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THE PRESENCE OF THE TROPICAL ALGA *CAULERPA TAXIFOLIA* IN THE MEDITERRANEAN SEA

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INTRODUCTION

Since 1984, an alga of tropical origin, *Caulerpa taxifolia*, has been spreading rapidly in the northwestern Mediterranean. From 1992 onwards, 150 scientists from 34 Mediterranean research centres have been studying this phenomenon, within the framework of national programmes and a large programme of the European Union (Directorate General XI "Environment, nuclear safety and civil protection"). The results of these studies illustrate an unprecedented environmental risk for the Mediterranean.

These results are summarized in this document. There are also proposals for the formulation of a strategy to control the phenomenon. The document was prepared for the United Nations Environment Programme, Coordinating Unit of the Mediterranean Action Plan by Professor Charles-F. Boudouresque (University of Aix-Marseille, France), Dr. Panayotis Panayotidis (National Centre for Marine Research, Greece) and Vincent Gravez (GIS Posidonie, France).

It is divided into two parts:

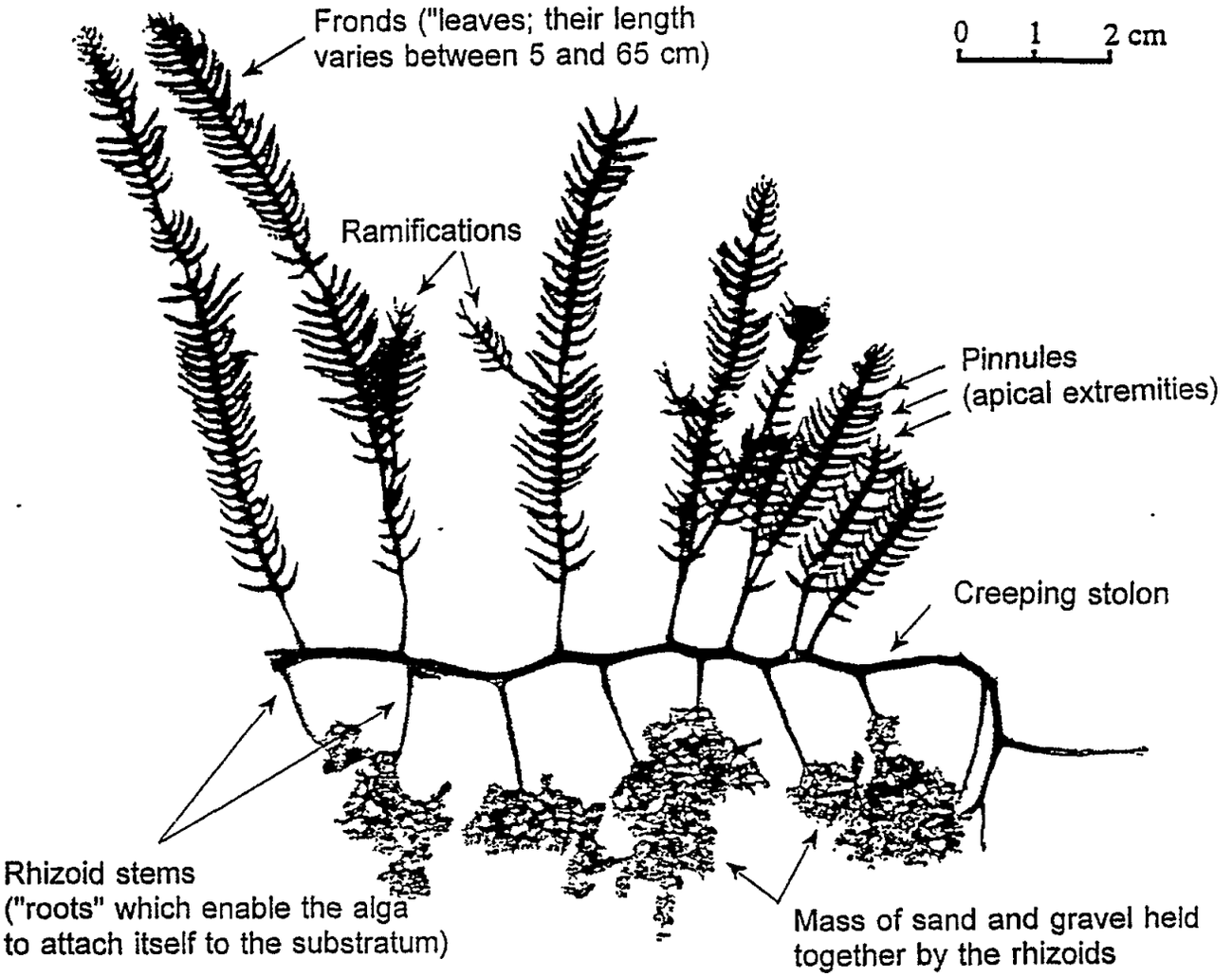
I - The current situation, which is a synthesis of the data on the spreading of the tropical alga *Caulerpa taxifolia* in the Mediterranean. The data have been collected within the framework of national research programmes (France, Italy, Spain) and the international programme of the European Union (LIFE, DG XI).

II - Proposals for a control strategy, which outline the main axes of an international strategy to prevent and/or slow down the spreading of *Caulerpa taxifolia* in the Mediterranean. These proposals reflect the personal views of the authors and not necessarily the official policy of the United Nations Environment Programme.

I - THE SITUATION

Caulerpa taxifolia, the rapid spreading of an alga in the Mediterranean

The alga *Caulerpa taxifolia* (Fig. 1) was observed for the first time in the Mediterranean offshore Monaco in 1984. In 1990, it was found for the first time in France, at Roquebrune-Cap Martin (Alpes-Maritimes; 5 km East of Monaco), where reportedly local divers had spotted it since 1987. In the same year, 1990, *Caulerpa taxifolia* was spotted at Toulon (Var). From that point on, it spread rather rapidly. In 1991, it was spotted at Cap d'Ail and Menton (Alpes-Maritimes), at Saint Raphaël and Lavandou (Var) and at Saint-Cyprien (Pyrénées-Orientales). In 1992, it was spotted in Italy at Livorno and Imperia (Porto Maurizio) in the Alpes-Maritimes at Villefranche-sur-Mer, in the Var at Hyères, Six-Frour-les Plages and Saint-Cyr-sur Mer and also at Cala Dor (Majorca, the Balearic Islands). In 1993, *Caulerpa taxifolia* was spotted in Sicily at Messina, near the island of Elba, in various ports of Liguria (San Remo, Bordighera, Diana marino, San Bartolomeo al Mare) and in the Alpes-Maritimes at Saint-Jean-Cap-Ferrat and Théoule-sur-Mer. In 1994, it was spotted at Cannes (Alpes-Maritimes), at Hyères (Pesquiers and in the National Park of Port-Cros; Var), at Capo Berta, Ventimiglia, Marina d'Andora in the Italian district of Liguria. In January 1995, *Caulerpa taxifolia* was spotted for the first time in the Adriatic, in Croatia (Figs. 2 and 3). Generally speaking, probable installation of the alga precedes by 1-3 years the date of first sighting.



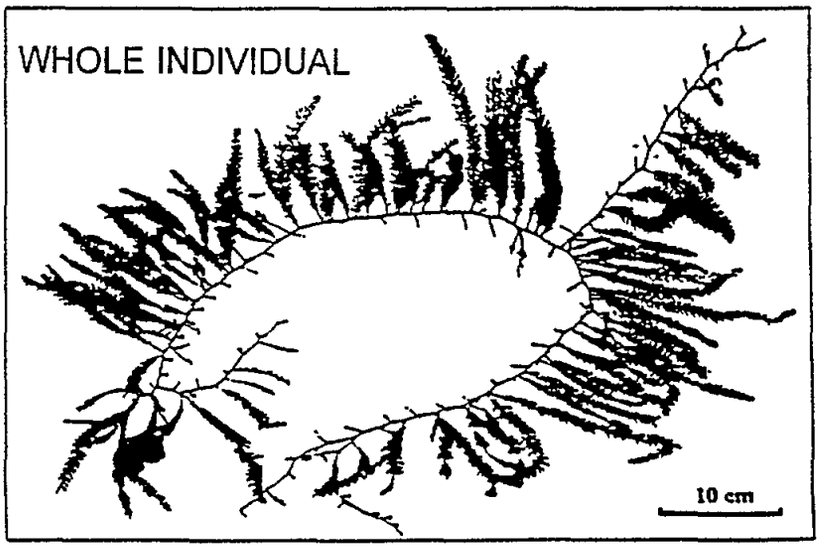


Fig. 1 General appearance of the tropical alga *Caulerpa taxifolia*. (After Meinesz et al., 1995)

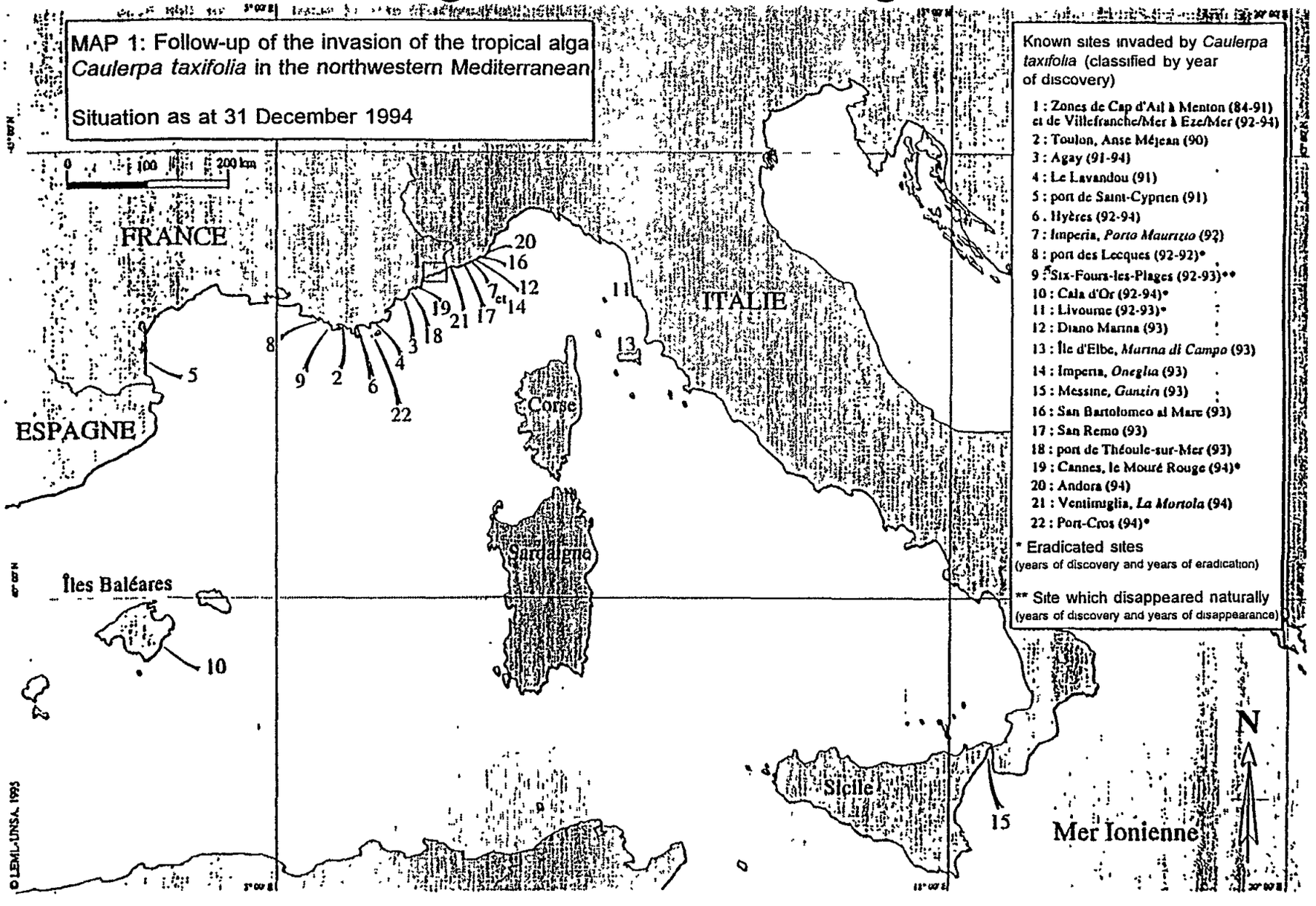


Fig. 2 Spreading of *Caulerpa taxifolia* in the Mediterranean at the end of 1994. (After Meinesz et al., 1995)

The area occupied by *Caulerpa taxifolia* was of the order of 1 m² in 1984. The affected area was estimated to be 3 ha in 1990, 30 ha in 1991 and 470 ha in 1992. In 1993, the affected area was estimated to be 1300 ha and at the end of 1994 approximately 1500 ha (Fig. 4). The latter figure does not however reflect a slowing down of the rate of spreading; within the "partially colonized" areas, the rate of occupation has progressed and in the oldest colonized area (between Villefranche-sur-Mer and Menton), the alga has reached its maximum expansion and cannot therefore continue to progress (Fig. 5). In the recently colonized regions (Var, Italian Liguria, Elba island, Messina) even if the progression rate is very rapid, the areas affected are still relatively small. From 1984 to 1994, the ratio of annual progression of the total covered area was of the order of 6.

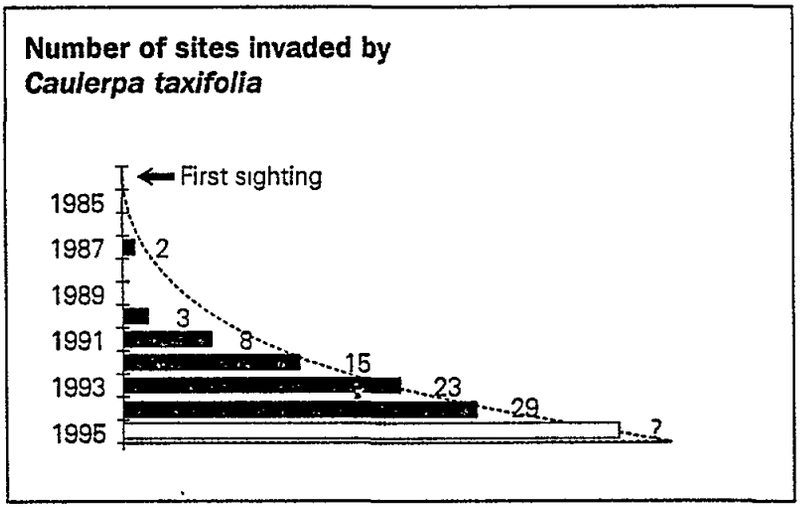


Fig. 3 Number of sites where *Caulerpa taxifolia* has been spotted since 1984. The full number of areas affected in the Mediterranean is certainly not yet known

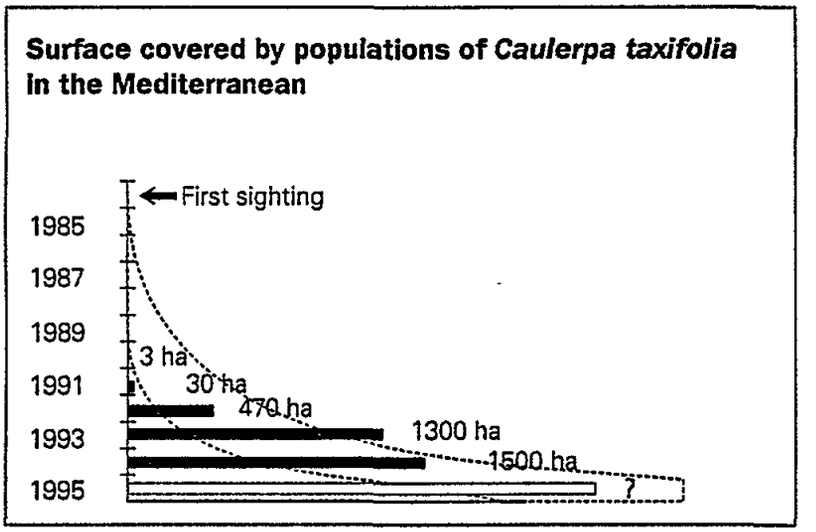


Fig. 4 Surface area covered by populations of *Caulerpa taxifolia* in the Mediterranean since the time of first sighting in 1984 (in hectares)

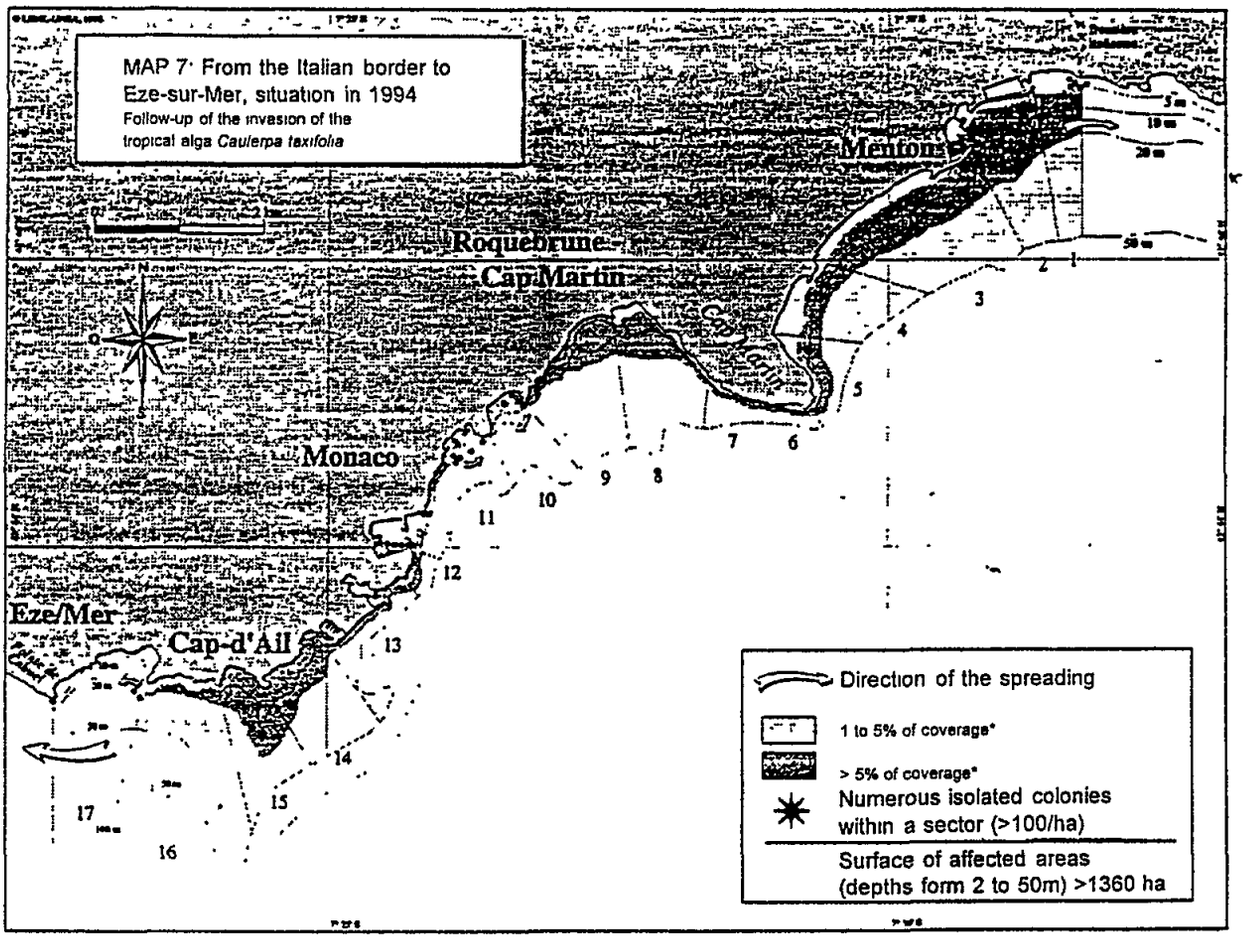


Fig. 5 State of *Caulerpa taxifolia* spreading in the most heavily colonized area (Eze-sur-Mer to the Italian border) in 1994. (After Meinesz et al., 1995)

No slowing down in the advancement of *Caulerpa taxifolia* has yet been evidenced; no natural regression, even localized, has been spotted, with the exception of the Brusc lagoon (Var), where a small patch, sighted in 1992 did not survive the very low winter temperatures.

The new *Caulerpa taxifolia* colonies have always been observed at depths between 1 and 14 m. Initially they spread along the coast. Then they spread towards greater depths. Short distance spreading is carried out mainly through cuttings hydrohynamically transported (sexual reproduction has not been observed so far). This phenomenon is important, since a small fragment of this alga is sufficient for the formation of a new patch; at that point, the advancement becomes very rapid (Fig. 6).

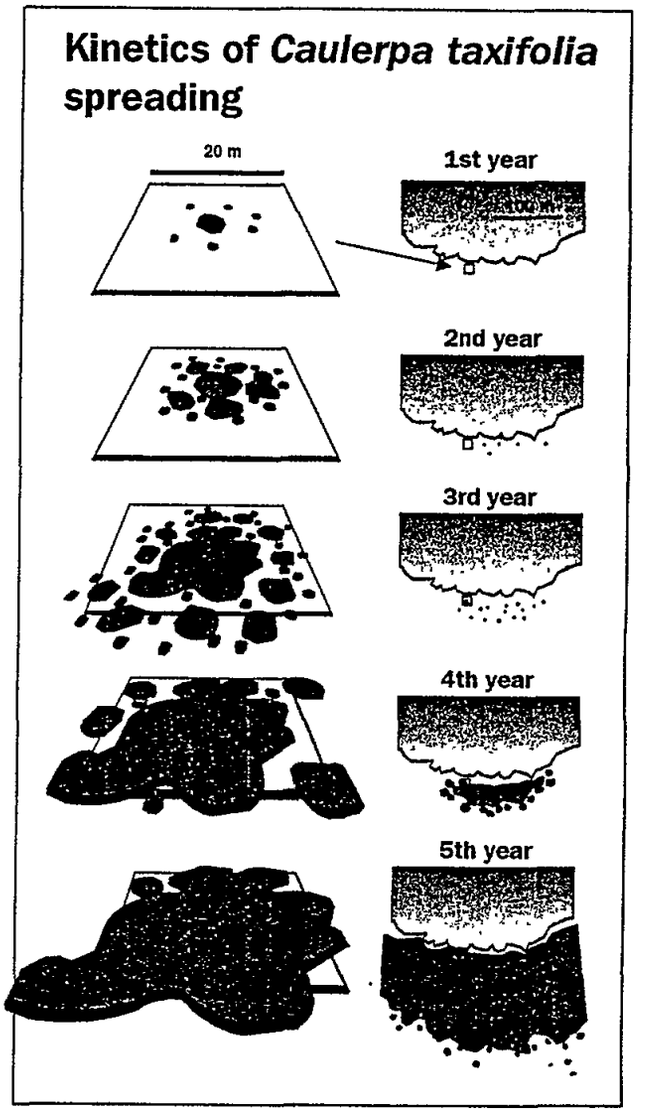


Fig. 6 Kinetics of *Caulerpa taxifolia* spreading in the Mediterranean, starting from an isolated patch. This diagram was elaborated on the basis of observations carried out at Cap-Martin (Alpes-Maritimes; Meinesz et al., 1995)

However, this diagram, illustrating the spreading of *Caulerpa taxifolia* through the dissemination of thallus cuttings near existing patches, cannot account for the presence of that alga in all the sites and especially those far away from the sites where *Caulerpa taxifolia* settled many years ago (Monaco, Alpes-Maritimes) and where it forms large populations.

It is noteworthy that these far away sites, currently colonized, are either mooring sites for pleasure craft or small fishing ports. This observation gave rise to the working hypothesis that the alga could spread along great distances because of fragments which remain on fishing nets or anchors of boats.

In order to test this hypothesis, research into the resistance of *Caulerpa taxifolia* to drying was carried out (box 1, p.20). The results obtained agree with the assumption which explains the dissemination of the alga to great distances, for instance its arrival at Hyères, the Balearic islands, the island of Elba, within the National Park of Port-Cros or in Croatia. Therefore rapid spreading throughout the Mediterranean is possible and must be examined with the greatest possible care.

The identity of *Caulerpa taxifolia*

The genus *Caulerpa* comprises over one hundred species living in the temperate and especially the tropical seas. *Caulerpa taxifolia* is a green alga widely distributed throughout the tropical seas : it is found in Brazil, Venezuela, Colombia, Costa Rica, Antilles, the Gulf of Guinea, the Red Sea, Somalia, Kenya, Tanzania, Madagascar, Maldives, Seychelles, Pakistan, India, Sri Lanka, Bangladesh, Malaysia, Indonesia, Philippines, Vietnam, China, Japan, Hawaii, Fiji, New Caledonia, Australia, etc. (Fig. 7). We are certain that two species of *Caulerpa* are indigenous to the Mediterranean: *Caulerpa prolifera* and *Caulerpa olivieri*. Three *Caulerpa* species are lessepsian immigrants, i.e. Red Sea species that have entered the Mediterranean through the Suez Canal: *Caulerpa scalpelliformis* (Turkey and the Levantine coast), *Caulerpa mexicana* (Levantine coast) and *Caulerpa racemosa*. *Caulerpa taxifolia* is not a Mediterranean species; it was never observed before 1984 (cf. box 2, p.20).

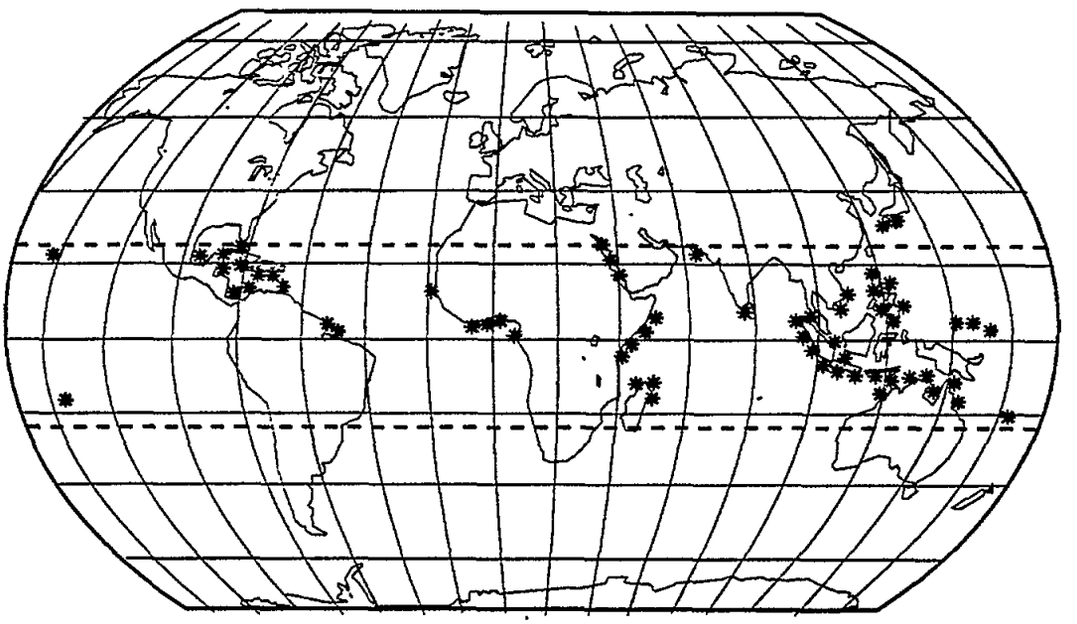


Fig. 7 The distribution of *Caulerpa taxifolia.* Note that the world distribution of this species is basically limited to the intertropical regions

How did *Caulerpa taxifolia* arrive in the Mediterranean ?

Even though no hypothesis can be discarded *a priori*, it is likely that the *Caulerpa taxifolia* a colonizing the Northwestern Mediterranean have come from tropical aquaria. Indeed, since about 1980, this alga has been used for decorative purposes in many tropical aquaria in Germany, France and later in Monaco. At that time, *Caulerpa taxifolia* was sold in France and Spain in shops specializing in aquaria or from catalogues. The discovery of tropical corals (*Porites somaliensis*) at the centre of the small patch of *Caulerpa taxifolia* which grew at the bottom of a jetty at the Lecques port (Var, France) - which was subsequently eradicated - confirms the possibility that the contamination started from the dumping of the contents of tropical aquaria into the sea.

Ecology of *Caulerpa taxifolia*

The strain of *Caulerpa taxifolia* which colonizes the Mediterranean has some unusual morphological and physiological characteristics with respect to those inhabiting the areas of origin. For instance, the average length of fronds (="leaves") ranges in the tropical seas, from 2 to 15 cm, whereas the fronds in the Mediterranean can exceed 60 cm in length; in autumn 1993, fronds of even 80 cm long were observed. In the Mediterranean, the *Caulerpa taxifolia* meadows may be of exceptional density (up to 14000 leaves/m^2), whereas in the tropical seas they are very sparse.

Research has been carried out on the growth of the Mediterranean strain of *Caulerpa taxifolia* as a function of light: the results show that there is adaptation to various lighting conditions in the marine environment and that moreover *Caulerpa taxifolia* can survive up to a depth of 90 m in the clearer waters of the central Mediterranean. Observation campaigns using a video camera or by means of the Griffon submarine have shown that *Caulerpa taxifolia* can be found, attached to the substrate in a depth of up to 100 m in the Alpes-Maritimes, which is totally coherent with the results of laboratory experiments.

The growth curves as a function of water temperature show adaptation of this strain to a large spectrum of temperatures. Lethal temperatures have been measured in the laboratory: <+7°C and >+30°C. Generally speaking, both growth and development increase as a function of water temperature. The growth of stolons (=creeping stems/stalks) begins in May-June (13.5 to 16°C). Optimal conditions are observed between 20°C and 30°C and it is in August-September that their growth is the fastest (5-14 mm/day). Over a one-year period total growth of a single stolon ranges between 88 and 186 cm. 350 m of stolons per m^2 have been measured which is an amazing figure.

The temperatures compatible with the development of *Caulerpa taxifolia* show that it is possible for this alga to due to a very cold winter or a warming of the water (in the Mediterranean, in the open sea, water temperature ranges between +10°C and 28°C) and is very likely and that *Caulerpa taxifolia* has great capacity for living in most of the Mediterranean. This is an amazing conclusion concerning a "tropical" alga, as it confirms that the strain that colonized the Mediterranean displays characteristics which are very different from those of the tropical regions of its origin.

No link seems to exist between the localization of the *Caulerpa taxifolia* populations and water quality; field observations (divers, video camera towed by oceanographic vessel) have confirmed the lack of connection between the *Caulerpa taxifolia* distribution and pollution sources (the mouth of underwater outfalls, ports).

Experiments have shown that enrichment with nutrients increases the productivity of *Caulerpa taxifolia* by a factor of 1.5 to 2.5 in April and September, when the productivity of the controls (Mediterranean algae) is at its annual minimum. In the other seasons growth is not limited by the nutrients, regardless of their levels. This is a surprising result, since nutrients are a limiting factor in the summer for Mediterranean algae when on the other hand temperature and light are best for growth. Eventhough no convincing explanation has been advanced so far for this behaviour of *Caulerpa taxifolia*, this could prove to be an important parameter for understanding the success of *Caulerpa taxifolia* in its competition with most of the Mediterranean algae and phanerogams.

The various biological characteristics of *Caulerpa taxifolia* (adaptation to a wide range of light intensities, temperature, types of substrates and rapid growth) indicate that it is likely to colonize several types of seabed in the Mediterranean. In fact it colonizes all types of substrate: rock, sand, mud, dead *Posidonia* "matte", *Posidonia* meadows (cf. box 4, p.21).

How can the particular characteristics of the *Caulerpa taxifolia* meadows in the Mediterranean be explained?

Generally, when an exotic species is introduced into a region, it finds itself away from all its natural enemies (diseases, parasites, predators, competitors) which normally limit its spreading in the area of origin. Therefore, in the case of *Caulerpa taxifolia*, the phenomenal success of its establishment can be explained first and foremost by the absence of factors limiting its spreading. Indeed, no predator (herbivore species) has so far been observed to limit its development and the indigenous plant species show no ability to compete with it in such a way as to confine it to a specific ecological niche.

However, it must also be added that *Caulerpa taxifolia* shows extraordinary vitality in the Mediterranean. There are various working hypotheses to account for this vitality and for the particular characteristics of the alga vis-à-vis those of the tropical populations.

Concerning the probable origin of its introduction, it is likely that the strain of *Caulerpa taxifolia* which colonizes the Mediterranean is the result of genetic modifications (cf. box 3, p.20) which occurred in aquaria to adapt to the particular conditions of temperature, light, and water quality for instance. Such phenomena of artificial modifications of the genetic make up of individuals are relatively well known and are sometimes induced in experiments for scientific or commercial purposes, since they are likely to provide species of commercial interest with the vitality of hybrids.

Impact of *Caulerpa taxifolia* on the natural environment

When *Caulerpa taxifolia* establishes itself on seabeds dominated by algae (generally implanted on rocks), a drastic decrease of algal populations is observed: most species tend to disappear and only a few (entrenched) species seem to resist longer. The decrease is greatest when the vitality of *Caulerpa taxifolia* is also greatest (summer-fall): its creeping stolons and leaves screen off the light and trap the sediment, the substrate becomes muddy and the other algae practically disappear.

When *Caulerpa taxifolia* invades such a population, its creeping stolons and rhizoids rapidly form a compact cover which traps the sediment and stops the light. The rock gradually becomes inaccessible to other organisms that would attach themselves to it. When the *Caulerpa taxifolia* meadow has been established to the detriment of the other algae, its cover and biomass remain rather stable throughout the year. Such dominant colonization leads to considerable decrease in terms of biodiversity.

The rate of impoverishment of a population colonized by *Caulerpa taxifolia* reaches 75% if we count only the main algae of the original Mediterranean plant cover (Fig. 8). From a quantitative point of view, most of the indigenous algae regress and tend to disappear as the drastic decrease of their biomass, which can reach 100%, clearly shows.

In this way, more than twenty communities and facies dominated by algae may be replaced by a monotonous and paucispecific *Caulerpa taxifolia* meadow. If it is taken into consideration that the sublittoral level, which generally is the northwestern Mediterranean extends from approximately the middle level of the sea to 30-40 m of depth, being the habitat of most algae, it becomes clear that if the spreading of *Caulerpa taxifolia* progresses unchecked, it would lead to the great scarcity of many species. The possibility cannot be excluded that some species, endemic in the Mediterranean and established in the sublittoral level, may be threatened with extinction: this is especially the case of several species of the *Cystoseira* genus for which protection has been sought (UNEP-IUCN-GIS Posidonie, 1990).

At the same time, the small invertebrate fauna which live in the Mediterranean algal populations have been strongly disrupted by the installation of *Caulerpa taxifolia.*

Observations on Mollusca, Amphipoda and Polychaeta show that their respective populations have been markedly reduced in numbers of individuals in the *Caulerpa taxifolia* meadow vis-à-vis the indigenous populations. As with algal species, the number of Polychaeta and especially Amphipoda species decreased; on the other hand, the species diversity of Mollusca may increase (Figs. 9-11).

Furthermore, *Caulerpa taxifolia* competes with the *Posidonia oceanica* meadows, which is one of the most important ecosystems of the Mediterranean (cf. box 4, p.21). However, the settling of *Caulerpa taxifolia* in the *Posidonia oceanica* meadows is less rapid than in algal populations on rocky substrate. Indeed, the density o the bundles of Posidonia leaves is considerable (up to 800 bundle/m^2) and the leaves which are long in the spring do not make up a favourable environment for the colonization of *Caulerpa taxifolia* which then colonizes only the sparser areas of the Posidonia meadow and establishes itself sporadically within the dense meadow. In the fall however, the Posidonia leaves are shorter and the *Caulerpa taxifolia* fronds attain maximum development; this means that the long fronds of the *Caulerpa taxifolia* patches established in the dense meadow screen off the light and this has a negative impact on the growth of the young Posidonia leaves. At that point, the direct competition between these two plants (for space and light) turns to the advantage of *Caulerpa taxifolia.*

When *Caulerpa taxifolia* and Posidonia are in direct competition, the size thickness, number and the longevity of the Posidonia leaves all decrease. Necrotic areas appear on the Posidonia leaves and the stems which bear the leaves die.

COMPARISON OF SPECIES RICHNESS AND TOTAL NUMBER OF INDIVIDUALS IN REFERENCE POPULATIONS AND IN THE CAULERPA TAXIFOLIA MEADOWS

Reference population

Caulerpa taxifolia population

ALGAE

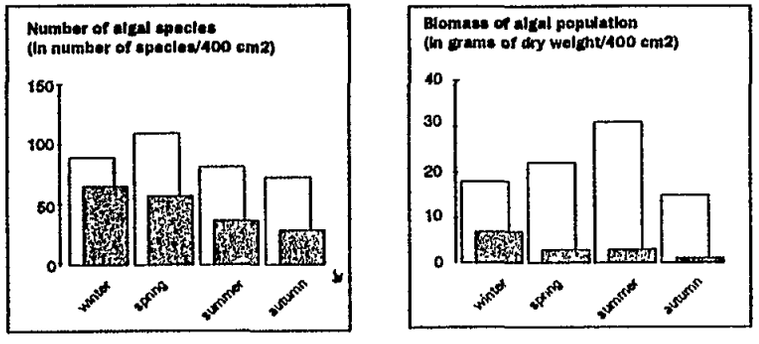


Fig 8 Comparison of species richness and algal biomass in reference populations and the *Caulerpa taxifolia* meadows in the Mediterranean. (Data from Verlaque *et al.*, 1994)

POLYCHAETES

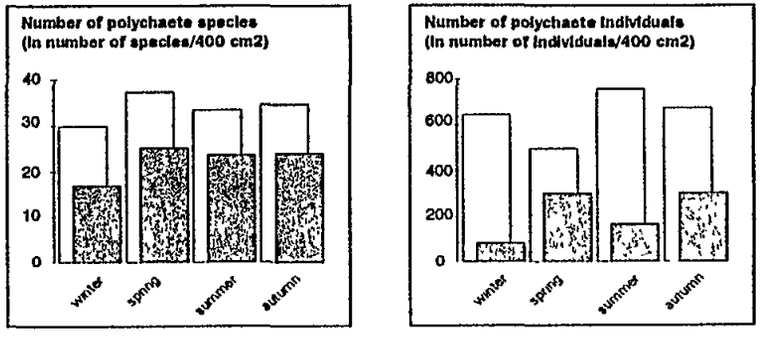


Fig 10 Comparison of species richness and number of polychaete individuals in reference populations and the *Caulerpa taxifolia* meadows in the Mediterranean. (Data from Bellan-Santini *et al.*, 1994)

MOLLUSCS

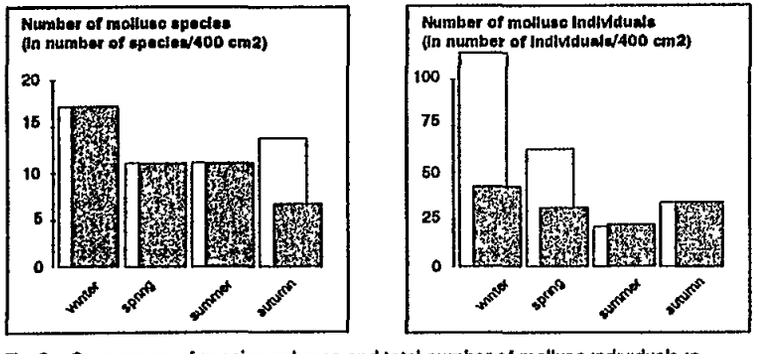


Fig 9 Comparison of species richness and total number of mollusc individuals in reference populations and the *Caulerpa taxifolia* meadows in the Mediterranean. (Data from Bellan-Santini *et al.*, 1994)

AMPHIPODES

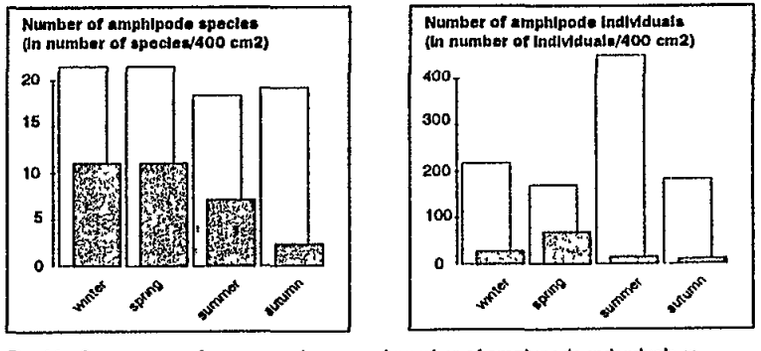


Fig 11 Comparison of species richness and number of amphipode individuals in reference populations and the *Caulerpa taxifolia* meadows in the Mediterranean. (Data from Bellan-Santini *et al.*, 1994)

The competitive success of *Caulerpa taxifolia* vis-à-vis indigenous species is without doubt due to its size, the density of the populations it establishes, the rapid growth rate, the physico-chemical and biotic changes it brings about, but also due to the toxic metabolites it synthesizes.

Toxicity of *Caulerpa taxifolia*

Several plant species and among them many algal species synthesize toxic substances which protect them from predators - herbivores - or competitors. Such metabolites are well known in the Caulerpa genus. Researchers have identified, for *Caulerpa taxifolia*, 9 toxic substances (cf. box 5, p.22) of which Caulerpenyne, specific to the Caulerpa genus, is predominant. It makes up 0.3 to 1.3% of fresh weight in the Mediterranean strain of *Caulerpa taxifolia*, but only 0.15% in tropical caulerpas.

However, the effects of the minor toxic compounds should not be overlooked; their impact on living organisms may be specific and/or synergistic. Thus it seemed important to assess the risk for human health and the physical environment. An extraction and dosage method has been developed to quantify caulerpenyne (down to very low levels of concentration: 5 ng per sample).

The concentration of caulerpenyne produced by *Caulerpa taxifolia* ranges between 3 mg/g of freshweight in the spring and 13 mg/g in the summer and goes down to 7 mg/g in the autumn. We have confirmed in aquarium conditions that the production of caulerpenyne increases as temperature goes up. Such studies in aquaria have shown that caulerpenyne can be diffused in seawater. However, this has not yet been confirmed in the natural environment.

To test the toxicity of a substance, experimental "models" are used (cells or live organisms) to study the response to different compounds (and varying doses). The metabolic extracts of *Caulerpa taxifolia* are active on the fibroblasts of the hamster, on mice and on the eggs of the sea urchin *Paracentrotus lividus*. It has also been shown that certain ciliates[1] are sensitive to low doses (0.5-1.0 µg/ml) of the various terpenes synthesized by *Caulerpa taxifolia*; it is thought likely therefore that in regions densely populated with *Caulerpa taxifolia*, there is a gap in the food chain leading to large size species.

It is probable that it is the presence of these secondary toxic metabolites which explains why the two main macro-herbivores of the Mediterranean, the fish *Sarpa salpa* and the edible sea urchin *Paracentrotus lividus*, strongly avoid *Caulerpa taxifolia* in summer and autumn. In those two seasons, when the toxicity of *Caulerpa taxifolia* is greatest, *Paracentrotus lividus* only eats *Caulerpa taxifolia* if no other food is available. The food rations, measured during experiments, progressively decrease to zero; absorption rate is then extremely low and sea urchins die after 3 months.

In spring and winter, when the toxicity of *Caulerpa taxifolia* is lower, *Paracentrotus lividus* eats this alga, but after a few weeks there is a negative physiological impact. The general weakening of the animals, in addition to the general antimitotic activity of

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[1] Ciliates are mono-cellular organisms at the very base of marine food chains and for that reason they play an important role - eventhough not fully understood yet - in the way ecosystems work.

caulerpenyne on sea urchin eggs (preventing the division of the eggs during the various phases of reproduction) could affect the recruitment and density of *Paracentrotus lividus* in the sites invaded by *Caulerpa taxifolia*. Such a population decrease was indeed observed in an area heavily colonized by *Caulerpa taxifolia*: Cap Martin between 1993 and 1994 when the number of sea urchin individuals decreased by three quarters. In such cases sea urchins crowd in areas that have not yet been settled by *Caulerpa taxifolia* (such as algal populations, Posidonia patches); and thus overgrazing takes place which may indirectly contribute to the spreading of *Caulerpa taxifolia*.

We should note however, that in sea urchins that have eaten *Caulerpa taxifolia* no accumulation of caulerpenyne has been found at least not in the gonads which are the part eaten by humans, eventhough caulerpenyne was detected there.

Concerning fish populations, after a three-year follow-up and more than 1000 counts carried out by divers *in situ*, it appears that the average number of species, the number of individuals, the biomass and the average weight per individual are significantly lower, both in the fall and in the spring, in the *Caulerpa taxifolia* meadows.

Finally, no serious irreversible damage, appears in the animals that are forced to consume *Caulerpa taxifolia*, during the weeks that experiments last; however, organ lesions and localized pathological phenomena become apparent which might mean that, in the natural environment, the animals become weaker and thus more vulnerable to either pathogens and/or predators.

Is there a risk for humans ?

For the moment, no potential toxicity risk for humans has been proven. Certain species of Caulerpae are eaten in some parts of the world (*Caulerpa lentilifera* and *Caulerpa racemosa*, mainly in Asia), but *Caulerpa taxifolia* is not known for its organoleptic qualities which *a priori* excludes the risk of intoxication by ingestion.

For the moment, no toxin accumulation risk along the food chain has been proven; the herbivore species which humans consume, mainly *Sarpa salpa* and the edible sea urchin *Paracentrotus lividus*, avoid *Caulerpa taxifolia* and in any case eat it only during the season when it has low toxin concentration. Todate, no case of intoxication could be blamed on *Caulerpa taxifolia*.

However, we should not forget that our knowledge of the toxins which are metabolized by *Caulerpa taxifolia* is still incomplete; most research has so far focused on the main toxin, Caulerpenyne. The impact of the other toxins, as well as the impact of the byproducts of their degradation must be investigated while bearing in mind that the quantities of synthesized metabolites are of fundamental importance; in spring, when the biomass of *Caulerpa taxifolia* reaches its peak, 70 kg of caulerpenyne are found in each hectare of meadow densely populated with *Caulerpa taxifolia*.

Finally, let us stress that to determine whether a substance is dangerous one should not take into consideration only its potential acute toxicity; several substances are currently banned because of chronic toxicity risks.

The most serious risk is upsetting the ecological balance, which is illustrated by the dominant and vigorous characteristics of *Caulerpa taxifolia*. Most of the types of coastal seabed found in the Mediterranean are in danger of being colonized by *Caulerpa taxifolia*.

The dominance of the populations it forms along with the drastic decrease in the indigenous populations, both in terms of biodiversity and ecodiversity as well as in terms of species of commercial interest must be seriously considered.

If the spreading pattern currently observed on the French Côte d'Azur were to become generalized throughout the Mediterranean it would lead to a major upset of its ecosystems. Such a scenario which predicts spreading to the whole Mediterranean coast and a large-scale environmental catastrophe is, on the basis of current knowledge quite plausible; it is known, in different parts of the world, such cases of spreading, which continues for a long period until all areas favourable to the particular species, have been invaded (e.g. the case of the water hyacinth *Eichhornia crassipes*).

A worst case scenario: its environmental, social and cultural consequences

It has thus become apparent that the current studies on the phenomenon must continue; several research goals must be pursued as a matter of priority: the characteristics of the species, its environmental needs, its physiology, the limiting factors existing in the Mediterranean, the toxicity and fate of its metabolites and the behaviour of the new ecosystem created by *Caulerpa taxifolia*. Moreover, the monitoring of the spreading at international level should continue by calling on the public to report the presence of *Caulerpa taxifolia*, as well as through active research in potential sites of introduction (anchoring areas, fishing ports).

Will *Caulerpa taxifolia* continue spreading ?

The future development of the *Caulerpa taxifolia* populations in the Mediterranean cannot be predicted as yet:

(1) Natural regulation may occur in the future, for instance in the form of a predator whose population may explode in a few years. Indeed, it is not rare that species introduced into a new area, after an initial phase of spectacular spreading, settle down and become integrated into the indigenous ecosystems. In such a case, it is possible for the algae populating on rocky substrates to recover; on the other hand, the destruction of *Posidonia oceanica* meadows must be considered irreversible on the human life scale (it is known that natural regeneration of such meadows is extremely slow and takes several centuries).

(2) Spreading may continue; since *Caulerpa taxifolia* is a tropical alga and the Côte d'Azur not the warmest area in the Mediterranean, one can imagine that it may overrun the whole Mediterranean and even that it will spread at a faster rate now that it has reached warmer waters (Balearic islands and the South of Italy). One should also keep in mind that one of the vectors of its dissemination along great distances, yachting, is a leisure activity in boom; the Mediterranean coasts are linked with one another in a network of maritime routes heavily travelled in the summer. More over it is likely that certain countries are already contaminated by *Caulerpa taxifolia* without anyone having become aware of it yet.

One question that can be raised now is the following: the strain of *Caulerpa taxifolia* which colonizes the Mediterranean and which seems to be original, dow not have the ability to colonize other regions of the world and especially the tropical seas?

Is there a risk that another species will be introduced into the Mediterranean and create a similar problem ?

Caulerpa taxifolia is not the first alga accidentally introduced into the Western Mediterranean since the beginning of the 20th century. Some have not caused problems, but were integrated into the indigenous ecosystems and occupied a precise and limited niche. However, never before did a species introduced into the Mediterranean have all the characteristics of *Caulerpa taxifolia* : dominance, toxicity, occupation of all the biotopes of the sublittoral areas, absence or scarcity of predators, longevity, etc.

However, it is true that the case of *Caulerpa taxifolia* must be put into the general context of species' introduction. Indeed there has been a tremendous increase of this phenomenon in the Mediterranean since the beginning of the '60s (Fig. 12). Just for the Mediterranean, it is estimated that approximately 350 species have been introduced, which means 3 to 7% of the total species, depending on the taxonomic group considered. For instance, just for plant species, the introduction rate since the beginning of the century can be adjusted to an exponential model. According to this model the number of species introduced would, by the year 2050, range between 250 and 1000. If the latter figure were correct, the number of plant species introduced into the Mediterranean would equal the number of indigenous species (Boudouresque & Ribera, 1994).

It is imperative today to reverse this trend if we do not wish to see in the 21st century a uniformity at world level of underwater communities and landscapes which would have incalculable consequences for the coastal Mediterranean populations. In the Mediterranean, the present national and international legislative provisions are totally inadequate to deal with the current risks of species' introduction (aquaculture, aquaria, ballast waters). Such legislation ought to be revised urgently in order to delay the rate of introductions.

The problems of species introduction can be extremely costly: a committee of the U.S. Congress determined that since the beginning of the century the species introduced into that country (both into the terrestrial and the marine environment) have cost U.S. $ 97 billion of damage. It has also been calculated that 15% of the species introduced into the U.S. have caused economic or environmental damage. For these reasons, some countries like the U.S. and Australia have enacted very strict legislation against the risks of species' introduction.

II - PROPOSALS FOR A STRATEGY TO CONTROL THE SPREADING OF *CAULERPA TAXIFOLIA* IN THE MEDITERRANEAN

On the occasion of the "International Seminar on *Caulerpa taxifolia*" held in Barcelona in 1994 which concluded the study programme sponsored by the European Union, the participating scientists adopted a text (cf. Annex I) which concludes as follows: "Scientists carry out research and undertake the responsibility to alert the authorities. It is now up to the governments of the countries concerned and the international organizations in the field of environmental protection to implement the precautionary principle (referred to in the Biodiversity Convention of Rio de Janeiro[2]) and to define a coherent international strategy suited to the problem".

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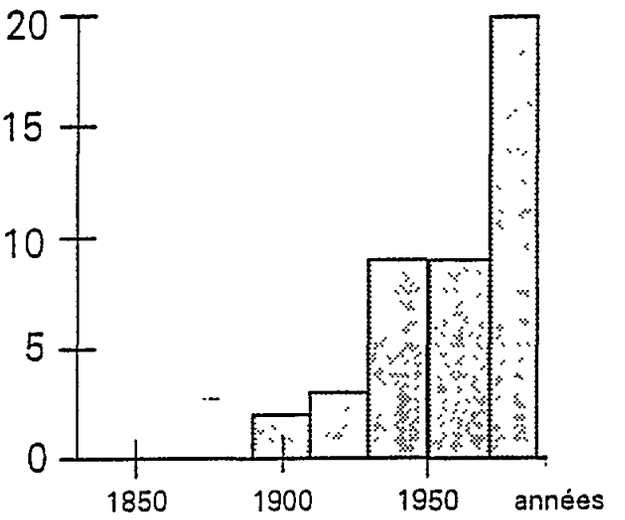
[2] «... *when there is the threat of considerable decrease or loss of biological diversity, the absence of total scientific certainty should not be advanced as a reason for postponing the adoption of measures which would prevent the danger or mitigate its effects, ...*» From the Preamble to the Convention on biological diversity, UNEP, Rio de Janeiro, 5 June 1992.

Number of macro-algae introduced into the Mediterranean
(lessepsian species included)



Number of invertebrates introduced into the Mediterranean
(lessepsian immigrants excluded)

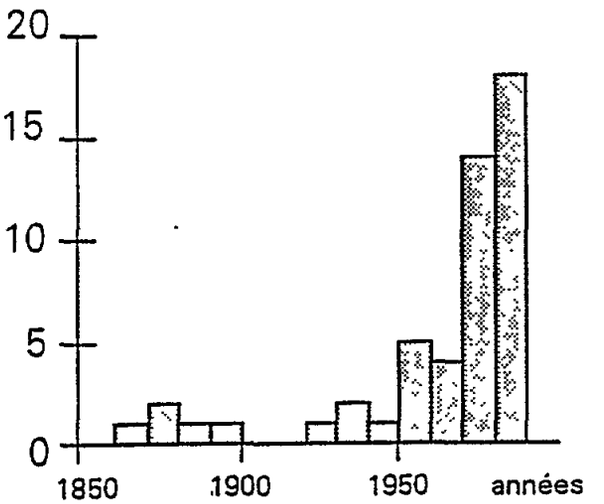


Fig. 12 Above - Number of macro-algae introduced into the Mediterranean (lessepsian species included). Bellow - Number of invertebrates introduced into the Mediterranean (lessepsian immigrants excluded). Note the dramatic increase since the '50's. (After Boudouresque et Ribera, 1994)

A - Prevention measures

Obviously it is not scientists that make the decisions. However, there is a consensus on the strategy that should be adopted at least in the short term: measures to control the spreading of *Caulerpa taxifolia* must be closely associated with concrete preventive measures which would aim at:

(i) preventing new phenomena of contamination from private or public aquaria; and

(ii) preventing *Caulerpa taxifolia*'s dissemination over great distances from existing populations.

In order to prevent new phenomena of contamination, it is imperative that all coastal Mediterranean States adopt legislation banning the selling, buying, transport and possession of *Caulerpa taxifolia* (similar to the ones adopted in Catalunya and France; cf. Annex II). Such legislation should be accompanied by real, concrete measures; indeed almost two years after such legislation was enacted in France and Catalunya, *Caulerpa taxifolia* can still be bought there. It is obvious that the banning measures adopted should receive the widest possible publicity.

In order to avoid long distance dissemination from already existing stations, it is important on the one hand to inform yachtsmen of the need to clean the anchor of their boat immediately after mooring in a contaminated area, and on the other hand to ban or regulate the mooring of boats in the most contaminated sites. Moreover, in such sites fishing activities (trawler or nets) should be prohibited.

Information material for the public at large exists already: it should be translated into the various Mediterranean languages, published and disseminated widely by the authorities in charge of marinas and fisheries. Information should also be disseminated by the specialized media. At the same time, spotting of *Caulerpa taxifolia* in sites that were not contaminated before should be brought to the attention of either the relevant authorities or the laboratories already involved in the relevant research programmes. Furthermore, appeals should repeatedly be made to the members of national federations and clubs of scuba divers, as well as through the specialized press to monitor the spreading of *Caulerpa taxifolia*, while insisting on precise localization of findings.

B - Strategy for slowing down the rate of spreading

Finally, it is imperative to implement a strategy for slowing down the rate of spreading throughout the Mediterranean Basin, through eradication programmes of the smaller patches already localized or those that will be discovered outside the heavily contaminated areas of the Menton - Cap d'Ail sector (Eastern part of the Alpes-Maritimes, France) and of Imperia (Italy).

On the basis of the techniques currently available, total eradication of the *Caulerpa taxifolia* strain which colonizes the Mediterranean is not realistic. Manual eradication by divers, successfully implemented at Mallorca in the Balearic islands, is the only technique which has been effective against populations covering a small surface.

For the moment no method provides total and definitive eradication or is effective for surfaces larger than one hectare; effective eradication methods are at the experimental stage and should be accordingly funded, always under scientific control. A scientific and technical mission should be entrusted with the task of reviewing the various techniques for the control of species spreading used around the world and determine their adaptability for possible use against *Caulerpa taxifolia*. Finally, it is important to continue follow-up programmes on the plant's spreading, as well as the on-going research programmes which might lead to suitable population control techniques: characteristics of the Mediterranean strain, environmental requirements, physiology and modalities of regeneration, multiplication and reproduction, limiting factors in the Mediterranean and among the natural populations.

Certain general principles have already been established:

(i) it is useless to eradicate a patch partially: lateral recolonization from the non-eradicated part is very quick;

(ii) since eradication cannot ever be total, regular revisiting of the eradicated areas is mandatory in order to eliminate regrowth;

(iii) manual uprooting is particularly well suited (speed, cost) to areas where most of the *Caulerpa taxifolia* populations have been previously eradicated (perhaps through another method).

The teams of underwater divers (National Marine, professional divers) which might be used for eradication must be reviewed. Firstly, and before any intervention they must receive some specific training. Specifications guaranteeing the quality of eradication and its follow-up must be adopted. The responsibilities (at decision-making levels) of managing the budgets, technical coordination and prevention and eradication operations must be clearly defined. Adequate financial instruments must be put in place.

It is crucial to insist on good coordination between the time of discovery of new *Caulerpa taxifolia* patches and the time of eradication. From experience it is believed that the newly sighted patches have already been in existence for 2-3 years. Under the best conditions, the newly discovered patch covers an area of several square metres. The investment needed to eradicate it grows proportionally to the speed of *Caulerpa taxifolia* development (annual spreading of approximately a factor of 6).

The measures to be adopted by the countries within the framework of the Barcelona convention must be effective in a very short time; each spring the speed with which the *Caulerpa taxifolia* populations grow increases the intervention cost to control the phenomenon. The multiplication of movements of pleasure craft in the summer increases the risks of dissemination in the coastal areas that are not monitored. We estimate that the geographical distribution of the *Caulerpa taxifolia* in the Mediterranean is currently relatively well known; it is quite probable that by the year 2000, the situation will be totally out of control.

A timetable for the actions proposed above must therefore be adopted by the different states. Finally, an international workshop seminar, whose principal objective would be to define the practical modalities of the strategy to control the spreading of *Caulerpa taxifolia* in the Mediterranean must be organized.

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BOX 1

Under the conditions of desiccation prevailing on board a ship (70-75% air humidity, temperature 22-24°C), the resistance of *Caulerpa taxifolia* is of short duration: *Caulerpa taxifolia* has very few chances of surviving transport in the open air which lasts for more than an hour. However, in desiccation conditions prevaling in an anchor casing for instance (obscurity, 85-90% air humidity, 18°C temperature), *Caulerpa taxifolia* resists well for more than 10 days. If it is re-immersed in the water it survives and can then grow. Thus the long distance transport of living fragments of *Caulerpa taxifolia* in anchor casings, but also in fishing nets or a diver's bag appears possible.

BOX 2

Natural migration ?

The great distance that separates the newly colonized sites in the northwestern Mediterranean from the tropical areas (natural distribution) shows that the entry into the Mediterranean of *Caulerpa taxifolia* as a natural migration of the species is unlikely.

Caulerpa taxifolia could not have entered the Mediterranean through Gibraltar; in fact the alga is not found in the neighbouring Atlantic regions and no intermediate station exists (between Gibraltar and the French Côte d'Azur) to validate such an itinerary.

The lessepsian origin (which refers to the species introduced through the Suez Canal from the name of the engineer who oversaw its opening, Fernand Lesseps) of *Caulerpa taxifolia* is equally unlikely: (i) more than a century after the opening of the Canal, no lessepsian alga has yet been found west of Sicily; (ii) the itinerary followed by lessepsian plants can be retraced with the help of several intermediate stations which is not the case with *Caulerpa taxifolia*; finally (iii), the characteristics of the Côte d'Azur *Caulerpa taxifolia* seem to be closer to those of the populations of the tropical Atlantic areas in the Americas than to those of the Indo-Pacific populations. The possibility that it was introduced into the Mediterranean as an individual attached to the hull of a ship (as is perhaps the case with very small species) does not appear likely.

BOX 3

A hybrid vigour...

Often, during hybridization, the addition of two different chromosomatic stocks, gives the new organism a vitality which is superior to that of the parents: this property is called "hybrid vigour".

There are several possible explanations for the exceptional vitality (hybrid vigour) of the strain of *Caulerpa taxifolia* found in the Mediterranean; this strain may come from:

- a selection by man (while collecting it in its original natural environment) of a particular strain, whose properties (e.g. aesthetic aspect, rapid growth, resistance to thermic shocks, etc.) were appreciated by those dealing with aquaria;

- a mutation, i.e. accidental modification of the genetic material (information contained in the DNA) of a spontaneous or provoked character by mutagenic agents (ultraviolet rays or chemical substances for instance). Indeed, it is common practice to use ultraviolet light in aquaria for protection against parasites; mutation could thus have been provoked by such mutagenic agents;

- hybridization, i.e. crossing in the aquarium of two strains of *Caulerpa taxifolia* of different origin, or of *Caulerpa taxifolia* with another species of the Caulerpa genus which might have produced a new organism (=hybrid) bearing the original characteristics;

- polyploïdal process, i.e. the modification of the genetic material through addition of a new batch of chromosomes (2n -> 3n, 4n, 5n, etc), either through accidental multiplication of the *Caulerpa taxifolia* chromosomes (autopolyploïds 2n -> 4n, 8n, etc) or through hybridization with a different strain or species then through polyploïdal process (allopolyploïdal process).

These various hypotheses can only be validated through genetic studies on the Mediterranean strain of *Caulerpa taxifolia* and comparison with the tropical strains.

BOX 4

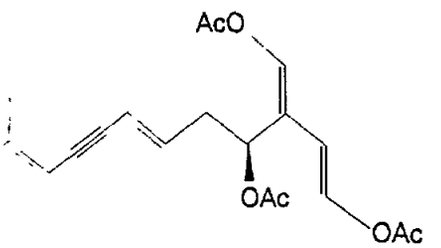
The Posidonia meadows

Posidonia oceanica is a flower-bearing plant which forms very large underwater meadows between the surface and 30 to 40 m in depth. Such underwater meadows are protected by a Directive of the European Union and regional and national legislations (European Directive "on habitats" of 21 May 1992 - 92/43/CEE; Decree for the protection of *Posidonia oceanica* in France and regional legislations in Valencia and Catalunya); *Posidonia oceanica* plays a fundamental role in the balance of the Mediterranean sea (high rate of biological production, nursery for the juveniles of many species, high biodiversity, etc.). As with forests on land, the *Posidonia oceanica* meadows must be protected as a heritage common to the Mediterranean countries. The spreading of *Caulerpa taxifolia* represents a major risk for the Posidonia meadows.

BOX 5

A cocktail of toxins

Caulerpenyne is a major secondary metabolite of *Caulerpa taxifolia*; it is an acetylenic sesquiterpene specific to the Caulerpa genus.



$C_{21}\ H_{26}\ O_6$

Caulerpenyne may have the following effects:

- antibacterial, antiviral, antifungal;
- cytotoxic (toxic for cells);
- ichtyotoxic (toxic for fish);
- repellent or anti-appetizing for herbivores (chemical defense).

Eight more metabolites are synthesized by *Caulerpa taxifolia*:

- Oxytoxin 1 ;
- 10-11 Epoxy-caulerpenyne ;
- Taxifolial A ;
- Taxifolial B ;
- Taxifolial C ;
- Caulerpenynol ;
- Taxifolial D ;
- Taxifolion ;

which could be either precursors or derivatives of caulerpenyne.

The above metabolites are found in smaller quantities, but some are very toxic. Moreover, the by-products of the degradation of all these metabolites have not yet been sufficiently studied; they should constitute one of the priorities for future research projects.

ANNEX I

«The Barcelona Appeal»

*The Barcelona Appeal**

Caulerpa taxifolia: a major risk for the coastal Mediterranean ecosystems is confirmed.

In ten years the tropical alga *Caulerpa taxifolia* has widely spread in the Mediterranean. At Monaco, where it was observed for the first time and in the Eastern section of the Alpes-Maritimes (in the South-East of France), the algal populations have reached (non-continuous coverage of the substrate) nearly 1350 hectares. In Italy, 150 ha are now affected in the vicinity of Imperia. Outside these areas, nearly 15 smaller sites have been discovered (ranging from tens of m^2 to 1 ha), but each one of these colonies spreads at the same rate as that of the first sites affected. These colonies are spread along the Northern coast of the Mediterranean, from the Baleanic Islands to Sicily. All these far-flung colonies are probably due to the transport of thallus cuttings in the anchor casings of pleasure craft or fishing equipment.

The alga grows on all types of substrate (rock, sand, mud, meadows) at depths ranging between 1 and 30 m. It has also been found at -30m down to -99m, but the density is much lower. It adapts to all environmental conditions (in waters far from any source of pollution but also in harbours) and to all coastal configurations (in front of capes, in sheltered bays). It can survive for 3 months at a 10°C temperature. This physiological characteristic distinguishes the *Caulerpa taxifolia* introduced into the Mediterranean from the *Caulerpa taxifolia* of the tropical seas. Its survival and development in the Mediterranean are not therefore linked with the possible warming of the waters or climate.

The spreading of the alga continues until it covers the whole substrate. This permanent vegetation rapidly displaces most of the other alga and modifies the Posidonia meadows. The fauna associated with the original vegetation undergoes profound changes: regression of certain species, which works in favour of other species. From a global point of view in the ecosystems which are typical of the Mediterranean and have been taken over by *Caulerpa taxifolia*, a decrease in biodiversity is evident.

The alga contains toxins that play a repelling role vis-à-vis the herbivorous fauna and may have an impact on the spores, eggs, microflora and microfauna. The environmental impact is amplified by the dominant character of the alga.

This is a summary of the results of four years of research which were just presented at the Second International Workshop on *Caulerpa taxifolia*.

Even if we cannot yet predict all the consequences of the spreading of *Caulerpa taxifolia* in the coastal Mediterranean environment, and even if some assumptions would not be confirmed by later research, the data collected to date confirm that there is a major risk for biodiversity, ecological balance and natural resources.

Scientists carry out research and undertake the responsibility to alert the authorities. It is now up to the governments of the countries concerned and the international organizations in the field of environmental protection (UNEP, Barcelona Convention, IUCN etc.) to implement the precautionary principle (referred to in the Rio de Janeiro Convention) and to define a coherent international strategy suited to the problem.

Barcelona, 16 December 1994

* Text adopted in Plenary Assembly by the scientists participating in the Second International Workshop on *Caulerpa taxifolia* convened at Barcelona, Dec. 15-17, 1994.

The Steering Committee for the LIFE Programme of DG XI

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ANNEX II

Decree concerning the fight against the species *Caulerpa taxifolia* (France)

Decree of 4 March, 1993 concerning the fight against
the species *Caulerpa taxifolia*
NOR: ENVN9320129A

The Minister for the Environment and the State Undersecretary for the Sea,
Considering Book II of the Rural Code on the protection of nature, especially articles L.211-1, L.211-2 and L.212-1;
Considering the opinion of the National Council on the protection of nature,

Decree:

Art. 1 - At all times and on the whole of the French territory the offering for sale, the selling, buying, use and dumping into the sea of all or parts of the specimens of the alga *Caulerpa taxifolia* (Vahl) C. Agardh is prohibited.

Art. 2 - At all times and on the whole of the French territory, the collection and transport of all or parts of the specimens of the alga *Caulerpa taxifolia* (Vahl) C. Agardh are subject to a system of authorizations granted by the relevant department in application of Art. R.212-2 of the Rural Code.

Art. 3 - Each application for authorization contains:
- the identity of the petitioner;
- the names(s) of the person(s) entrusted with the operation;
- the parts of the plant collected;
- the quantity envisaged (number of stems or patch surface etc.);
- the precise location of collection;
- the collection method envisaged;
- the dates and hours scheduled for the operation;
- the method, length and conditions of transport;
- the destination of material collected and commitment of the petitioner to destroy it.

Art. 4 - The provisions of this decree shall apply for a period of 5 years from its publication.

Art. 5 - The Director of nature and landscapes and the Director of maritime fisheries and cultures are entrusted, each within the sphere of his competence, with the enforcement of this decree, which shall be published in the Official Journal of the French Republic.

Done at Paris, 4 March 1993

The Minister for the Environment
For the Minister and on his authority:
The director of nature and landscapes
G. SIMON

The State Undersecretary for the Sea
For the State Undersecretary and on his authority:
Because the Director of marine fisheries and cultures
was prevented (from signing), the Deputy Director
B. BOYER