



# United Nations Environment Programme



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> ENGLISH Original: FRENCH

# MEDITERRANEAN ACTION PLAN

Workshop on invasive Caulerpa species in the Mediterranean

Heraklion, Crete, Greece, 18-20 March 1998

# THE PRESENCE OF INVASIVE CAULERPA SPECIES 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, nearly 250 scientists from 63 research centres and Mediterranen organizations have joined efforts in studying this phenomenon, within the framework of national programmes and two large projects supported by the European Commission (LIFE programme of Directorate General XI). A large bibliography is now available on *Caulerpa taxifolia* (Boudouresque *et al.*, 1998); furthermore, it is likely that never in the past has another invasive marine species been so widely documented.

Caulerpa racemosa (Forsskål) J. Agardh (Fig. 1) is a pan-tropical (temperate-warm) species widely distributed throughout the world (Fig. 2). In the literature, this species is described as having several different varieties and forms, which in fact could be distinct species as implied in some recent studies (Benzie et al., 1977). C. racemosa is expanding in the Eastern Mediterranean and recently it has been observed in Genova, Italy and Marseilles, France.

# Distribution

The first sighting of *Caulerpa racemosa* in the Mediterranean was made by Hamel (1926) who collected it in the port of Sousse in Tunisia. Later, it was spotted in Lebanon (Rayss, 1941), Egypt (Aleem, 1950, 1992), Syria (Huvé, 1957), Israel (Rayss & Eldestein, 1960) and Turkey (Cirik and Ozturk, 1991). *C. racemosa* is widely distributed in the Red Sea (Nasr, 1947). *C. racemosa* has a wide distribution in the Red sea (Nasr, 1947). From the beginning of the 90s, in addition to the discovery of new sites in the Middle Eastern countries (Fig. 3), *C. racemosa* is discovered in the lagoon of Bizerte (North of Tunisia, Djellouli *et al.*, in press) in Sicily (Alongi *et al.*, 1993), Sardinia, Tuscany (Piazzi *et al.*, 1994; Cossu & Gazale, 1997), Liguria (on the Italian side) (Bussoti *et al.*, 1997) and in Marseilles France (Gravez *et al.*, unpublished; Fig. 4). The frequency of recent discoveries, the distance form the Eastern Mediterranean colonies and the small size of the new colonies seem to indicate a rapid spreading of the distribution areas of the particular species in the Mediterranean.

The alga Caulerpa taxifolia (Fig. 5) was observed for the first time in the Mediterranean in 1984 at Monaco. 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, it was spotted in another French region, the Var, at Toulon. From that point on, it spread rather rapidly. In 1991, it is again spotted in several sites in France in the Alpes Maritimes, Var and Pyrénées-Orientales near the border with Spain. In 1992 while new sites are discovered in France, Caulerpa taxifolia is spotted, for the first time in Italy (Livorno and in Tuscany) and in the Balearic islands in Spain (Majorca, Cala d'Or). In 1993 it is spotted in Sicily (Messina), near the island of Elba, while new sites are discovered in the Italian and French sections of Liguria. In January 1995, Caulerpa taxifolia is spotted for the first time in the Adriatic, in Croatia. In the beginning of 1997, 5 countries are affected by this phenomenon (Monaco, France, Spain, Italy and Croatia). Seventy seven sites in all are counted (double the 1994 figure) and it is very likely that several colonies have not been discovered as yet (Figs 6-7). Corsica, continental Spain and Sardinia, do not seem to be affected from what is known so far. The sites, where Caulerpa taxifolia was found are basically west of Monaco, in mooring sites and marinas and to the East in fishing areas and fishing ports. The far away sites (Balearic islands, Croatia) are also ports and mooring areas (Meinesz et al., 1997).



Caulerpa racemosa

Fig. 1 General appearance of the alga Caulerpa racemosa

The area occupied by *Caulerpa taxifolia* was of the order of 1m² in 1984. The affected area was estimated to be 3 ha in 1990, 30 ha in 1991, 470 ha in 1992, 1300 ha in 1993 and 1500 ha in 1994. In early 1997, the total affected area was estimated to be 3100 ha (Fig. 8). Fourteen years after the probable date of introduction, 99% of the areas colonized by *Caulerpa taxifolia* cover a region extending between Toulon in France and Alassio in Italy. In the regions colonized first (between Villefranche-sur-Mer and Menton), the alga has reached its maximum spreading and can no longer spread further (Fig. 9). In all other sites, the progression rate of colonization is considerable and no slowing down is for the moment observed. No natural regression, even if only localized, has ever been observed.

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 toward greater depths. Short distance spreading is carried out mainly through cuttings transported by currents (the possible success of sexual reproduction has not been established as yet). Reproduction through cuttings is important, since a small fragment of this alga is sufficient to form a new colony; at that point the expansion rate of this new colony becomes very high (exponential growth of a factor between 2 and 10; Fig. 10). After about 10 years, when the expansion area has reached the deeper limit of the spreading of the alga (between -40 and -50 m), there is a levelling off in the annual growth of the affected area. In effect, colonization can only spread horizontally on both sides of the affected area (Fig. 11), the progression being faster on the side under the dominant current.

It is noteworthy that these far away sites, currently colonized, are either mooring sites for pleasure craft marinas, fishing areas or fishing ports. This observation gave rise to the working hypothesis that the alga could spread in areas far away from other colonies due to fragments which remain on fishing nets or anchors of boats.

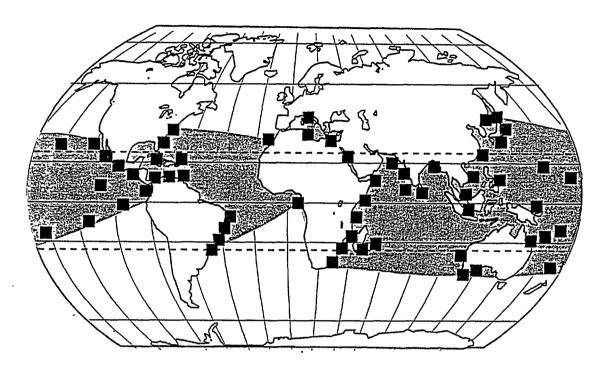


Fig. 2 World distribution of Caulerpa racemosa sensu lato (after Verlaque, unpublished).

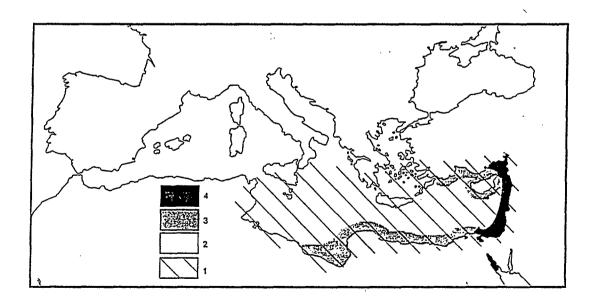


Fig. 3 The lessepsian province. The relative importance of lessepsian immigrants is shown by different density lines (After Por, 1978).

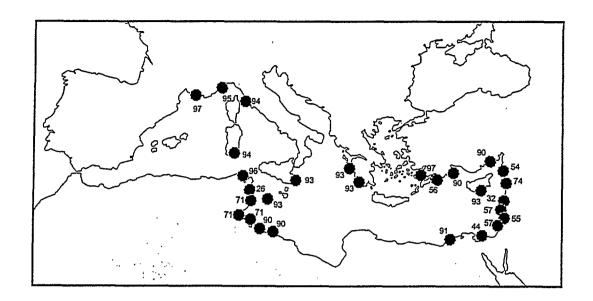


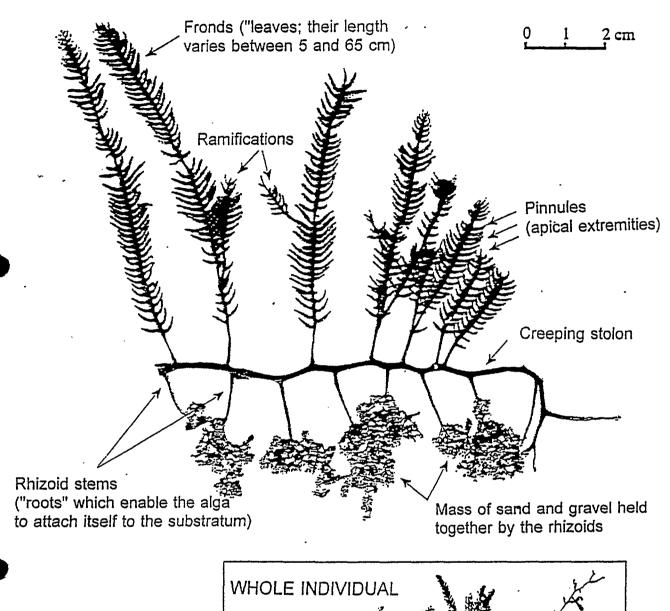
Fig. 4 State of Caulerpa racemosa spreading in the Mediterranean. The station of Marseilles, France is new.

In order to test this hypothesis, research into the resistance of *Caulerpa taxifolia* to drying was carried out. 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.

Regardless of how far back or how exactly *C. racemosa* was implanted in the Mediterranean, the rapidity with which is has spread in the Mediterranean should be explained.

The success of plant migrants in implanting themselves in a favourable environment depends on their competitiveness vis-à-vis other species (interspecies competition), their resistance to grazing (predation) and the existence of vacant ecological niches. Verlaque (1994) states that the plant species, which were introduced into the Mediterranean and are a nuisance, have one or more of the following characteristics: large ecologic valency, population persistence, high degree of competitiveness, resistance to grazing, high rate of reproduction or high rate of multiplication (e.g. many gametes, intense propagation through cuttings). *C. racemosa* has all of these (cf Cossu & Cazale, 1997).

On the other hand, the kinetics of spreading depend on the dissemination vectors. The dissemination pattern for macrophytes slowly slowly resembles rather well that of the surface water circulation (Verlaque, 1994). The fact that *C. racemosa* has appeared in sites far away from well known colonies suggests the presence of anthropogenic vector such as mooring equipment of boats and ships (Cossu & Gazale, 1997; Djellouli *et al.*, in press), and shellfishgrowing activities (Djellouli *et al.*, in press). Moreover, introduction through aquarium-related activities, perhaps as in the case of *C. taxifolia*, remains a possibility, since *C. racemosa*, *C. taxifolia* and *C. ertularioides* (Gmelin) Howe, are all caulerpa species used frequently in aquaria (Verlaque pers. comm.).



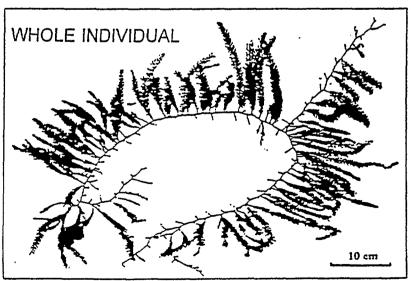
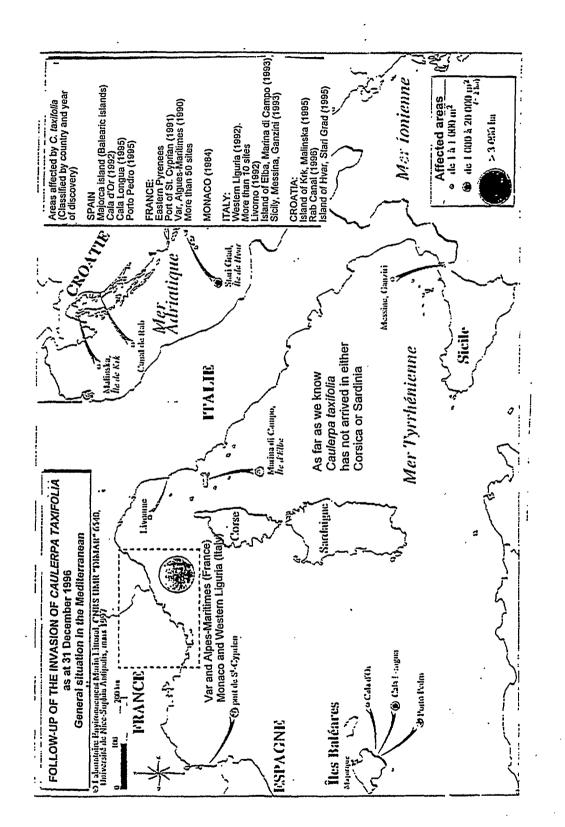


Fig. 5 General appearance of the tropical alga Caulerpa taxifolia (After Meinesz et al., 1997).



Spreading of Caulerpa taxifolia in the Mediterranean at the end of 1996 (After Meinesz et al., 1997). Fig. 6

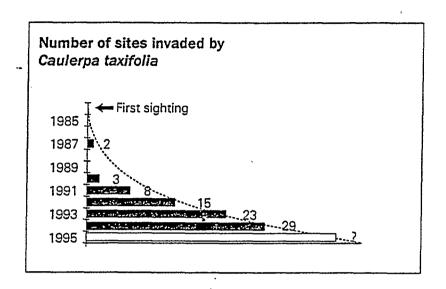


Fig. 7 Number of sites where *Caulerpa taxifolia* has been spotted since 1984. The total number of areas affected in the Mediterranean is certainly not yet known (After Meinesz et al., 1997).

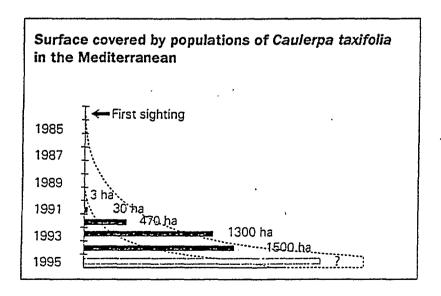


Fig. 8 Surface area covered by populations of *Caulerpa taxifolia* in the zones affected since 1984 (After Meinesz *et al.*, 1997).

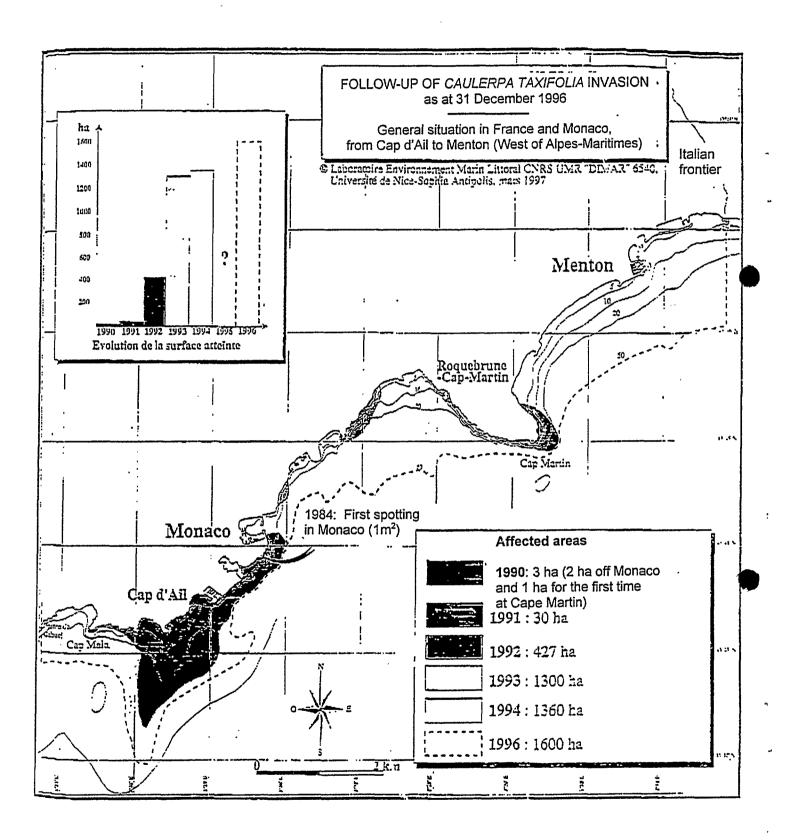


Fig. 9 State of Caulerpa taxifolia spreading in the most heavily colonized area at the end of 1996 (from Meinesz et al., 1997).

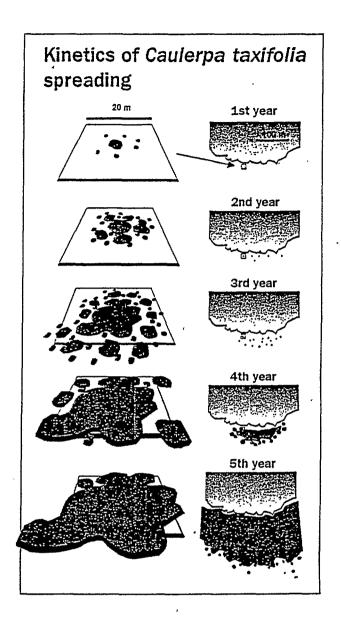


Fig. 10 Kinetics of 5-year *Caulerpa taxifolia* spreading in the Mediterranean, starting from an isolated patch. This diagram was prepared on the basis of observations carried out at Cap Martin (Alpes-maritimes, France; Meinesz *et al.*, 1995).

# The identity of Caulerpa

The genus Caulerpa comprises over one hundred species living in the temperate and especially in tropical seas. Caulerpa taxifolia (Valh) C. Agardh 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. 12). Two species of Caulerpa are certainly indigenous to the Mediterranean: Caulerpa prolifera (Forsskål) Lamouroux and Caulerpa olivieri Dostal. Two other Caulerpa species are probably lessepsian immigrants, (which refers to the species introduced through the Suez Canal from the name of the engineer who oversaw its opening, Fernand Lesseps): Caulerpa scalpelliformis (Brown ex Turn.) C. Agardh (Turkey and the Levantine coast) and Caulerpa mexicana Sonder ex Kützing (Levantine

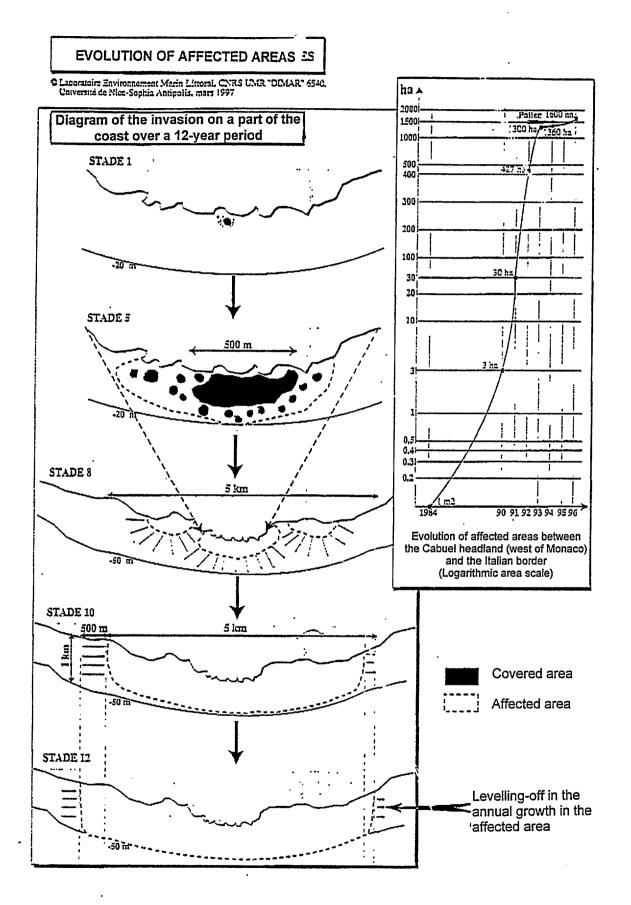


Fig. 11 Diagram of the invasion on a part of the coast over a 12-year period (in Meinesz et al., 1997).

coast). Caulerpa racemosa (Forsskål) J. Agardh, is generally considered as a Lessepsian immigrant, but is could also be a pre-Lessepsian immigrant, even an indigenous species (Verlaque, 1994). C. racemosa in the last 10 years, has extended its pressure in the Mediterranean since initially spotted in Tunisia (in Sousse, 1926), then found in the Eastern Mediterranean and later off the Northwestern coasts.

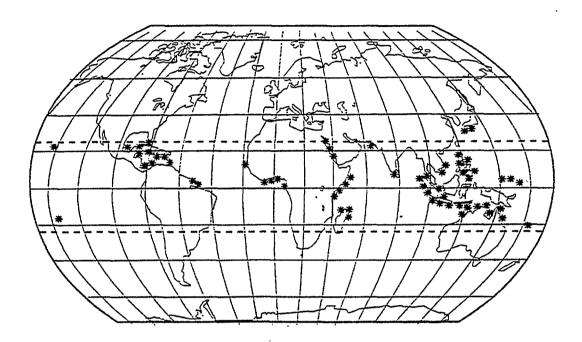


Fig. 12 The distribution of Caulerpa taxifolia. Note that the world distribution of this species is basically limited to the intertropical regions.

Several working hypotheses have been advanced on the origin of *C. racemosa*, in the Mediterranean: natural origin, lessepsian or pre-lessepsian introduction:

- It is rather unlikely that *C. racemosa* is an autochthonous Mediterranean species. It is widely accepted now that few Indo-Pacific algae could have sustained the salinity crises of the Messinian era (5-6 million years) (Por, 1978). The species of tropical affinity, which have a good probability to belong to this stock of remnants of the tertiary, are the species of Eastern Mediterranean which can also be found in the Western Basin and/or the near Atlantic (Verlaque, 1994). Eventhough no assumption should be rejected a *priori*, this does not seem to be the case of *C. racemosa* which was only spotted in the Western Basin in the 90s.
- Repeatedly, in the era of the Pharaohs and then in Roman times and during the Arab conquest, man has opened seaways between the Mediterranean and the Red Sea, by establishing communications between the gulf of Suez and a branch of the Nile river (Por, 1978). On such occasions, Red Sea species could have entered or transported into the Mediterranean. This process, if proven correct, would doubtless be the oldest example of introduction by man into the Mediterranean of marine species (Verlaque, 1994). This type of migration, which is called pre-lessepsian is highly probable for the Caulerpa species, and especially C. racemosa.

Finally, even if it is possible that the migration between the Red Sea and the Mediterranean took place before the digging of the Suez Canal (1869), most scientists believe that *C. racemosa* came into the Mediterranean through the Suez Canal (Lessepsian migration). In the Mediterranean, the lessepsian migrants make up the largest contingent of species introduced into that sea. In respect of marine macrophytes, Verlaque (1994) estimates at 34 the number of lessepsian species, while Ribera & Boudouresque believe the number to be between 22 and 26 species.

In the latter case, it is worthnoting that *C. racemosa* would be the only lessepsian macrophyte to be discovered west of the Sicilo-Tunisian shelf and the one with the northern-most distribution.

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 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.

### 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/m²), 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 m² have been measured which is an amazing figure.

The temperatures compatible with the development of *Caulerpa taxifolia* show that it is unlikely that this alga disappears 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 thus very likely 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.

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 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 racemosa and Caulerpa taxifolia on the natural environment

For the moment, it has not been proven that the spreading of *C. racemosa* has a marked effect on the indigenous Mediterranean populations, or that it is a nuisance for human activities. Caution should however be exercised since:

- the implantation of the species in newly spotted sites seems recent;
- several of the characteristics of the species (ecological valence, high competitiveness, rapid dissemination, absence of predators) make it a potentially dangerous one;
- migrants from the Red Sea are generally successful in establishing themselves in the Mediterranean; lessepsian species are not incidental but develop large populations which are often dominant and stable (Por, 1978; 1990)
- acclimatization and spreading of this species of tropical affinity may be facilitated and/or be linked with the beginning of a climatic warming phase (Verlaque, 1994);
- the latest observations from Turkey show that the species tends to establish continuous and dense populations (Evirgen, 1997).

It would seem evident therefore that the spreading of *C. racemosa* should be monitored. The evolution of the populations that it establishes and their possible impact on ecodiversity, biodiversity and human activities must be studied.

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 of ecodiversity and 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. 13). 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.

Thus, 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. 14-16).

Furthermore, Caulerpa taxifolia competes with the Posidonia oceanica meadows, which is one of the most important ecosystems of the Mediterranean. However, the settling of Caulerpa taxifolia in the Posidonia oceanica meadows is less rapid than in algal populations on rocky substrate. Indeed, the density of the bundles of Posidonia leaves is considerable (up to 800 bundle/m²) 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. Thus, 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 such a direct competition, the size, thickness, number and the longevity of the Posidonia leaves decrease. Necrotic areas appear on the Posidonia leaves and the stems which bear the leaves die.

Sedimentary seabeds also lend themselves to *C. taxifolia* colonization. This in turn drastically alters the physico-chemical and biotic characteristics of such environments.: silting, enrichment in organic matter, decrease of the redox potential leading to anoxic conditions; modification of endogenous fauna. The impact of colonization on these seabeds which are important for coastal balance (sediment transfers, nutrient recycling) and on demersal species have not been adequately studied as yet (Romero, 1997).

Finally, it must be pointed out that in the oldest colonized areas, the specific richness, the density and the biomass of fish populations, are now on average, significantly lower than in the nearby reference areas. Moreover, in the same areas, a drastic decrease of the populations of the edible sea urchin *Paracentrotus lividus* is observed. This species disappears completely from the dense meadows of *C. taxifolia*. The urchins then gather in the areas not yet colonized by *C. taxifolia* (algal populations, Posidonia patches) where they are responsible for overgrazing which indirectly contributes to the spreading of *C. taxifolia*. Preliminary observations on coral also seem to show a considerable impact. Generally, one can predict greater impact on the fauna, as time goes by and as colonization spreads such as decrease in the resources necessary for herbivores and thus for carnivores, loss of habitats, and homogenization of the landscape.

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

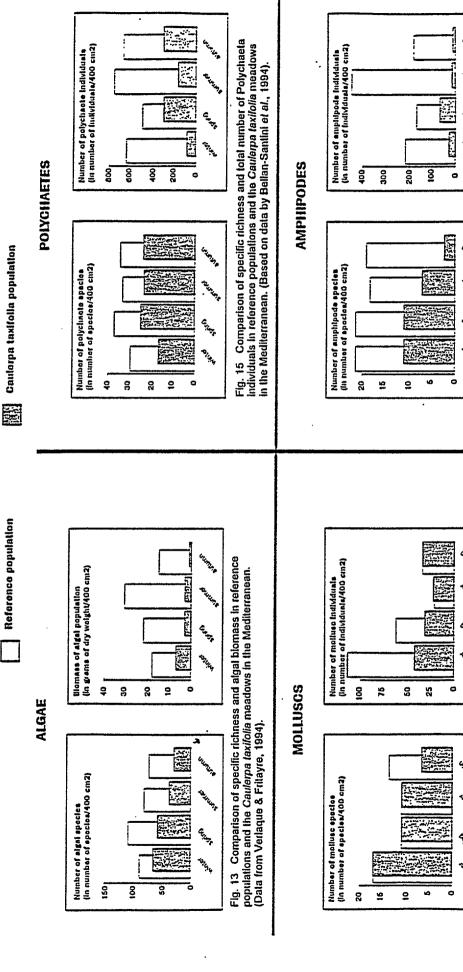


Fig. 16 Comparison of specific richness and total number of Amphipoda individuals in reference populations and the *Caulerpa taxifolia* meadows in the Mediferranean. (Based on data by Bellan-Santini *et al.*, 1994). Fig. 14 Comparison of specific richness and total number of mollusc individuals in reference populations and the *Caularpa taxifolia* meadows in the Mediterranean. (Based on data by 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.

# The toxicity of Caulerpa taxifolia

Several plant species, and among them many algal species, synthesize toxic substances which protect them from predators (herbivores) or competitors (other sessile species). Such metabolites are well known in the *Caulerpa* genus. Thus, it is important to assess the risks for human health and the natural environment which are linked with the secondary metabolites contained in the Mediterranean strain of *Caulerpa taxifolia*.

Researchers have identified, for *Caulerpa taxifolia* 9 toxic substances, sesqui- and mono-terpenes (Guerriero *et al.*, 1992, 1993), among which caulerpenyne (which for a long time had been considered specific to the *Caulerpa* genus) is predominant. It makes up 0.1 to 13% of dry weight in the Mediterranean strain of *Caulerpa taxifolia*, but only 0.1 to 2% in tropical strains. All the other secondary metabolites together make up a little less than 0,004% of dry weight of the alga in the Mediterranean. However, these metabolites cannot be disregarded, since their impact on living organisms may be either specific in small doses and/or synergistic.

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. Caulerpenyne has antibacterial, antiviral, antifungal, cytotoxic, ichthyotoxic, repulsive effects and inhibits the activity of certain enzymes. The aqueous extracts of *Caulerpa taxifolia* are active on the fibroblasts of the hamster and on the eggs of the sea urchin (*Paracentrotus lividus*). Epoxycaulerpenyne 10,11 is the metabolite most active on the fibroblasts of mice and hamsters, whereas the main metabolite, caulerpenyne, is the most active on the eggs of the sea urchin (Lemée *et al.*, 1993). The latter metabolite specifically affects the development of sea urchin eggs, by inhibiting the phases of the cell cycle close to the mitosis (during the pseudo-metaphase; Pesando *et al.*, 1996 and paper submitted for publication).

It has also been shown that certain ciliates¹ are particularly sensitive to low doses (0.5-1.0 μg.ml⁻¹; Dini et al., 1996) of the various terpenes synthesized by Caulerpa taxifolia. Moreover, the sensitivity of various gram positive strains of marine bacteria to the toxic effects of secondary metabolites brings about considerable modification of the bacterial populations in the colonized sites (Giannotti et al., 1996). 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. Finally, eventhough it has been shown using extracts of C. taxifolia or purified caulerpenyne that the growth of microalgae is inhibited (Lemée et al., 1994), similar results have not so far been obtained on the productivity of microalgae indigenous to the Mediterranean (Ferrer et al., 1994).

The continuous expansion of *Caulerpa taxifolia* and the large production of caulerpenyne lead to the assumption that the quantity of this metabolite may be considerable in the water around the thick *C. taxifolia* meadows. *In vitro* studies have shown that caulerpenyne may diffuse in seawater, but it degrades quickly (total degradation in 25 hours) giving rise to a series of toxic components for the sea urchin model. *In situ*, similar biological

<sup>&</sup>lt;sup>1</sup> - Ciliates are mono-cellular organisms at the very base of the marine food chains and for that reason they play an important role, eventhough not fully understood yet, in the way ecosystems work.

activities are also observed with substances extracted from planctonic microorganisms filtered on *C. taxifolia* meadows (Amade *et al.*, in press). On the other hand, it has been shown that caulerpenyne degrades rapidly in the light, in the presence of air and of chlorophyll or phaeophytin. The degradation in these circumstances leads to the detoxification of the above mentioned substances. What we must still prove is whether this phenomenon as well as the detoxification actually occur *in situ* in the debris and the senescent parts of the alga (Pietra, 1997).

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 the summer and autumn (Ruitton & Boudouresque, 1994). In those two seasons, when the toxicity of *Caulerpa taxifolia* is greatest, *P. 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 (Lemée *et al.*, 1994).

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 caulerpenyne on sea urchin eggs, could affect the recruitment and density of *P. lividus* in the sites invaded by *Caulerpa taxifolia*. Such a population decrease was indeed observed in the areas most heavily colonized by *C. taxifolia* (e.g. Menton, France).

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 is the part eaten by humans, eventhough caulerpenyne was detected there.

### 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 is at present being investigated bearing in mind that the quantities of synthesized metabolites are quite large; in spring, when the biomass of *Caulerpa taxifolia* reaches its peak, nearly 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. Such cases of spreading, which continue for a long period until all areas favourable to the particular species, have been invaded are known in different parts of the world, (e.g. the case of the water hyacinth Eichhornia crassipes).

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). In addition, a prevention strategy should be developed in order to avoid colonization of new sites and countries not affected yet.

# 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, does it 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. 17). 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, (approximately 95 species introduced into the Mediterranean), 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 2,050, 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 and the prevention and control measures 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.

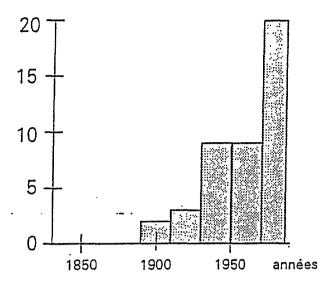
The problems of species' introduction can be extremely costly: a committee of the U.S. Congress determined that since the beginning of the century the species introduced into that country (both into the terrestrial and the marine environment) have cost U.S. \$ 97 billion of damage. It has also been calculated 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.

# <u>Proposals for a strategy to control the spreading of Caulerpa taxifolia in the Mediterranean</u>

Indeed, the phenomenon of *C. taxifolia* spreading is mostly a transboundary phenomenon. Because of that, the implementation of the precautionary principle and the adoption of a coordinated international strategy for controlling the phenomenon must become official. Such a strategy however cannot be developed, unless it is strongly supported by the coastal States, in an accepted regulatory framework and under the aegis of supra national institutions.

The first step towards implementing such an international strategy is raising the awareness of and informing both the scientists and the decision makers of the Mediterranean coastal states. Since 1991, when the first paper on *Caulerpa taxifolia* in the Mediterranean was published (Meinesz & Hesse, 1991), considerable research work was undertaken on the basis

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



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

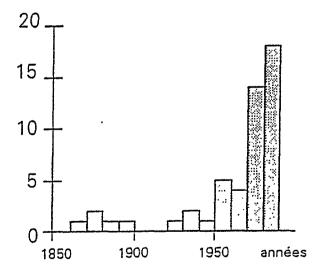


Fig. 17 Top - Number of macro-algae introduced into the Mediterranean (lessepsian species excluded); Bottom - Number of invertebrates introduced into the Mediterranean (lessepsian immigrants included). Note the dramatic increase since the '50s. (After Boudouresque & Ribera, 1994).

of which a large number of relevant papers was published. It is even likely that it is the best documented case in the world of an invading marine species. The work carried out, especially within the framework of the European programmes, and the relevant experience acquired allow the formulation of the main axes of a coherent strategy for the control of *Caulerpa taxifolia* in the Mediterranean.

The challenge presented by the spreading of introduced species on the impact on biodiversity and habitats has been officially recognized and formulated at the highest levels (De Klemm, 1997). The Convention on biological diversity (Rio de Janeiro, June 1992), stipulates specifically that each Party "prevents the introduction, controls or eradicates the exotic species which threaten ecosystems, habitats or species" (Art. 8h of the Convention). More specifically, the SPA Protocol of the Barcelona Convention (June 1995), which all Mediterranean States have signed, stipulates that the Parties must take "all appropriate measures to regulate the voluntary or accidental introduction into the natural environment of non-indigenous species .... and ban those that might have harmful effects on the ecosystems, habitats or species (Art. 13.1)". The Parties must also undertake "to implement all the necessary measures to eradicate the species already introduced, if on the basis of scientific evaluation, it appears that such species cause or are likely to cause damage to ecosystems, habitats or species in the area of application of this protocol" (Protocol on specially protected areas and Biodiversity in the Mediterranean).

Today, fourteen years after the introduction of *C. taxifolia* in the Mediterranean, and despite repeated appeals on the part of the scientists studying this phenomenon, it must be admitted that very few concrete actions have been taken. They mostly emanate from research organizations and local or regional bodies (studies, awareness campaigns, isolated actions for the control of spreading in Croatia, the Balearic islands and the National Parc of Port-Cros).

Already in late 1992, scientists stated that the total eradication of the particular strain of *Caulerpa taxifolia* from the Mediterranean is not a realistic goal. The strategy thus to be adopted must aim at (i) continuing the monitoring of the spreading and studying the phenomenon, (ii) preventing the contamination of new sites and countries, (iii) preserving the sites of particular heritage importance, (iv) slowing down the rate of expansion through the control of existing colonies. This strategy must be adapted on a country by country basis, depending on the risks or the current contamination state by this invading species. The assessment of the gravity of the risk must be carried out separately for each country/site through the study of dissemination vectors, mostly anthropogenic. Finally, it is imperative to adopt an agreement based on a timetable for the adoption and implementation of the measures outlined below.

# To continue studying and monitoring the phenomenon and to identify poles of expertise

It is important to continue the programmes of cartographic follow-up of the expansion and the on-going research which would lead to applications for the control of *C. taxifolia* populations: characteristics of the Mediterranean strain of *C. taxifolia*, ecological demands, physiology and modalities of regeneration, multiplication and reproduction, limiting factors in the Mediterranean and in terms of the natural populations.

The strategy for the control of *C. taxifolia* spreading must be based on a network for the study and follow-up of spreading, on awareness and, if necessary, on operational validation structures, when a new colony is spotted. Indeed, the international network put in place in 1992, within the framework of two European programmes is an effective structure which should be continued and enlarged with increased support. The key points of the network, which would

expand to include the whole of the Mediterranean, must be bodies with communication means, logistics and operators at sea for each Mediterranean region (national and regional institutes, university laboratories, staff at specially protected marine areas). A significant contribution can be made by NGOs.

# 2. To adopt prevention measures

Combatting the spreading of *Caulerpa taxifolia* is unthinkable without official and long-term measures of prevention which aim at (i) preventing new contamination episodes from private or public aquariums and (ii) preventing anthropogenic dissemination from existing colonies.

# 2.1 To adopt regulatory and control measures

To avoid new cases of contamination, it is imperative that regulatory provisions banning the sale, transport and use of *Caulerpa taxifolia* (similar to those adopted in Catalonia and France be adopted by all Mediterranean coastal states. It is obvious that such measures should be given the widest publicity possible. The staff entrusted with applying such provisions in the field must receive formal instructions as well as specific training.

# 2.2 To promote awareness and disseminate information

Both activities targeted at the sea users and the public at large is the main tool in the strategy for slowing down spreading. Such actions, especially aimed at those involved with yachting (short distance near shore cruising or long-distance cruising), fishing and diving, should highlight the role of humans as dissemination vectors for the algae. The message, which should be kept simple, must encourage the users to check and clean mooring equipment (anchors, chains), fishing gear (nets, ganguis) and diving equipment in order to avoid any discharges into the sea and harbours.

Furthermore, it is evident that users play a fundamental role in spotting patches thus in ensuring the follow-up of the algal spread. Diving throughout the Mediterranean has become very important, Thus, there is a tremendous potential of observers which must continue being encouraged to follow the phenomenon. Such awareness campaigns are carried out in the Northwestern Mediterranean every year since 1991, by University laboratories, in the framework of regional, national and European programmes. Coastal States, through the relevant institutions must make such actions official, continuing and of a wider scope. Users will be informed by the media, but especially through brochures and posters distributed in ports, clubs, local authorities and professional organizations. In addition, port authorities and maritime police must also be entrusted with information, counsel and if necessary enforcement activities.

# 3. To adapt and reinforce prevention measures in sites of particular heritage importance

Taking into consideration the impact of *Caulerpa taxifolia* on biodiversity and ecological diversity, special attention must be paid to those sites of particular heritage importance. The specially protected Mediterranean areas contain a marine component; those listed by RAC/SPA are 45 in number, or 553614 ha (RAC/SPA, 1997). New sites, especially those emanating from the "Habitat Directive" of the European Union will be shortly added (Nature 2000). A specific strategy, adapted to the prevention and conservation of these sites in respect of the danger of contamination and spreading of invasive exotic species, such as *C. taxifolia*, must be adopted. Such a strategy has been developed for the National Park of Port-Cros (France). It is based on staff training, awareness campaigns for visitors, systematic forecasting of the main sectors

at risk and on control operations when contamination has been verified. The strategy must also include regulation and control of mooring sites and fishing areas for these two sectors in danger.

# 4. To set up control measures

# 4.1 Management of heavily colonized sites

In order to avoid further contamination by humans from existing colonies, in addition to awareness campaigns, it is important to inform users on the state of colonization, and to regulate, ban or manage the colonized sites. Instructions can be included in ships' logs with yearly updating of the affected areas. Special attention must be paid to dredging of harbours contaminated with *C. taxifolia*. Such preventive measures - in order to be enforced - must be respected by users and local authorities, thus be accompanied by specific information activities.

# 4.2 To develop techniques for the control of existing colonies

For the moment, with the exception of certain isolated experiments, no technique leads to total and definitive eradication of *C. taxifolia* from surfaces larger than a few square meters. Control operations must at present, aim at slowing down the spreading of managed colonies. Such control operations must be carried out regularly. The cost of operations, borne by the community, is therefore recurrent. However, it must be considered when taking decisions, that without intervention the problem will become unmanageable in the short term (5 years) and will have an impact on human activities and the natural heritage in the medium term (>10 years).

More effective techniques are for the time being developed on an experimental basis. The necessary financial and technical support should be made available for the full scientific development of these techniques. A particular effort should be made by the countries already contaminated in order to develop reliable control techniques and thus acquire experience which can be transferred elsewhere. More particularly, the effort should focus on defining practicable techniques for port areas. It would be necessary to make a list of the various control techniques for invasive aquatic species used throughout the world and study the possibility of adapting them to combatting *Caulerpa taxifolia* spreading.

A strategy for slowing down the rate of spreading of the alga through the control of small size patches already spotted or that will be spotted outside the heavily contaminated areas can be put in place. Coordinated efforts should focus in priority on well-defined lines of attack which should be based on the following 3 general principles:

- (i) to intervene at an early stage on the colonies which are still small in size;
- (ii) the partial treatment of a colony is futile: lateral recolonization from the untreated area is very rapid;
- (iii) to return regularly to treated areas in order to eliminate possible regrowth.

# 4.3 To register the operational teams for controlling operations

Teams of professional divers that might participate in control operations must be identified. Such teams must receive training, before they take part in any operation. Specifications for the optimization of control services and follow-up operations must also be adopted.

# 4.4 To set up coordination structures

The time between the discovery of new *Caulerpa taxifolia* colonies and the control operations should be taken into serious consideration. In the best case scenario the new colonies spotted will only cover a few square meters. The investment needed to control them grows proportionally with the speed of *Caulerpa taxifolia* spreading (annual rate: a factor of between 2 and 10).

# 4.5 To study the possibility of biological weapons

The occurrence of several invasions of introduced species which are harmful to the biodiversity of marine ecosystems has led several teams of researchers to envisage the possibility of biological weapons. In the case of *C. taxifolia*, some Ascoglossal molluscs feed exclusively on algae of the *Caulerpa* genus and might thus prove useful as a possible controlling agent. Aquarium experiments offer a ray of hope, but controlled *in situ* experiments will be needed to assess effectiveness. The introduction of exotic agents to combat *C. taxifolia* should be based on international expertise whether to use or not this weapon (Meinesz *et al.*, in press). To control in the long term invasive exotic species, such as *C. taxifolia*, a world-wide and coordinated programme of biological control might be the solution.

# 5. To adopt a timetable in the framework of an international agreement

The measures to be adopted by the Mediterranean coastal states must yield very rapid results. Each spring, when the growth of the *Caulerpa* populations starts again, the cost of control interventions increases. During the summer with the increased movements of pleasure crafts the dissemination risks increase. A calendar for the actions proposed in this document must be adopted quickly by the States around the Mediterranean. The areas of responsibility (decision-making levels) for adoption of budgets, contracting the work and carrying out control operations must be clearly defined. Appropriate financial tools must be provided.

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