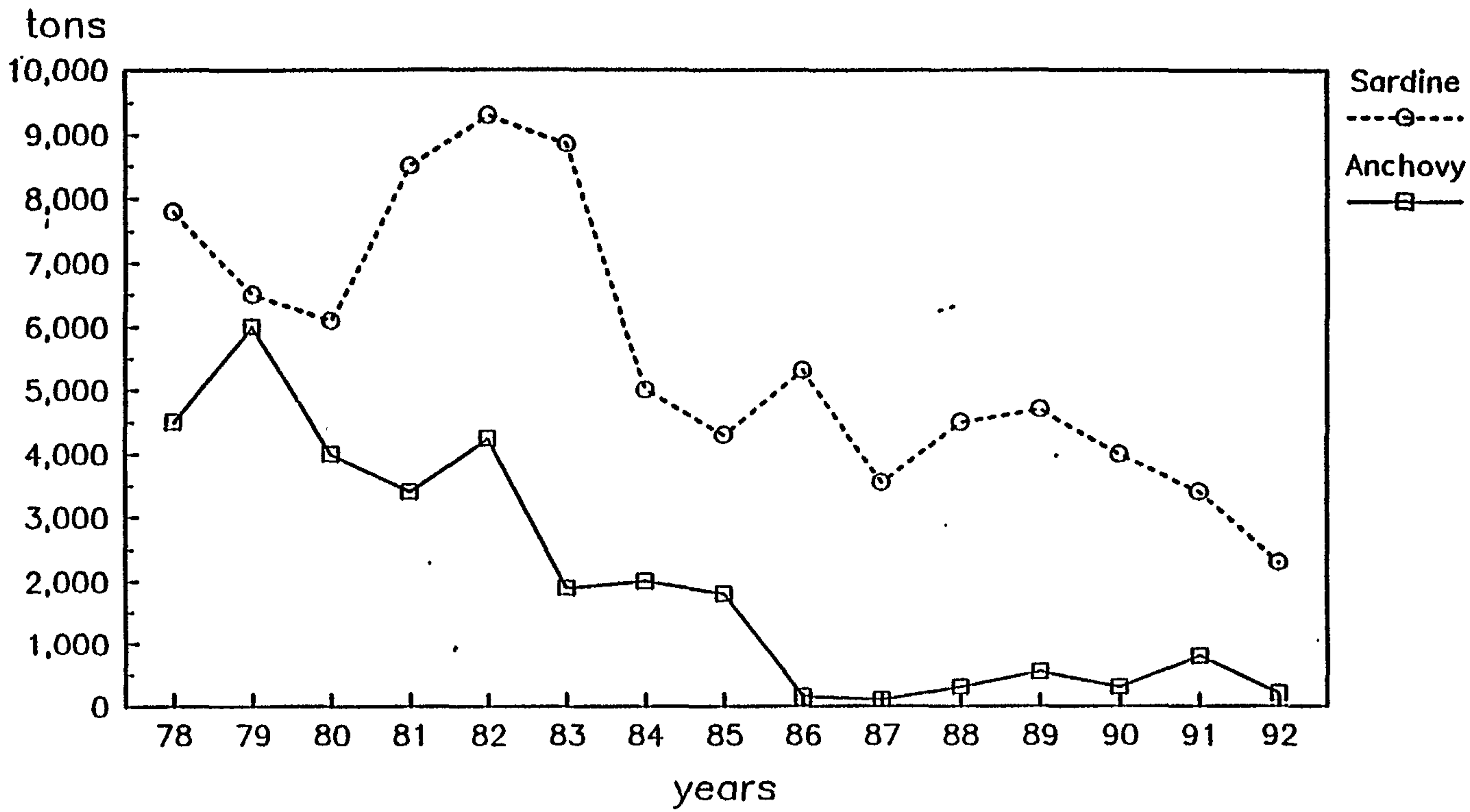


Fig. 17 Evolution of landings of sardines (*Sardina pilchardus*) and anchovy (*Engraulis encrasicholus*). Commercial fisheries data of the community of fishermen of Cesenatico (Northwest Adriatic)

in spring and remain there until August. Therefore these species, like others with similar migratory behaviour, are particularly vulnerable to oxygen deficiencies on the seabed (Piccinetti, 1986; Mancini and Sansoni, 1986). However, even for the benthic fauna, although the linkage with algal blooms is clear, there are still large difficulties in discriminating between the damage caused by eutrophication and that derived from overfishing.

6.3.2 Effects on tourism in regions and subregions of the Mediterranean Sea

During the summer, the coastal areas of the Mediterranean are visited by over a hundred million foreign visitors (one third of all international tourism), who spend their holidays there, attracted by the natural beauty and the leisure facilities, and also to a certain extent by the wish to admire the treasures of the ancient civilizations found throughout the Mediterranean area.

Since one of the main reasons for tourism is the promotion of health, any potential risks to the health of tourists are immediately important.

The bacteriological quality of the coastal waters was the first alarm signal for health, leading to plans for the study of subsequent intervention at an international level. In 1975 MED POL Phase I was approved in the framework of the Mediterranean Action Plan prepared by the governments of the countries which border the sea. In the context of this programme, the MED VII project is concerned with the quality control of coastal waters, and has been implemented in coordination with UNEP and the World Health Organization (Saliba, 1989).

The evaluation of the environmental impact of chemical pollutants has therefore acquired great interest; in fact, current estimates attribute 80-85% of the total chemical pollution of the Mediterranean to terrestrial sources. These chemical pollutants are today considered to be toxic trace metals (Cd, Pb, Hg etc.), non-metals (As), synthesized organic substances (for example DDT, PCB), hydrocarbons of petroleum origin and radionuclides, and nutrients (organic and inorganic compounds containing nitrogen and phosphorus) (Saliba, 1989).

Of the chemical pollutants, only the nutrients responsible for eutrophication may have a direct impact on tourism. In general there is little information for the Mediterranean on the effects of eutrophication on tourism.

The Italian part of the North Adriatic is the only area where tourist numbers have been monitored with some attention, because of the serious events that have occurred repeatedly over the last fifteen years, and the importance of tourism in the economy of this region.

The linear coastal area of the Emilia-Romagna region is a continuously developed strip of almost unprecedented compactness and uniformity. This urban agglomeration along the Adriatic coast has grown up from a few resorts established during the last century or the early 1900s, such as Cesenatico, Rimini, Riccione etc., all existing towns which developed tourism alongside their main activities (Chicchi, 1990; Benzi, 1990).

During the post-war years, the concomitance of mass demand for summer holidays with plenty of accommodation and skilled labour with only limited alternative employment in agriculture or industry, led to the gradual urbanization of the entire coastal strip, starting with expansion around the original points and eventually saturating every available space (Giordani, 1990 a-b).

In the summers of 1975 and 1976, large numbers of dead marine fauna, mainly bottom-dwelling fish, appeared on the foreshores of this linear coastal town (Bisbini, 1976; Turci, 1976; Viviani, 1976).

This environmental disturbance of unprecedented dimensions not only caused serious problems for the municipal authorities responsible for public health and keeping the beaches clean, but also had a severe psychological impact on the mass-media and those making their living from tourism (Goldoni, 1976).

The press, misled by the many illogical comments flying around, carried gloomy warnings of disaster, often blaming the fish kill on dangerous pollutants or highly toxic microalgae.

The Emilia-Romagna region was able to draw on all the research performed by the University of Bologna from 1966 to 1975 on heavy metals (Hg, Cd, Pb) and chlorinated hydrocarbons (DDT, PCB) contents in the tissues of molluscs and fish in the North Adriatic (Viviani, 1977; 1988; 1989), which demonstrated levels incapable of inducing acute toxicity or causing mortality, as well as the initial results indicating the absence of algae toxic to fish. Thanks to this information, the authorities were able to rule out the existence of "dangerous pollutants" and fish toxins in the North West Adriatic.

These data directed public opinion towards the new concept of eutrophication, "coloured tides" and the consequent oxygen deficiencies.

After detailed investigations, the doubts disappeared and the true cause of the disaster was identified: the death of the benthic fauna was related to the oxygen shortage in the bottom waters due to the sedimentation of the phytoplankton biomass during mineralization (Turci, 1976; Viviani, 1977).

How did tourism respond to these events? An analysis of tourist numbers does not seem to show any reduction in bummers as a consequence to the problem of "coloured tides" due to eutrophication. No one can deny that the availability of precise information has helped to shed light on the real causes of the phenomenon and the negligible level of the health risks involved.

The response of tourism to the problem of mucilages has been different. It should be remembered that this phenomenon has occurred in an invasive form in 1988, 1989 and 1991 (see chapter 5.2.5).

During July and August of these years, widespread masses of mucilaginous material tended to approach the coast, and in some cases the beach. Apart from the "dramatic" nature of the phenomenon and the large area affected (10,000 km^2 in summer 1989), long stretches of coastal water were unsuitable for bathing. This triggered social tensions and negative psychological effects, deriving mainly from the uncertainty over the future of the area's tourist industry. These worries were partly worsened by the lack of clear answers regarding the causes and mechanisms of formation of the phenomenon.

Although tourist numbers had consolidated during the previous years, in spite of a considerable loss of water quality because of the current algae blooms, the mucilages caused significant drops in visitor figures in 1989 and 1990 (Agertur, 1992) (see Fig. 18).

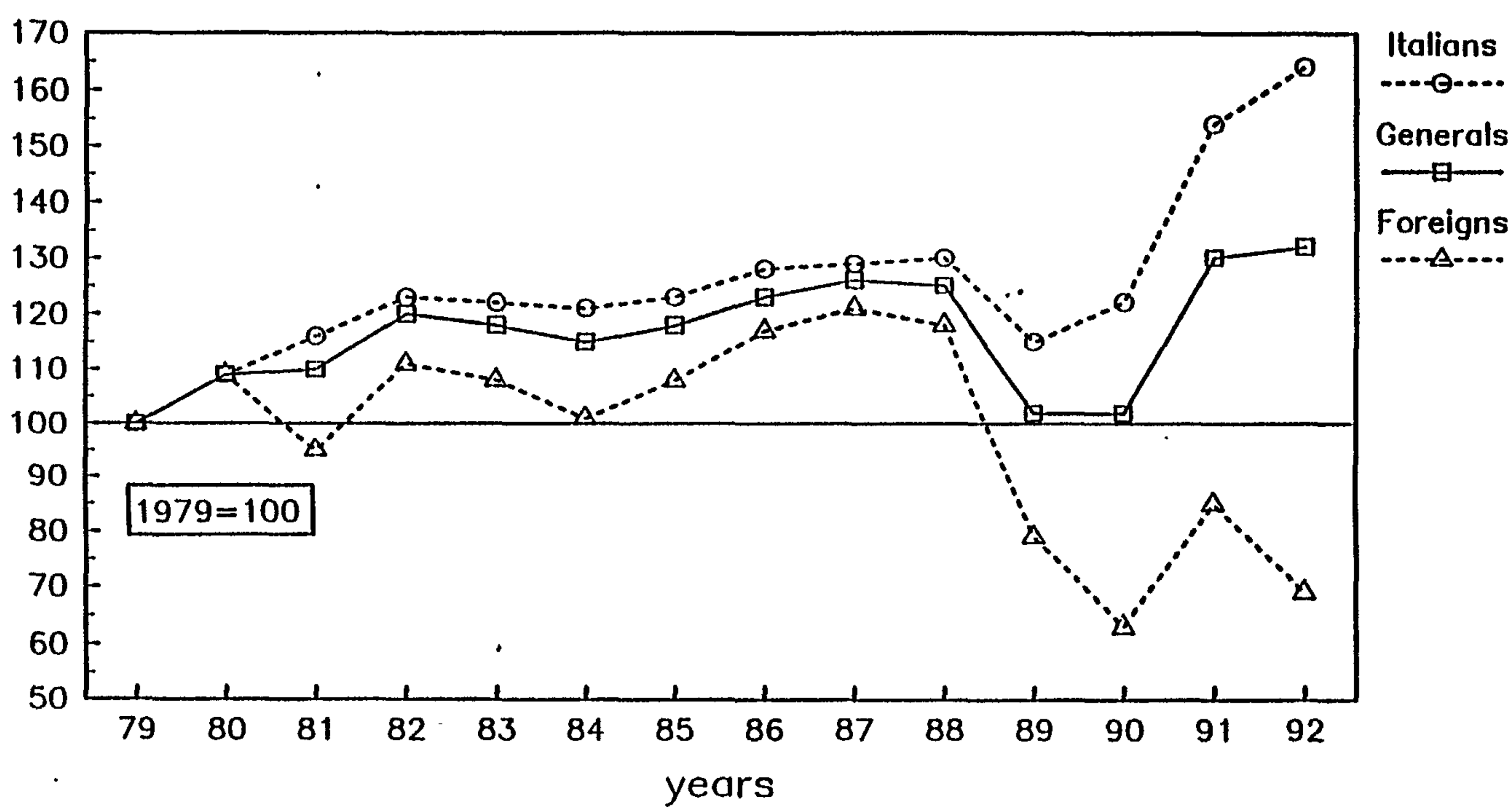


Fig. 18 Evolution of tourist arrivals along the coast of Emilia-Romagna (Data from Agertur, 1992)

7 HUMAN HEALTH ASPECTS

7.1 General facts on harmful algal blooms, marine biotoxins in seafood and human biointoxications

In this chapter the role played by the eutrophication phenomenon on human health aspects has been considered. Eutrophication phenomena in marine coastal water can today be explained on the basis of natural or anthropogenic causes. These causes may also be simultaneously present in the same areas.

The main characteristic which distinguishes the two types of eutrophication is the length of time before its appearance. Natural eutrophication is a relatively slow process (time scale 10^3-10^4 years). Anthropogenic eutrophication which occurs more frequently in coastal areas due to man's contribution of nutrients appears in a short space of time, 10 years or less on a time scale.

In order to further clarify anthropogenic eutrophication, an important role is played by the eutrophic phenomena which have appeared in recent decades and will be studied together with the determined lengths of time for the occurrence of eutrophication owing to the creation of new urban settlements including touristic, zootechnic and aquaculture areas. The first and most important indices of natural and anthropogenic eutrophication phenomena are given by the visible characteristics of water: abnormal growth of macroalgae and/or increase in the phytoplankton biomass, which is indicated by the terms "colored sea" and "red tides". Undesirable effects and also sanitary problems in both types of eutrophication are often produced, but they may differ greatly in frequency and significance.

From data at present available it results that the health conditions of man can be affected through the digestive, respiratory and cutaneous apparatus, therefore eutrophication may have an environmental impact affecting coastal inhabitants, fishing workers and bathing. The information currently available indicates that some biotoxins are synthesized in the phytoplankton, in the phytobentos and in macroalgae and produce their effects on man as such or after being modified during metabolism in the food chain. The ingestion by man of biotoxins present in aquatic plants or animals produces disorder called biointoxication.

Biointoxication is differentiated from pathological conditions caused by food poisoning from virus and bacteria, radioactive contaminants, aromatic polycyclic hydrocarbons (PAH), toxic metals, persistent chlorinated hydrocarbons, parasites and allergies resulting from the consumption of fishing products. The seafood toxins are preformed toxins that are present in the product when it is taken from the marine environment. Although can undergo some transformations that can increase their toxicity, they do not propagate like bacterial contaminants. Sanitary treatment of sea food for eliminating bacterial contaminants and which largely deactivates the proteinaceous toxins produced by bacteria, is not reliable to destroy the marine toxins.

At present, toxins from blooms or red tide dinoflagellates are known to be responsible for four biointoxications: paralytic shellfish poisoning (PSP), neurotoxic shellfish poisoning (NSP), diarrhoeic shellfish poisoning (DSP), venerupin poisoning. Another biointoxication is due to a diatom bloom: "amnesic shellfish poisoning" (ASP). Also some marine green and red algae are responsible for human biointoxications. Pathologic phenomena in the respiratory tract are present in association with NSP. Other biotoxins produced by blue algae blooms have effects on the skin.

In regard to considering the harmful effects of algal blooms, it was suggested that toxic algal blooms fall into three categories: eutrophication dependent on a large number of cells; associated with a large number of cells but not with anthropogenic nutrient enrichment of coastal waters; toxicity associated with low cell numbers (as for *Dinophysis* blooms or *Alexandrium* cystes) (Smayda, 1990).

It is important to recognize that the various seafood toxin syndromes depend not only from natural nutrient enrichment of coastal waters or anthropogenic or due to agricultural runoff, aquaculture (anthropogenic eutrophication), but also through the dispersion of the resting cyst of toxigenic dinoflagellates by dredging, discharge of ship ballast water, or transplanting of shellfish.

In this chapter the attention is paid to PSP and DSP (the most widespread biointoxication from toxic marine dinoflagellates in the world and for which the EEC is proposing health legislation such as tolerance limits and methods for official analysis) since these are today the known seafood toxin syndromes in the Mediterranean sea. In this chapter are also considered marine algae potentially toxic for seafoods and for respiratory or cutaneous symptoms of poisoning in the Mediterranean sea (*Prorocentrum minimum* and venerupin poisoning; *Nitzschia spp.* and ASP; Chlorophyta and Rhodophyta toxins; *Gymnodinium spp.* blooms; marine Cyanophyta blooms); and general effects on eutrophication, bacteria and human health.

From data currently available on PSP, DSP and other biointoxications attention is paid, on the basis of available literature, to: organisms producing toxins, chemistry of components, occurrence in the world, occurrence in Mediterranean, compromised seafood, detoxification in bivalve molluscs, methods of analysis, human intoxication and mechanism of action, tolerance levels and remark on safety. References documentation (general) and references cited are also reported.

7.2 The known seafood toxin syndromes in the Mediterranean sea

7.2.1 Paralytic Shellfish Poisoning (PSP)

In certain coastal areas oysters, mussels, clams and gastropod molluscs as well as some crustaceans become toxic sporadically or constantly in some month of the year and produce in man a neurotoxic syndrome known as "paralytic shellfish poisoning" (PSP) (Steidinger and Baden, 1984).

7.2.1.1 PSP-producing or potentially toxic dinoflagellates

In the sea world. The water-soluble toxins of PSP type (a family of closely related toxins known as saxitoxins) are produced in temperate water by members of the genus *Alexandrium* (Taylor, 1984), also known as *Gonyaulax* or *Protogonyaulax*, including: *A. tamarensis* (*A. excavata*), *A. catenella, A. acatenella, A. fundyense, A. minutum* and *A. cohorticula*. The dinoflagellate recognized to be source of PSP toxins in tropical waters is *Pyrodinium bahamense* var. *compressa* (Taylor, 1984). Other dinoflagellates reported to produce PSP include *Gymnodinium catenatum* and probably *Cochlodinium spp.* (Krogh, 1989). *G. catenatum* collected for first time in Galician rias (Spain) in October 1976 and also in 1991 produces PSP toxins, but not brevetoxins (Estrada et al., 1984). *G. catenatum* has a wide geographic distribution (Pacific coast of America, Japan, Australia, and the Atlantic coast of Spain) (Campos et al., 1982; Hallegraeff and Summer, 1986; Hallegraeff et al., 1988) and also in the Mediterranean coast of Spain (Bravo et al., 1990) and in Southern Tyrrhenian coast (Carrada et al., 1988; 1991). The dinoflagellates are propelled by two flagellae; some are bioluminescent. In addition to the motile form, such as *A. tamarensis*, they produce resting cysts (hypnozygotes), as a result of sexual reproduction. Thus there are two sources for contamination of shellfish with PSP: (a) motile cells of *Alexandrium* species; (b) resting cysts of *A. tamarensis* in the sediment-water interface; the latter example is thus not associated with a bloom phenomena.

Recent evidence indicates that bacteria present in *A. tamarensis* may be a source of saxitoxin (see 7.4.4).

In the Mediterranean sea. Species of the genus *Alexandrium* and strains of *Gymnodinium catenatum* producing PSP (Delgado et al., 1990; Bravo et al., 1990) or potentially toxic are present in the Mediterranean sea.

The monitoring of blooms of dinoflagellates in the Adriatic sea, occurring from 1970 on the ex-Yugoslav coasts, and from 1975 on the coasts of Emilia-Romagna, have focused on the existence of a potential danger for this area not only because the blooms are supported by species in the genera *Gonyaulax* and *Gymnodinium*, a group with many toxic species, but also because in August 1982 a new species appeared, similar to *Gonyaulax tamarensis* (Boni et al., 1983), a variety found in the Atlantic Ocean and in other seas, but never before found in the Mediterranean sea. This dinoflagellate was afterwards identified as *Protogonyaulax tamarensis* sensu Fukuyo (Boni et al., 1986) because of the presence of a ventral pore near the middle of the upper right margin of the first apical plate (now called *Alexandrium tamarensis* (Lebour) Balech) and linked in different zone of the world to PSP. This fact poses not only an ecological problem, but a health problem as well.

In 1985 in the same environment a new red tide was noticed caused by a species with resemblance to an *Alexandrium fundyense* Balech, since it lacked the ventral pore (Boni, 1992). After these data other *Alexandrium spp.* potentially toxic were found, but not in red tides, in the Adriatic sea and in the Gulf of Trieste: *A.* cf. *fundyensis, A. pseudogonyaulax, A. eusitanicus* (Honsell et al., 1992), *A. minutum* (Honsell, 1991), although PSP has never been detected in the Adriatic sea until now (Viviani et al., 1992).

Before the discovery of *Alexandrium spp.* in the Adriatic sea, *A. tamarensis* had been found in the Northern Tyrrhenian but not in the red tide (Innamorati et al., 1989a-b). Also in the Gulf of Naples and Salerno *A. tamarensis*, *A. minutum* and *A. balechi* are present (Montresor et al., 1990). Only in the Gulf of Salerno *A. balechi* caused red tide. PSP toxicity of *Alexandrium spp.* in the Tyrrhenian sea is not known.

The first report of a bloom of *Gymnodinium catenatum* for the Mediterranean sea and for a coastal lagoon was observed in early September 1987 at Fusaro lagoon, located on the Southern Tyrrhenian coast (Carrada et al., 1988). Previous observations in the same lagoon (1985, unpublished data) indicate the presence of this species from June through September. Despite the lack of information regarding the PSP toxicity of its population, the presence of *G. catenatum* in the Fusaro lagoon may represent a possible complication for the reclamation programme aimed at restoring in the lagoon ecological conditions compatible with its century-long tradition (Roman times) in shellfish farming (Carrada et al., 1988). In the Mediterranean sea the first toxic bloom (PSP) of *G. catenatum* has been reported along the coast of Spain (Bravo et al., 1990) (see 7.2.1.4).

7.2.1.2 Chemistry of the PSP components

The 18 PSP components make up three groups: carbamate, N-sulfocarbamoyl, and decarbamoyl components (WHO, 1984) (Fig. 19). Generally, they have chemical properties comparable to saxitoxin. The carbamate toxins are the dominant components in shellfish, whereas the N-sulfocarbamoyl are the dominant group in dinoflagellate cells.

R1	R2	R3	Carbamate Toxins	N-Sulfocarbamoyl Toxins	Decarbamoyl Toxins
H	H	H	STX	B1	dc-STX
OH	H	H	NEO	B2	dc-NEO
OH	H	OSO_3^-	GTX I	C3	dc-GTX I
H	H	OSO_3^-	GTX II	C1	dc-GTX II
H	OSO_3^-	H	GTX III	C2	dc-GTX III
OH	OSO_3^-	H	GTX IV	C4	dc-GTX IV

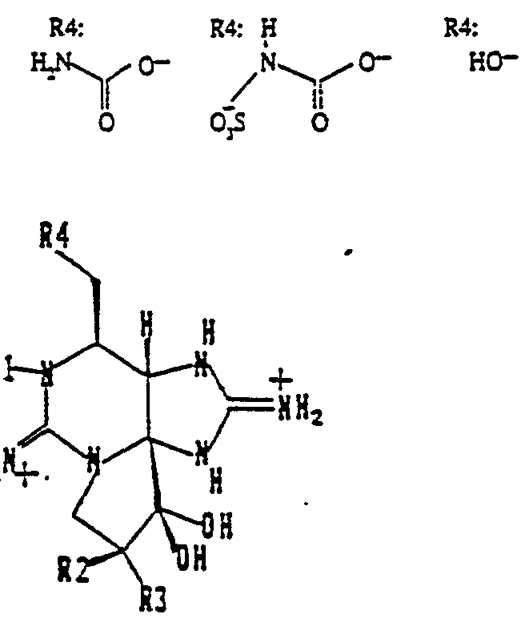


Fig. 19 Structures of 18 naturally occurring PSP components. STX: saxitoxin; FEO: neosaxitoxin; GTX: gonyautoxin

Stability of the toxins. Two reactions relating to the pH to which the toxins are exposed relate directly to the evaluation of analytical techniques. At a low pH (approximately < 2.0) the C-21 sulfo group is hydrolyzed from the sulfamate toxins to yield the corresponding carbamate form (i.e., B1 hydrolyzes to STX). High temperatures greatly accelerate the reaction, and quantitative conversion of the sulfamate toxins to their carbamate counterparts can be achieved in 5 min at 100°C at a pH of 2 or less (Hall et al., 1980; Hall, 1991). Exposure of the toxins to elevated pH (>about 7) likewise has a degradative effect and may be due to the oxidative reaction. This alkaline reaction completely destroys their characteristics (Schantz et al., 1961).

Ionic properties. Owing to the presence of several charge-bearing functional groups on the PSP toxin molecules, a variety of net charges are possible for the various toxins. By controlling the pH, it is possible to drastically alter the separation characteristics of the various toxins in ion-exchange schemes and conduct quantitative separation chemistries that otherwise would not be possible.

Chemical properties and toxicity. The specific toxicity of the various saxitoxins is reported in Fig. 20.

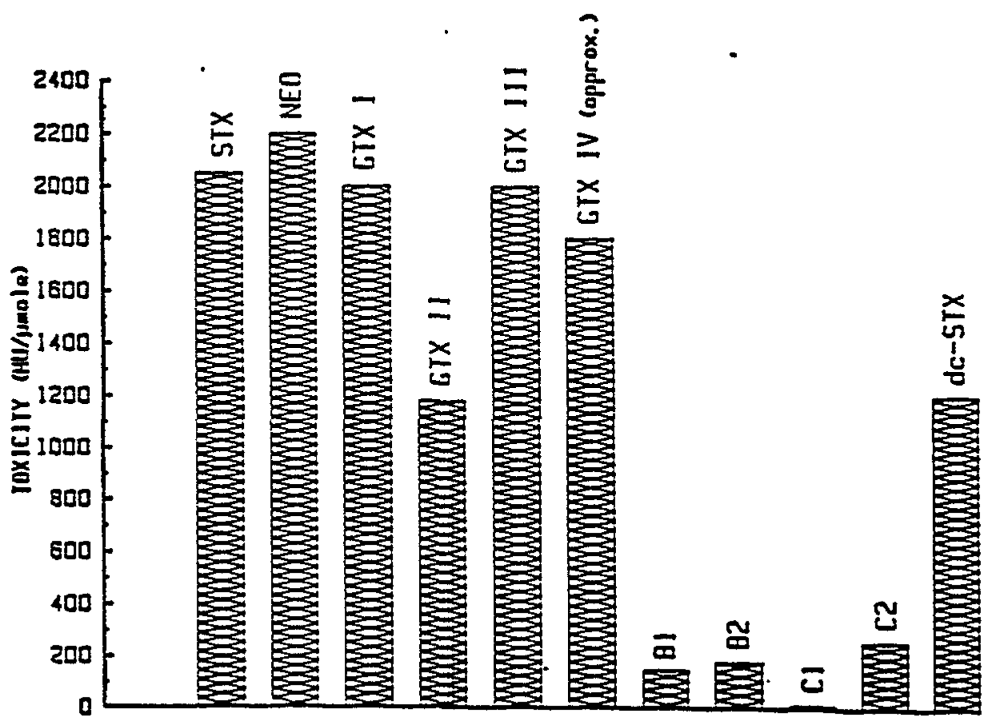


Fig. 20 Specific toxicity of the various saxitoxins. One mouse unit (MU) is the quantity of toxin needed to kill a 20-g mouse in 15 min.

7.2.1.3 PSP occurrence in the world

PSP was recorded in Canada as long ago as 1793 and over the past 20 years has become common throughout the world. Between 1969 and 1983, 905 cases were documented, with 24 deaths. PSP is now being reported much more widely. In 1983, for example, PSP was recorded for the first time in the Philippines. It resulted in 21 deaths along with 300 cases of illness, and a ban extending for 18 months imposed on the harvesting and sale of shellfish. Countries with a long history of PSP are Canada, USA and several states bordering the North Sea. In Europe the first PSP-induced fatal cases in humans reported in the medical literature occurred in 1855 in Germany (Salkowski, 1885). In the Atlantic coast of Portugal, Spain, England, Norway and Faroe Island cases of PSP have been described since 1960.

Shellfish toxins associated with PSP have been demonstrated also annually since May 1968 when 78 people were affected after consuming mussels from the north-east coast of England (Ayres and Cullum, 1978). In October and November 1976, an epidemic of PSP was recorded in Spain (63 cases), France (33 cases), Italy (38 cases), Switzerland (23 cases) and Germany (19 cases) (Viviani et al., 1977, 1978; Lüthy, 1979). This incident in western Europe was caused by mussels (*Mytilus edulis*) originating from Vigo and Pontevedra (Atlantic coast of Spain) (Lüthy, 1979). During the last two decades PSP has been observed in temperate and tropical areas throughout the world, with increasing frequency. It is not clear whether this is a true increase, or it results from improvement in surveillance, detection and reporting systems.

The introduction of toxic dinoflagellates into all seas of the world can be made through their cysts in the ballast water of vessels collected during a red tide. Episodes of PSP caused by red tides attributed to hypertrophication of terrestrial origin and to marine aquaculture in the last ten years are reported. So, for example, the population of Shatin and Tai Po in Honk Kong's coast has increased from 70,000 in 1973 to 600,000 in 1988, and an ultimate population by 1990 of more than 1 million is anticipated (Morton, 1989). The surface waters of the Tolo Harbour system, polluted by these cities and from agricultural waters have experienced a progressive increase in phytoplankton standing crop and in the incidences of red tides. The occurrence of red tides as well as fish kills due to red tides, algal blooms and oxygen depletions in Tolo Harbour have shown a progressive increase since 1979, and have become regular phenomena in recent years (Morton, 1989).

Paralytic shellfish poisoning (PSP) from *Protogonyaulax* toxicity levels in shellfish collected from Tolo Harbour have, on average, tripled from 1984 to 1987 (Morton, 1989). The intensification of aquaculture has also influenced the quality of the water leading to an increase of toxic phytoplanktonic blooms and of the appearance of cases of PSP (Mortensen, 1985; Eng et al., 1989; Phillips and Tanabe, 1989). This has occurred both in the coastal waters of the Atlantic and in countries of the Far East. In the Faroe Islands in 1984, the first proof was found that a relationship exists between aquaculture pollution and red tides caused by *Gonyaulax excavata*, which results in massive fish kills and cases of PSP in man after having eaten mussels from the same area (Mortensen, 1985). Similar cases have occurred in the Far East (Eng et al., 1989; Phillips and Tanabe, 1989). Management measures to mitigate deteriorating coastal water quality and the adverse environmental impacts of aquaculture development are now required as a matter of urgency.

7.2.1.4 PSP in the Mediterranean sea

In 1989-1990 there are the first reports of PSP in shellfish in north-western Mediterranean. Routine toxicity testing of *Venus verrucosa* of Andalucian origin revealed rising levels of PSP toxins during January 1989. Toxin levels in the clam *Cytherea chione* reached 200 g equiv. saxitoxin/100 g meat in February and collecting of shellfish was forbidden. Plankton samples taken along the coast between Malaga and Bahia de Algeciras just north of Gibraltar (the northern shore of the Alboran sea) an area subject to continuous inflow of Atlantic waters, revealed *Gymnodinium catenatum* in concentrations up to 3000 cells L^{-1} in mid February. No other species were found (Bravo et al., 1990). A bloom of *Alexandrium minutum*, reaching concentrations up to 28 x 10^6 cells L^{-1}, was observed in the harbour of S. Carles de la Rapita (northwestern Mediterranean) on 4th May 1989. During the following days, PSP toxicity was detected in mussels exposed to harbour waters and in mussels from the neighbouring bay of Els Alfacs, where extensive cultures of bivalves are located. In El Fangar, the other by of the delta, *A. minutum* was recorded in lower concentrations and no toxicity was detected in mussels. Shellfish extraction was stopped in the delta region and no human illnesses occurred (Delgado et al., 1990).

7.2.1.5 PSP compromised seafoods

Saxitoxin and related toxins which cause PSP usually have little effect on shellfish but are potent neurotoxins to vertebrates, including man, causing respiratory paralysis and death by asphyxia. DSP are a group of toxins produced by certain species of dinoflagellates, present in phytoplankton or in resting cysts. The toxins are taken up by predators feeding on plankton, such as bivalve mollusc, but also as fish plankton feed. Human exposure is brought principally about by consumption of PSP-containing shellfish which accumulate the toxins. The highest concentrations of PSP have been found in these digestive organs, but PSP is also present in other soft tissues. Since the sulfamate toxin is far less potent than its corresponding carbamate-sulfgroup it is easy to convert sulfamate to carbamate, the sulfamate toxins, when present in bivalves constitute a reservoir of latent or cryptic toxicity (Hall and Reichardt, 1984).

7.2.1.6 PSP depuration of live stock of bivalve molluscs and of fish plankton feed

Owing to the importance of detoxification of toxic live shellfish, the effects of ozonation, thermal shock, cation exchange and chlorination have been studied on the biological process of detoxification (Viviani, 1981). Ozonation appears to be the most viable procedure to remove low levels of the toxins from soft-shell clams (Blogoslawski and Neve, 1979), but is ineffective when they have retained the toxins for long periods (White et al., 1985). Several observations and studies in the past suggest that industrial processing (canning) may be a way of utilizing contaminated shellfish resulting in a pronounced decrease in PSP concentration (Viviani, 1981). In seafood a particular problem concerns finfish. Since finfish, unlike shellfish, are unable to accumulate the toxins in their flesh, there would seem to be no problem in terms of the suitability of fish plankton feed for human consumption, except possibly in instances where whole fish are consumed without processing (White, 1984).

7.2.1.7 Methods of analysis for PSP

The most commonly employed methods is the mouse bioassay. All PSP components are measured by this procedure (Viviani, 1981; WHO, 1984). The biological analysis is based on the dose of PSP (expressed as the equivalent amount of saxitoxin), that provokes a fixed death time in mice (from 1 to 60 minutes) injected intraperitoneally with an acid-soluble extract of bivalve molluscs (Helrich, 1990; Hall, 1991). The mouse bioassay will be banned in Europe in the comins years due to the public outcry over the use of animal in testing. Identification of numerous saxitoxin derivatives during the last two decades has led to consider the mouse bioassay for PSP detection a not entirely satisfactory assay for potentially contaminated food sources in various PSP toxins. The development of assay procedures alternative to the in vivo animal bioassay has gained increasing support (Shimizu and Ragelis, 1979). An improved high pressure liquid chromatographic procedure (HPLC) for the PSP toxins has been developed by Sullivan and Wekell (Sullivan and Wekell, 1984).

At present a radioimmunoassay (RIA) and indirect enzyme-linked immunoabsorbent assay (ELISA) were developed only for the detection of saxitoxin but not for all PSP toxins (Carlson et al., 1984; Chu and Fan, 1985). It appears that a relatively less expensive analytical method is needed, simple to use and which can be employed in the field, comparable to or better than the mouse bioassay in sensitivity and accuracy, and have the ability to cross react among all the toxins likely to be present in marine shellfish affected by PSP.

7.2.1.8 Human intoxication: clinical toxicology

PSP causes a widespread inhibition of impulse-generation in peripheral nerves and skeletal muscles, by blocking the sodium channel, which may result in respiratory paralysis leading to death. Saxitoxin is one of the most lethal non-protein toxins known for man (fatal dose 1-2 mg) and approaches botulinum toxin in its lethal effects (Viviani, 1981; Steidinger and Baden, 1984; WHO, 1984). The symptoms of PSP usually appear in man in 30 min following the consumption of toxic bivalve molluscs: paraesthesia affecting the mouth, lips, tongue, and finger tips, profound muscular asthenia, inability to maintain an upright posture, ataxic gait, loss of balance. Other symptoms rapidly develop, including lightheadedness, disequilibrium, incoordination, weakness, hyperreflexia, incoherence, dysarthria, sialorrhea, dysphagia, thirst, diarrhea, abdominal pain, nausea, vomiting, nystagmus, dysmetria, headache, diaphoresis, lose of vision, a sensation of loose teeth, chest pain, and tachycardia (Auerbach, 1988). The gastro-intestinal symptoms in PSP due to *Alexandrium*, such as nausea, vomiting, diarrhea and abdominal pain are less common or do not appear at all. Unless there is a period of anoxia, the victim will often remain awake and alert, although paralyzed. Up to 25% of victims expire from unsupported respiratory arrest within the first 12 hr. In milder cases, alcohol ingestion appears to increase toxicity (Acres and Gray, 1978). In the most severe forms, the clinical setting is dominated by a progressive muscular paralysis beginning from the legs, and this paralysis prevents standing and results in death due to respiratory paralysis. Consciousness is rarely compromised. In lethal cases the evolution is very fast and the death occurs within 8 hr on an average, due to respiratory or cardiocirculatory deficiencies. The prognosis is favourable in cases of survival in the first 12-24 hr (Auerbach, 1988). The mortality index is equal to about 8-10% in the paralytic syndrome due to molluscs (Bagnis et al., 1970; WHO, 1984).

7.2.1.9 Mechanism of action

Ion channels in plasma membranes are primary targets of marine toxins. These channels are important regulators of a cell's physiology, and many of the pathophysiological effects of toxins arise from actions on ion channels. The voltage-gated Na^+ channel, as it exists in excitable cells, as an example of a receptor with multiple binding sites for different types of toxins. Occluders, activators and stabilizers are considered as modes for toxins binding to and acting directly on the ion channel. Saxitoxin acts by inhibiting the temporary permeability to Na^+ ions, and this has made a considerable contribution to the hypothesis that the Na^+ and K^+ ions move independently through the cell membrane by separate channels and not by a single common channel (Steidinger and Baden, 1984). At the molecular level all PSP toxins are water-soluble non depolarizing toxins (Catterall, 1980). The saxitoxin as guanidinium toxin is regarded a "blocking" agent that reduces the number of conducting Na^+ channels by occupying some site near the outer opening (Richie and Rogart, 1977). Saxitoxin binds to specific receptors in the nerve membrane in a 1:1 stoichiometry with high affinity (K_D = 2 nM) (Catterall, 1979). The potent inhibition of ion flux is not due to a plugging phenomenon but is rather the result of a lid on the sodium channel, occupying a flat arrangement bound to the anionic surface of the membrane (Kao and Walker, 1982) (Fig. 21). The recent experiments suggest that this action is not independent from the presence of other toxins (Strichartz et al., 1987). So channels modified by lipophylic toxins has to be altered in respect to STX also.

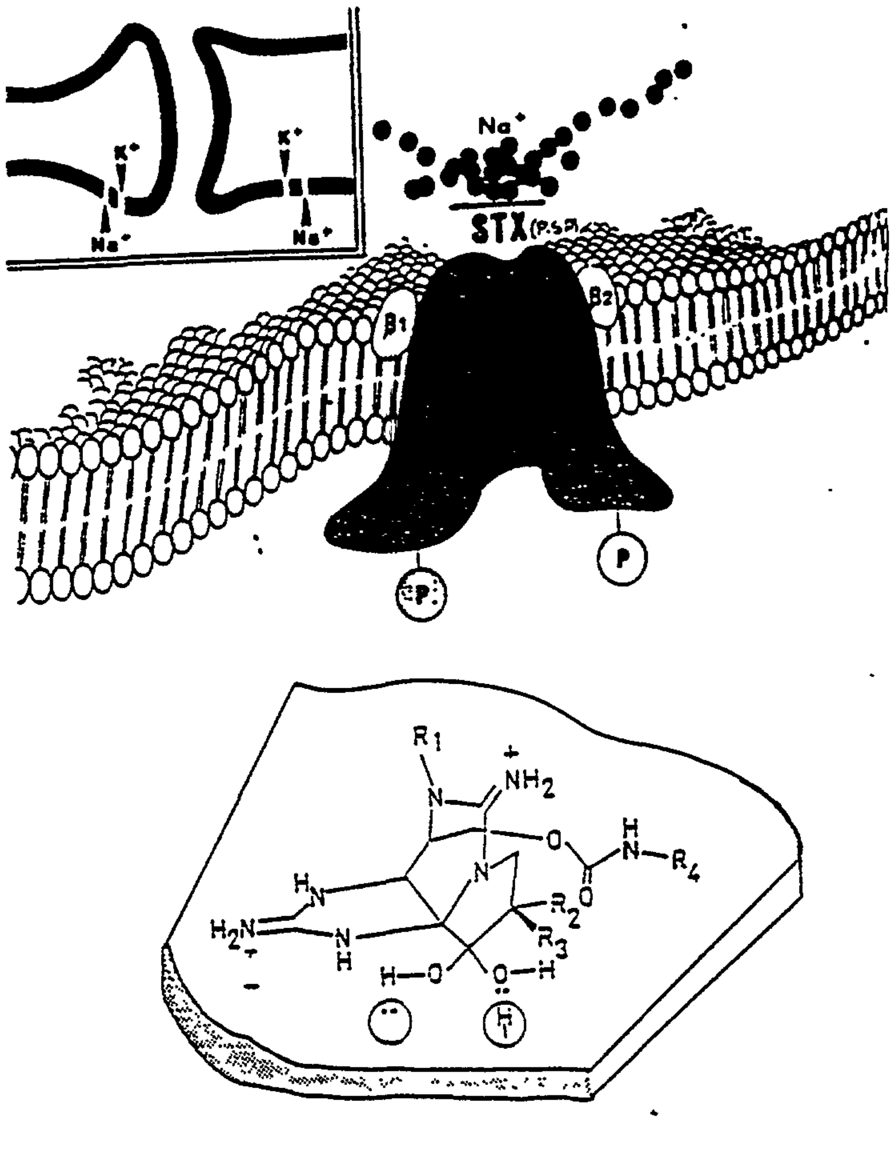


Fig. 21 The "lid" hypothesis of saxitoxin action on sodium channels: a saxitoxin-receptor model (Shimizu, 1987)

7.2.1.10 Therapeutical notes

In cases where humans eat saxitoxin-contaminated shellfish, symptoms appear within minutes of ingestion, while death can occur anywhere from 1-12 hr later (Kao, 1966; Halstead, 1978). This should give sufficient time to intervene with an injection of antiserum. Studies of Davio (1985) examined antiserum neutralization of saxitoxin in greater detail. The effect of antiserum injected i.v. must be essentially immediate, since saxitoxin injected s.c. normally kills mice within 5-10 min. While the data demonstrate that antiserum A can counteract saxitoxin in vivo, this particular antiserum may not be effective against the many other "saxitoxin-like" paralytic shellfish poisons produced by *Alexandrium* dinoflagellates and associated with toxic shellfish. Thus, a true antidote for the paralytic shellfish poisons must have a broader reactivity.

Current research is directed at the development of polyclonal and monoclonal antibodies directed against saxitoxin and neosaxitoxin, for the purposes of diagnosis and therapy. Since up till now effective antidotes against all biotoxins are not available, therapy remains essentially symptomatic (Southcott, 1977; Auerbach, 1988). Therapy is supportive and based upon symptoms. If the victim comes to medical attention within the first few hours after ingestion, the stomach should be emptied with gastric lavage and then irrigated with 1 liter of a solution of 2% sodium bicarbonate. The administration of activated charcoal (50-100 g) and a cathartic (sorbitol, 30-50 g) makes empirical sense but is not documented as effective in the literature. Some authors advise against the administration of magnesium-containing solutions, such as certain cathartics, with the explanation that hypermagnesemia can contribute to suppression of nerve conduction. The use of neostigmine to counteract the curarelike effects is empirical (Auerbach, 1988). The greatest danger is respiratory paralysis.
The victim should be closely observed in the hospital for at least 24 hr for respiratory distress. Supplemental oxygen should be administered and mechanical assistance applied if appropriate. With prompt recognition of respiratory failure, endotracheal intubation and mechanical ventilation will prevent anoxic myocardial and brain injury (Auerbach, 1988).

Researches on antidotes for PSP are directed to natural active substances. In this respect during a red tide episode caused by *Pyrodinium bahamense* var. *compressa* in Western Samar, Philippines in 1983, those who were taken ill after ingesting the green mussel, *Perna viridis*, were reported to hate taken coconut milk (gata, Pilipino) with brown sugar or unpurified sugar lumps (*tagapulot*, Pilipino) as a temporary palliative pending medical attention. Many victims felt relief after this drink. It has been demonstrated in mice that substances active in detoxification of *Pyrodinium* toxins are present in coconut milk and in brown sugar (Gacutan, 1986).

7.2.1.11 Tolerance levels and remarks on safety

The Task Group of the World Health Organization recognized serious difficulties in establishing the dose associated with the appearance of signs and symptoms and death (WHO, 1984) with reference to bioassay of contaminated food. The human dose resulting in death ranges from 500 g and up to 12,400 µg. The USA and Canada more than 30 years ago have adopted the tolerance level of 80 g PSP/100 g (exercised on fresh shellfish at production site). In Europe most countries have adapted the 80 µg/100 g tolerance; however, within the European Community (EC), three countries, the Federal Republic of Germany, Italy (Ministero della Sanità, 1978) and The Netherlands have established a lower tolerance of 40 µg/100 g. During the PSP outbreaks in Italy in 1976, caused by imported mussels from the Atlantic coast of Spain, the lowest level causing symptoms was 566 µg/100 g (Viviani et al., 1977; 1978).

The most recent PSP outbreak in Europe, published in the scientific literature took place in Norway in 1981. Eight out of 10 persons, who consumed mussels containing about 1600 µg, total PSP/100 g became affected. Two persons developed no symptoms of intoxication at all, having ingested an estimated total dose of 320 µg (Langeland et al., 1984).

Since the tolerance employed in USA and Canada (80 µg/100 g) is more than 10 times lower than the lowest level that has caused intoxications, as observed during the most recent PSP outbreak in Europe (Viviani et al., 1977, 1978; Langeland et al., 1984) in order to harmonize the PSP tolerance in the EC, it is recommended that EC adapt a tolerance of 80 µg PSP/100 g for shellfish. In relation to the common methods to be used, in addition to the bioassay method, the fluorometric HPLC procedure (Sullivan and Wekell, 1984) has been proposed, but the use of this procedure requires the availability of reference material for at least six PSP components (Krogh, 1987), and that kind of reference material is not yet commercially available. In addition, studies should be undertaken to elucidate PSP distribution in shellfish under several environmental conditions such as blooms of PSP-producing dinoflagellates and absence of dinoflagellates but presence of resting cysts.

7.2.2 Diarrhetic Shellfish Poisoning (DSP)

Diarrhetic shellfish poisoning (DSP) has become known only in recent years as a shellfish poisoning distinctly different from the paralytic shellfish poisoning (PSP) and neurotoxic shellfish poisoning (NSP) in both symptomatology and etiology (Yasumoto et al., 1978). The clinical symptomatology is of gastrointestinal type, consisting of nausea, vomiting and diarrhea, and unlike PSP no fatal cases have been reported (Yasumoto et al., 1978; Krogh, 1989). The first studies on DSP were carried out in Japan and continued in Western Europe, in Italy and Canada.

7.2.2.1 DSP producing or potentially toxic dinoflagellates

In the sea world. In Japan *Dinophysis fortii* has been incriminated as the organism producing DSP toxins (Yasumoto et al., 1980). On European Atlantic coasts other dinoflagellate species are involved in DSP intoxications: *Dinophysis acuminata* in Spain (Campos et al., 1982); *D. acuminata, D. sacculus, Prorocentrum lima* in France (Berthomé et al., 1986); *D. acuminata, Prorocentrum redfieldii, P. micans* in The Netherlands (Kat, 1979); *D. acuminata, D. norvegica, P. micans* in Scandinavia (Krogh et al., 1985). Until now, eight *Dinophysis sp.* have been shown to be toxic (DSP): *D. acuminata, D. acuta, D. fortii, D. mitra, D. norvegica, D. rotundata, D. sacculus* and *D. tripos* (Yasumoto, 1990; Sampayo et al., 1990). Also other species of *Dinophysis* and *Prorocentrum* should be regarded as shellfish contaminant that may have caused diarrhetic poisoning.

In the Mediterranean sea. Various species of the genera *Dinophysis* and *Prorocentrum* are present in the Mediterranean sea. Many *Dinophysis* species are present in the Italian seas, but never form red tides (Rampi, 1951; Solazzi and Andreoli, 1971; Innamorati et al., 1989a-b; Honsell, 1990). Some *Dinophysis* species have always been encountered in the Adriatic phytoplankton (Schroeder, 1911; Jørgensen, 1923; Ercegovic, 1936; Revelante et al., 1984). During the DSP intoxication which occurred in 1989 (Boni et al., 1992), the presence was noticed of *D. fortii, D. tripos, D. caudata* and another species similar to *D. acuminata*. In phytoplankton of the Northern and Central Adriatic sea were also observed *D. rotundata* Clap. et Lachm., *D. acuta* Ehrb., *D. diegensis* Kof. (Boni et al., 1990, 1991; Ammazzalorso et al., 1991; Della Loggia et al., 1991). Many *Dinophysis* species are also present in the Tyrrhenian sea but DSP was never detected in the local shellfish. However it is not known whether the species *D. circumsuta* (Karsten) Balech, *D. infundibulus* Sch., *D. umbosa* Sch. and some others (Innamorati et al., 1989 a-b) found in the Tyrrhenian sea are toxic or not.

In the genus *Prorocentrum, P. lima* (Ehr) Dodge is considered causative agent of DSP and also of ciguatera. This dinoflagellate has been found in the Tyrrhenian sea since

1978 (Innamorati et al., 1989 a-b), and recently in the Adriatic sea, Grado-Marano Lagoon (Moro and Andreoli, 1991) and Gulf of Trieste (Honsell, 1992).

7.2.2.2 Chemistry of the components of DSP toxins

DSP toxins biosynthesis occurs in various species of *Dinophysis* (Yasumoto et al., 1980; Kat, 1983; Undertal et al., 1985; Lassus et al., 1988; Kat, 1989; Marcaillon-Le Baut and Masselin, 1990) and in *Prorocentrum lima* (Murakami et al., 1982). The chemically defined toxins, isolated from these dinoflagellates and mussels and in other bivalve shellfish fall into three structural classes, all being lipophilic compounds. The first (acidic toxins) consisting of okadaic acid, dinophysis toxin 1 (DTX-1) and DTX-3, the second (neutral toxins) of pectenotoxins (PTX) 1, 2, 3, 6 and the third of yessotoxin (YTX) and 45-hydroxy yessotoxin (45-OH YTX) (Murakami et al., 1982; Murata et al., 1982, 1986; Yasumoto et al., 1984; Kumagai et al., 1986; Yasumoto, 1990; Yasumoto and Murata, 1990) (Fig. 22). Confirmation of toxigenicity of suspected species of *Dinophysis* was possible using high sensitivity of fluorimetric HPLC determination in small number of *Dinophysis* cells purified under a microscope by capillary manipulation (Yasumoto, 1990). *Dinophysis fortii, D. acuminata, D. acuta, D. norvegica, D. tripos, D. rotundata* were confirmed to produce okadaic acid or DTX-1. In addition *Dinophysis fortii* produced DTX-2, but not other DTXs (Yasumoto and Murata, 1990).

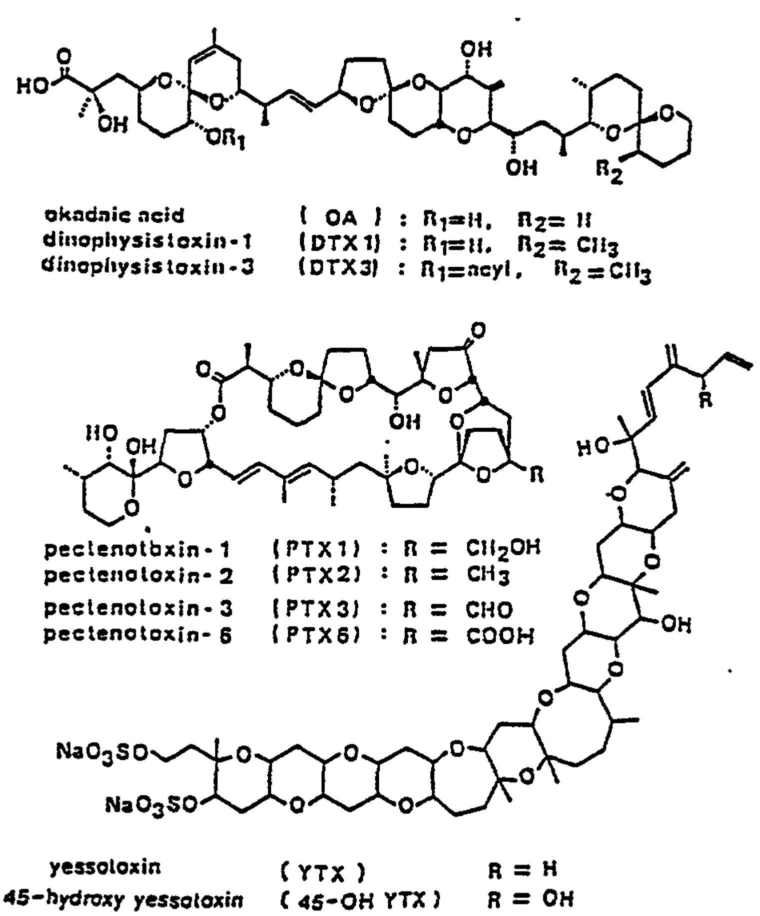


Fig. 22 Diarrhetic shellfish toxins (DSP)

The chemical properties and toxicity of DSP compounds are reported in Table 20. Only the acidic components (okadaic acid, DTX-1, DTX-3) have been shown to cause diarrhea in experimental animal studies. The remaining four components have not been reported to have a diarrheagenic effect. Intraperitoneally administered in mice PTX-1 causes liver damage (Murata et al., 1987).

Table 20

Toxicity and chemical properties of diarrhetic shellfish toxins

Toxin	Mw(m/z)	Molecular formula	UV(nm)	20 á D	Toxicity[1]	Pathological effect
DA	804	$C_{44}H_{68}O_{13}$	end[2] abs.	+23.0 (c.0.34,$CHCl_3$	200	diarrheagenic
DTX1	818	$C_{45}H_{70}O_{13}$	end abs.	+28.0 (c.0.46,$CHCl_3$	160	diarrheagenic
DTX3	-	-	end abs.	-	ca.500	diarrheagenic
PTX1	874	$C_{47}H_{70}O_{15}$	236	+17.1 (c.0.40,MeOH)	250	hepatotoxic
PTX2	858	$C_{47}H_{70}O_{14}$	235	+16.2 (c.0.05,MeOH)	230	hepatotoxic[3]
PTX3	872	$C_{47}H_{68}O_{15}$	235	+2.2 (c.0.19,MeOH)	350	hepatotoxic[3]
PTX4	874	$C_{47}H_{70}O_{15}$	235	+2.1 (c.0.19,MeOH)	770	hepatotoxic[3]
PTX6	888	$C_{47}H_{68}O_{16}$	235	+37.1 (c.1.49,$CHCl_3$	500	unknown
YTX	1186	$C_{55}H_{80}O_{21}S_2Na_2$	230	+3.0 (c.0.45,MeOH)	100	unknown
45-OH YTX	1202	$C_{55}H_{80}O_{22}S_2Na_2$	-	-	ca.100	unknown

[1] Intraperitoneal injection to mice
[2] No absorption maxima above 220 nm
[3] Presumed from the toxicity of PTX1

7.2.2.3 DSP occurrence worldwide

Not all DSP outbreaks are accompanied by macroscopic blooms of *Dinophysis sp.* or *Prorocentrum sp.* The first studies on DSP were carried out in Japan and continued in Europe in which diagnostic investigations were made for differentiate gastrointestinal disorders caused by some foodborne bacteria and virus from DSP. In Japan more than 1300 cases of DSP intoxication have been reported in the period 1976-1982 (Yasumoto et al., 1984). In Spain approximately 500 cases of gastrointestinal disorder was encountered in September 1981 (Fraga et al., 1984). Bacteria were ruled out as a cause, but no attempts were made to detect DSP components in the shellfish, as no methodology was available at that time. In France outbreaks of DSP intoxication amounted to about 2000 cases in both 1984 and 1986, with only 10 cases in 1985 (Lucas, 1985; Belin and Berthomé, 1988). Similar disease descriptions have been reported from outbreaks of DSP-intoxication in The Netherlands (Kat, 1983). In Scandinavia 300-400 cases were encountered in the DSP outbreak in the fall of 1984 (Underdal et al., 1985). In August 1990, at least 16 people developed symptoms of DSP shortly after eating cultured mussels from the Mahone Bay area in Nova Scotia (Eastern Canada) (Quilliam et al., 1991).

7.2.2.4 DSP occurrence in the Mediterranean sea

In June 1989 the presence of *Dinophysis fortii* cells in hepatopancreas of mussels and of lipid soluble toxin of DSP type in mussel tissue collected in the coastal water of the Emilia-Romagna region (Boni et al., 1990; 1992) allowed to prove that the cause of certain cases of diarrhea in consumers of molluscs was not due to bacteria or virus but to biointoxication by DSP. This phenomenon, brought to light by the Research Centre of Marine Biological Resources of Cesenatico (University of Bologna, Italy), has subsequently extended over the coastal areas of Marche, Abruzzo, Veneto and Friuli-Venezia Giulia. The existence of the enterotoxin in seafood was initially revealed by the McFarren et al. (1965) method (biological test for the research into fat-soluble algal biotoxins, according to the previsions of Italian Law) (Ministero della Sanità, 1978). In the second stage the Yasumoto et al. method (1984) was used. Although coloured water and algae blooms have regularly been seen since 1975 in the areas of the Adriatic Sea off Emilia-Romagna (Viviani, 1981, 1983, 1988; Viviani et al., 1985), the appearance of molluscs toxic because of DSP in 1989 and 1990 was not preceded or accompanied by evident phenomena of this kind (Boni et al., 1990, 1992; Viviani et al., 1990).

The toxicity of mussels was correlated, in 1989 and 1990, with the presence of *D. fortii, D. sacculus, D. acuta, D. caudata, D. rotundata, D. tripos* and species similar to *D. acuminata* in quantities of 2,000 cells/litre and even only 40 cells/litre (Viviani et al., 1990; Boni et al., 1992). These species of dinoflagellates had not previously given rise to mono-specific blooms, but their presence had been detected by the analysis of the phytoplankton carried out by the Cesenatico Centre since 1976. From the data on the presence of phytoplankton in the seawater off the coast of Emilia-Romagna (Table 21) and in the hepatopancreas of mussels (*Mytilus galloprovincialis*) it can be seen that there are many species of *Dinophysis* and that the toxic material is related to the following species: *D. fortii* is dominant in June and July, *D. sacculus* in August and September, *D. fortii* and *D. caudata* in October and November, *D. tripos* in December.

Table 21

Monthly distribution mean (cell/dm^3) of *Dinophysis spp.* along the Emilia-Romagna coast during 1989 (Boni et al., 1990; Viviani et al., 1990)

Month	*D. acuminata*	*D. caudata*	*D. fortii*	*D. sacculus*	*D.sp.*	*D. tripos*
June	8	0	136	16	8	32
July	0	0	130	0	80	50
August	0	0	0	640	0	0
September	0	10	90	615	90	5
October	0	124	148	80	0	48
November	3	43	89	11	6	54
December	0	6	29	6	0	63

Also along French Mediterranean coast (Sete) in June 1989, during *Dinophysis spp.* blooms it has been reported the presence of DSP toxins in mussels (Lassus, 1991). In addition few cases of poisoning due to ingestion of mussels contaminated by *Dinophysis spp.* were encountered in the region situated outside the Etang the Thau (Thau Lagoon) (Leveau et al., 1989).

7.2.2.5 DSP compromised seafoods

Causative shellfish in Japan were the mussels *Mytilus edulis* and *M. coruscum*, the scallops *Patinopecten yessoensis* and *Chlamys nipponensis akazara,* and the short-necked clams *Tapes japonica* and *Gomphina melaegis*, while in European Atlantic coasts *M. edulis* and in Adriatic and French Mediterranean coast *M. galloprovincialis*. In Japan and in the Atlantic coast of Spain and France the infestation period ranges from April to September and the highest toxicity of shellfish is observed from May to August, though it may vary locally (Yasumoto et al., 1978, 1980, 1984; Campos et al., 1982; Berthomé et al., 1986). In Scandinavia, on the contrary, oysters in February and mussels in October have caused DSP (Edebo et al., 1988). According to the provisions of the Italian Laws on toxic shellfish (1978, 1990) the level of DSP biotoxins present in mussels from intensive farms and natural beds along the coasts of Emilia-Romagna made them unsuitable for sale for human consumption for a duration of 8 months (Viviani et al., 1990) for two consecutive periods, from June 1989 to January 1990 and from June 1990 to January 1991. Okadaic acid and DTX-1 have been found in Western European shellfish (Dahl and Yndestad, 1985), while YTX was detected in Norwegian blue mussels in addition to acidic components (Lee et al., 1987). PTXs have not been reported from European shellfish but few attempts have been made to detect these components because of lack of routine methodology for their detection (Krogh, 1989). In hepatopancreas of highly toxic mussels of Adriatic sea the presence of okadaic acid has been evidenced through ^{1}H NMR spectroscopy. In addition, structural elucidation of the components of two further toxic fractions is still in progress (Fattorusso et al., 1992).

The method of cooking did not alter toxicity of the causative shellfish but intoxication could be avoided if the digestive glands were eliminated beforehand (Yasumoto et al., 1978; 1990). Comparative analysis for DSP in various shellfish collected from the same area was conducted in Japan and the highest toxicity was found in the blue mussels, with less toxicity in scallops, and very little in oysters. The differences were noted between mussels cultivated at different depths, with concentrations differing by factors of two to three (Yasumoto et al., 1978; 1980). Also the first results obtained in the Adriatic sea (Boni et al., 1990; 1992) show that not all species of bivalve molluscs, living in the same habitat infested by the microalgae, manifest an analogous functional attitude towards the absorption and concentration of the enterotoxin in their tissues. In particular, although they were drawn out of the same habitat, while values of 4 MU (calculated according to IFREMER) were found in mussels, in *Tapes semidecussatus* the risk level was never passed, in *C. gallina, O. edulis* and *V. verrucosa* DSP was not detected.

7.2.2.6 DSP detoxification in bivalve molluscs

Two DSP depuration experiments have been undertaken in 1989: one in laboratory conditions and the other one in an oyster culture pond (Lassus, 1991). Two different sets of diarrheic toxins (DSP) contaminated mussels have been used, respectively at high (3 MU) and low (1 MU) initial toxic levels. These two sets were contaminated during two *Dinophysis spp.* blooms, which occurred in June 1989 respectively along French Mediterranean (Sete) and West-Brittany coasts (Douarnenex Bay). Depuration rates have been estimated simultaneously by mouse test and high pressure liquid chromatography (HPLC). For highly toxic mussels, results evidence a better depuration rate in the oyster culture pond with 0.5 MU g^{-1} in digestive glands after 20 days and 1.0 MU g^{-1} in laboratory conditions after 42 days. For lower initial toxic level (1 MU), time needed for depuration is of course shorter but similar disparity is observed between laboratory and pond experiments are assumed to be the causative factors for observed differences in depuration rates (Lassus, 1991).

Transplantation of mussels (*Mytilus edulis*) contaminated by okadaic acid (0A) from a more toxic environment in the northern part of the Swedish west-coast to a less toxic environment in the southern part showed a decrease in 0A content of 12 mg 0A/100 g mussel meat per day (Haamer et al., 1990). Transplantation of less toxic mussels from the south to the north did not show a rapid uptake of 0A. Toxic mussels from the north were reimmersed in two basins. One of them contained ordinary sea water, and the other one boiled baker's yeast was added. Decreases of 4-5 µg 0A/100 g mussel meat per day were observed. The 0A-data showed a more consistent behaviour when boiled yeast added. Without yeast, decreases alternated with increases.

7.2.2.7 Methods of analysis for DSP

The bioassay of all DSP components is based on the dose that provokes a fixed death time in mice injected intraperitoneally with a toxic residue extracted from shellfish with acetone (Yasumoto et al., 1984). The acetonic phase is evaporated and the residue resuspended in 4 ml of 1% Tween 60. Aliquots of 1 ml of this solution were injected i.p. into 18-20 Swiss albino mice. This procedure is the official method in Japan and in several other countries. In France toxicity is expressed differently than the official Japanese biological test. In Italy, the method established by the Ministerial Decree dated 1.9.1990 (Ministero della Sanità, 1990) is similar to the official method used in France (Marcaillon-Le Baut and Masselin, 1990). The mices were observed for 24 hr and positive tests consists in T < 5 hr. Other bioassay methods are: suckling mouse bioassay (Hamano et al., 1985), rat bioassay (Kat, 1983), Tetrahymena test (Shiraki et al., 1985).

The fluorometric determination of okadaic acid and DTX-1 has been developed using HPLC (Lee et al., 1987). The bioassay and the HPLC method have not been studied collaboratively, and no attempts have been made to study the scientific parameters, such as precision, sensitivity and specificity. The intercalibration procedure is not appliable since at present only okadaic acid and DTX-1 as reference material for the DSP components are commercially available. A "second stage" analysis is the identification of okadaic acid and other DSP toxins through ^{1}H NMR spectroscopy of fractions positive to official mouse lethality test, obtained by repeated chromatographic separation (Fattorusso et al., 1992). Although it is a more laborious test than fluorimetric HPLC assay (Lee et al., 1987), which has a similar scope, it has two important advantages: it does not need any standard sample of the toxins, which may be difficult to purchase for toxins other than okadaic acid and DTX-1 (Krogh, 1989); only literature NMR data are required. In addition this method is not limited to known toxins, but can potentially work with new ones, also allowing their structural elucidation, provided a large enough quantity of toxic material can be obtained.

7.2.2.8 Human intoxication: clinical toxicology

Frequency of signs and symptoms of DSP in patients were as follows: diarrhea (92%), nausea (80%), vomiting (79%), abdominal pain (53%), and chill (10%). The incubation period ranged from 30 min to several hours but seldom exceeded 12 hr. Around 70% of the patients developed symptoms within 4 hr. Suffering may last for 3 days in severe cases but leaves no after-effects (Yasumoto et al., 1978; Lucas, 1985). Thus no fatal case has ever been recorded. The minimum amount of DSP required to induce disease in adults has been estimated from analysis of left-over food to be 12 MU (Yasumoto et al., 1984). In Scandinavia mussels associated with the outbreak contained approximatively. 17 MU per 100 g (Underdal et al., 1985). In an inventory of phytoplankton perturbation along the French coast in 1986 the higher DSP levels in shellfish were 10.6 MU/100 g (Belin and Berthomé, 1988).

7.2.2.9 Mechanism of action

Okadaic acid, being hydrophobic (Shibata et al., 1982), can enter cells and operate on particulate as well as cytosolic fraction of various mouse tissues (Suganuma et al., 1989). It is a very potent inhibitor of protein phosphatase 1 (PP1) and protein phosphatase 2A (PP2A), two of the four major protein phosphatases in the cytosol of mammalian cells that dephosphorylate serine and threonine residues (Cohen, 1989). Of the other two major protein phosphatases, the Ca^{2+}/calmodulin-dependent protein phosphatase 2B (PP2B) is far less sensitive, while the Mg^{2+}-dependent protein phosphatase 2C (PP2C) is unaffected (Bialojan et al., 1988). Okadaic acid probably causes diarrhoes by stimulating the phosphorylation that controls sodium secretion by intestinal cells as in the disease cholera caused by a toxin secreted by *Vibrio cholerae*, but with another mechanism. One of the subunits of cholera toxin can permanently activate the Gs protein, leading to continuous adenylate cyclase activity (Johnson, 1982). The resulting increase in cAMP activates cAMP-dependent protein kinase, which then phosphorylates one or more proteins that control sodium secretion by intestinal cells. Since cAMP or Ca^{2+}/calmodulin dependent protein kinases or protein kinase C (Terao et al., 1986; Takai et al., 1987; Haystead et al., 1989) are unaffected by okadaic acid, the inhibition of PP1 and PP2 is probably responsible for phosphorylate control of ion channels.

Recent data indicate that okadaic acid may function, not only as tumor promoter, but is also capable for the reversing cell transformation by some oncogenes. It was found, employing the two-stage carcinogenesis model, that okadaic acid (Suganuma et al., 1988) and DTX-1 (Fujiki et al., 1988) acted as promoters of skin tumors in the mouse, using dimethyl-benz(a)anthracene (DMBA) as tumor initiator. Whether this implies a risk for human health, it remains to be clarified (Hall, 1991). In addition NIH3T3 cells transformed by either the raf or ret-II oncogenes partially revert to the normal phenotype after incubation for two days with 10 nM okadaic acid (Sakai et al., 1989).

7.2.2.10 Therapeutical notes

Fluid secretion (diarrhea) occurs in patients with DSP. The secretory state is a result of okadaic acid and DTX1 biotoxins which probably stimulate the phosphorylation that controls sodium secretion by intestinal cells.

The luminal plasma membrane contains a transport system that facilitates a tightly coupled movement of Na^+ and D-glucose (or structurally similar sugars) which is not regulated by protein kinase. Modern oral treatment of cholera takes advantage of the presence of Na^+-glucose cotransport in the intestine. In this case, the presence of glucose allows uptake of Na^+ to replenish body NaCl. Composition of solution for oral treatment of cholera patients is glucose 110 mM, Na^+ 99 mM, Cl^- 74 mM, HCO_3 39 mM, and K^+ 4 mM (Carpenter, 1980). A similar oral treatment in DSP could be experimented.

7.2.2.11 Tolerance levels and remarks on safety

The established tolerances vary greatly from country to country since no evaluation of DSP tolerances has yet been made by international organizations, like the World Health

Organization (Krogh, 1989). In the interim before issue of these definitions a surveillance plan for DSP has been introduced in several European countries and also in Italy comprising the analysis of seawater of phytoplankton and shellfish for detection of *Dinophysis sp.* in digestive tract as well as for toxin analysis (Krogh, 1989; Ministero della Sanità, 1990 a-c). A number of EC countries have established tolerances for DSP, applicable for domestic shellfish production sites as well as for importation of shellfish. In Denmark, The Netherlands and Spain the mouse bioassay method established "no detectable amount". In France the mouse bioassay established a tolerance of 0.044 MU/g digestive glands (Marcaillon-Le Baut and Masselin, 1990), while in Japan with another principle for MU calculation the level is 5 MU/100 g soft tissue and this so in Norway (Yasumoto et al., 1984; Underdal, 1988). Sweden is the only country in Europe which monitors shellfish for DSP by the HPLC procedure (Lee et al., 1987) and is maintaining a tolerance level of 60 µg/100 g soft tissue (as okadaic acid and DTX-1). In Italy DSP toxins were determined using the method established by the Ministerial Decree dated 1.9.90 (Ministero della Sanità, 1990) similar to the official method used in France. The mice were observed for 24 hr and positive test consists in T<5 hr. As no case of DSP contamination is known without the presence of at least one of the acidic components, an EEC tolerance level for DSP is suggested to include the acidic components, such as okadaic acid and DTX-1, which can be monitored by chemical procedures such as HPLC (Report CEC, 1989). JUPAC is presently organizing a collaborative study of the HPLC procedure by Lee et al. (1987). However, it is essential that the EEC makes reference material of DSP components, in addition to the acidic components (okadaic acid, DTX-1) available to member countries.

The data, which show the presence of okadaic acid in hepatopancreas of *Mytilus galloprovincialis* of Adriatic sea, but also of two other biotoxins with the separation method used (Fattorusso et al., 1992), provide the following conclusions as far as the public health and economic aspects are concerned Mediterranean sea:

- The validity of the biotoxicological tests carried out so far under Italian law is demonstrated by the presence of okadaic acid, one of the most toxic components of DSP.

- Research has indicated the presence of two more toxins and it is not yet possible to say whether these are compounds in the okadaic acid group or pectenotoxins or yessotoxins or others.

- So far, mouse bioassay is the only method which can detect all DSP components, both the group of acid derivates (okadaic acid, DTX-1 and DTX-2) which are diarrhogenic components (Hamano et al., 1985), and pectenotoxins and yessotoxins, which cause mouse mortality by other mechanisms not connected to gastrointestinal symptoms (Terao et al., 1986; Murata et al., 1987).

7.3 Marine algae potentially toxic for seafood and for respiratory and cutaneous symptoms of poisoning in the Mediterranean sea

7.3.1 *Prorocentrum minimum* and Venerupin Shellfish Poisoning

Venerupin poisoning is a non paralytic human biointoxication different from the DSP. Venerupin shellfish poisoning is caused by the Japanese lake-harvested oyster (*Crassostrea gigas*) and clam (*Venerupis semidecussata* or *Tapes semidecussata*), which feed on toxic dinoflagellates of the genus *Prorocentrum*.

7.3.1.1 Organisms producing toxins

Prorocentrum minimum var. *mariae lebouriae* and var. *triangulatum*, which co-occur in blooms (Okaichi and Imatomi, 1979) have been incriminated in Japan in venerupin poisoning. *Prorocentrum minimum* Schiller, probably responsible for the shellfish poisoning in Norwegian coasts, is a phytoplanktonic species so common that, if it is the source of the highly toxic "venerupin", toxin must be only in rare strains (Tangen, 1983).

7.3.1.2 Research on the components of venerupin poisoning

The toxic principles were found in the digestive glands (hepatopancreas, liver or dark gland) of the bivalves (Akiba and Hattori, 1949). Toxicity of 75% methanol extracts of cultured *Prorocentrum minimum* var. *mariae-lebouriae*, which is supposed to produce venerupin poisoning (Okaichi and Imatomi, 1979) was determined using mice as test animals. The chemical nature of the toxins is not established. The toxin was found to be soluble in water, methanol, acetone and acetic acid. It was insoluble in benzene, ether and absolute alcohol.

7.3.1.3 *Prorocentrum minimum* and venerupin occurrence worldwide

Venerupin poisoning was first reported in Nagai, Japan, in 1889, following the ingestion of the oyster *Crassostrea gigas*. Of the 81 persons poisoned, 51 died (Halstead, 1965). A second outbreak occurred in 1941, when of 6 patients, 5 died, and from 1942 to 1950 there were 455 additional cases involving the eating of oysters and the short-necked clam *Tapes japonica* (Nakajima, 1965). Several hundred cases have been reported in the area of Lake Hamana with more than 100 deaths (Nakajima, 1968). So also in Norway symptoms of venerupin poisoning have been described in 70 persons after consumption of mussels collected close to the centre of the massive bloom of *P. minimum* in the autumn 1979 (Tangen, 1983).

Prorocentrum minimum Schiller red waters have often been observed in Obidos Lagoon (Portugal) and have caused toxicity of bivalves there. Particular attention is given to two of those blooms, separated by about 10 years, in May-June 1973 and in January-February 1983 (Silva, 1985). A comparative study of environmental conditions during the two red water of *P. minimum* indicates that the instances of *P. minimum* red water in 1973 and 1982-83 were both preceded by long periods of heavy rain. Phosphate in the lagoon waters increased during the observed phytoplankton blooms, with the two maximum peaks found during the *P. minimum* bloom. Also nitrate and ammonium proved to be important for the start of *P. minimum* growth in 1982-83. The sudden occurrence and massive blooming of *P. minimum* also in Kiel Fjord on the Baltic sea in 1983 can serve as a case study of typical coastal eutrophication (Kimor et al., 1985). This species was previously recorded in Oslo Fjord in 1979 (Tangen, 1980), and in subsequent years it expanded its area of distribution throughout the Skagerrak and Kattegat into Danish and Swedish coastal waters under conditions of intense eutrophication (Granéli et al., 1983). This was the first record of *P. minimum* in Kiel Fjord, and it fits well with the progressively eastward expansion of this euryhaline and eurythermal species into the Baltic sea. The development of the bloom was enhanced by favourable weather conditions, unusually high temperatures of the water (> 20°C) and prevailing winds as well as by high levels of phosphate-P and nitrate-N compounds. While the phosphate was derived mainly from anoxic sediments, the nitrate was delivered from river runoff and originated from agricultural fertilizers.

A number of papers (Bodeanu and Usurelu, 1979; Mihnea, 1979, 1992; Petrova-Kardzhova, 1984, 1986; Bodeanu, 1992) reported frequent summer blooms in the Black sea, the main cause of which was held to be *Exuviaella cordata* (similar to the *P. minimum* from Sibenik Bay in the Adriatic sea). These summer blooms were due to progressive eutrophication of the Black sea during the seventies and eighties of this century.

7.3.1.4 Occurrence of *Prorocentrum minimum* in the Mediterranean sea

Changes in the phytoplankton composition due to the occurrence and progressive proliferation of species previously rare or unknown have been reported from the Adriatic during recent years by Marasovic (1985) and Pucher-Petkovic and Marasovic (1988). In their view, these changes often accompanied by a substantial increase in primary productivity, both in inshore and offshore waters of the central Adriatic, were due primarily to increased eutrophication resulting from urban development and riverborne wastes. A case in point noted by Marasovic (1986) is the increasing occurrences in recent years of the potentially toxic dinoflagellate *Prorocentrum minimum*. During the summer months of 1983 *P. minimum* (Pavillard) Schiller, not earlier recorded from the Adriatic, constituted a considerable proportion of the regular summer bloom in the Sibenik Bay (eastern Adriatic coast). During the subsequent years (1984, 1985, 1986) the proportions of *P. minimum* in summer blooms in the Sibenik Bay was constantly increasing (Marasovic et al., 1990). At the same time its presence was reported from the other parts of the Adriatic coast, attributed to the intensified eutrophication of the Adriatic waters (Viviani et al., 1985, 1992; Pucher-Petkovic and Marasovic, 1987; Marasovic et al., 1988; Pucher-Petkovic et al., 1988; Marasovic, 1989). Also red tides of *P. minimum* occurs in coastal lagoon (etaug de Berre) in French Mediterranean coastal area (Leveau et al., 1989). Venerupin shellfish poisoning have never been detected in the Adriatic sea and in etaug de Berre.

7.3.1.5 Methods of analysis for venerupin

Toxic principles of *P. minimum* inducing venerupin poisoning were tested by intraperitoneal injection into mice of 75% methanol extracts of samples of bivalves (Okaichi and Imatomi, 1979; Tangen, 1983). If toxins are present mice died within 24-48 hr. The relationship between the dose and the time of death was not ascertained (Okaichi and Imatomi, 1979).

7.3.1.6 Human intoxication

The poisoning is characterized by a long incubation (24-48 hr), and sometimes longer (Halstead, 1965; Okaichi and Imatomi, 1979; Tangen, 1983). The heat-stable toxin induces the rapid onset of nausea, vomiting, diarrhea, headache and nervousness. In serious cases, jaundice may be present, and petechial haemorrhages and ecchymosis may appear over the chest, neck, and arms. Leucocytosis, anaemia, and a prolonged blood-clotting time are sometimes observed. The liver is usually enlarged. In fatal poisoning, acute yellow atrophy of the liver, extreme excitation, delirium and coma occur with death reported in up to 33% of victims (Auerbach, 1988).

7.3.1.7 Therapy

Therapy is based upon symptoms and is supportive. Any victim who shows the early symptoms of gastroenteritis should be placed on a low-protein diet and observed for 48-72 hr for signs of liver failure. There is not yet clinical experience with exchange transfusion, chemotherapy, hemoperfusion, or liver transplantation in the management of profound liver failure associated with this disorder (Auerbach, 1988).

7.3.2 *Nitzschia spp.* and Amnestic Shellfish Poison (ASP)

As regards eutrophication phenomena, diatoms were not considered as problematic as dinoflagellates until the end of November 1987, when 153 cases of acute intoxication related to ingestion of toxic mussels were documented in Canada. Symptoms included vomiting and diarrhea, which in some cases were followed by confusion, loss of memory, disorientation and even coma. The term Amnesic Shellfish Poison (ASP) has been proposed for this new shellfish toxin (Wright et al., 1989; Todd, 1990).

7.3.2.1 Chemistry of ASP component

It was established that the mollusc toxin was domoic acid, a relatively rare neurotoxic amino acid (Wright et al., 1989) (Fig. 23).

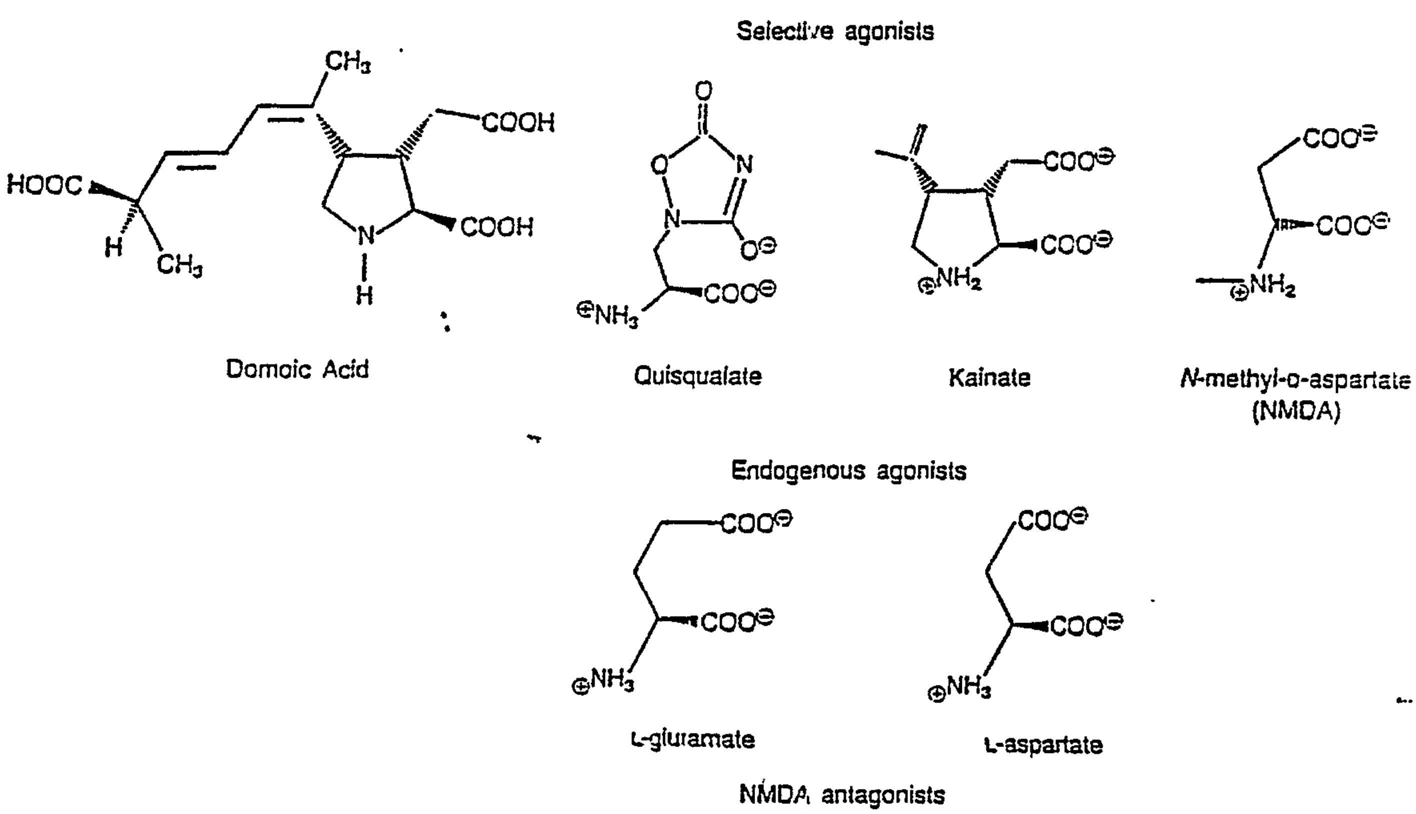


Fig. 23 Structural formulas for domoic acid, other selective agonists and for the excitatory amino acids neurotransmitters (endogenous agonists)

7.3.2.2 ASP producing organisms

Collaborative work in Canada led to identification of the pennate diatom, *Nitzschia pungens* Grunow forma *multiseries* Hasle, as a source of domoic acid. Samples of the diatom were taken from the water through the ice in the vicinity of the Cardigan River estuary and were found to be associated with domoic acid levels as high as 1% dry wt following the toxicity outbreak (Subba Rao et al., 1988).

7.3.2.3 Methods of analysis for ASP

The biotoxicological method of analysis on mice for ASP is the same as that used for PSP. Mice injected intraperitoneally with a dilute hydrochloric acid extract of mussel tissue containing domoic acid showed that the relative potency of domoic acid is lower than that of PSP (Subba Rao et al., 1988). The observation time was extended to 24 hr. Within 3 hr it is possible a simultaneous testing for PSP and domoic acid. Chemical methods have also been defined for the demonstration and quantification of domoic acid in molluscs (Lawrence et al., 1989). In order that both domoic acid toxicity and PSP may be identified in shellfish in Atlantic Canada, half the dilute hydrochloric acid extract, from shellfish being tested, is used for mouse bioassay and the other half for HPLC tests (Lawrence et al., 1989).

7.3.2.4 ASP occurrence in the world

Only in Canada, now. Concentrations of the *N. pungens* in Cardigan Bay were 10 million cells per litre in 1987. Some suggest that the proper mix of nutrients, sunlight and stratification due to fresh-water runoff contributed to the diatom blooms (Waldichuk, 1989).

7.3.2.5 *Nitzschia spp.* and mucilaginous aggregates in Adriatic sea

With regard to health, particular attention must today be paid to the diatoms, in the high and middle Adriatic sea, in relation above all to the appearance in August 1988, in July 1989 and in July and August 1991 of the "mucilaginous aggregates", which seems to originate from the diatoms among which there is a species of *Nitzschia.* This phenomenon has in fact created considerable ecological problems and has given rise to worries over health in Italy and former Yugoslavia. The monitoring of biotoxins ASP, PSP, NSP, DSP in "mucilaginous aggregates" and in mussels in a coastal area of the Northern Adriatic sea facing Emilia-Romagna in the summer months (in particular June, July and August) of 1988, 1989 and 1991 has been reported (Viviani et al., 1992). Using the PSP method which, according to current Canadian legislation is valid for ASP monitoring, the presence of domoic acid has been excluded. Also PSP, NSP and DSP are absent from "mucilaginous aggregates" (Viviani et al., 1992).

7.3.2.6 Human intoxication: clinical toxicology

Domoic acid is a mild neurological poison compared to PSP. When mussels contaminated by domoic acid were eaten in eastern Canada they produced 153 cases of gastrointestinal distress with nausea, vomiting and diarrhea within 24 hr; but added to that disorder, they also caused neurological illness within 48 hr in older victims (ca. over 60 years old). Three elderly patients died. In the most severely affected cases neurological symptoms still persisted (Wright et al., 1989; Waldichuk, 1989).

7.3.2.7 Toxicology

The mechanism of action of domoic acid is actually known on excitatory amino acid receptors and synaptic transmission. Excitatory amino acids, most notably L-glutamate and L-aspartate, have long been considered to be the most likely neurotransmittors (Collingridge et al., 1987) (Fig. 23). These amino acids are known to act on several receptor types, the best characterized of which are named after the selective exogenous excitants N-methyl-D-aspartate (NMDA), kainate and quisqualate (Fig. 24). Glutamate and also NMDA subclass act to open membrane channels permeable to Na^+, leading to a Na^+ influx and membrane depolarization (Collingridge and Bliss, 1987). Only the channel opened by NMDA receptor accessible to kainate, quisqualate and to domoic acid are, in addition, highly permeable to Ca^{2+} and induced lethal cellular Ca^{2+} entry.

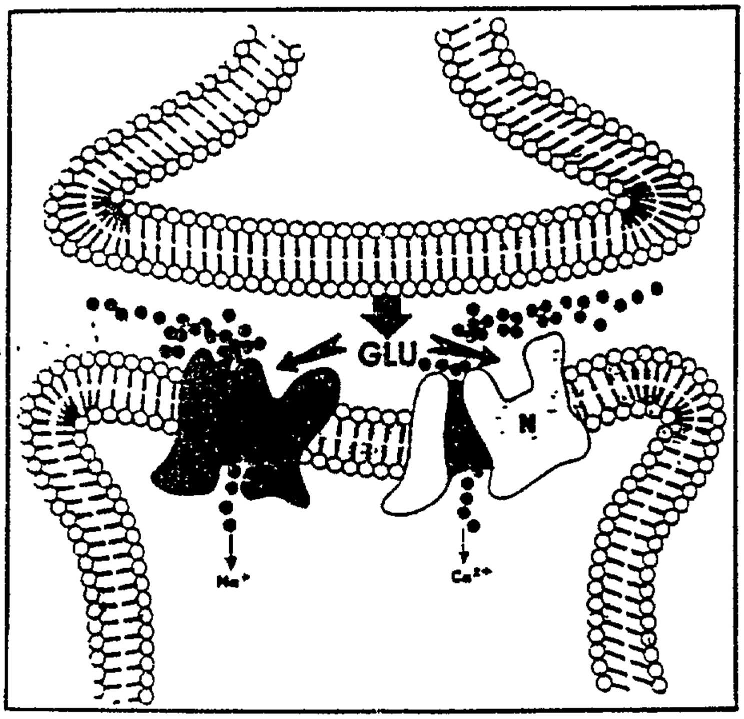


Fig. 24 Postsynaptic receptors of neurotransmitter amino acids and channels of sodium and calcium.
N = NMDA receptor
KQ = Kainate and quisqualate receptors

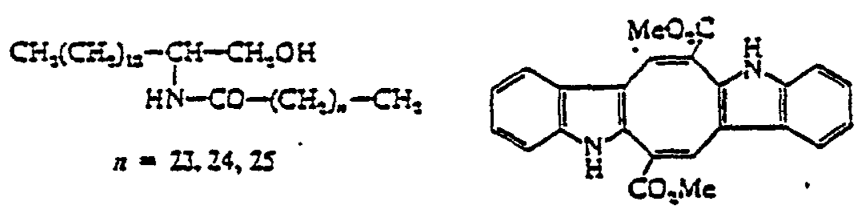
7.3.2.8 Tolerance levels and remarks on safety

An effect on certain consumers of domoic acid contaminated shellfish was inferred at an estimated concentration of 200 $\mu g.g^{-1}$ wet wt. So an application factor of 0.1 was applied for safety, and a concentration of 20 $\mu g.g^{-1}$ wet wt was set as the level of domoic acid above which a shellfish operation should be closed (Waldichuk, 1989). This compares with 0.8 $\mu.g^{-1}$ for saxitoxin in shellfish, above which an area is closed for shellfish harvesting owing to PSP. With regard to health safety, a concentration of 20 $\mu g/g$ of domoic acid in a fresh weight of molluscs is considered tolerable (Waldichuk, 1989).

7.3.3 Chlorophyta toxins and seafood

The marine benthic green algae (Chlorophyta) are the organisms usually responsible for blooms in coastal anthropogenic eutrophication. The relationships between the blooms of Chlorophyta and human health concern some toxins introduced by man, directly or through the trophic chain, with the food. Several species, such as dried algae, provide part of man diet, particularly in various parts of the Orient.

A usually edible benthic genus from the Philippines, *Caulerpa sp.*, is known to be toxic during the rainy months, and injury to the plant thallus causes extrusion of toxins. Caulerpicin and caulerpin (Aguilar-Santos and Doty, 1968; Maiti et al., 1978) are the toxins isolated (Fig. 25). The two toxins are also transferred through the food-chain, to soft corals, and sea snails. In humans, the toxins produce paresthesia around the mouth, tongue and terminal parts of the extremities, often as a feeling of coldness. Vertigo, ataxia and respiratory distress may also occur. The clinical manifestations are self-limiting and usually disappear within 12 hr.



Caulerpicin Caulerpin

Fig. 25 Chlorophyta toxins

The green alga *Chaeatomorpha minima* is toxic to fish and has haemolytic activity (Fusetani et al., 1976). Another green alga, *Ulva pertusa*, also has several haemolytic fractions (Fusetani and Hashimoto, 1976), two being water soluble and one fat soluble. However, there is no known relationship between these toxins and human health.

Caulerpa prolifera is present in all the coast of the Mediterranean sea, with the exception of the coasts of the Adriatic sea, Israel and Turkey where *Caulerpa racemosa* (Riedl, 1991) is found. *Caulerpa racemosa* came from the Red Sea from 1960. In the Mediterranean *Chaetomorpha aerea* and *C. capillaris* are also diffused. Of the *Ulva* genus, the most diffused species is *Ulva lactuca* in nutrient rich coastal areas. On these species of the genus *Chaetomorpha, Ulva,* research is lacking on the presence of toxins.

7.3.4 Rhodophyta toxins and seafood

Also some marine red algae (Rhodophyta) are responsible for human intoxication (Hashimoto, 1979). PSP components (gonyautoxin I, II, III) have been detected in a red macroalga *Jania sp.* (Oshima et al., 1984). These red algae are eaten by crabs and snails, and PSP has been detected in crustaceans, such as *Zosimus paeneus*, which has been implicated in human PSP intoxications in the Far East.

The big question was the source of domoic acid. It was originally discovered some 30 years ago in red alga *Chondria armata* in Japan (Daigo, 1959) and later identified in a Mediterranean species *Alsidium corallinum* of the family of Rhodomelaceae (Impellizeri et al., 1975).

7.3.5 *Gymnodinium spp.* blooms: Neurotoxic Shellfish Poisoning (NSP) and respiratory irritation

All red tides reported in Florida are associated with mass mortality in marine animals. Health problems caused by consumption of neurotoxin infested shellfish (NSP) and by inhalation of the windsprayed cells were evidenced (Steidinger and Baden, 1984).

7.3.5.1 NSP producing or potentially toxic dinoflagellates

The red tide-forming dinoflagellate, *Ptychodiscus brevis* (= *Gymnodinium breve*), is one of the most notorius species for its mass fish kills and destruction of other marine life along the coast of Florida. Resting cysts of *P. breve* are not present in sediment-water interface. The motile form of *P. brevis* produces several neurotoxins, collectively called brevetoxins (or *P. brevis* toxins). These accumulate in filter-feeding shellfish (oysters, clams) causing neurotoxic shellfish poisoning (NSP) when consumed. An atoxic strain of *G. breve* was found in the Inland sea, Japan (Okaichi et al., 1978). *P. brevis* appears to be not only restricted to the gulf of Mexico, the east coast of Florida an North Carolina coast (Pierce, 1987), but in reports of some blooms from northern Spain, Japan (Steidinger, 1983) and the eastern Mediterranean coast (Steidinger, 1983; Pagou and Ignatides, 1990).

7.3.5.2 Chemistry of NSP components

P. brevis neurotoxin consisted of an eleven-number heterocyclic oxygen containing fused ring system culminating in an unsaturated lactone at one end and an unsaturated aldehyde at the other, designated brevetoxin-B (BTX-B). Other brevetoxins were characterized too (Chou and Shimizu, 1982; Nakanishi, 1985; Shimizu, 1987). Figure 26 illustrates the structures of brevetoxins according their nomenclature in the PbTX series (*Ptychodiscus brevis* toxins) and two structural backbones a and b (Poli et al., 1986; Baden, 1988).

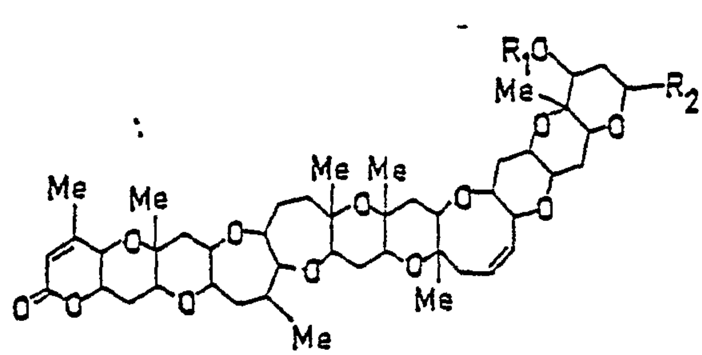
7.3.5.3 Brevetoxin compromised seafoods

The major seafoods containing brevetoxins are shellfish (Cummins et al., 1971). There is little qualitative data on rates of accumulation and depuration of brevetoxins in bivalves. Oysters accumulate the toxins in less than 4 hr in the presence of 5000 cells/ml, and depurate (60%) of the accumulated toxin in 36 hr (Cummins and Stevens, 1970). Potency of depuration is species-specific and highly variable, even under controlled laboratory conditions (Ray and Aldrich, 1965). So commercial bivalves are generally safe to eat 1-2 month after the termination of any single bloom episode. Channing cannot be a way for decreasing NSP concentration in bivalves. The fishing industry also suffers due to adverse publicity concerning dead fish washed ashore. The fish usually start dying when *P. brevis* counts reach the 250,000 cells/litre range.

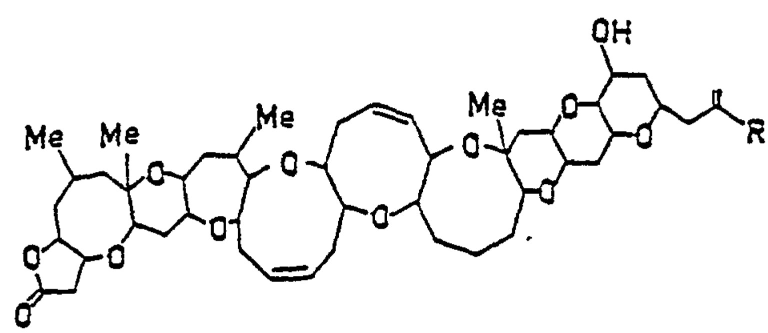
7.3.5.4 Methods of analysis for NSP

Toxicity of contaminated shellfish is determined by the mouse bioassay, which evaluates the cumulative effects rather than determining concentrations of individual toxins.

The bioassay is based on the dose that provokes a fixed death time in mice injected intraperitoneally with a crude toxic residue extracted from bivalves with ethyl ether (McFarren et al., 1960). The relative toxicity of the residue of crude lipid extracted from bivalve molluscs which on average kills 50% of the test animals (of 20 g body weight) in 30 min. Recently, methods using high performance liquid chromatography (HPLC) have been developed for the qualitative and quantitative analysis of the *P. brevis* toxins (Baden and Mende, 1982; Pierce et al., 1985). The potency of *P. brevis* blooms is determined by an ichthyotoxicity assay of either the contamined seawater or crude and purified toxin extracts (Viviani, 1981).



(a)



(b)

Fig. 26 The brevetoxins. (a) PbTX-2 [R_1=H, $R_2=CH_2C(=CH_2)CHO$]; PbTX-3 [R_1=H, $R_2=CH_2C(=CH_2)CH_2OH$]; PbTX-5 [R_1=Ac, $R_2=CH_2C(=CH_2)CHO$]; PbTX-6 [R_1=H, $R_2=CH_2C(=CH_2)CHO$], 27, 28-epoxide; PbTX-8 [R_1=H, $R_2=CH_2COCH_2C1$]. (b) PbTX-1 [R=CHO]; PcTX-7 [R=CH_2OH]. PbTX-4 structure is unknown (Redrawn from Poli et al., 1986)

7.3.5.5 Toxicology

The two intoxication phenomena that occur in man during Florida red tides are NSP and respiratory irritation. NSP is a milder neurotoxic form showing similarity in some aspects to ciguatera.

Mechanism of action. While the PSP toxins act as non-depolarizing agents in the membrane of the excitable cells, lipid-soluble neurotoxins, brevetoxins, responsible for NSP, act as depolarizing substances. One of the toxic fractions (T_{47}) acts to open membrane channels permeable to Na^+, leading to a Na^+ influx. ^{42}K analysis precludes the effect of T_{47} acting on the K^+ channels (Risk et al., 1979).

According to more recent research, the lipophilic toxins profoundly affect Na^+ channels, modifying virtually every aspect of their physiology and also the interaction of the channel with nearly every other known class of active drug, including polypeptide toxins, local anaesthetics, and the guanidinium toxins (Strichartz et al., 1987).

P. brevis cell fragments, upon becoming airborne in sea spray, elicit nonproductive sneezing and coughing when inhaled (Music et al., 1973). The inhalation of the windsprayed cells contaminated by the toxin(s) cells of *P. brevis* caused the opening of sodium channels by the toxin(s), releasing acetylcholine and causing smooth tracheal muscle contraction. The effects are only temporary (Krzanowski et al., 1981). All toxins isolated from *P. brevis* possess this activity and during purification, if they become airborne on silica gel particles, cause the same effect.

7.3.5.6 Tolerance levels and safety considerations

The Florida Department of Natural Resources (DNR) has run a general control program since the mid 1970's. Only in 1984 were *Ptychodiscus* blooms specifically noted in control regulations. Closures are made when the dinoflagellates exceed 5000 cells/litre near harvesting areas. Closures have lasted between a few weeks and six months. Two weeks after *Ptychodiscus* concentration drop below 5000 cells/litre, the first mouse bioassays of shellfish are carried out. When levels are below 20 MU/100 g, the grounds are reopened (Beverly, 1985). Also in Italy provision of law was based on this bioassay but established "no detectable amount" (Ministero della Sanità, 1990c).

7.3.5.7 *Gymnodinium spp.* in the Mediterranean sea

The annual periodicity of *Gymnodinium sp.* indicated as *Gymnodinium breve* (Davis) in an inshore eutrophic environment (Saronicos Gulf, Aegean Sea) from January 1977 to December 1983 as well as during January to December 1985 has been studied. Spectral analysis of the data as well as auto and cross correlation analysis confirmed the existence of 12 month cyclic variation of *G. breve* populations, regulated mainly by temperature (Pagou and Ignatiades, 1990). Identification of *G. breve* Davis (1948) = *Ptychodiscus brevis* (Steidinger, 1979) using electron microscopy was not performed. Effects on health implications (NSP) and fish kills are to date not reported in the Aegean sea.

The other dinoflagellate of interest in the Mediterranean (Adriatic sea) is *Gymnodinium sp.* responsible for "green tides" in 1976-77 (Viviani, 1981; Viviani et al., 1985, 1992) along the Emilia-Romagna coast and in 1984 and 1988 in the Northern Adriatic sea (Artegiani et al., 1985; Honsell et al., 1989; Regione Emilia-Romagna, 1985, 1989; Centro Cesenatico, 1985, 1989). This species, at first regarded as similar to *G. corii* and distinct from the toxic *G. breve* of Florida and also described in Japan, is now being studied in order to define its taxonomy. The NSP and PSP toxins have never been detected in the cells (Viviani, 1983). During the blooms of this *Gymnodinium sp.* in September 1977 symptoms of respiratory irritation were reported in people, both along the coast and in the sea probably related to the presence of seawater aerosols containing cell fragments or substances of this naked dinoflagellate (Viviani, 1983; Sacchetti, 1983). A *Gymnodinium spp.* bloom also occurred in the gulf of Olbia (Sardinia) during the fall 1985. This case of eutrophication may be due to the discharge of untreated sewage into the gulf. The alga did not produce exotoxins (Sechi et al., 1987).

It is fundamentally important to carry out deeper taxonomic studies for obtaining evaluation not only of a biological, biochemical, physiological and ecological kind for each individual species of genus *Gymnodinium*, but also to consider possible properties that might prove toxic for man.

7.3.6 Cyanophyta toxins causative agents of respiratory irritation and contact dermatitis

Cyanophyta in the freshwater are the main organisms responsible for eutrophication effects and for producing toxins (Viviani, 1981). Esotoxins are also produced by planktonic bloom forming genera of marine cyanobacteria belonging to the Oscillatoriaceae family, which poses potential public health problems for the respiratory tract (*Trichodesmium erytraeum*) (Sato et al., 1963-64) or cutaneous (*Lyngbya majuscula*) symptoms of poisoning (Grauer, 1959). As far as *Trichodesmius erytraeum* respiratory symptoms are concerned, these are related to the presence of sea water aerosol containing fragments of this cyanophita during blooms in coastal waters of Brazil (Sato et al., 1963-64) and in the Thailand Gulf (Hungspreugs, 1989). The filamentous cyanophita *L. majuscula* that grows abundantly in many areas of the sub-tropical and tropical Pacific basin and also in Caribbean, is the causative agent of a severe contact dermatitis that affects swimmers and bathers at the beaches (Grauer, 1959; Moore, 1984).

7.3.6.1 Chemical structures

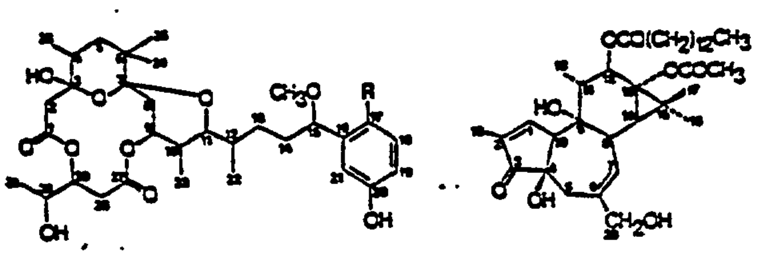
The active principle of the blue-green alga *L. majuscula* have been isolated and identified as two phenolic bis-lactones, aplysiatoxin and debromoaplysiatoxin (Kato and Scheuer, 1975), and an indole alkaloid, lyngbyatoxin A (Cardellina et al., 1979) (Fig. 27). All of these three substances have been showed to be potent irritants, producing erythema, blisters and necrosis when applied to the skin (Solomon and Stoughton, 1978; Cardellina et al., 1979).

7.3.6.2 Human intoxication

A total of 86 cases were reported to the Hawaii State Department of Health. The most recent major outbreak of this severe contact dermatitis that affects swimmers and bathers at beaches on the windward side of Oahu occurred in August 1980, at Kailua, Kalama, and Pilapu beaches. The severe contact dermatitis was described as similar to a burn and generally involved the genital and perinatal areas. The initial symptoms, which appeared after a few hours, were erythema and a burning sensation, followed by blister formation and deep desquamation which lasted for several days (Moore, 1984).

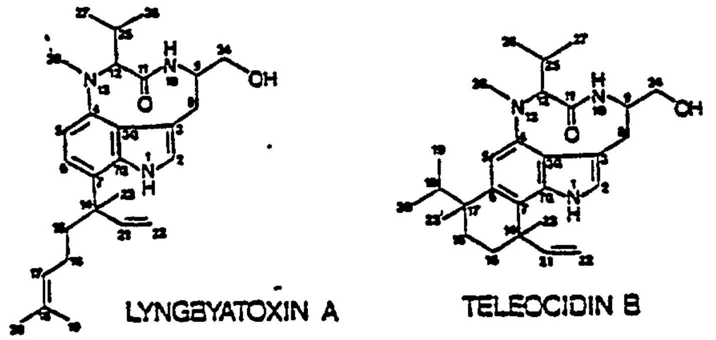
7.3.6.3 Toxicology

The mechanism of action at cutaneous and respiratory organs level can be explained on the basis of knowledges about tumor-promoting properties, in that lyngbyatoxin A, debromoaplysiatoxin, and aplysiatoxin induce irritation in mouse skin to the same degree as TPA (Fujihi et al., 1981). Carcinogenesis involves at least two stages, namely initiation and promotion. The tumor initiation stage is caused by agents that produce damage in DNA. The most well known promoter is 12-o-tetradecanoylphorbol-13-acetate (TPA), a deterpenoid ester from Croton oil (Fig. 27). Unlike carcinogens which act directly on the cellular DNA, tumor promoters exert their effects by binding to receptors.



APLYSIATOXIN, R = Br
DEBROMOAPLYSIATOXIN, R = H

TPA



LYNGBYATOXIN A

TELEOCIDIN B

Fig. 27 Structures of toxins of *Lyngbya majuscula* which behaved like the phorbol esters (TPA) and teleocidin B typical tumor promoters

Recent studies suggest that the phorbol ester, teleocidin, and aplysiatoxin tumor promoters operate by activating a phospholipid and calcium ion dependent phosphorylating enzyme, protein kinase C (Castagna et al., 1982). In a search for new antineoplastic agents from blue-green algae, a cytotoxic substance active against P-388 lymphocytic mouse leukemia from a deep-water variety of *L. majuscula* showed that it was identical to debromoaplysiatoxin (Mynderse et al., 1977). In the same species of Cyanophita there is a molecule which, depending on whether it contains Br or not, shows either tumor-promoting or antineoplastic properties.

7.3.6.4 Cyanophyta in the Mediterranean sea

In the Mediterranean sea 150 species of Cyanophyta are described (Riedl, 1991). Among these, species of *Trichodesmus* are not present, but seven species of *Lyngbya* are present. Between the species of *Lyngbya* of the Mediterranean sea, *L. majuscola* is not reported, but another filamentous Cyanophyta is present: *L. confervoides*. In the Nile estuary (Halim, 1989) and in the lake of Tunisis (Kelly and Naguib, 1984) and more recently also in the North Adriatic sea (Kaltenböck and Herndl, 1992) blooms of *Cyanobacteria* have been reported. Effects on human health are not described.

7.4 General facts on eutrophication, bacteria and human health

Relationships between eutrophication, bacteria and public health are very complex and little studied. In order to understand them it is necessary to take into consideration the nutrient effects of bacterial growth, the effects of bacteria on algae, the effects of phytoplankton, phytobenthos and macrophytes produced by eutrophication on sea bacteria.

7.4.1 Bacterial cycle eutrophication

Among the schemes and patterns of cyclical processes that characterize the cultural eutrophication of coastal areas, in the study of bacterial role Aubert (1986, 1990, 1992) distinguished between a planktonic cycle (eutrophication) and a bacterial cycle eutrophicatin.

The bacterial cycle eutrophication, in which the bacteria play a major role, begins with sea green because of phytoplankton growth, followed by its disappearance and transparent water; then a development of sulpho-reducer, sulphade-reducer (*Desulphovibrio*) and sulphite-reducer (*Clostridium*) bacteria begins with production of H_2S and a decrease in 0_2, in results of which we shall have transition waters, then whitish waters, precipitation of sulphur on bottom with whitish deposits, and, soon afterwards, appearance of red colonies of flavobacteria on bottom and red water through the action of sulphur-oxidative bacteria: reappearance of sulphites, sulphates, increase in O_2 and clarified water. Unfortunately, we have no precise knowledge about this scheme. It can't be excluded that some phenomena of red water observed without dinoflagellates are due to a process of this kind.

7.4.2 Marine bacteria and the red tide link

In addition to the promoting blooms of dinoflagellata from the vitamins B_{12}, thiamin and biotin and chelating agents produced from marine bacteria (Provasoli, 1979), recent research has suggested that a strong link may exist between the activity of marine sediment bacteria and the accelerated phytoplankton growth resulting in red tides. Various researchers have revealed that plant growth hormones can be produced by a wide range of marine bacteria. 45-55% of sediment bacteria tested were found to produce cytokinin, a principle plant growth hormone. The algae responsible for the "red tide" phenomena are known to be dependent on substances exuded from the sediment surface, and the phytoflagellates causing red tides have been shown to respond to cytokinin. Heterotrophic bacterial activity is influenced by nutrient supply, and an influx in nutrients or rise in temperature will accelerate this activity. It is possible that observed correlations between nutrient/temperature increase and red tide outbreaks may be at least partially due to increased hormone production by marine bacteria. Translocation of hormones from the sediment bacteria to the phytoflagellates may be accounted for by upwelling currents in deep water or by simple diffusion or stratification in shallow.

7.4.3 Effects on bacteria of active principles produced by phytoplankton

It is possible to separate substances produced by phytoplankton and macroalgae bioactive on bacteria into three large groups: (1) antibiotics (Duff et al., 1966; Allen and Dawson, 1969); (2) growth promoting substances (Lelong et al., 1980); (3) compounds that are inhibitors of mineralization processes of organic substances (biomass) (Chriost, 1975a-b).

Particular researches on this reactive principle have been carried out not only in laboratory and in various marine ecological conditions, but also with hygienic-sanitary aims in coastal areas. Active principles produced by algae during their bloom or released by cells during the degradation process at the end of the bloom, can carry out antibiotic functions of functions promoting the growth of bacteria. In particular these principles can promote the growth of those bacteria which are used as indicators of sea areas bathing possibility (in national and at EC level legislation) and of other bacteria which can arrive to man through the food trophic chains of fishing products.

Besides negative effects related to eutrophic phenomena that affect aesthetics and some economic activities, such as fishery and tourism, there are some more effects that seem to lead to an apparent environmental "purification". Thus, during the summer period, along Emilia-Romagna coasts the levels of hygienic-sanitary indicator bacteria for bathing increased after a *Gymnodinium sp.* bloom, while they decreased during the maximum bloom period of *Gonyaulax polyedra* (Volterra et al., 1986) and during diatoms blooms of *Chaetoceros sp.* and *Skeletonema costatum* (Bonadonna et al., 1985; Mancini et al., 1989). On the other hand, it is known that dinoflagellates of *Gonyaulax* genus produce metabolites that carry out inhibitory functions, particularly selective on *Staphylococcus aureus* (Burkholder et al., 1960).

While the bloom of *G. polyedra* is accompanied by an "apparent purification" with a decrease in usual titration of "coliforms" as well as a "streptococci", also in areas constantly contaminated by the same bacteria, because of production of substances with an activity of antibiotic kind, other researches have demonstrated that an extract of *G. polyedra* cultivated in laboratory has properties which stimulate the growth limitedly to *Streptococcus faecium* (Piretti et al., 1989). This shows that in various phases of dinoflagellates physiology there is a formation of substances that can have different effects on specific bacteria. While antibiotic effects on usual indicators of faecal pollution (coliforms and enterococci) seem to be related to ectocrin production during algal maximum development phase, the effects which promote the growth, obtained from cell extracts; perhaps can be considered related to final phase of algal bloom and to decomposition of cells that, from the ecologic point of view, is supported by an intense bacteriological development.

7.4.4 Direct and indirect effects of bacteria on human health

In case of bacterial cycle or planktonic cycle eutrophications the effects of bacteria on human health known at present are direct or indirect. The unique direct effects are the respiratory ones, i.e. these related to production of H_2S, that strikes the coastal population. The indirect effects are much more numerous and regard the relationships between bacteria and phytoplankton. In fact, marine bacteria can stimulate the production of toxic red tides, whose toxins reach man via food-channel through contaminated fishing products (PSP, NSP, DSP, vennerupin poisoning, ciguatera) or, in case of *P. breve,* can have effects at cutaneous and respiratory levels. In the first case the health hazards concern all potential consumers of fishing products containing biotoxins, in the second case only population living on sea, fishermen, tourists and bathers of the area where red tide developed.

Recent evidence indicates that bacteria may be a source of tetrodotoxins (TTX) and saxitoxin (STX) (Tamplin, 1990). Bacteria have been proposed as the source of STX in marine dinoflagellates (Kodama and Agata, 1988; Kodama, 1989, 1990a-b).

Sousa and Silva (1962) provided early suggestions of a bacteria source of STX, subsequently STX and neo STX were isolated from strains of a cyanobacterium *Aphanizomenon flos-aquae*, a procaryotic organism (Alam et al., 1978) and more recently Kodama (1989) has reported that a bacterium (*Vibrio*-like sp.) cultured from *A. tamarensis* produces STX.

Relationships between anthropogenic eutrophication, marine bacteria present in *Alexandrium* species and saxitoxins biosynthesis are at present not studied in the coastal area of the Mediterranean sea.

8. MANAGEMENT OF EUTROPHICATION

In summing up the previous chapters, the question arises about the guiding principles that address the translation of this information into practical management. There are essentially two different though interconnected outlooks under which the problem must be visualized, each one involving different concepts and approaches that are of equal importance. Their relationship is depicted in Figure 28 in the form of a flow diagram with two cycles.

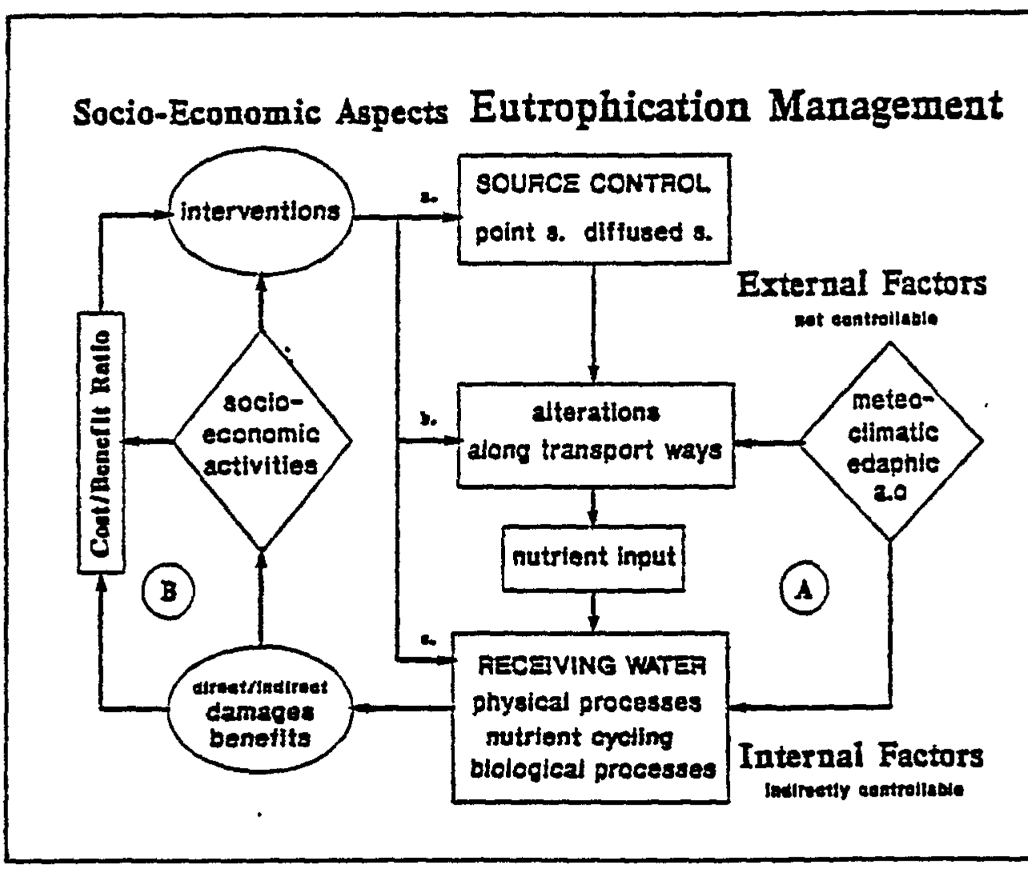


Fig. 28 The concepts of eutrophication management

<u>Cycle A:</u> refers to aspects that relate to the physical side of the system to manage and trace the principal relationship between the basin (sources, path) and the receiving water body. These calls firstly for a sound knowledge of the functioning of the system, and in particular of those conditions and processes that control eutrophication; secondly for a knowledge about the critical points of the system at which interventions into the process are potentially feasible with technological means, and thirdly for a knowledge about those technologies themselves.

Cycle B: refers to aspects that relate to human ie the socio-economic environment, within which eutrophication is perceived as a problem that affects, either directly or indirectly, the socio-economic activities and interests of the resident population, and within which decisions are made.

While these two outlooks are often treated as distinct (the scientific aspects addressed separately from the socio-economic involving two communities, scientists versus administrators/politicians), they must be linked as soon as large scale remedial interventions are at stake. Whether these are technological, or administrative, the matter at this point becomes inevitably political and requires a model different from that discussed in Chapter 3, ie a consolidated integrative model. This is the meaning of the two cycles (A and B).

The scientific/technological aspects that relate to cycle (A) have been discussed in details in previous chapters of this report. Little instead has been said about the second aspect. With regard to the first it is important to recognize a) that there are conditions and processes external to the system, which cannot be altered at all (e.g., meteo-climatic conditions; natural background supply of nutrients from the drainage basin); b) that the critical points of possible attack by which the system processes can be manipulated are essentially only three:

- control at source,
- interventions along the transitional paths,
- interventions into the receiving water body.

Each of these major points of attack involve different approaches that are defined by the properties of the physical system, and by the available technologies and resources.

With regard to the second aspect, it is further important to recognize that the decision to do something about eutrophication, and if so, that the actual choice between options, will not be determined by science alone, but will rather depend on the degree of societal perception of the problem, i.e., the kind of damages and its seriousness in terms of socio-economic activities, as well as on the perceived intent of intervention, i.e., the purpose, and scope to be achieved dealing with the problem. In practice, as with any societal problem that requires solution, the choice between technologically and administratively feasible options is primarily driven by the cost/benefit ratio connected with each particular type of intervention versus the expected gains.

If carried out correctly, the evaluation process that considers all the available alternatives, and in which, both scientists and administrators/politicians are involved, will have to run repeatedly through cycle (B), whereby for each alternative the potential effect on cycle (A) is to be evaluated. The final selection will then be determined by the c/b ratio that is considered optimal in terms of societal expectations. Clearly, the selected alternative will not always be that of the highest c/b ratio; in the contrary most often it is not. Also, the optimal ratio is rarely defined by one unique technological option, but rather by a judicious mix of different technological/administrative options, each one with its own c/b ratio. It must be noted that the c/b ratio includes also time, i.e., the time to realize the intervention, and most importantly, the time that lapses between implementation and the time when benefits will materialize.

Taking as example the reduction of the phosphorus load: this may involve reduction at source (e.g., reduction of polyphosphates in detergents; relatively rapidly to achieve by industries, and indeed already implemented in many countries), precipitation of phosphorus in urban treatment plant effluent (upgrading of treatment procedures in existing plants, relatively rapidly; building of treatment plants if not already existing, medium time terms), reduction of the use of fertilizers on crop land, application of animal manure on land, restriction of the number of animals (e.g., cattle, etc.) per unit of available surfaces of pastures, adoption of strip farming, etc. (requiring often long-term education and administrative regulation about farm practice).

Further, it should not be ignored that cost evaluations are not always straight forward. E.g., installation and running costs for urban sewage treatment plants are relatively easy to estimate within the usual uncertainty range of changing costs, although actual cost over-runs are frequent. On the other hand, the cost evaluation of sludge deposition is much more uncertain because sludge deposition may create new and unexpected problems, such as limited site availability for deposition, undesirable environmental impact such as toxicity if used in agriculture, a.o. Reformulation cost of detergents will be hidden in consumer costs, as are installation and running costs of say food processing industries that are required to clean their effluent, etc. On the other hand, there may also be gains, say to farmers using less fertilizers, which however may be offset by required changing work procedures.

With the same token, benefits cannot always or exclusively be evaluated in terms of tangible money gains. Benefits such as increased amenities e.g., cleaner bathing beaches, reduced health risk, augmented sport fisheries, etc. are still desirable societal objectives for themselves regardless of the extent of connected economic gains. Economic returns are often indirect, and as such difficult to evaluate.

For further worthwhile reading on the subject, in which details are elaborated on several aspects touched upon, refer to Fole and Kåberger (1991).

B. REMEDIAL ACTIONS AND CONTROL MEASURES

9. MONITORING, PREDICTION AND DECISION MAKING

Monitoring, in the context of the assessment and protection of the marine environment, is here defined as the repeated measurement of an activity or a contaminant or of its direct or indirect impact (Villa, 1989; Rinaldi, 1989; Vollenwieder, 1992; Bonalberti et al., 1992; Bucci et al., 1992; Volterra, 1992).

In practical terms, monitoring can fall within the following three categories :

- monitoring for regulation purposes (control);
- monitoring of levels and trends;
- monitoring for scientific purposes.

The monitoring for scientific purposes is generally the main step for establishing monitoring of levels and trends which in turn provides useful information for defining the parameters of control (monitoring for regulation purposes).

In order to define the monitoring programmes of the marine environment, the following operational objectives, which have a high degree of universality, must be taken into consideration:

- Protection of human health;
- Protection of marine life and its environment;
- Assessment of levels and trends.

9.1 Monitoring of eutrophication

In the pelagic domain, monitoring of eutrophication should be relatively straightforward. The only difficulty may be in finding the most cost-effective strategy. Two major strategies are available, namely remote sensing and direct measurements in the field.

a) Remote sensing

Remote sensing may be successfully employed when eutrophication extends over large areas such as the northern Adriatic Sea or the Aegean Sea. Although the satellite operated Coastal Zone Colour Scanner (CZCS) has been terminated, the possibility of using LANDSAT's thematic mapper or SPOT's sensors still exists: their low sensitivity is overcome by the high chlorophyll content of the upper layers of the eutrophic areas. Another possibility is the use of airborne spectral scanners or even aerial photographs to monitor the extent of eutrophication (Zevenboom et al., 1989).

b) Direct measurements made in the field

No single analytical tool is adequate to measure the degree of eutrophication of a given body of water. Instead, most experts believe the best approach is to measure many different parameters and to synthesize the results into a general model providing an overall, somewhat integrated degree of eutrophication for the water. Unless proper selection of the parameters to be measured is made, the amount of work required to assess the extent and intensity of eutrophication may be rather costly.

Much in the same way that the information provided by remote sensors on the surface layer is used to evaluate the entire water column, direct measurement of surface variables may be used to infer what is happening at deeper layers. However, subsurface and near bottom waters should also be monitored, particularly in relation to monitoring of the benthic domain.

Direct observations by SCUBA or underwater TV can also be very useful in detecting changes in benthic populations, especially in the early stages of deterioration.

9.2 Major variables to be sampled

Various parameters such as suspended solids, light penetration, chlorophyll, dissolved oxygen, nutrients, organic matter, etc. may be determined either at the surface or at various depths.

If only limited means are available, determination of those parameters that synthesize the most information should be retained. Chlorophyll determinations for example, although not very precise representations of the system, are data which provide a great deal of information. Reliable data on nutrients are extremely useful indicators of potential eutrophication. Turbidity and water colour may also be a good measure of eutrophication, except near the mouths of rivers where inert suspended solids may be extremely abundant. Dissolved oxygen is one parameter that integrates much information on the processes involved in eutrophication, provided it is measured near the bottom or, at least, below the euphotic zone where an oxycline usually appears.

- Nutrients

The concentrations of plant nutrients reflect the balance between a large number of physical and biotic processes. Therefore nutrient concentrations (N, P, Si) in every form (organic, inorganic, dissolved, particulate) should be determined. Although phosphorus has been the most popular nutrient determined in fresh water systems, there are good reasons to believe that nitrogen in any of it forms may play a more important role in most, though not all, marine systems. Silicate is a good indicator of fresh water dispersion and of the potential for diatom blooms.

- Bacteria

It is suggested to monitor the total aerobic, and possibly anaerobic, bacterial count using sampling opportunities of sanitary monitoring programmes.

- Standing crop of algae

Volume or dry weight-of plankton in a vertical haul of a plankton net from bottom to surface can provide an estimate of potential grazing intensity by mesoplankton.

- Dissolved oxygen

One of the more frequently used parameters for assessing the eutrophication of water bodies is the oxygen concentration in the lower layers. Oxygen depletion in the lower layers, particularly when there is a strong stratification, is probably the most widely used index that distinguishes between eutrophic and oligotrophic waters. The rate of depletion of oxygen from the lower layers depends, of course, to a large degree on the hydrodynamics of the region.

- Turbidity

Light penetration, an inverse function of water turbidity, is one of the most widely used measurements in aquatic monitoring. If possible a light profile should be determined, or at least a Secchi disk reading must be taken. Although sometimes criticized because of its simplicity, the Secchi disk is an important tool in marine studies on eutrophication, and the determination of water colour (Forell scale) is also important.

9.3 Sampling and analytical techniques

Sophisticated instrumental techniques exist for the automatic measurement of the above variables. Normally however, some of the analyses are carried out in the laboratory, therefore field sampling and sample preservation are required.

Most scientists use fully comparable techniques, some of which have become practical standards. However, an effort should be made to harmonize those sampling and analytical techniques considered to be the minimum required to monitor eutrophication phenomena. This alone can enable comparison of the results obtained by various research groups.

9.4 Location of sampling sites and frequency of sampling

The location of the sampling stations should be selected on the basis of previous knowledge of the morphological and hydrodynamic characteristics of the area. Good coverage of the sources of eutrophicants is extremely important, as is the choice of stations. These should cover the full range of environmental conditions from near-shore eutrophic waters to offshore, more oligotrophic waters.

Direct measurement by moving ship of many of the variables previously mentioned, when used in conjunction with computerized date acquisition, allows for a practically real time display of the conditions encountered in the area.

In order to estimate the variation of potential eutrophication, a monthly frequency is recommended. The frequency should be increased during critical periods, which may be identified during sanitary monitoring programmes.

Because of the great variability of the pelagic system, strongly correlated to meteorological changes, bursts of intensive sampling and/or measurements (round the clock) during one day periods, may be preferable to more sparse sampling campaigns.

Monitoring of long-term changes over at least 5 to 10 years is necessary, and must concentrate of selected variables which are easy to estimate. On a long-term scale it is most useful to measure changes in the area in which the surface chlorophyll concentration is above a certain value, and the oxygen concentration in the lower layers is below a certain value.

9.5 Policy analysis

Environmental managers seek advice on which policies to pursue in managing the problem of eutrophication. Modelling, field data collection, and laboratory and field experimentation have an important role to play in the evaluation of these policies. The results of policy analyses presented to the manager can be regarded, in their simplest form, as a table or score care on a single page which facilitates the complex trade-off which must be made in the making of a decision.

The score card consists of an array of squares. Each square contains a number or qualitative index, which measures, or scores, the performance, impact, cost or benefit of each policy option under chosen political, economic, social, legal and environmental headings. Models, whether they are socio-economic or ecological mathematical models solved on a computer, or mesocosms in the laboratory are the tools which provide the entries in the score card. Hence, modelling should always be governed by a question to be answered, in this case: what value should be entered in a given square of the score card and what is its uncertainty ?

When there are significant aspects of the eutrophication phenomenon which are not understood (for example, the response of plankton species to a new nutrient control technique) a combined application of mathematical modelling, field data collection, laboratory and field experimentation can be recommended. In this case also, modelling is governed by a question to be answered.

Since models are always a simplification of reality, the question posed guides the simplification. The resulting model is a limited set of working hypotheses to be confronted with laboratory and field experiments which are designed to test them. The qualitative and quantitative comparison of model predictions with field and laboratory data may force a revision of the model, and the emergence of new hypotheses.

9.6 Environmental capacity

According to the definition given by GESAMP Reports and Studies No. 30, the use of the marine environment for waste disposal should be based on an assessment of the local capacity to accommodate a rate of waste discharge, without unacceptable impacts on the environment. The acceptability of the impact is a subjective judgement which should be reflected in environmental standards set nationally or internationally. From a purely scientific point of view, following again the GESAMP definition of marine pollution, any discharge which has no deleterious effects on the important components of the ecosystem or on the various uses of the marine environment., is acceptable.

Assessment of this capacity must take into account such physical processes as dilution, dispersion, sedimentation and upwelling, as well as chemical, biological and biochemical processes which lead to the degradation or removal from the impacted area of eutrophicants, until they lose their potential for unacceptable impact.

The environmental capacity of an area for eutrophicants may be calculated, appropriate models providing a preliminary assessment that can be progressively refined by the inclusion of more parameters and variables and by experimentation.

9.7 Mathematical models

Mathematical models provide a means for synthesising available knowledge and for testing control hypotheses.

The models should elucidate the most important factors affecting the ecosystem, and the principle of parsimony should be advocated in order to reduce the large number of physical, chemical and biological state variables to an essential and sufficient number compatible with the questions to be answered.

A careful choice of space and time scales, boundaries and boundary conditions, should be made in relation to the morphology and stratification of the area and the nature of the problem.

Models of eutrophication may be based on the following principles:

- conservation of mass, momentum and energy,
- process kinetics,
- stoichiometry.

See for example O'Kane et al. (1990), Betello and Bergamasco (1991), Rajar and Certina (1991), Bragadin et al. (1992), Giovanardi and Tromellini (1992), Guidorzi et al. (1992) and O'Kane et al. (1992).

From these, a set of simultaneous non-linear differential equations is derived in terms of the chosen state variables. "Process kinetics" provide some of the terms of the right hand sides of the chemical and biological equations, for example, specific growth and death rates of populations of plankton and bacteria. Laboratory and field experimentation is essential for the precise specification of their dependence on forcing functions such as temperature and light. When the model contains several subsystems interconnected by mass flows due, for example, to ingestion and excretion, the stoichiometric conversion factors must also be determined.

Forcing functions such as inflows of nutrient, light, temperature and wind, drive the model. Those forcing functions which are subject to alteration by man are called control variables or functions.

Some of the following control variables will always be present in a eutrophication model:

- mass discharge rate of nutrients from point and diffuse sources;
- location of the discharge points;
- harvesting or biomass;
- dredging of nutrient-rich sediments;
- burial of nutrient-rich sediments with inorganic material;
- biocide inputs, etc.

"What-if" experiments can be made with the model in order to support the decisions of the manager. If the chosen control strategy achieves the predicted response, the hypotheses or the model stand. Any disagreement between predicted and observed response of the trophic system necessitates a revision of the model. Clearly, the approach presented here demands the co-operation of many different specialists and provides a focus for it (Fedra, 1988).

10. POSSIBLE PREVENTIVE AND REMEDIAL ACTIONS

There is evidence that in some coastal areas of the Mediterranean Sea the inputs of eutrophying substances, particularly phosphorus and nitrogen, exceed the capacity of the receiving environment.

Since the Mediterranean is generally an oligotrophic sea, small sewage discharges on open coastline shores, if properly spaced, can normally be disposed into the sea without extensive treatment, via submarine pipelines with diffuser outlets at an appropriate depth and distance from shore.

For larger discharges or the concentration of several small ones in an area, especially when situated within bays, additional treatment or other measures for reduction of nutrient loads, e.g. reuse of waste waters, recycling in aqua culture, is required.

The type of treatment and disposal design depends on the overall inputs and environmental receiving capacity (for pertinent Guidelines etc. see UNEP/WHO, 1982. *Waste Discharge into the marine Environment.* Pergamon Press, Oxford.). This should be decided on a case-by-case basis, taking into consideration all the load of existing and planned discharges versus the receiving capacities.

When important loads, carried by rivers and originating from point and distributed sources, overwhelm the point discharges along the shores, the simple control of the latter is not sufficient. In these more complex situations, such as in the North Adriatic, it is essential to add appropriate interventions in order to reduce the inland nutrient loads.

The fundamental steps in the decision-making processes at the strategic planning and policy level can only be performed through the improvement of knowledge, as obtained by research, assessment and monitoring.

The definition of most appropriate strategies of intervention requires a preliminary cost-effectiveness evaluation, in an overall policy analysis framework, of the role of the different factors which relate to the origin, transport and dispersion of nutrients. The analysis of the point sources (urban and industrial effluents), of the distributed sources (diffuse and linear erosion, fertiliser run-off, etc.), of the transport and diffusion mechanisms, as well as the biological and ecological processes which are driven by the meteorological, hydrological, hydrochemical conditions, is of increasing complexity.

If eutrophication is to be contained, an initial evaluation is needed to establish which nutritional substances provide the main stimuli for algal growth, and which tend to limit it. Research must also identify any concurrent factor (limited circulation of water, imbalances in the food chain, etc.) and the ways in which some of these might be corrected.

These elements of information must come from an exhaustive investigation of the system. A reliable monitoring program and an expert analysis of the data collected will provide the ultimate basis for decision on any actions to be taken.

In the case of coastal marine areas, where the organisms involved in eutrophication are primarily phytoplankton, the most effective remedial action will be reducing the growth limiting factor. Around the Mediterranean situations of eutrophication dependent, in terms of growth limitation, on either or both, nitrogen and phosphorus. Nitrogen limitation is likely the prevailing condition in open offshore waters, prevailing phosphorus limitation has been identified in the Northwest Adriatic. Both factors are supplied by sewage discharges, which reach the sea either directly, or via rivers.

The situation in lagoons and deltas differs from open coastal areas. Such systems are characterised in most instances by brackish, shallow waters of limited communication with the open sea; they will often be affected by invasive accumulations of macroalgae (mainly ulvaceae). Here, eutrophication is generally the result of an excessive influx of nutrients (nitrogen compounds in particular) coming mainly from the drainage of farmlands. In addition to the trophic component, due consideration must be given to man-made structures (harbour walls, docks, breakwaters, etc.) which in many instances isolate stretches of water from the sea and thus give rise to stagnation.

In respect of nutrients, the scope for preventive and remedial action is relatively wide. The possibilities include:

a) <u>Elimination of nutrients at source</u>. Preventive measures can be taken to rationalize the methods of cultivation used in farming; control the distribution of livestock husbandry activities over the territory; reduce or replace tripolyphosphates in detergents; encourage the adoption of manufacturing technologies with a low trophic impact.

b) <u>Reduction of nutrients in effluent</u>. Implementation of advanced treatment in sewage purification plants (tertiary treatment) to reduce phosphorus and nitrogen levels in sewage effluents is recommended for treatment systems on coastal sites, or anywhere near to eutrophic bodies of water.

c) <u>Isolation of nutrients from coastal water systems</u>. Effluent discharged from treatment plants may be recycled back into agriculture (fertirrigation) or carried by submarine pipelines to outflow points removed from the immediate coastline.

10.1 Elimination of nutrients at source

10.1.1 Agriculture and livestock

Measures that can be taken in agriculture to reduce the burden of nutrients (nitrogen compounds in particular) are preventive in character, and concerned foremost with the rational use of fertilizers. In essence, kind and amounts of fertilization should be reconciled with and proportioned to the nature of the soil and the nutritional requirement of the crops to be cultivated. A rational approach in this regard will not only reduce losses of useful fertilizer simply being washed away due to surplus amounts used, but also bring considerable economic benefits to farmers by saving on unnecessary consumption. Another important avenue of improvement that might be pursued is the adoption of "slow release" synthetic fertilizers.

Also, irrigation systems should be improved in order to minimize losses by wash-out and erosion. Erosional losses can also be minimized by various methods of strip farming, like protective strips of grassland around crop land; alternation and systematic rotation of crop types in adjacent strips; etc. Particularly important in hilly regions is ploughing and cultivation across to, not with the slops of terrains.

Radically different from simple fertilizer and soil control is the option of selecting "genetically improved" crops having a greater capacity for assimilation and accumulation of nutrients (which will therefore be taken up from the soil more rapidly and efficiently), or having a lower requirement in respect of certain nutrients (allowing a reduction in the use of fertilizers).

In the area of livestock husbandry, action can be taken both to treat sewage and to encourage manure-spreading. Sewage purification by appropriate treatment is possible in theory, but costly, and often impracticable because of lacking technical know-how by rangers regarding the running of treatment plants. Spreading is easier, and widely practised. Whilst the manuring of agricultural land is certainly beneficial, in view of the fertilizing and organic substances that are added to the soil, local and regional development authorities must take care nonetheless to avoid excessive concentrations of livestock in a given territory, preferably at the planning stage, since extensive spreading may carry the risk of contaminants washing into surface waters and percolating down to the aquifers, and in case of continued excessive spreading to damage soils.

10.1.2 Detergents

Utilized as a tripolyphosphate in detergents, phosphorus has the advantage of being a relatively cheap additive for enhancing the capacity of a washing product to remove dirt; conversely, it has caused serious problems in aquatic ecosystems, and indeed continues to do so. Many researchers from the 'fifties onwards have shown how instances of eutrophication began to increase in number as the use of such washing products became widespread. On the prevention front, polyphosphate elimination at source can be achieved by adopting alternative substances which perform the same function. Polyphosphate reduction and substitution has already been effected in many countries, with the introduction of new zeolite formulations containing salts of nitrilotriacetic acid (NTA) or citrates.

10.1.3 Industry

The preferred action is without doubt to adopt production cycles which reduce or prevent the formation of nutrient-rich liquid waste. At all events, the industrial processes presenting the greatest risk are those connected with food processing, such as dairies, slaughterhouses, canneries, breweries, etc., and manufacturing industries, such as paper and leather products. In these instances, all waste should be treated before being discharged into any body of water.

10.2 Reduction of nutrient levels

10.2.1 Sewage treatment plants

There are various methods of purifying raw sewage from urban and industrial complexes. According to the level of purification to be achieved, one distinguishes between primary (simple gross sedimentation and clarification), secondary (biological treatment, oxidation and clarification), and advanced or tertiary treatment to substantially reduce phosphorus and nitrogen levels. Standard biological treatment will reduce phosphorus and nitrogen by some 20 or 25%. In the case of phosphorus greater reduction is achieved by chemical precipitation, adding salts of aluminium and iron at certain stages of the treatment process. With to-day standard technology as much as 90% reduction can be easily achieved; using advanced technology another 5 to 9% can be gained, but treatment costs will substantially increase.

Nitrogen can be reduced by biological methods based on processes that occur spontaneously in nature, namely nitrification and denitrification. The methods most widely used consist in a sequential process chain (aerobic-anoxic, anoxic-aerobic, alternated aeration), designed to modify the oxidation state of nitrogen to obtain its release ultimately in volatile form. Nitrogen reduction technology is relatively costly, and only warranted, where nitrogen load from urban areas makes out a substantial fraction of the total nitrogen load.

A somewhat different but corresponding biological method has also been developed for phosphorus, but the technique has not generally been adopted yet.

10.2.2 Other forms of purification

In addition to methods of nutrient reduction in conventional treatment plants, there are other methods available (Merrill, 1991), such as phytopurification, lagooning and fertirrigation, to cut down the nutrient load. These are usually applicable only downstream of the plant. Methods of this kind have been tried in many parts of the world. The first two are based on the capacity of growing plant biomass (either naturally growing or introduced as in the case of water lilies) to absorb large amounts of nutrients, and thus to abstract them from the body of water.

Biomass grown in this way in lagoons, like the macroalgae (ulvaceae) that often tend to amass in their relatively still and/or shallow waters, must be removed periodically to minimize the risk of new release of nutrients that would otherwise accumulate in the basin by mineralization, and hence upset the rational for the procedure. Among the main drawback which frequently disallow the adoption of such a solution are discernible in the costs of transporting the large volumes of biomass collected, the lack of suitable storage sites, and the lack of opportunities to rationally utilize the biomass stored for other purposes.

Composting and utilization for soil conditioning is limited by reason of the high salt content of marine macroalgae. Biomass of e.g., water lilies has been utilized for feed of hogs, biogas, and paper. However, this otherwise highly efficient water plant for removing nutrients presents also a risk, particularly in warmer climates, because of its invasive potential; indeed, water lilies have become a pest in water courses, lagoons and reservoirs throughout the world.

In fertirrigation, by contrast, partly purified water is returned to agricultural lands and reused (Rismal, personal communication). Besides watering the soil, fertirrigation serves to recycle nitrogen, phosphorus and organic matter in a cultivation environment (accordingly, elimination of nutrients from sewage in this instance would be illogical and counterproductive). Naturally, the risk of contamination from bacteria or other pathogens (viruses, fungi) must dictate caution in the use of any such procedure for crops like salad and fruit; on the other hand there is practically no health hazard in applying fertirrigation for others crops destined to be stored and processed (cereals in general, sugar beet, etc.).

10.3 Other courses of action

Alternatively to full sewage treatment, lagooning and otherwise, there is the option of discharging urban and/or industrial sewage effluents by way of pipelines laid on the sea bed out to deeper offshore waters. This arrangement is not recommended for shallow inshore waters and sheltered bays, where the discharged waste can resurface, or accumulate progressively by reason of insufficient dispersion. A further often used but questionable procedure is to transport solid and/or liquid waste by tanker out into the oceans, far from land, where the risk to contaminate the coastline appears attenuated.

10.4 Monitoring programmes

As an essential complement to interventions, monitoring programmes should be established.

Monitoring programmes are in fact an essential prerequisite for the assessment and the control of the evolution of phenomena as well as of assessment of the effectiveness of the interventions.

For their establishment the following point should be considered:

- Monitoring objectives must be reformulated in a more coherent way in order to make planning more comprehensible and effective (Reuss, 1990);

- It is necessary to reaffirm the crucial role of the monitoring of pollution sources (UNEP, 1988);

- The first essential phase is to establish beyond the shadow of doubt the baseline contamination levels, before establishing permanent programmes for certain areas of special concern;

- A biological effects monitoring programme must be devised and implemented within the MED POL framework (UNEP, 1992);

- The quality assurance programme for results must not only be continued but reinforced as well;

- At the same time, all research efforts which might add to the general knowledge of the marine environment must be encouraged in order to promote the optimisation of monitoring programmes.

Once the phenomenon to be monitored or studied has been identified, it is of the uppermost importance to plan a monitoring programme, that both in terms of frequency in time and distribution and typology of sampling in space will be representative of the phenomenology under study as well as of the sequential mechanisms and the triggering effects: in other words it is necessary to establish a redundant programme that will allow for grasping the most relevant events happening during the process. From the correct interpretation of the phenomena and the implications in terms of environmental and hygienic-sanitary impact, may descend the correctness of the interventions.

Among the general objectives that should characterise the control and management programmes for the coastal zones, the following items should be considered:

- Control of the environmental conditions and the qualitative status of the water body:
- climatic variations (Stravisi, 1991; Cacciamani et al., 1992);
- definition of the trophic state;
- presence of algal blooms;
- presence of dystrophic conditions (anoxia of bottom waters);
- pollution levels;
- presence of toxic micro-algae;
- quality standards for recreational activities.
- Time evolution of phenomena under control using long time series (see Penna, 1989; Aertebjerg, 1991; Beukema, 1991; Braun, 1991; Cescon, 1989; Dooley, 1991; Eleftherion, 1991; Heip, 1991; Herman, 1991; Kendal, 1991; Skoidal, 1991):
- trends of parameters indicating the trophic levels (nutrients, chlorophyll, etc.);
- trends of traditional pollutants (heavy metals, pesticides, radio nuclides, etc.);
- changes in the typical phitoplanctonic species (Hichel, 1991);
- dynamics of the fauna (particularly in terms of bentonic ecosystem) and flora (Hutchinson, 1991, Neiland, 1991);
- assessment of rehabilitation plans and validation of the effectiveness;
- applied scientific research.

Therefore, the quality management of coastal waters should be derived from an integrated monitoring programme and process modelling which will include:

- "point" and "distributed" nutrient sources;
- transport of nutrients;
- hydrodynamics of the coastal waters;
- a description of the ecological and physiological processes triggered by the excessive nutrient loading and the consequent environmental degradation phenomena.

10.5 Mathematical models

In addition to the monitoring programmes and the establishment of geo-related data bases, the use of models is essential on the one hand for the coherence analysis of the available data and the understanding of phenomena, and on the other hand for the planning and the evaluation of the effectiveness of the management policies such as:

- point source control by means of sewage treatment and disposal;
- distributed source control by means of soil conservation techniques and fertiliser restrictions;

- inland water management;
- improvement of dispersion of nutrients in the coastal waters; - waste water reuse and recycling in aqua culture.

11. EXISTING NATIONAL AND INTERNATIONAL LEGAL MEASURES

11.1 National legal measures

The authors were able to record the following information for France, Italy and Spain. The new draft of the document will include information from all countries to be submitted by the MED POL National Coordinators.

France

- The law of May 2, 1930 relating to sites protection (Coulet, 1990);
- The law of December 16, 1964 relating to water regulation and the fight against pollution, that concerns the various categories of water and the control of pollution through the technique of discharge authorizations: besides, it aims, thanks to the receipt of fees by water Agencies from polluters, at financing equipment to fight against pollution;
- The law of July 10, 1976 relating to the protection of nature;
- The law of July 19, 1976 relating to classified installations for environmental protection grants administration with a police power in order to fight against damages provoked by polluting installations, especially industrial;
- The decree of November 25, 1977 relating to protection of biotopes;
- The law of January 7, 1983 on sea development schemes in order to settle fundamental policies of protection, exploitation and coastal improvement;
- The law of January 3, 1986, relating to the coast, establishes rules aiming at the protection of zones still spared by equipment;

  The decree of April 25, 1988 relating to natural regional parks.

  In addition special provisions have been adopted concerning products issued from the lagoons exploitation. Several texts define conditions of dispatching or selling to the consumers (decree of August 20, 1939, modified by the decree of June 12, 1969, decisions of October 20, 1976 and of December 21, 1979).

Italy

- Norms for prevention of water pollution (Merli bill) Law May 10, 1976, n. 319 (Official Gazette n. 141 of May 29, 1976) which concerns:
- Discipline for the effluents of any kind, public or private, direct or indirect, into surface and subsurface waters, internal or marine;
- Formulation of general criteria for the use and discharge of waters in relation to urbanisation;
- Organisation of public services for water distribution, sewers and water treatment.
- Formulation of a general water rehabilitation plan;
- Systematic monitoring of qualitative and quantitative characteristics of the water bodies.
- Rules for the protection of the sea (prevention and protection of the sea resources). Law December 31, 1982 n.470 (Official Gazette Ordinary Supplement n. 16, January 18, 1983, which concerns:
- Organisation of a sea environment protection service and coastal waters control;

- Organisation of a prompt intervention service for the protection of sea waters and coastal zones from pollution caused by accidents;
- Penal law norms against the discharge of forbidden substances from merchant ships;
- Institution of marine reserves.
- Urgent dispositions for the protection of areas of particular environmental interest. Decree June 27, 1985 n.312, converted into law August 8, 1985, n. 431 on the protection of coastal areas. (Official Gazette n.152, June 29, 1985)
  Regional Governments are urged to prepare urban and land use plans in order to define and to exploit areas of special environmental interest.
- Urgent dispositions for the containment of eutrophication phenomena.. Decree November 25, 1985 n.667, converted into law January 24, 1986, n. 7 on the limitation of nutrients load. (Official Gazette n.277, November 25, 1985).
  The law aims at the reduction of sea and lake eutrophication of anthropic origin on the basis of norms finalized at the reduction of phosphorous and of other eutrophying substances released by communities, agricultural and industrial activities and by promoting the use and the spread of appropriate water treatment plants.
- Institution of the Ministry of Environment and norms relevant to environmental damages. Law July 8, 1986 n.349 (modified by Law 305 of August 28,1989) (Official Gazette n.162, July 15, 1986).
  Scope of the Ministry is to assure, in a comprehensive framework, the promotion, the conservation and the rehabilitation of the appropriate environmental conditions essential for the fundamental interest of the Community and for the quality of life. In addition the Ministry must operate in order to preserve and exploit the national natural patrimony and to protect the natural resources from pollution.
- Dispositions for the fulfilment of obligations deriving from Italy being a member of EC.Law December 29, 1990 n.428 (EC Law for 1990 - Item 12 "environmental protection"). (Official Gazette n.10, January 12, 1991) concerning:
- Rehabilitation and conservation of environmental quality standards in order to protect the fundamental interests of the community, the quality of life, the conservation and valorisation of natural resources by means of:

  - measures for health and environment protection;
  - measures for environmental watch and control;
  - measures aiming to the prevention from environmental damages and restoration;
  - measures for the elimination, and recycling of polluting substances.

For a comprehensive bibliography see the publication edited jointly by CISDCE and MAE, 1991 and Capria, 1988.

Spain

- Spanish Shores Act (1988) aimed to:

  a) Define the portion of the cost which should be legally considered as public property and ensures its integrity and adequate preservation.
  b) Guarantee the public use of the sea, the shores and the remaining portion of the coastal public property.
  c) Regulate the rational use of said properties under terms which suit their nature and purpose and which respect the landscape, environmental and historical heritage.
  d) Achieve and maintain an adequate level of water and shores quality.

The Shores Act, therefore, does not regulate and order all aspects that affect the coastline. The distribution of responsibilities that the Spanish Constitution establishes in this respect between the various Public Administrations gives the arrangement for territorial and urban planning to the Regional Governments and to Town Councils (Montoya, 1991).

11.2 International legal measures

Information on the EC Environment directives can be found in Capelli and Friz (1987). More recently, the following directives were issued:

- Council Directive of December 12, 1991 concerning the protection of waters against pollution caused by nitrates from agricultural sources (Official Journal of the European Communities No L 375/3 - 31/12/91).

  This Directive has the objective of:

  - reducing water pollution caused or induced by nitrates from agricultural sources and
  - preventing further such pollution.

  The Member States are compelled to:

  - Identify the polluted water bodies;
  - Designate as vulnerable zones all the draining areas contributing to pollution.

In addition the Directive dictates a number of actions to be taken by the Member States in order to set up action programmes aimed at improving agricultural practices and taking additional measures and reinforced actions in order to meet the objectives of the Directive.

- Council Directive of May 21, 1991 concerning urban waste water treatment (Official Journal of the European Communities No L 135/40 - 30/5/91).

This Directive concerns the collection, treatment and discharge of urban waste water and the treatment and discharge of waste water from certain industrial sectors.

The objective of the Directive is to protect the environment from adverse effects of the above mentioned waste water discharges.

12. RATIONALE FOR ESTABLISHING CONTROL MEASURES

In order to establish control measures aimed to the reduction of the eutrophication phenomena, it is essential to recognise the following points:

(a) the phenomenon known as eutrophication is affecting, to a greater or lesser extent, many coastal areas all around the Mediterranean Sea, particularly in shallow or land-locked basins;

(b) the causes are the discharges into coastal waters, either directly or through the catchment basins, of substances originating from land, mainly nutrients (phosphorus, nitrogen, etc.) and biodegradable organic matter containing nutrients;

(c) the effects of intense events, even if temporary, often cause massive mortalities of marine organisms due to anoxia and consequent production of toxic H_2S, and a foul smell of the waters and shorelines due to decomposing materials, therefore seriously impairing the legitimate uses of the sea by threatening the living resources, the natural inheritance including genetic resources, and the recreational and aesthetic amenities;

(d) there is ample scientific evidence of the increase in the expanse and intensity of eutrophication in some areas that might also endanger natural equilibria in larger areas of the Mediterranean Sea,

(e) there are nevertheless clear gaps in the scientific knowledge of the important physical, chemical and biological processes which control the intensity of the phenomenon in the various areas,

(f) methods already exist for the abatement of the intensity and extension of the phenomenon through proper policy analysis and the use of legal, technical and other measures envisaged inter alia in the Mediterranean Action Plan.

13. RECOMMENDED ACTION

13.1 Monitoring

It is essential to extend the monitoring component of the Programme for Pollution Monitoring and Research in the Mediterranean (MED POL Phase II) within the framework of the Mediterranean Action Plan, to cover those areas showing clear signs of eutrophication, to cover the inputs of eutrophicants and the physical, chemical and biological parameters and variables cited in the guidelines. In establishing this extension, the spatial characteristics of the impacted areas, the ease of assessment, and the appropriateness of the measures taken should be considered.

In addition the monitoring of and research on eutrophication, which are being carried out or planned, should be mutually supportive and beneficial to each other.

13.2 Assessment of present status

It is suggested that a detailed assessment of the state and extent of eutrophication in the Mediterranean Sea is prepared by region giving special attention to the monitoring of coastal areas.

The ecological assessment of the state and the extent of eutrophication requires an investigation of community structure and diversity, which should consider the different compartments of plankton and benthos, and indicate the activity at different levels: species, populations and communities.

13.3 Inventory of land based sources

There is an urgent need for a survey and for the establishment of a geo-related inventory of land based sources to be linked to the monitoring programmes.

When producing an inventory of land based sources of pollution in the Mediterranean Sea attention should be paid especially to those substances which cause eutrophication and, whenever possible, to the effects they cause around the discharge sites.

13.4 Scientific action

It is necessary to complement the already ongoing monitoring and assessment efforts, and to provide scientific information as required for modelling and control policies, by conducting specific research focused on the following objectives :

(a) Factors controlling eutrophication processes;

(b) The structure and function of eutrophic ecosystems and the relevant hydrodynamics as the basis for the determination of their receiving capacities for eutrophicants;

(c) Classification of the stages and degrees of eutrophication on the basis of quantitative parameters;

(d) Investigation of the recovery processes in ecosystems that have been modified due to anoxia and mortalities induced by eutrophication;

(e) Further development of scientific methods as needed, particularly for the monitoring and ecological assessment programmes.

It is recommended that the policy analysis of eutrophication be strengthened in order to take into account the complex socio-economic, legal and political factors which influence both the perception of the problem and what should be done about it. Reliance on legal instruments alone, e.g. the protocol on land-based sources of pollution, in some cases may not be an effective means of managing the open-access common-property marine resources of the different parts of the Mediterranean.

It is also recommended that a problem-oriented approach be pursued in addressing particular cases of anthropogenic eutrophication.

Mathematical modelling should be used as a means for:

(a) co-ordinating the work of multi disciplinary teams of physical, chemical and biological specialists in the interpretation of the phenomenon;

(b) improving the design and operation of monitoring networks;

(c) testing control techniques.

However, mathematical modelling should not be regarded as a substitute for the scientific approach; on the contrary, an integrated programme of data collection, field and laboratory experimentation and modelling which addresses specific and concrete questions is the best way forward.

Appendix I

MEASUREMENTS OF BIOMASS

It is not the place here to enter into methodological questions of how to measure biomass in the aquatic environment. The reader can find pertinent information in several Handbooks. Nevertheless, it seems advisable to give at least some hints to current measurement concepts, as an comprehension of this matter is crucial from both, theoretical and practical considerations.

Among the respective problems to mention are those regarding, e.g., consistency, comparability and interpretability of data gathered. This entails several elements: a) statistical significance of the one sample; b) variability of replicates; c) variability of the surroundings; d) data cohesiveness over a larger spatial segments (which are either homogenous, inhomogeneous, or characterized by gradients); e) variability in time over the same spatial unity.

These points are pertinent all measurements, regardless of whether the biological entities in question are phytoplankton, macrophytes, zooplankton, bottom fauna, fish, bacteria, or else.

Further it is to be noted that non of the various measurements listed below is a perfect expression of biomass. Indeed, biomass per se is not measurable. Biomass can only by interpreted from substitute measurements of miscellaneous quality and comparability. Because of limited analytical capability (either in terms of instrumentation, manpower and logistic resources), actual measurements are often restricted to some components; others are estimated indirectly using conversion factors. This may, or may not be justifiable in the light of present knowledge. To note: simple conversion from one dimension to another does not add new information. Therefore uncritical use of converted data can be misleading.

The following are a view remarks to the most common measurement types and respective notions.

(a) Gross measurements
- (i) Counts and biomass volume
- (ii) Wet and dry weight

(b) Substitute measures
- (iii) Pigments
- (iv) Elemental composition (POC, PON, POP)
- (v) ATP
- (vi) Biochemical components

The classical methods to quantify biomass is counting of numbers of specimens per species found per unit of volume or surface. This presupposes, of course, identification of species. This latter is primarily a taxonomic problem that requires experience. Taxonomic expertise is diminishing among aquatic biologist; still, specialists in various fields of taxonomy exist around the world, which in case of uncertainty have to be contacted.

a) Plankton and bioseston

A common praxis to estimate partial phytoplankton volume per species is to multiply the number of cells or colonies counted per ml (or lt, or m^3) by the volume estimates of single cell units or colonies for each species. Single cell volume is obtained from (simple or composite) geometric approximations of the cell shape (spheres, ellipsoids, cylinders, cones, etc.). Unit measures are "u^3" (10^{-9} mm^3 or 10^{-12} cm^3). Counts are made using Utermöhl techniques (inverted microscope). The total phytoplankton volume is than obtained from summing all partial species volumes. Total volumes are in the order of cm^3/m^3 (approximately g/m^3 wet weight).

Dry weight is obtained from gravimetric measurements of aliquots of washed filtered or centrifugated phytoplankton sample of known total plankton volume, dried at 105 °C, and ash content from the sample incinerated at 450 °C to constant weight.

There are several limitations regarding these three fundamental biomass estimates. A main uncertainty about volume estimates regards the question whether one has sized correctly all component species as to occurrence and actual numbers present in the sample. Strongly buoyant species (e.g., many cyanobacteria), and very small species (picoplankton) are easily underestimated. Another source of error is large variability in cell numbers in colony forming species. Dry weight and ash content estimates can be affected by several circumstantial facts. Unlike laboratory batch cultures of which the species content is known, natural samples for the most are mixtures of active and inactive phytoplankton, dying more or less disintegrating cells, organogenic detritus, and mineral turbidities of various nature. Separation of these components is virtually impossible; hence, measurements obtained refer to the seston, not to phytoplankton.

Chlorophyll a is the most important reference pigment, as it is found in all photoautotrophic organisms. Other chlorophylls (b, c, d) are restricted to certain classes, as are a large variety of supplementary pigments known under the group name of xanthophylls, phycobilins and carotenes. Lutein, e.g. is found in chlorophyceae, chrysophyceae and rhodophyceae; myxoxanthophyll, e.g., is a component pigment in cyanobacteria; phycobilins are restricted to cyanobacteria and rhodophyceae; peridinin and other xanthophylls are found in dinophyceae, fucoxanthin and others in diatoms and phaeophyceae, etc. Carotenes are less class specific.

The analytical methodology for chlorophyll determination is well established, and it is now common praxis to determine chlorophyll a routinely. Values found rang in the order of 0 to over 200 mg/m^3. Nevertheless, chlorophyll measurements are not without problems. Apart of the diurnal and seasonal variation of chlorophyll per cell per species and origin, acetone extraction (which is commonly used) works well for certain algal groups, but extraction is often incomplete in certain chlorophyceae species. Further, the presence of degradation products (phaeophytin) may distort the values.

Accessory pigments are more difficult to measure routinely. In oceanography a complex is often measured under the term SPU, but consistent information on accessory pigments is largely lacking.

Determination of the particulate organic carbon, nitrogen and phosphorus (POC, PON, POP), respectively, is relatively easy and apt for routine measurements. POC, PON and POP are operational terms, and as measures refer to the (millepore filterable) total biogenic sestonic organic material. Biogenic organic seston enters the food-chain (zooplankton, detritus feeders), is settleable (by sedimentation), mineralizeable (through autolysis and bacterial activity), and its oxygen consumption potential can be estimated relatively reliably.

Adenosine-Tri-Phosphate (ATP) ATP is practically found only in living cells, and represents a measure of stored biologically available energy, and hence, of active biomass. The ratio between organic cell carbon and ATP is fairly constant in unicellular and multicellular organisms (bacteria, phytoplankton, zooplankton), varying around 265. Therefore, ATP is not a specific measure for phytoplankton, but if the bulk of zooplankton can be filtered off, and the bacteria content vis-a-vis that of phytoplankton is small, it gives a fairly reliable measure of the active particular organic carbon (aPOC) in phytoplankton even in the presence of high amounts of detrital POC.

Main biochemical components. Information about the biochemical composition of sestonic material is important for evaluating its nutritional value. There is a large number of biochemical components, such as proteins, carbohydrates, lipids, amino acids, RNA, DNA, o.a., that can be measured directly by standard biochemical analytical techniques. Total proteins are often estimated indirectly from PON, using a factor of 6.25. Carbon in proteins is about 3.3*PON. Carbohydrates (present as assimilates and storage products, as components in cell walls (crude fibres), and as gelatinous involucra of cells or colonies) plus lipids are estimated by difference. However, the relative composition in these main compartments depends on a number of factors: species specificity, age, activity phase, nutritional conditions, etc., and therefore can vary significantly. Therefore, interpretation of indirect estimates has to be made with caution.

Beside the components mentioned above, knowledge about, and measurement of other biochemic compounds, such as toxins produced by algae, have become over recent years of increasing practical interest. This field is in full progress of development (cf. Chapter 3.6).

b) Macrophytes and macroalgae

Much of what has been said above applies also to these categories albeit with variation in methodology and relative importance. Still, the quantitative assessment of macroalgae and macrophytes is by far more difficult than the quantification of phytoplankton, and hence, the respective figures are subject to more uncertainties.

c) Zooplankton, nekton and bottom fauna

Biomass assessment of the typical zooplankton (like e.g. *Calanus*) follows similar principles as exposed for phytoplankton, except, of course, that there is no pigment, like chlorophyll, common to all component species, which could be used as a crude mean to quantify the amounts of zooplankton present.

Macro-nekton, like nektic Cnidaria (Medusae), etc., and the vast array of bottom fauna require special techniques that are not of direct interest in the context of the present report. For the assessment of biomass of pelagic fish, instead, sonar techniques have given reliable figures.

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