AFFIDAVIT OF SUSAN L. WILLIAMS, PH.D.

Sworn statement to the United States Department of Agriculture
on the Seaweed Genus *Caulerpa*
in support of noxious weed listing petitions

of

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Susan L. Williams, being duly sworn, hereby deposes and says:

1. I am a Professor of Environmental Science & Policy and the Director of the Bodega Marine Laboratory at the University of California at Davis. I did my doctoral research on the biology and ecology of the seaweed *Caulerpa* in its native habitat. I also have published on the deleterious effects of non-native species on native eelgrass in southern California, and am a technical advisor on the *ad hoc Caulerpa* management team in southern California (SCCAT: Southern California *Caulerpa* Action Team). I am also a member of the California Caulerpa Control Committee established by the California Department of Fish & Game. I submit this affidavit in support of noxious weed listing petitions for *Caulerpa* on a volunteer basis and I have no financial stake in this matter.

2. It is my scientific opinion that species in the seaweed genus *Caulerpa*, including *Caulerpa taxifolia*, pose a realized and future threat to the marine communities in the United States, as well as a regulatory challenge. The body of scientific evidence and scientific opinion from the Mediterranean supports the exceptional invasiveness of *Caulerpa taxifolia*. Since its introduction in 1984 in Monaco, it spread around the Riviera into Italy and Spain and northward into colder waters of Croatia, and most recently, Tunisia (Meinesz et al. 2001).

Because species of *Caulerpa* produce toxins making them distasteful to animals (Lemee et al. 1996), *Caulerpa* provides less food web support than diverse native communities of seaweeds and seagrasses. Field studies in the Mediterranean have demonstrated that *Caulerpa taxifolia* can overgrow native seagrass beds (Ceccherelli and Cinelli 1997, 1998, 1999), although not every seagrass bed that *Caulerpa* invaded in the Mediterranean is demonstrably harmed (Jaubert et al. 1999) and not every published study provides unequivocal evidence that *Caulerpa taxifolia* causes ecological damage. These are only a few examples from the extensive scientific literature on the Mediterranean invasion. In addition to ecological effects, *Caulerpa taxifolia* has interfered with marine tourism and fishing (Boudouresque 2002). In a recent invasion of *Caulerpa taxifolia* into non-native habitat in Australia, it also overgrew native seagrass (Dr. Alan Millar, Royal Botanical Garden of Australia, pers. comm.).

3. We unfortunately now have witnessed a similar capacity for non-native *Caulerpa taxifolia* to spread rapidly in California. *Caulerpa taxifolia* was identified in Huntington Harbor in 1998, before it was federally listed as a Noxious Weed based on the recommendation of over 100 scientists. At this site, *Caulerpa* now grows patchily over 6 acres of two artificial ponds. Between the first survey and a second one a few months later, CDFG personnel observed *Caulerpa* outside of the ponds in the main harbor connecting to Newport Bay, which was subsequently eradicated. A local biologist reported observing *Caulerpa taxifolia* in Aqua Hedionda four years ago. It now covers at least 3,000 sq. feet. The rate of *Caulerpa taxifolia* expansion has been modeled at 1 acre/year (Aussem and Hill 1999).

4. The *Caulerpa taxifolia* that invaded the Mediterranean and California originated in aquaria, based on DNA analyses (Jousson et al. 1998, Jousson et al. 2000, Wiedenmann et al. 2001, Schaffelke et al. 2002, Meunsier et al. 2002). Until there is a reliable, rapid, and routine means of identifying the invasive Mediterranean aquarium strain of *Caulerpa taxifolia* from other *Caulerpa taxifolia*

strains and other *Caulerpa* species, there is a risk of re-introducing it to California's waters. Species of *Caulerpa* are some of the most difficult seaweeds to identify, either by morphology or current molecular (DNA) methods (Olsen et al. 1998). Researchers have concluded that "*C. taxifolia* is clearly a complex of genetically and ecologically differentiated sibling species or subspecies" (Meusnier et al. 2002). The genus *Caulerpa* is known as one of the most morphologically variable, with morphology strongly influenced by the environment (Taylor 1972, Carruthers et al. 1993, Meinesz et al. 1995, Ceccherelli and Cinelli 1999, Collado-Vides and Robledo 1999; diagrams excerpted from the literature are appended). *Caulerpa cupressoides*, as an example, has at least 9 varieties, one of which can be mistaken for *C. taxifolia* (*C. cupressoides* var. *lycopodium*). *Caulerpa taxifolia* is very similar to *C. scalpelliformis* from Australia and the Mediterranean, another species reported to be invasive (Davies et al. 1997). In the Mediterranean, morphs intermediate between *C. taxifolia* and *C. mexicana* were confused and debated (the 'taxmex' morph), to the detriment of taking efforts to eradicate *C. taxifolia* (Olsen 1997). In the past, *C. mexicana* was once considered the same as *C. taxifolia*, indicating how similar the species can appear (Taylor 1972).

5. Currently, expensive DNA analysis is the only unequivocal means of separating the invasive aquarium strain of *Caulerpa taxifolia* from other strains, and there are only a handful of experts to call upon. This creates a problem with interpretation of the Noxious Weed listing and with regulation. *Caulerpa taxifolia*, the invasive Mediterranean aquarium strain, is the only strain listed as a noxious weed, and molecular techniques are required to identify it. *Caulerpa taxifolia* also recently invaded 3 new locations near Sydney, Australia, 800 kilometers from the nearest native population (Schaffelke et al. 2002). None of these introductions represent natural range expansions (Schaffelke et al. 2002) and each is considered to be the result of an independent introduction of a distinct genetic strain. One strain (Lake Conjola) is very closely-related yet slightly different (based on DNA) from the Mediterranean aquarium strain. This difference was not

great enough to exclude it from the Mediterranean aquarium strain, which is identical with the Californian strains. Depending on interpretation, the noxious weed listing would not protect California from this invasive Australian strain that is now considered part of the Mediterranean-aquarium-California complex. One other strain was similar to native Australian populations, although its introduction is not considered natural. The origin of the third strain is currently unresolved by DNA analysis. The noxious weed listing would not protect California from these two additional strains, both of which have exhibit the robust morphology and cold-tolerance of the Mediterranean-aquarium-California-Lake Conjola Australia complex (Scheffelke et al. 2002). Additional molecular markers need to be developed for improved identification, and currently scientists are uncertain how many strains exist (Meusnier et al. 2001, 2002).

6. *Caulerpa taxifolia* apparently underwent a genetic change while being maintained in aquaria (Jousson et al. 1998). This change is hypothesized by scientists to contribute to its invasiveness. The invasive aquarium strain tends to be larger and more tolerant of colder waters than tropical native strains of *Caulerpa taxifolia*. It is reasonable to expect that other *Caulerpa* species have undergone genetic changes while being maintained in aquaria, although this awaits DNA comparisons for verification.

7. In addition to the risk of re-introducing *Caulerpa taxifolia*, there is the risk of introducing other rapidly-growing non-native species of *Caulerpa*. No *Caulerpa* species is native to California or the west coast of the U.S. (Abbott and Hollenberg 1976). All *Caulerpa* species have the capacity to be invasive because of their phenomenal growth rates (up to 3"/day) and their ability to regenerate from very small fragments, even when buried (Williams et al. 1985, Smith and Walters 1999). Other species in addition to *Caulerpa taxifolia* have become invasive. Examples include *C. racemosa* in the Mediterranean (Olsen et al. 1998, Ceccherelli et al. 2000, Piazzi et al. 2001, Dr. Enrico Sala, Scripps Inst. Oceanography, pers. comm.), *C. scalpelliformis* (Davis et al. 1997), as well as

already very harmful invasions of *C. verticillata* in the Florida Keys (Science News, Vol. 157:373, 6/10/00), and *C. brachypus*, also in the Florida Keys (Drs. Mark Littler and Steven Murray, pers. com.) and on reefs off the Palm Beaches and Treasure Coast (Lazaroff 2003). In a replicated experimental study in two different habitat types, *Caulerpa racemosa* completely covered the habitat within 6 months after introduction, overgrowing native seaweeds within 4 months (Piazzi et al. 2001).

8. *Caulerpa racemosa* and other species are grown in aquaria in this country, and hence risk being released into our marine environments. At least 16 varieties are known to be sold in California (Dr. Steven Murray, a marine botanist and Professor at California State University at Fullerton, pers. com. of results from a study funded by Sea Grant; Withgott 2002). A recent survey by the San Diego Regional Water Quality Control Board (appended) of 70 aquarium and pet shop stores in San Diego and Orange Counties revealed that, in the 4 stores (5%) reported handling *Caulerpa*, employees stated that they did not know the kind of *Caulerpa* they handled. A survey of internet sites (e.g., www.ipsf.com, www.qualitymarineusa.com/ inverts/plants.html) reveals that *Caulerpa* is not typically identified by species, but instead referred to by common names ('smooth leaf, 'notched leaf', 'assorted', example appended). The capacity for rapid proliferation of these species of *Caulerpa* is recognized by aquarists in their trade publications (Scott, Marine Fish and Reef, Vol. 2, Number 2). In summer 2000, enthusiasts communicating on chat rooms at the Indopacific Sea Farms web site (www.ipsf.com) noted problems with overgrowth by *Caulerpa* in their aquaria.

9. Until identification of strains and species of *Caulerpa* is definitive and economical, it will be very difficult for the public and government officials to know what species of *Caulerpa* they encounter and whether the specimen is a federally-listed noxious weed. I urge USDA to weigh the risk of further introductions of *Caulerpa taxifolia* to other locales, the risk of introducing many other species of rapidly-growing non-native *Caulerpa*, and the difficulty of making

a correct species identification required for enforcement of the Noxious Weed Act. Certainly the Mediterranean clone limitation should be removed from the current *Caulerpa taxifolia* noxious weed listing. Further, the entire *Caulerpa* genus should be listed as prohibited noxious weeds, otherwise in my opinion the USDA will be courting ecological and economic invasive species disasters.

Dated this 19 day of February, 2003, at Davis, California.

Susan Lynn Williams
Signature

State of California
:ss.
County of Yolo

Subscribed and sworn to before me this 19 day of February, 2003.

Christina J. Faltis
Notary Public

CHRISTINA J. FALTIS
COMM. #1345831
NOTARY PUBLIC - CALIFORNIA
YOLO COUNTY
My Comm. Expires Mar. 9, 2006

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Variations in the Structure, Morphology and Biomass of *Caulerpa taxifolia* in the Mediterranean Sea

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Year-round morphological variations were investigated in *Caulerpa taxifolia* collected from the invaded regions of the northern Mediterranean Sea at different depths and from different types of substrates as well as from monthly samples in a dense unshaded meadow at a depth of 9 m at Cap Martin, Alpes-Maritimes, France. This tropical Ulvophyceae, recently introduced into the Mediterranean, shows pseudoperennial growth: although no single part of the thallus persists for more than a year, the individual persists by means of indefinite vegetative development. The length of the stolons varies from 150 to 352 m m^{-2} over the year. The rhizoid pillars are regularly spaced, showing no seasonal variation, with one pillar along the stolon every 1.63 ± 4.3 cm in average. The density of primary fronds varies from 5100 to 13920 m^{-2}, with the maximum number of ramifications, 25000 m^{-2}, occurring in spring. From late summer to autumn, primary fronds were without necrosis and with few ramifications. From winter to spring, however, these fronds often displayed numerous ramifications and their distal ends were usually broken or necrosed. These variations counterbalanced one another so that the vegetation cover at 9 m depth was always 100% with a relatively constant total biomass (average of 500 g dw m^{-2}).

Introduction

The tropical alga *Caulerpa taxifolia* (Vahl) C. Agardh was first found in the Mediterranean Sea in 1984: it then covered an area of 1 m^2 in front of the Oceanographic Museum of Monaco (Meinesz and Hesse 1991). Since then, its spreading in the neighbouring coastal regions has been regular and very rapid. Pleasure boat anchor systems have disseminated the alga far from the site of introduction (Meinesz 1992). *Caulerpa taxifolia* was discovered in France (1990), Italy (1992) and Spain (1992) as far as Messina (Italy, Sicily) and Mallorca (Spain, Balearic Islands) (Meinesz *et al.* 1994). The area affected has been estimated at 3 ha in 1990, 30 ha in 1991, 427 ha in 1992 and more than 1300 ha in 1993 (Meinesz *et al.* 1993, 1994). *Caulerpa taxifolia* develops on a wide range of substrates and depths, mainly from 0 to 50 m (Belsher and Meinesz 1995).

Marked seasonal variations occur in the morphology of *C. taxifolia* (Meinesz and Hesse 1991). In order to define the seasonal vegetative cycle and the morphological and structural adaptations of the different components of the thallus, year-round collections of *Caulerpa taxifolia* from the invaded regions of the Mediterranean Sea at different depths and on different types of substrates were made and monthly samples, from a dense meadow at a depth of 9 m, were analysed.

Materials and Methods

Observations have been made and samples examined since 1989 at most of the sites now known to be colonised.

To complete and rationalize this existing set of investigations, monthly samples were taken by SCUBA diving from March 1992 until April 1993, from within a 100 m^2 *C. taxifolia* meadow at a depth of 9 m on the eastern side of Cap Martin, Alpes-Maritimes, France (42°45'15" N; 7°29'40" E). This zone supports a complete, even cover by the alga throughout the year. The soft substrate is slightly sloping and is composed of coarse sand with mud. Each sample was collected by cutting out a square of *C. taxifolia* contained within a 500 cm^2 metal quadrat. It is the same sample area that was used for the study of the Mediterranean *Caulerpa prolifera* (Forsskål) Lamouroux in 1978 (Meinesz 1989 a, b, c). All proportions of *C. taxifolia* were then stored in a herbarium, allowing subsequent determination and description of seasonal morphological changes, as previously carried out for *Caulerpa prolifera* (Meinesz 1979 a).

Biometrical methods were used on thalli to determine the following parameters (Fig. 1A):

(i) the total length of stolons;
(ii) the number of rhizoid pillars;
(iii) total frond length;
(iv) number of primary fronds;

(v) number of secondary fronds (i.e. ramifications of primary fronds);
(vi) number of fronds of order higher than 2 (i.e. ramifications on secondary or higher-order fronds).

Whole fronds were counted separately from those in which the apex was broken or necrosed (chlorosis).

Herbarium storage induces reduction in the size of different parts of the thallus by a fairly uniform 3% of the length. Our results report the herbarium dimensions. Herbarium specimens are deposited at the Laboratoire environnement marin littoral of the University of Nice-Sophia Antipolis.

Results

Stolon

The stolon represents the morphological element on which the thallus is based. This principal axis bears fronds and pillars of rhizoids.

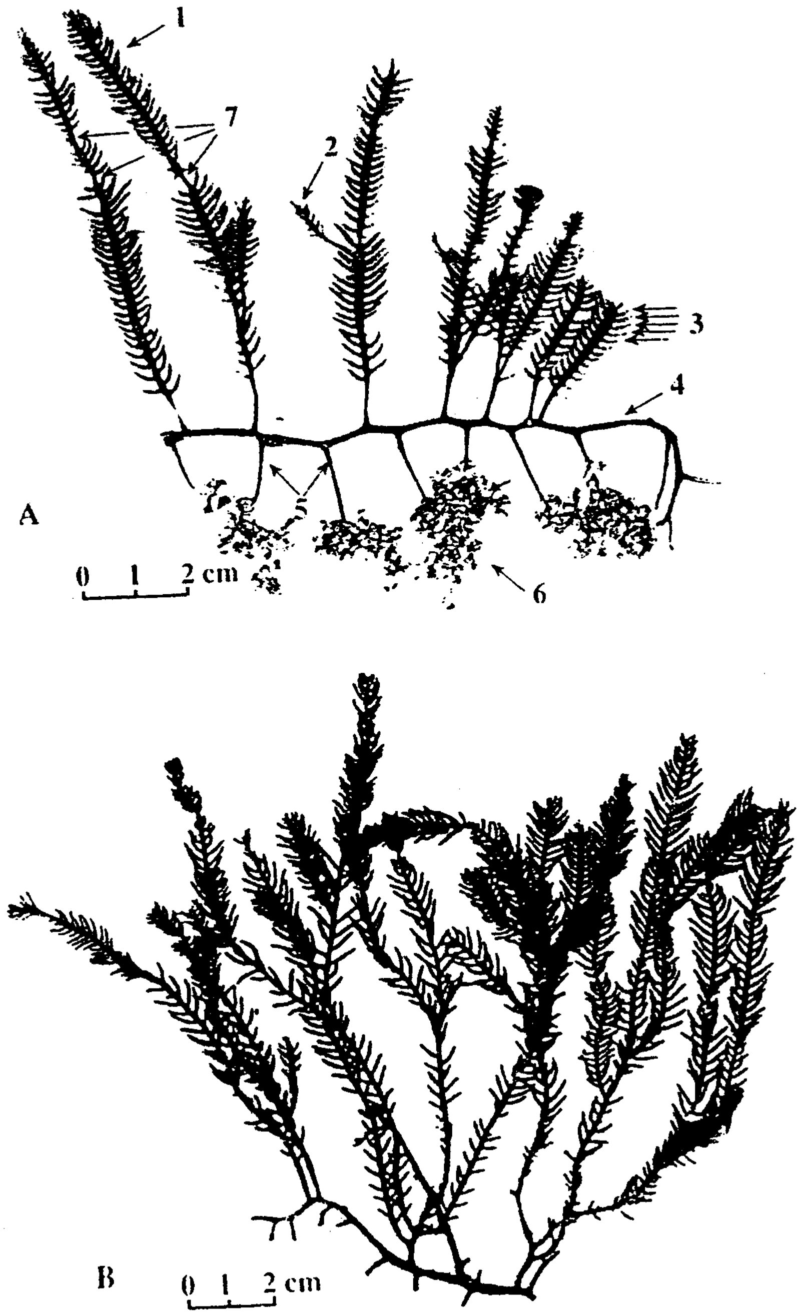


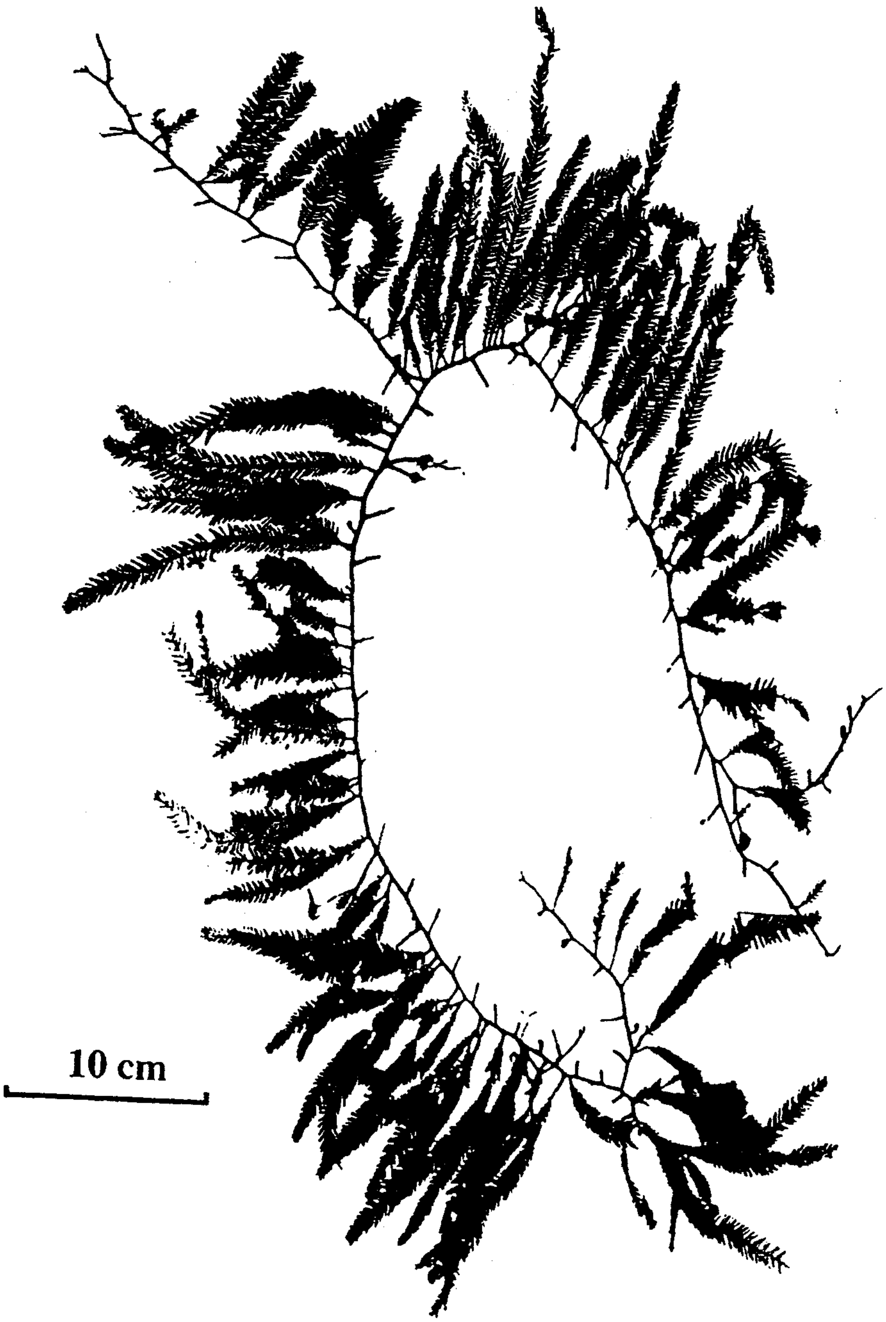
Fig. 1. (A) The different structural elements in *Caulerpa taxifolia* (in autumn). 1: primary frond. 2: secondary frond (ramification). 3: pinnules. 4: stolon. 5: pillar of rhizoids. 6: bunch of rhizoids fixing sediment particles. 7: interruption in the distribution of the pinnules. Cap Martin. 26/07/1994. Herb. A. Meinesz N° 658. (B) Winter structure of the thallus of *Caulerpa taxifolia* (numerous ramifications and widely spaced pinnules at the base of the fronds). Cap Martin. 15/03/1990. Herb. A. Meinesz N° 551.

Meinesz et al.

...xifolia meadows, the stolons constitute an ...ble network. Measurements of stolon length ...ated thalli have shown that they can reach 1 to ...n in autumn, with up to six ramifications (Fig. ...he maximum stolon length found for one thallus ...8 m (bearing 285 primary fronds and 196 pil... of rhizoids). In winter, the stolon suffers necrosis, ...icularly of the parts most exposed to water move...t or to the cold.

...rom April to July, the remaining stolons or fronds ... survived the winter give rise to new stolons, ...h project above the substrate without clinging to ...ue to the important variations in stolon length throughout the year, whole individuals of *C. taxifolia* could not be sampled in the 500 cm^2 quadrat (22.36 × 22.36 cm). Our results thus give the cumulative length of the collected fragments (Fig. 3).

The cumulative length of the stolons varies slightly throughout the year. It tends to stabilise around an equilibrium value of 220 m m^{-2}. In June and July, the addition of newly formed stolons to the older ones, infers a total stolon length that may exceed 350 m m^{-2}. During late July and August, the old stolons necrose rapidly while the young ones are still short, so the total length of stolons may decrease during this period to around 160 m m^{-2}.



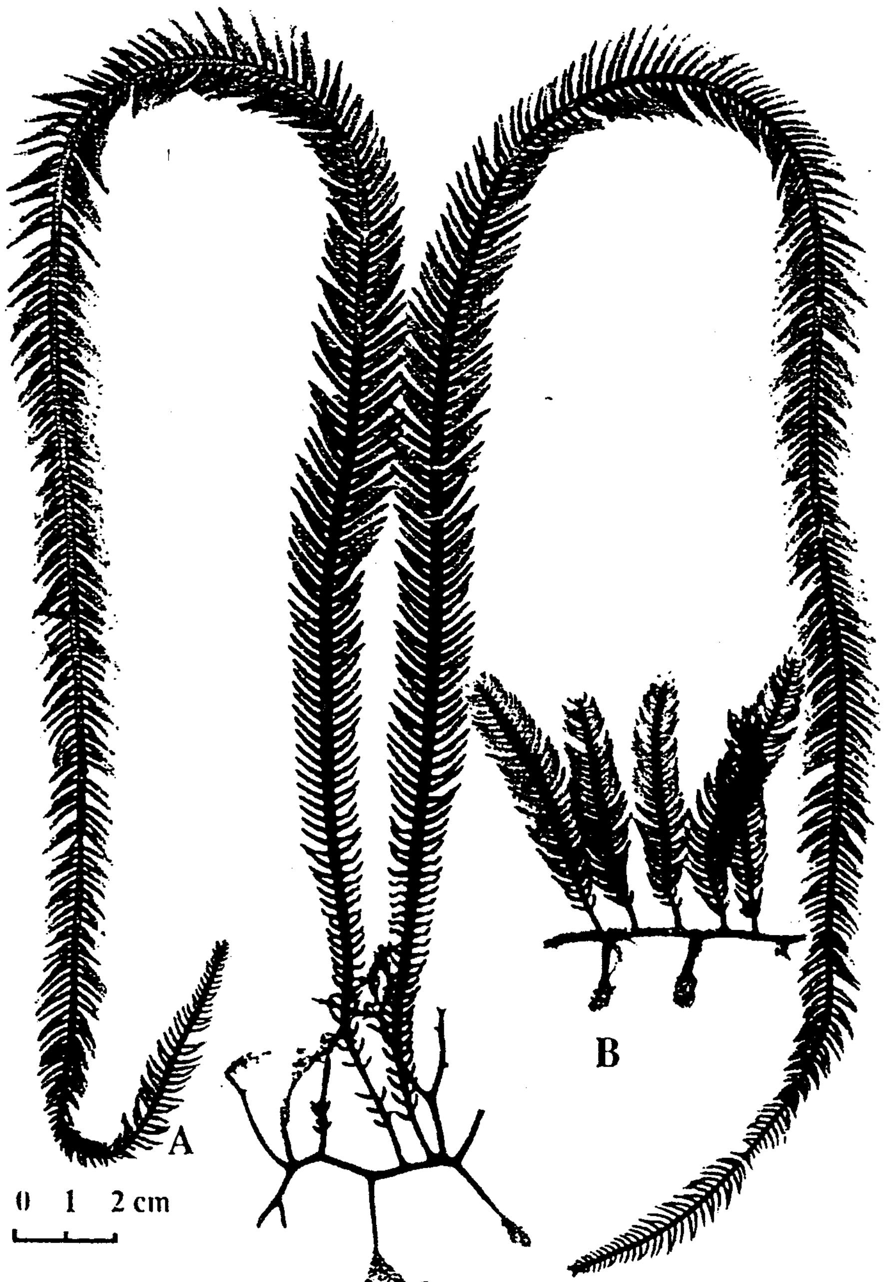
...*xifolia* thallus, composed of a stolon 1.5 m long, ramified 5 times bearing 55 fronds and 35 pillars of rhizoids ...-sur-Mer, 05/10/1993, at 4 m. Herb. A. Meinesz N° 672).

…of pinnules (Fig. 6A, B, C). Fronds also div-…hotomously (Fig. 6D), in which case we have …ned one of the two dichotomous parts as one …anch.

The number of ramifications of primary fronds (of [illegible] or higher orders) is lowest in summer and autumn (minima: 250 m^{-2} in September) (Fig. 9). Their number increases in winter, reaching a maximum at the end of spring (May), with more than 24 500 m^{-2}. In early winter, the total length of the branches may even exceed that of the primary fronds. In some samples we have found successive branches with their apices as far as 83 cm from the stolon (Cap Martin, at 15 m, 12/12/1993; Herb. A. Meinesz N° 666). In February the total length of all the fronds (primary and branching fronds) reaches its maximum (901 m m^{-2}). In May and June the higher order ramifications are even more numerous than the first order ones (Fig. 9). At this time of the year, these multiple branches are small in size, resulting in a dense, smooth texture of the *C. taxifolia* meadow.

Frond axis and pinnules

The compressed frond axes (rachis) are morphologically identical throughout the year. The variation of



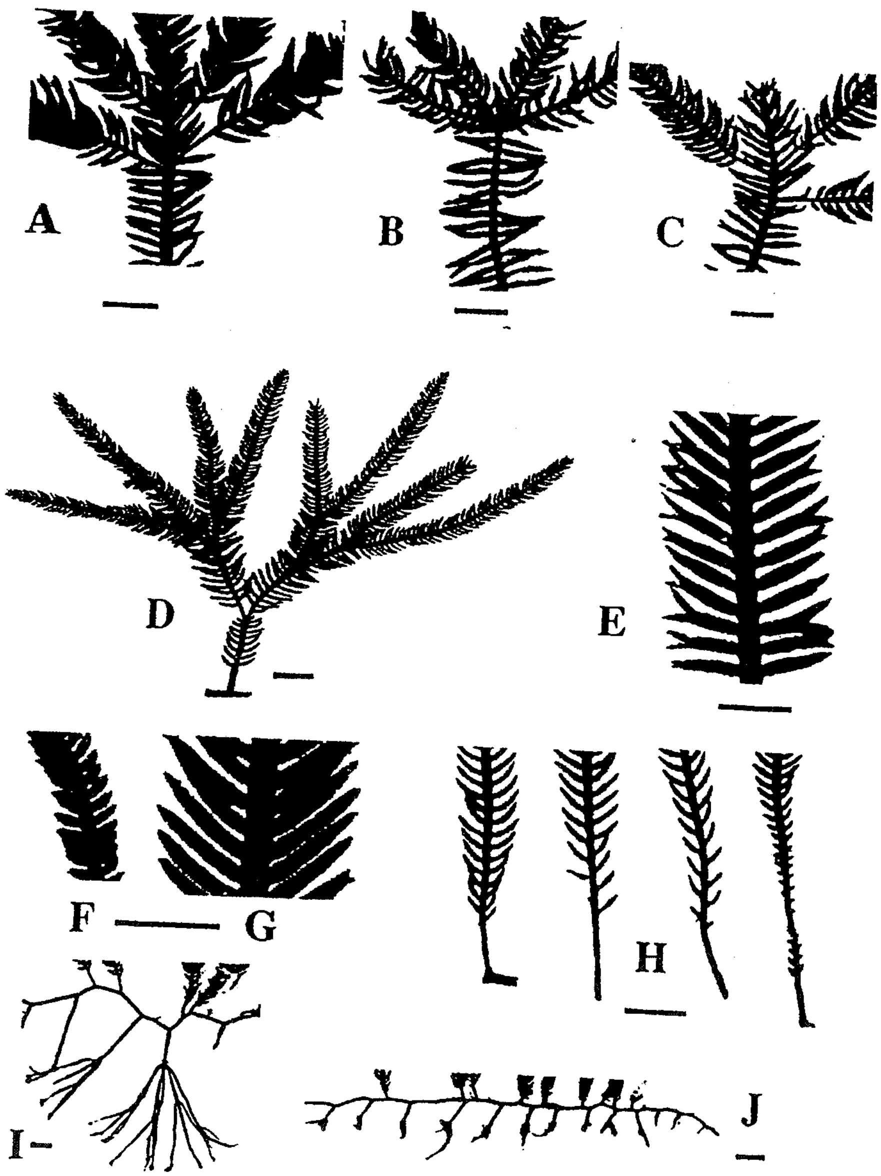
[illegible]. Differences in the length of primary fronds. (A) in low light conditions (Cap Martin, at 11 m, 18/12/1992, Herb. A. Meinesz N° 569). (B) in high light conditions (Cap Martin, at 5 m, 8/11/1990, Herb. A. Meinesz N° 561).

the width of the frond axis (between 0.8 and 2.5 mm) cannot be correlated with the length of the fronds, the length of the pinnules or the seasons.

As for all the *C. taxifolia* of tropical seas, pinnules are usually upcurved, strongly compressed, slightly contracted at the base, tapering toward the tip and mucronate. The pinnules are most often opposite and closely approximated (4 to 7 pinnules cm^{-1} on one side of the frond axis), but at the end of the winter, they can be alternate and widely spaced at the base of the fronds (2 to 3 pinnules cm^{-1} on one side of the frond axis) (Fig. 6H). Some of the pinnules can be bifurcate at their extremity (Fig. 6E).

Occasionally fronds may be found in which the distribution of pinnules is interrupted (Fig. 1A). The length of the pinnules varies from 0.5 to 1 cm, reaching at least three times, and more often five to ten times, their width (between 0.5 and 1.7 mm) (Fig. 6F and G).

Size and spacing of pinnules along the frond axis depend on light intensity. Under low light conditions such as autumn and winter, in deep water, in the shade of rocks or in *Posidonia oceanica* meadows their pinnules become long (0.8 to 1 cm) and approximate along the axis (4 to 5 pinnules cm^{-1} on one side of the frond axis) (Fig. 1B). In summer, however,



ig. 6. Variation in morphology of different parts of the thallus of *Caulerpa taxifolia*. (A) ramifications along the axis (rachis) of a primary frond, (B) ramifications on the distal end of a primary frond, (C) ramifications from the distal end of pinnules, (D) dichotomous ramifications of the primary frond, (E) bifurcate pinnules, (F) short, wide and closely placed innules (exceptionally found at Hyères, at 1 m in summer), (G) long, linear pinnules characteristic for the species, (H) lternate and spaced pinnules at the base of fronds (winter), (I) long and ramified pillars of rhizoids (*C. taxifolia* in *osidonia oceanica* beds), (J) short, regularly spaced pillars of rhizoids (*C. taxifolia* on rocks). Scales A to J: 1 cm. (Herb. A. Meinesz N° 594, 659, 672 and 674.)

Table 1. List of sampling sites, collectors, codes, ITS1 lengths and GenBank Accession numbers for all the sequences considered in the analysis. The master alignment of all clones is available via GenBank. Example of nomenclature: LI.1.A refers to Livorno population, individual 1, clone A.

Sampling station and code		Collector and date	Individuals per sampling site	Sequences per individual	ITS1 lengths (min.–max.)	GenBank Accession nos.
France	Marseille (MA)*	GenBank	MA.1	3	114–116	AJ228997 AJ228998 AJ228999
Italy	Livorno (LI)*	L. Piazzi, 1998	LI.1	18	115–127	AF256088–105
			LI.2	9	114–118	AF256106–114
	Salerno (SA)*	P. Famà and G. Procaccini, 1999	SA.1	4	116	AF256115–118
			SA.2	3	116–127	AF256119–121
	Capo Miseno (CM)*	P. Famà and G. Procaccini, 1999	CM.1	5	116–118	AF256122–126
			CM.2	4	116–118	AF256127–130
			CM.3	2	116–118	AF256131–132
	Capo Molini (CO)*	P. Famà and M. Vicinanza, 1999	CO.1	2	116	AF256133–134
			CO.2	1	118	AF256135
	Capo Rizzuto (CR)*	L. Mazzella, 1998	CR.1	1	115	AF256136
			CR.2	1	115	AF256137
			CR.3	2	116–118	AF256138–139
Greece	Rhodes Is. (RH)**	M. van Rijssel, 1998	RH.1	4	116–118	AF256140–143
Spain	Gran Canaria (GC)[n.a.]	H.J. van de Strate, 1998	GCa.1	1	116	AF256144
			GCs.1	3	114–118	AF256145–147
			GCl.1	2	118	AF256148–149
	Fuerteventura (FU)[n.a.]	H.J. van de Strate, 1998	FU.1	4	140–146	AF256150–153
Panama	Panama (PAN)[n.a.]	W.H.C.F. Kooistra, 1997	PAN	1	131	AF256154
Australia	Perth (WA)***	M. Polifrone and S. Williams, 1999	WA.1	5	116–120	AF256155–159
			WA.2	2	116	AF256160–161

* var. *occidentalis* (J. Agardh) Borgesen; ** var. *lamourouxii* (Turner) Weber-van Bosse; *** var. *laetevirens* f. *cylindracea* (Montagne) Weber-van Bosse; [n.a.] not available. See drawings in Fig. 2.

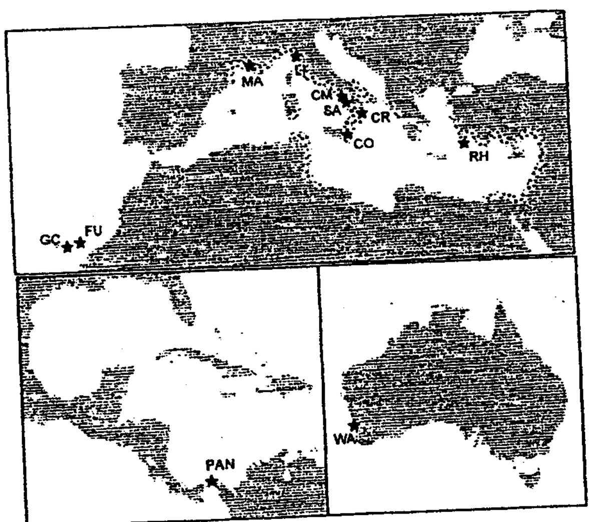


Fig. 1. Geographic location of collections. Stippled bands show the currently known distribution of *C. racemosa* in the Mediterranean. Note its absence from the Western Mediterranean.

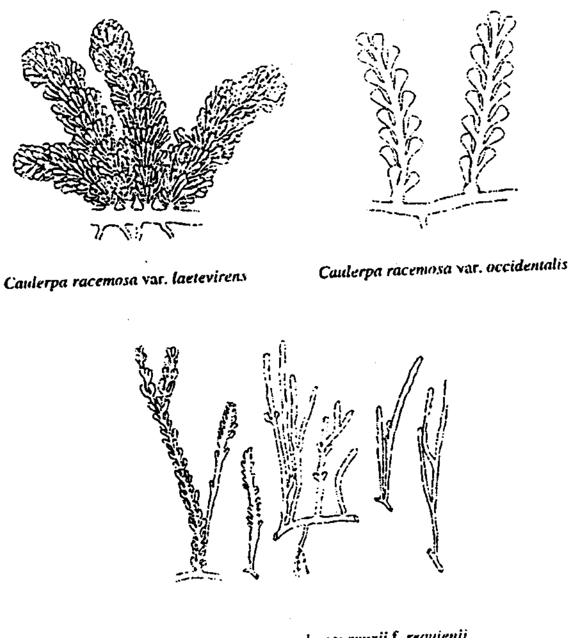


Fig. 2. The three morphological varieties described for the Western Australian, Western Mediterranean and Rhodes samples: *Caulerpa racemosa* var. *laetevirens* (J. Agardh) Borgesen; *Caulerpa racemosa* var. *occidentalis* (J. Agardh) Borgesen and *Caulerpa racemosa* var. *lamourouxii* f. *requienii* (Turner) Weber-van Bosse. Drawings are from Sartoni (1978), Panayotidis & Montesanto (1994) and Huvé (1957) respectively.

with and without gaps, and under polymorphism parsimony (see below). Bootstrap analyses (1000 replicates) were performed on the complete data.

Kimura 2-parameter distances were calculated in PAUP among different groups of sequences in order to estimate intra- and inter-individual variation.

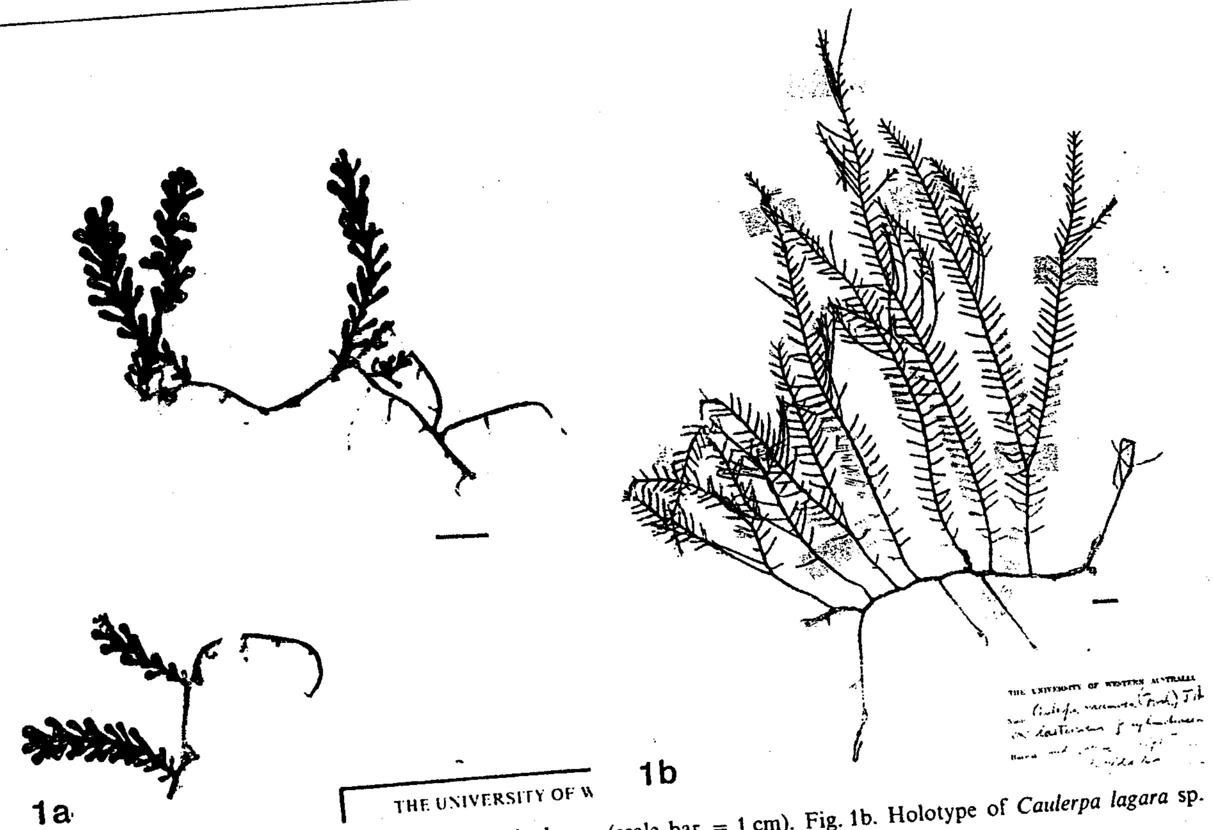


Fig. 1a. *Caulerpa racemosa* var. *laetevirens* form. *cylindracea* (scale bar = 1 cm). Fig. 1b. Holotype of *Caulerpa lagara* sp. nov. (UWA 3284) (scale bar = 1 cm).

Estuarine and coastal environments in south-west Western Australia display differences in several factors presently perceived as being important in the control of morphology in *Caulerpa*. Svedelius (1906) stated that substratum and light intensity (depth) can control morphology in *Caulerpa*. Morphological variation due to different light and temperature conditions was shown in laboratory culture by Ohba and Enomoto (1987). Coppejans and Beeckman (1989) suggested that the influences of salinity, water movement and emersion time may be valuable in further describing the relationship between environment and morphology in *Caulerpa*. The Swan River estuarine environment has mud substratum, seasonally brackish water (13–34.2‰), very low light (Sechii depths to 0.3 m) and cold temperatures (12.8–17.2 °C) (John 1983), whereas the coastal intertidal environment has solid limestone substratum, constant oceanic salinities, very high light intensities and potentially high temperatures.

This research was carried out to investigate whether estuarine and coastal growth-forms of *C. racemosa* are determined by environment factors or whether differences in morphology are fixed genotypically. Culture techniques were used to assess the short term morphological and performance (health) responses of both growth-forms to a range of environmental pa-

tus. The null hypothesis was that both growth-forms were of the same genotype. It was thus expected that the morphological variation observed was only a short term response to the environmental stimuli varying between the two habitats and that both growth-forms would perform equally well under the range of experimental conditions.

Material and Methods

Caulerpa racemosa (estuarine growth-form) was collected at 1.5–2.5 m depth from Pelican Point in the Swan River during the winter of 1990 (Fig. 2). These estuarine specimens were cut into portions with two to five assimilators and at least one growing tip. To lessen numbers of bacteria and epiphyte spores, specimens were washed in seawater containing 'Miltons' anti-bacterial solution. The algae were left for 6 days before culture, to allow the wounds to heal (see Dreher *et al.* 1978). Specimens were slowly acclimatised to their experimental salinities. *Caulerpa racemosa* (coastal growth-form) was collected from th intertidal/subtidal reef to the south of the main Cottesloe groyne (Fig. 2). Material preparation was th same as that of the estuarine material.

Light and salinity were varied in a 4 × 4 cultu experiment with five replicates of each treatmer

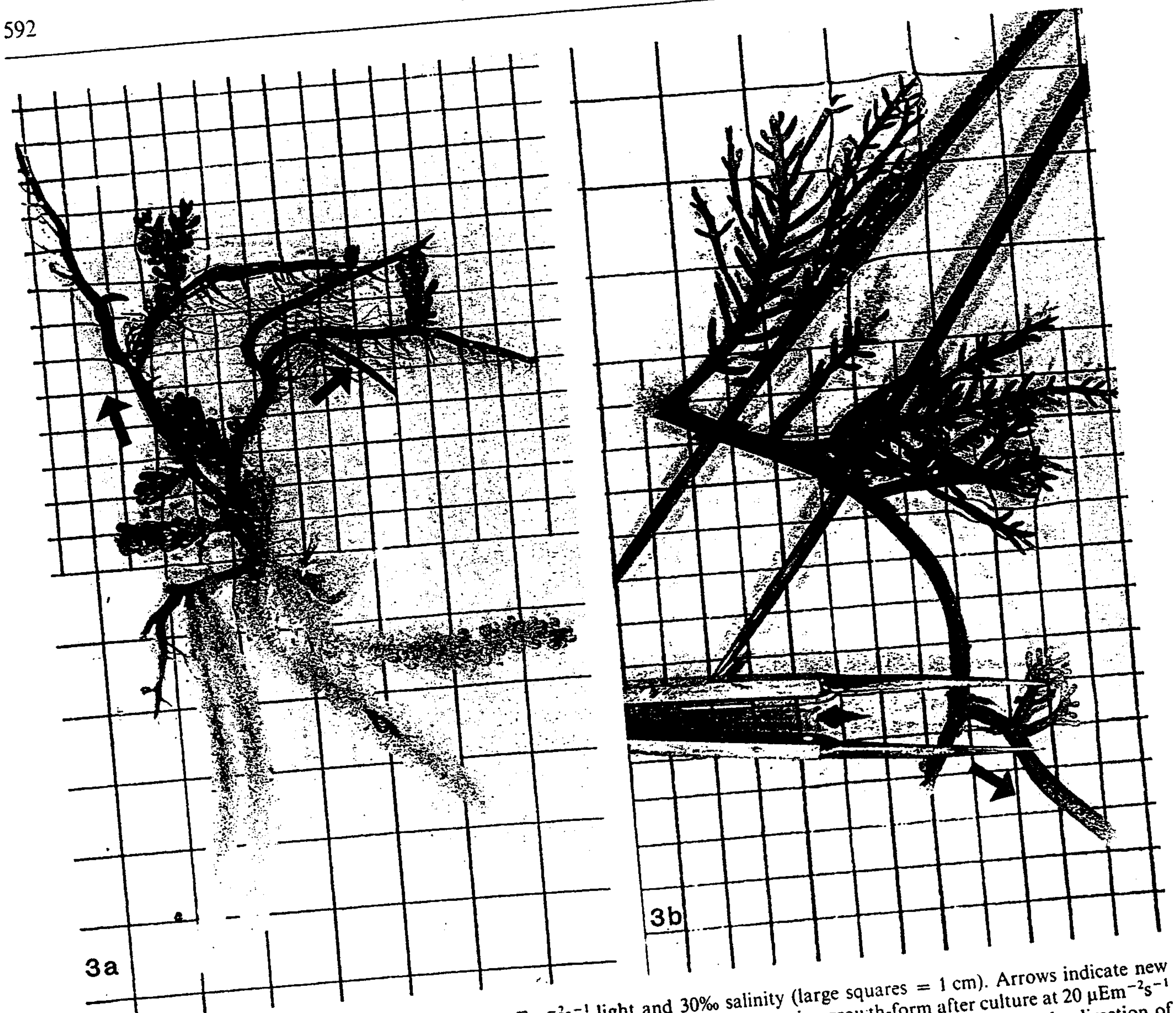


Fig. 3a. Coastal growth-form after culture at 60 μEm^{-2}s^{-1} light and 30‰ salinity (large squares = 1 cm). Arrows indicate new growth, starting at the base of the arrow, in the direction of the arrow. Fig. 3b. Estuarine growth-form after culture at 20 μEm^{-2}s^{-1} light and 30‰ salinity (large squares = 1 cm). Arrows indicate new growth, starting at the base of the arrow, in the direction of the arrow.

grew at 20‰ salinity. The coastal growth-form showed slightly higher growth at 20 and 60 μEm^{-2}s^{-1} light, but there was no apparent influence on the estuarine growth-form (Table III).

Both growth-forms of *C. racemosa* showed very low levels of morphological variation for a range of light and salinity conditions in culture. The new ramuli of coastal growth-form algae were clavate and distinctly radially arranged (Fig. 3a). The new growth on the estuarine growth-form had the robust appearance of the parent tissue (see stolon and stem of assimilator). The ramuli were terete, notice particularly the lowest (oldest) ramuli on the new growth of the estuarine growth-form and compare to the ramuli on the parent assimilators (Fig. 3b). The density of ramuli reduced slightly in the coastal growth-form under experimental conditions, however in the estuarine growth-form, ramuli density appears to have remained constant (Figs 3a and 3b).

Table I. Number of algae showing growth of different organs under culture conditions.

Growth-form	No. of scored algae	No. showing growth	Organ showing growth: Stolon	Assimilator	Rhizoid
	64	25	25	11	22
			6	7	1

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Short-term effects of nutrient enrichment of the sediment and interactions between the seagrass *Cymodocea nodosa* and the introduced green alga *Caulerpa taxifolia* in a Mediterranean bay

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Abstract

Nutrient addition experiments provide a means for testing ecological theories concerning the effects of nutrient availability on community composition and development. Here we present the results of two reciprocal short-term (4 months) experiments testing for the effects of nutrient addition in the sediment and competitive interactions between the seagrass *Cymodocea nodosa* and the tropical green alga *Caulerpa taxifolia*. This study was conducted from 1 June to 30 September 1995 in a bay on the south coast of Elba Island (Italy) recently impacted by the alga. Each experiment consisted of the manipulation of the level of nutrients (addition vs. control) and the manipulation of neighbours (presence vs. removal). Response variables were blade density and length of *C. taxifolia* in one experiment and shoot density and leaf length of *C. nodosa* in the other. Results indicate that during the growing season the canopy of *C. nodosa* did not have any effect on *C. taxifolia* growth while nutrient addition in the sediment favoured colonization (higher blade density) of this alga independently of the presence of the seagrass. Conversely, *C. taxifolia* had a negative effect on shoot density of *C. nodosa* independently of nutrient availability. Hence, colonization by the introduced alga *C. taxifolia* may provide an explanation for the regression of *C. nodosa* beds occurring at the site. Furthermore, we demonstrated that *C. taxifolia* was favoured by high nutrient loads in the sediment, while *C. nodosa* was not; this might affect the outcome of the interactions among these species in the long-term. © 1997 Elsevier Science B.V.

Keywords: Nutrient enrichment bioassay; *Caulerpa taxifolia*; Seagrasses; Porewater nutrients; Biological invasions; Species interaction; *Cymodocea nodosa*

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Threat to macroalgal diversity: effects of the introduced green alga *Caulerpa racemosa* in the Mediterranean

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ABSTRACT: *Caulerpa racemosa* is a tropical green alga introduced into the Mediterranean probably as an immigrant from the Red Sea. This study evaluated the modifications caused by *C. racemosa* invasion on the structure of the benthic macroalgal community near Leghorn (northwestern Mediterranean). To achieve this objective, we studied invasion by *C. racemosa* of algal assemblages on 2 different substrata by monitoring the structure of the phytobenthic community before and after the invasion of the alga and comparing our results with assemblages not invaded by *C. racemosa*. Results showed that the invasive alga *Caulerpa racemosa* completely covered the surface of both types of substratum 6 mo after the start of the invasion. With rapid horizontal elongation of the stolons (up to 2 cm d^{-1}) *C. racemosa* overgrew native macroalgal species within 4 mo. As a consequence, phytobenthic community structure deeply changed: species cover, number and diversity greatly decreased. Furthermore, the relative importance among vegetation layers of the macroalgal assemblage changed: turf and encrusting species were deeply affected while erect species survived after 1 yr of the invasion. During the period from December to May, when both cover and biomass of *C. racemosa* diminished, the macroalgal community did not return to initial conditions. Instead, the structural changes increased in the following season, indicating the importance of *C. racemosa* invasion on a local scale.

KEY WORDS: *Caulerpa racemosa* · Introduced species · Invasion · Macroalgae · Species diversity

INTRODUCTION

Biological invasions affect the integrity of natural communities in many different ecosystems (Vitousek et al. 1997). This adverse process is increasing, especially in the marine environment where it is linked to the intensification of international shipping, aquaculture and aquarium activity (Carlton 1989, Carlton & Geller 1993, Verlaque 1994, Ribera & Boudouresque 1995). However, in comparison to an extensive literature documenting the rate of spread and current distribution of non-indigenous species (Carlton & Scanlon 1985, Piazzi et al. 1994, Russell & Balazs 1994, Cohen et al. 1995, Piazzi et al. 1997), quantitative studies on the effects of marine invasions on resident communities are rare (but see Bertness 1984, Trowbridge 1995, Grosholz & Ruiz 1995, Abrams 1996, Ceccherelli & Cinelli 1997, Reusch & Williams 1998). In this regard, a further fundamental goal for ecologists is to estimate the vulnerability of different communities to invasions (Burke & Grime 1996, Rejmánek & Richardson 1996, Thébaud et al. 1996, Williamson & Fitter 1996, Dukes & Mooney 1999, Ceccherelli et al. 2000). Structural modifications of native communities may depend on the characteristics of both invaded systems and of introduced species (Stachowicz et al. 1999).

Displacement of existing flora by introduced algal species, intentional or accidental, has been widely reported (Sindermann 1991, Chambers et al. 1993),

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Molecular evidence for the aquarium origin of the green alga *Caulerpa taxifolia* introduced to the Mediterranean Sea

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ABSTRACT: Here, we present the first molecular evidence that the tropical green alga *Caulerpa taxifolia*, which is quickly spreading in the Mediterranean and out-competing native species, escaped to the sea from a public or private aquarium. Our data show that this alga is genetically identical to the strain cultivated in western European aquaria since the early 1970s. The facility with which this strain is obtained world-wide represents a potential danger of additional biological invasions.

KEY WORDS: *Caulerpa taxifolia* · Mediterranean · Aquaria · ITS rDNA

INTRODUCTION

The genus *Caulerpa* (Ulvophyceae) comprises about 70 species ubiquitous in coastal marine environments. One of these species, *C. taxifolia* (Vahl) C. Agardh, is common in tropical seas and has been reported along the Atlantic American coast (from Brazil to the Caribbean), in the African Atlantic (Gulf of Guinea), the Indian Ocean and the Pacific Ocean (Taylor 1977, Meinesz & Boudouresque 1996). Since the early 1970s, a strain assigned to this species, of unknown geographical origin, has been cultured to be used as a natural display in the tropical marine aquarium of the Wilhelma Zoologischbotanischer Garten (Stuttgart, Germany). Between 1980 and 1983, this strain was given to the tropical aquarium of Nancy (Northern France) and subsequently to the aquarium of Monaco, located on the Mediterranean shore (Meinesz & Boudouresque 1996). As a rapidly growing and decorative alga, it is particularly appreciated by aquariophiles (Artaut 1987).

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In the mid-1980s, an alga similar to the *Caulerpa taxifolia* strain cultivated in public aquaria was observed for the first time in the Mediterranean Sea, off the coast of Monaco (Meinesz & Hesse 1991). Since then, the species has rapidly spread in the Northwestern Mediterranean, invading most of the sublittoral environments and competing with native benthic species (Verlaque & Fritayre 1994, Villèle & Verlaque 1995, Bellan-Santini et al. 1996, Bartoli & Boudouresque 1997). Spectacular progression of this alga was observed on the French and Italian Riviera, where the affected areas increased from 1 m^2 in 1984 to 3 ha in 1990 and 3000 ha in 1996 (Meinesz et al. 1997). Isolated colonies were also discovered in French Catalonia (1991), Tuscany, the Balearic Islands (1992), Sicily (1993), and Croatia (1994) (Meinesz & Boudouresque 1996) (Fig. 1), possibly resulting from vegetative dissemination by pleasure boats and/or fishing nets (Sant et al. 1996). Morphological, ecological and physiological studies have demonstrated that the Mediterranean *C. taxifolia* differs from known tropical populations, exhibiting larger size, vigorous growth and resistance to low temperatures (Meinesz et al. 1995, Komatsu et

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J. Wiedenmann · A. Baumstark · T. L. Pillen
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DNA fingerprints of *Caulerpa taxifolia* provide evidence for the introduction of an aquarium strain into the Mediterranean Sea and its close relationship to an Australian population

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Abstract The occurrence of *Caulerpa taxifolia* in the Mediterranean Sea was reported for the first time in 1984. Since then the alga has spread rapidly and is now considered to be a potential threat to sublittoral ecosystems. Two hypotheses on the origin of the Mediterranean strain of *C. taxifolia* have been discussed in the literature. One hypothesis assumed migration of the alga from the Red Sea, the other introduction via a public aquarium. The hypothesis of a descent from an aquarium strain has been supported strongly by recent studies based on DNA sequences. The DNA fingerprints of *C. taxifolia* presented here also provide evidence for the descent of the Mediterranean *C. taxifolia* from an aquarium strain. Furthermore, the present study shows that a strain of *C. taxifolia* from Manly Harbour/Moreton Bay (Australia) is closely related to the aquarium/Mediterranean strain. The feasibility of detecting similar genotypes by restriction digests of total DNA is demonstrated, which will facilitate the ongoing search for further relatives of Mediterranean *C. taxifolia*.

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Introduction

Caulerpa taxifolia (Vahl) C. Agardh (Ulvophyceae: Caulerpales) is a common green alga of tropical seas (Meinesz et al. 1994). In 1984 a low-temperature-resistant strain of *C. taxifolia* was observed for the first time in the Mediterranean Sea, along the coast of Monaco (Meinesz and Hesse 1991; Meinesz and Boudouresque 1996; Meinesz et al. 1998). Since then the alga has spread rapidly by vegetative reproduction, and covered more than 4600 ha of sea-bed in 1997 (Meinesz 1992; Meinesz et al. 1998). With its potential to overgrow natural biotopes, it represents a major risk for Mediterranean sublittoral ecosystems (Boudouresque et al. 1995; Romero 1997).

Two hypotheses on the origin of the Mediterranean strain of *C. taxifolia* are posed in the literature: one assumes migration of *Caulerpa mexicana* from the Red Sea and metamorphosis into *C. taxifolia* (Chisholm et al. 1995); the other, an introduction of *C. taxifolia* via a public aquarium (Meinesz and Hesse 1991; Meinesz and Boudouresque 1996). The latter has been strongly supported by demonstrating that *C. taxifolia* from the Mediterranean Sea and from different aquaria have identical ITS rDNA sequences (Jousson et al. 1998) and that – also based on ITS rDNA sequences – Mediterranean *C. taxifolia* and *C. mexicana* are not conspecific (Jousson et al. 1998; Olsen et al. 1998). Knowledge of the origin of this invasive strain of *C. taxifolia* and on the population structure are fundamental for understanding the dynamics of the invasion.

In our study we used DNA fingerprinting to characterise strains of *C. taxifolia*, as this technique has an exceedingly high power for differentiating and identifying individual genotypes (Epplen et al. 1992; Housman 1995; Coffroth 1998). Moreover, as methods based on DNA sequences, like studies of ITS (internal transcribed

Invasive alga reaches California

The alga has been identified that threatens to smother Californian coastal ecosystems.

The recent discovery of the marine green alga *Caulerpa taxifolia* on the Californian coast[1,2] has raised public concern about the potential danger of a new invasion similar to the one endured by the Mediterranean Sea over the past decade. A small colony of *C. taxifolia* introduced into the Mediterranean in 1984[3,4] from a public aquarium[5] has spread to more than 6,000 hectares today, outcompeting native species and seriously reducing diversity in areas of the northwestern Mediterranean[6]. This invasive strain of *C. taxifolia* differs from tropical populations in that it is much larger, grows more vigorously, does not rely on sexual reproduction, and is resistant to low temperatures[4,6,7]. Here we evaluate the risk of invasion by Californian *C. taxifolia* by comparing it genetically with the Mediterranean and aquarium strain, as well as with native tropical populations. Our results show that the Californian alga is the same as the invasive Mediterranean strain, calling for its rapid eradication to prevent a new invasion.

Colonies of *C. taxifolia*, morphologically similar to the Mediterranean strain, have been reported from Carlsbad and Huntington Harbour, California. *C. taxifolia* covers 3,500 square metres at Carlsbad and is dispersed over 20,000 square metres at Huntington Harbour. We extracted DNA from algal samples collected in these areas in independent analyses in two laboratories (Geneva, Switzerland, and Fresno, California). The internal transcribed spacer of ribosomal DNA (ITS rDNA) was amplified by using the polymerase chain reaction and then cloned[5], and the sequence data were used to examine intraspecific variability within *C. taxifolia*. Five clones were sequenced for each isolate in Geneva; two specimens analysed in Fresno were sequenced directly. We compared Californian alga sequences with 148 ITS rDNA sequences obtained from 32 *C. taxifolia* specimens collected from all over the world.

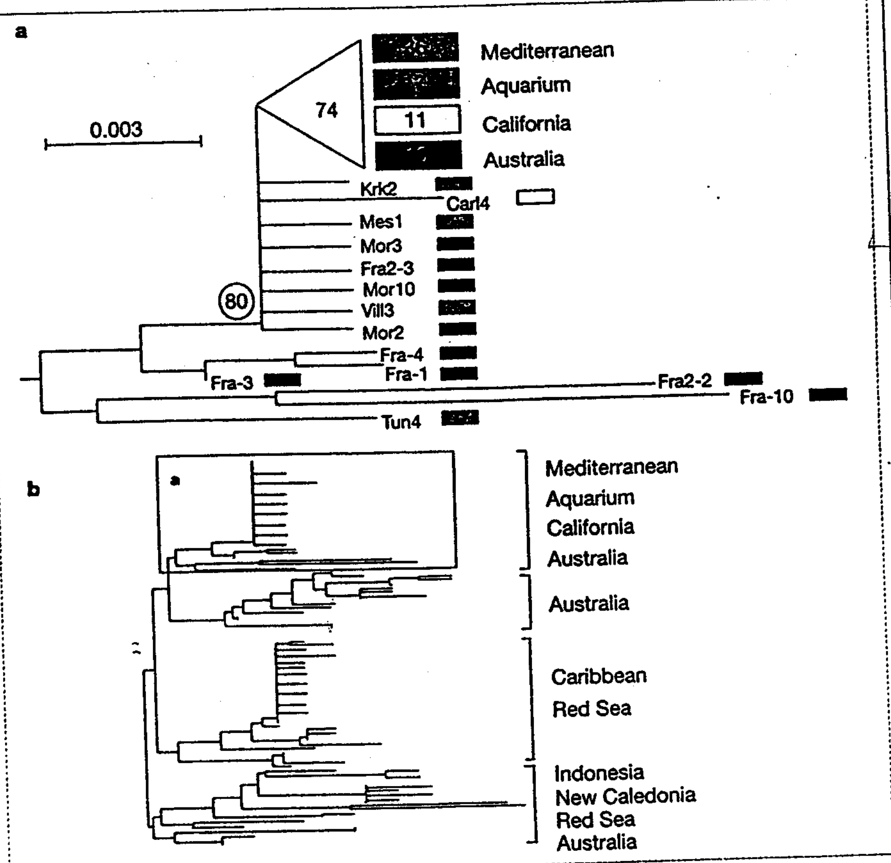

Figure 1 Phylogenetic tree of *C. taxifolia* inferred from 160 complete ITS (internal transcribed spacer) ribosomal DNA sequences by using the neighbour-joining method applied to pairwise sequence distances calculated by the Kimura two-parameter method. **a**, The clade containing all Californian sequences; the origin of different sequences is indicated by coloured rectangles containing the number of identical sequences; number 80 at the base of the clade corresponds to the bootstrap value based on 1,000 replicates. Abbreviations are: Krk (Krk Island, Croatia), Carl (Carlsbad, California), Mes (Messina, Italy), Mor (Moreton Bay, Australia), Fra (Fraser Island, Australia), Vill (Villefranche, France), Tun (Sousse, Tunisia). **b**, Total tree, presenting relationships between algae of different geographical origins. Sampling localities (number of analysed sequences in parentheses) in the Mediterranean Sea: Majorca (3), Saint-Cyprien (3), Le Brusc (3), Toulon (3), Port-Cros (3), Le Lavandou (3), Villefranche (5), Messina (3), Elba (3), Krk (3) and Hvar (3) Islands, Croatia; Sousse, Tunisia (5); aquarium samples: Nancy (3), Stuttgart (5), Geneva (3), Oahu (3), Enoshima (3); sampling localities off the Californian coast: Carlsbad (6) and Huntington Harbour (6); from Australian waters: Townsville[8] (1), Fraser Island (13), Moreton Bay (18), Port Hacking (5), Lake Conjola (9); in the Caribbean: Puerto-Rico (5), Martinique (7), Guadeloupe (3); from the Red Sea: Safaga, Egypt (21); from Indonesian waters: Djakarta[9] (1); and from New Caledonia: Noumea (8). Ethanol-fixed specimens of Californian *C. taxifolia* have been deposited in the herbarium of the Botanical Garden in Geneva and in the Jepson Herbarium at the University of California, Berkeley. EMBL accession numbers for the sequences analysed are AJ228960–AJ228999 and AJ299742–AJ299811.

Eleven out of twelve Californian sequences were found to be identical to all aquarium and most Mediterranean sequences (Fig. 1a). The single divergent sequence (Carl4), which differs by two nucleotide substitutions, branches with other Mediterranean sequences. This group is clearly separated from all sequences obtained from other isolates of *C. taxifolia*, including tropical strains of this species collected in the Red Sea, Caribbean Sea and the Indo-Pacific (Fig. 1b). Phylogenetic analysis of all *C. taxifolia* ITS rDNA sequences reveals a relatively robust clade (80% bootstrap value) grouping Californian, Mediterranean, aquarium and some Australian sequences. Thus, the Californian alga belongs to the aquarium–Mediterranean strain.

A particular feature of all aquarium, Mediterranean and Californian (AMC) specimens we examined is the low degree of intra-individual polymorphism of their rRNA genes: 64 sequences from AMC isolates were identical, whereas five were slightly divergent (from 0.4 to 1.1%). Such homogeneity contrasts with the high polymorphism of ITS copies in non-AMC isolates, characterized by intra-isolate sequence divergence ranging from 0.8 to 3.6%.

It is notable that the eastern Australian polymorphic isolates possess ITS copies that are identical to the AMC type (10 out of 46 Australian sequences). This may point to Australia as being the possible native land of the invasive alga, but this needs to be confirmed by analysis of other genetic markers to ascertain the similarity of the strains.

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QUALITY MARINE

PLANTS

Code	Common Name	Origin	Scientific Name
60000	CAULERPA, ASSORTED	-	*Caulerpa sp.*
60001	CAULERPA ON ROCK, ASSORTED	-	*Caulerpa sp.*
60002	CAULERPA, GRAPE	-	*Caulerpa sp.*
60003	CAULERPA ON ROCK, GRAPE	-	*Caulerpa sp.*
60004	CAULERPA, NOTCHED LEAF	-	*Caulerpa sp.*
60005	CAULERPA ON ROCK, NOTCHED LEAF	-	*Caulerpa sp.*
60006	CAULERPA, SMOOTH LEAF	-	*Caulerpa sp.*
60007	CAULERPA ON ROCK, SMOOTH LEAF	-	*Caulerpa sp.*
60008	GRASS ON ROCK	-	-
60010	HALIMEDA	Indian Ocean	*Halimeda sp.*
60011	KELP ON ROCK, RED	-	-
60012	KELP, RED	Hawaii	-
60009	HALIMEDA	Caribbean	*Halimeda sp.*
60013	MERMAID'S FAN	Caribbean	*Udotea sp.*
60014	SHAVING BRUSH	Caribbean	*Penicillus sp.*
60015	TURTLE WEED	Indian Ocean	*Codium sp.*
60016	XMAS TREE	Caribbean	*Penicillus sp.*
7822	CAULERPA PROLIFERA	-	*Caulerpa prolifera*
60018	LETTUCE	-	*Ulva sp.*

Report on Aquarium/Pet Shop Store Survey
March 23, 2001
San Diego Regional Water Quality Control Board

A total of seventy (70) aquarium and pet shop stores were surveyed between December, 2000 and March, 2001 (Fig 1). The stores are located in San Diego County (54) and in Orange County (16). Contact information was obtained through a phone directory listing.

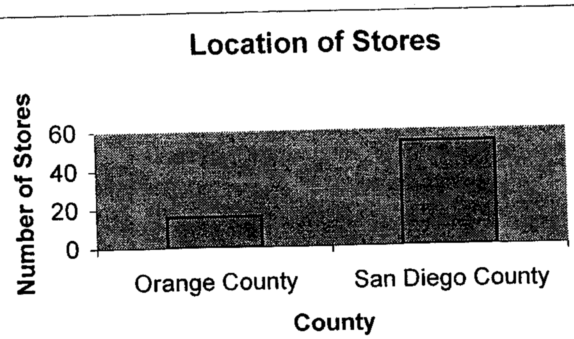


Fig. 1

Initially, the survey consisted of several questions regarding the handling of *Caulerpa* and other saltwater algae. Store managers and employees were also asked about their general knowledge of *Caulerpa*. Unfortunately, most of them were not cooperative in answering our survey. For this reason, we decided our survey would consist of only two questions, for most stores. Managers were only asked if they handled *Caulerpa* or any other type of seawater algae at their stores.

Four (5%) of the stores reported handling Caulerpa (Fig. 2), 3 in San Diego County and 1 in Orange County (Table 1). Only one store reported having the algae for sale at the time of contact. The other three reported not selling it at the moment, but they said that they could obtain it. When asked about the species of *Caulerpa* handled at their stores, they stated not knowing the kind. Except for the four stores selling *Caulerpa*, all stores reported they did not sell any type of saltwater algae.

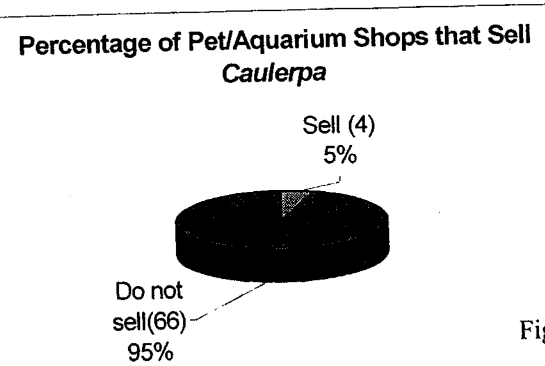


Fig. 2

Table 1. **Name and location of aquarium/pet store that sell *Caulerpa***

Store Name	Location
1. Octopuss' Garden	San Diego County
2. Fish Heaven	San Diego County
3. Pet Kingdom	San Diego County
4. Coral Island	Orange County

(see complete list of all stores contacted at bottom)

Not all stores in Orange County were contacted, since we were notified that a student from CSU Fullerton, as part of a thesis project, concurrently conducted a survey similar to ours in that area. Once we obtain data from the student, we will be able to make a better estimate of the total number of aquarium/pet stores selling *Caulerpa* in both San Diego County and Orange County.

All stores contacted by our agency were sent outreach materials, including brochures, posters, and a *Caulerpa* Fact Sheet.

Complete list of Stores Contacted

#	DATE	STORE NAME	AREA	Caulerpa	Other algae
1	12/28/00	Aquatic Warehouse	North C.	No	No
2	12/28/00	Petco	North C.	No	No
3	12/28/00	Pet & Vet Supply Center	North C.	No	No
4	12/28/00	Jungles and Oceans	East C.	No	No
5	12/28/00	Fountain's Aquarium	East C.	No	No
6	12/28/00	Lemon Grove Pets	East C.	No	No
7	12/28/00	PetRageous!	East C.	No	No
8	12/28/00	Mike's Aquarium & Pet	East C.	No	No
9	12/28/00	San Diego Pet Supply	East C.	No	No
10	12/28/00	Parkway Pet Center	East C.	No	No
11	12/28/00	Petco	East C.	No	No
12	12/28/00	Petco	East C.	No	No
13	12/28/00	Clairemont Tropical Fish	North C.	No	No
14	12/28/00	Tropical Fish World	North C.	No	No
15	12/28/00	Octopuss' Garden	East C.	Yes	No
16	1/3/01	Pet Kingdom	East C.	Yes	No
17	1/3/01	Pet Works	East C.	No	No
18	1/3/01	Pet Plaza	East C.	No	No
19	1/3/01	Plaza Pet	East C.	No	No
20	1/3/01	Crystal Blue Aquarium	East C.	No	No
21	1/8/01	Tri-City Tropical Fish	North C.	No	No
22	1/8/01	Fish Heaven	North C.	Yes	No
23	1/8/00	Encinitas Tropical Fish	North C.	No	No
24	1/8/00	Penasquitos Pets	North C.	No	No
25	1/16/00	The Pet Pantry	Orange	No	No
26	1/16/01	Aneheim Feed & Pet	Orange	No	No
27	1/16/01	Best For Pets	Orange	No	No
28	1/16/01	Coral Island	Orange	Yes	No
29	1/16/01	Harbor Fish & Pets	Orange	No	No
30	1/16/01	Lucky Pets Center	Orange	No	No
31	1/19/01	Noah's Ark Pet Kingdom	Orange	No	No
32	1/19/01	Pet Plus	Orange	No	No
33	1/19/01	Petco	Orange	No	No
34	1/19/01	Petco	Orange	No	No
35	1/19/01	Petco	Orange	No	No
36	1/19/01	Petco	Orange	No	No
37	1/19/01	Petco	Orange	No	No
38	1/19/01	Petco	Orange	No	No
39	2/2/01	Petco	Orange	No	No

40	2/2/01	San Bar Tropical Fish &	Orange	No	No
41	2/28/01	Petco	North C.	No	No
42	2/28/01	Petco	North C.	No	No
43	2/28/01	Petco	North C.	No	No
44	2/28/01	Petco	North C.	No	No
45	2/28/01	Petco	North C.	No	No
46	2/28/01	California Pets	North C.	No	No
47	3/1/01	A Bird Heaven	North C.	No	No
48	3/1/01	A Bird Heaven	North C.	No	No
49	3/1/01	Adams Valley View	North C.	No	No
50	3/1/01	The Animal Keeper	North C.	No	No
51	3/1/01	Animal Pharm	North C.	No	No
52	3/1/01	Bird Crazy	North C.	No	No
53	3/1/01	Birdland of San Diego	North C.	No	No
54	3/1/01	Borrego Feed Pet and	North C.	No	No
55	3/1/01	Live Cargo Reptile and	North C.	No	No
56	3/1/01	Escondido Feed And Pet	North C.	No	No
57	3/1/01	M & S Ponds and	North C.	No	No
58	3/1/01	The Pet Pawler	North C.	No	No
59	3/1/01	Petco	North C.	No	No
60	3/2/01	Raining Cats And Dogs	North C.	No	No
61	3/2/01	Lee's Aquarium Products	North C.	No	No
62	3/2/01	All For Pets	East C.	No	No
63	3/2/01	Alpine Animal Jungle	East C.	No	No
64	3/2/01	Animal House Pet Store	East C.	No	No
65	3/2/01	Fiesta Pet Shop	East C.	No	No
66	3/2/01	Birdland	East C.	No	No
67	3/6/01	Petco	East C.	No	No
68	3/6/01	Pet People	East C.	No	No
69	3/6/01	PetSmart	East C.	No	No
70	3/6/01	Alpine Fishery	East C.	No	No

ICTA

INTERNATIONAL CENTER FOR TECHNOLOGY ASSESSMENT

July 6, 2003

Alan V. Tasker, Ph.D.
Noxious Weed Program Manager, Unit 134
Invasive Species and Pest Management, PPQ
USDA APHIS, 4700 River Road
Riverdale, MD 20737

Re: Supplemental information in support of noxious weed listing petition for *Caulerpa taxifolia* (whole species) and *Caulerpa* (entire genus)

Dear Dr. Tasker,

Attached please find supplemental information submitted by the International Center for Technology Assessment (ICTA) and Susan L. Williams, in support of the above-referenced caulerpa noxious weed listing petitions. We did receive your letter dated June 2, 2003, acknowledging receipt of the petitions and indicating your intent to respond by July 30. We have obtained this supporting information since we filed the petitions:

1. A webpage, www.dep.state.fl.us/lands/invaspec/2ndlevpgs/nUNvasion.htm indicating that the Florida Department of Environmental Protection considers *C. brachypus* to be a serious new invader in Florida waters.

2. A Nov. 28, 2001, Press Release from the Harbor Branch Oceanographic Institution, Ft. Pierce, Florida, www.hboi.edu/news/press/nov2901.html indicating that *C. brachypus*, *C. verticillata*, and *C. racemosa* are becoming harmful invaders in the State's inshore waters.

3. An Apr. 25, 2003, editorial in the *Treasure Coast Palm*, a Florida paper, urging readers to lobby Congress to take steps against the ongoing *C. brachypus* invasion including listing it as an invasive species and ending its sale.

4. A webpage, www.inlandaquatics.com/prod/tr-algae.html indicating that the company Inland Aquatics is selling eight caulerpa species, notably including *C. taxifolia*, *C. brachypus*, and *C. racemosa*, all known harmful invaders.

Considering the attached information together with that submitted earlier with the two petitions, the evidence in support of our listing requests is abundant and convincing. You are strongly encouraged to exercise your judgment in a proactive way so as to avoid further caulerpa infestations. Unless USDA tackles these problems immediately, preferably genus-wide, further environmental degradation and economic harm is inevitable.

Finally, I would like to inform you that the list of endorsers has expanded. First, the national conservation

666 Pennsylvania Ave., S.E., Suite 302, Washington, D.C. 20003 • Phone: 202-547-9359 • Fax: 202-547-9429
www.icta.org • Email: info@icta.org

group Defenders of Wildlife has endorsed both petitions. Second, a coalition of environmental groups known as the National Environmental Coalition on Invasive Species (NECIS) has endorsed them. The endorsing members, consisting of groups active in invasives species issues, are: The Nature Conservancy, American Lands Alliance, National Wildlife Federation, Union of Concerned Scientists, ICTA, and Defenders. Each of those groups is also listed on the sheet sent with the original petitions as endorsing the petitions individually and now they have done so as voting members of the NECIS coalition.

Thank you for your consideration of this information. We look forward to your response.

Sincerely,

Peter T. Jenkins
Attorney/Policy Analyst

cc: Susan L. Williams, Ph.D.

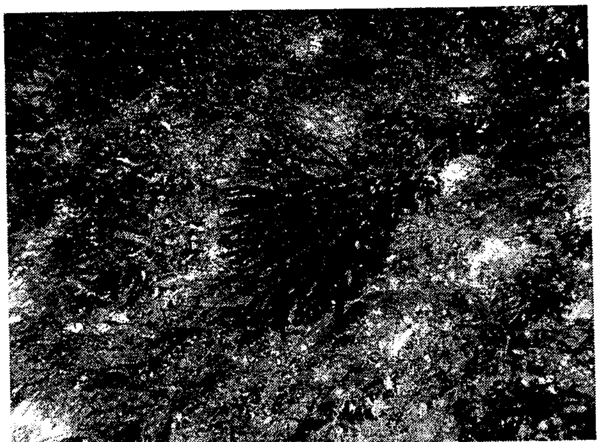
Enclosures

my Florida

Department of Environmental Protection

"More Protection, Less Process"

ISWG's New Invasive Species Reports in Florida's Natural Environments



Caulerpa brachypus, Harbor Branch photo

Caulerpa brachypus

Jupiter, Fla. According to recent reports from divers and fishers, the coral-smothering non-native seaweed known as *Caulerpa brachypus* has now become so thick on reefs in Florida's Palm Beach County, about an hour north of Miami, that it is forcing lobsters and fish away. The species has also now been spotted as far north as Ft. Pierce, Fla., about sixty miles away. For more information, please see the Harbor Branch Oceanographic Institute Press Release: http://www.hboi.edu/news/press/jan2303.html

Last Updated: 04/16/03

Green mussel (*Perna virdis*)

The green mussel has been found in Tampa Bay in late 1999. It is originally from the Indian and Pacific Oceans. For more information on the green mussel, please download the USGS fact sheet:

http://www.fcsc.usgs.gov/greenmussel2.pdf

Giant Salvinia (*Salvinia molesta*)

According to the USGS, the February 1999 discovery of giant salvinia, a native of South America, at a canal in Naples, followed months of chemically treating a small population that was initially thought to be *Salvinia minima* [*Jacqueline Smith s.n.* (FLAS)]. *Salvinia molesta* is now suspected to have been occurring in that canal for about three years. In January 2000, *Salvinia molesta* was again found in Naples, at a separate location. Plants were found just a few days after being placed by a homeowner into a 5 acre subdivision pond. For more information about giant salvinia, please see the USGS website or DEP's Weed Alert.

PRESS RELEASE

HARBOR BRANCH SCIENTIST SAYS AREA REEFS HEADED FOR POSSIBLE "ECOLOGICAL MELTDOWN"

SIX-MONTH STUDY SHOWS PROBLEM OF INVASIVE ALGAE BLOOMS IS WORSE THAN ORIGINALLY THOUGHT

FORT PIERCE, FLA., November 28, 2001 - A news conference will be held Thursday, Nov. 29th, at 9am at the dock at New Port Cove Marina, 255 E. 22nd Court in Riviera Beach, to announce the results of a comprehensive six-month study of invasive and harmful algal blooms appearing on reefs in the waters off Palm Beach County.

HARBOR BRANCH research scientist Dr. Brian Lapointe, an expert on invasive species of algae who first recognized the problem in the early 90s, says "the study pretty conclusively" points to treated human sewage making its way into Florida's waterways and near-inshore coastal areas as the culprit.

"I don't think it's too strong to say that we're headed for an ecological meltdown. The sewage runoff is raising the nutrient levels of our inshore waters, making an ideal habitat in which these invasive species of algae - notably Caulerpa brachypus, Caulerpa verticillata, and Caulerpa racemosa - can establish themselves and crowd out every thing else," Dr. Lapointe said.

The result is large patches of reef, often hundreds of yards across, where nothing else grows. Fish that rely on the native marine plants disappear as well, and the affected areas begin to resemble an underwater desert, Dr. Lapointe said.

State Senator Ken Pruitt, R-Port St. Lucie, was instrumental in providing direction and support in obtaining the funds for Dr. Lapointe's study.

Dr. Lapointe and several research assistants, including Dr. Peter Barile, Palm Beach resident Connie Gasque, and HARBOR BRANCH Public Relation's HARBOR BRANCH, made dozens of dives in seven areas along the coast from Deerfield Beach to Jupiter. Dives were made and samples of algae collected at depths ranging from 30-feet to 130-feet deep.

Dr. Lapointe said he was looking for elevated levels "N\15", a stable nitrogen isotope that in the right proportions is associated with human sewage. Sample results showed the highest "N\15" values in Boca Raton and the lowest in Juno Beach. The values were also higher in the shallower samples, suggesting that the closer to land or a source of sewage runoff, such as an outfall pipe or an inlet, the higher the nutrients that lead to invasive algae growth.

Dr. Lapointe said one shocking discovery was the presence of a particularly aggressive and damaging species of algae called "*Caulerpa brachypus*".

"Before now, this was only seen in a few small patches in the Caribbean and is really a Pacific species of alga, and it shouldn't even be here," Dr. Lapointe said. "The fact that it is means the problem is worse than we anticipated."

"The combination of an exotic species of alga that takes over the reefs, along with our belief that nutrient levels are rising as a result of pollution and other sources, means we'll be seeing more and more severe bloom events," Dr. Lapointe said.

Dives made in the spring and again in August and September produced more evidence that Caulerpa and Codium algae patches have grown from only a few square meters near the Lake Worth inlet, to an area that is now more than six miles long, extending north toward the Jupiter Inlet.

For more information please call HARBOR BRANCH at 772.465.2400.

HARBOR BRANCH Oceanographic Institution, Inc., is one of the world's leading nonprofit oceanographic research organizations dedicated to exploration of the earth's oceans, estuaries and coastal regions for the benefit of mankind.

Close Window

Editorial: The algae menace

Lobby Congress, the Legislature to save our waters, our lifeblood

April 25, 2003

An algae from the Pacific Ocean is doing serious damage to offshore reefs and the Indian River lagoon, and is threatening the lifestyle and economy of the Treasure Coast. Named *caulerpa brachypus,* the algae suffocates reefs and kills off the lower levels of the local marine food chain, which in turn will reduce both sport and commercial fishing along the Treasure Coast.

The *News* warned of the need to fight this foreign algae in an editorial on April 7, "Our lagoon imperiled."

More than 1,000 people are involved in marine-related businesses in the three-county area, and their jobs are now in jeopardy unless speedy action is taken by everyone — national, state and local governments, environmentalists, the business community and ordinary citizens.

Even more dire is the long-term impact on the brightest hope for the future of the Treasure Coast. Dead reefs and sterile waters populated only by parasites such as the algae will mean an end to the hope of developing a center for marine and agricultural research and education — and the many commercial spin-offs that would develop naturally if the water stays in a natural state.

The algae has no natural enemies in our region and feeds on nitrogen, which is unfortunately plentiful in run-off from farms, groves, golf courses, lawns, and sewage plants.

Floridians need to encourage state legislators and members of Congress to include the algae on lists of invasive species. Call for an end of the sale of the algae for use in aquariums, and support the efforts of the Harmful Algae Bloom Task Force, scheduled to meet this summer, and urge the Indian River Lagoon National Estuary Program board to lobby Washington for assistance in killing this pest.

It will be difficult to get the lawmakers' attention. The last thing they want right now in a tight economy is another "must appropriate." But if no action is taken to fight this algae, our region will lose most of its traditional sources of revenue.

Contact your legislators and tell them to make this battle for the survival of a traditional way of life on our coasts and waterways a top priority. And hurry.

INLAND AQUATICS

CHANGING THE WAY THE WORLD LOOKS AT AQUARIA

MACROALGAE

	Scientific Name	Refs.	Page #	Eac
Blue Ball	Ochtodes	MP/BA	176	
Carpet on a Rock	Derbasia	MP/BA	36	$24.
Caulerpa, Feeder		MP/BA		
Caulerpa, Grape	Caulerpa racemosa	MP/BA		
Caulerpa, Notched	mexicana, taxifolia, sertularioides	MP/BA	42	
Caulerpa, Razor Ribbon	Caulerpa serrulata	MP/BA	44	
Caulerpa, Umbrella	Caulerpa pasploides	MP/BA		$19.9
Caulerpa, Umbrella, Thin	Caulerpa pasploides	MP/BA		$9.9
Caulerpa, Undulated	Caulerpa prolifera	MP/BA	42	
Caulerpa, Undulated, Miniature	Caulerpa brachypus	MP/BA		
Dragons Tongue	Heymenia	MP/BA	134	$14.
Gracillaria, Brown	Gracilaria			
Gracillaria, Red	Gracilaria	MP/BA	190	
Gracillaria, Green	Gracilaria			
Green Branch	Codium sp.	MP/BA	62	$19.
Fan	Udotea flabellum	MP	74	$3.9
Fire Algae		MP/BA		
Halimeda, Clump	Halimeda opuntai	MP	94	$9.9
Halimeda, Rooted	H. incrassata, monile	MP	88	$3.9
Halimeda, Coin	Halimeda tuna	MP	90	$9.9
Irredescent	Dictyota	MP/BA	98	
Red, Dictyota	Dictyota			
Red on Rock, Bubble, Sparkling				$19.9
Red on Rock, Grape		MP/BA		$29.9
Red on Rock, Smooth		MP/BA		$27.9
Red on Rock, Verigated	Rhodophyton			$24.9
Sargassum. On Rock	Sargassum	MP/BA	124	$24.9
Sea Lettuce	Ulva fasciata	MP/BA	22	$2.9

Shaving Brush	Penicillus	MP/BA	80	$3.9
Spaghetti	Chaetomorpha	MP/BA	132	
Turtle Grass	Thalassia sp.			$4.9
Turf, Seed Screen				$9.9

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With questions or comments about this web site, send e-mail to: InlandAquatics@aol.com

Peter Jenkins
<peterjenkins@icta.org>

03/08/2004 03:14 PM

To: "'Alan.V.Tasker@aphis.usda.gov'" <Alan.V.Tasker@aphis.usda.gov>
cc: "'Polly.P.Lehtonen@aphis.usda.gov'" <Polly.P.Lehtonen@aphis.usda.gov>, "'Erich.S.Rudyj@aphis.usda.gov'" <Erich.S.Rudyj@aphis.usda.gov>, "'Elna.B.Hunter@aphis.usda.gov'" <Elna.B.Hunter@aphis.usda.gov>, "'Susan.A.Bright@aphis.usda.gov'" <Susan.A.Bright@aphis.usda.gov>, "'slwilliams@ucdavis.edu'" <slwilliams@ucdavis.edu>
Subject: Article re Caulerpa in FL

Further to my message of this morning on the caulerpa petitions, this article came to my attention today and I forward it to you for your information.

Peter Jenkins

http://www.tcpalm.com/tcp/local_news/article/0,1651,TCP_16736_2699579,00.html

Killer exotic algae skulks in Martin County waters

The invasive species, found in the Indian River Lagoon last year, was spotted tangled in a reef off of the House of Refuge.

By Suzanne Wentley staff writer
March 3, 2004

STUART — The killer lurked all winter.

Exotic, smothering algae — first found in the Indian River Lagoon almost exactly a year ago — has been spotted locally for the first time in nine months.

Research divers two weeks ago found what they thought were pieces of Caulerpa brachypus tangled in an artificial reef offshore of the House of Refuge. On Tuesday, the divers said state scientists confirmed the species is the Pacific Ocean native that's been plaguing coral reefs in Juno Beach.

"It's not good news," said Mark Perry, executive director of the Florida Oceanographic Society. "It is an invasive and it has the potential to really take over. We don't want that to happen to our reefs."

It hasn't been seen locally since June, likely because a strong upwelling of cold water last summer put the warmth-loving algae into dormancy, Perry said. Now rising ocean temperatures have brought it back, he said.

Last March, scientists found the algae for the first time in the Indian River Lagoon — and quickly organized a team of volunteers to search for the species.

The researchers feared the fast-growing exotic algae would feed off the nutrients from stormwater runoff discharged into the estuary and kill sea grasses and other marine life.

Instead, sightings of the algae slowly faded — until this latest dive.

Merle Stokes, coordinator of the Florida Oceanographic Society research dive team, said he was monitoring the county's artificial reefs with other dive team members when he spotted the small, bright- green leaves.

He shot underwater photos of the algae — which could have been another, native species — and sent them to state scientists, who confirmed his fears.

"There was a lot of it. It's not rooted. It was in pieces," Stokes said. "Last year, I don't remember seeing it in such large numbers."

Scientists suspect the algae, native to the Pacific Ocean, might have been carried to the area in ships' ballast water.

Perry said the new sighting of the algae is proof that it has established itself in Martin County waters.

"The reefs in Palm Beach County are pretty well covered in those Caulerpa algae," Perry said. "It completely looks like a golf course down there. We need to raise a caution flag for people to keep an eye out for it."

Perry said he plans to coordinate further monitoring work with Brian Lapointe, a scientist with Harbor Branch Oceanographic Institution, who is studying the subject.

- suzanne.wentley@scripps.com

Killer algae

•**Appearance:** Dark green with 2-inch blades on a vine reminiscent of lawn grass.

•**Growth pattern:** Aggressive growth in clumps that cling to just about anything under water; thriv nutrient-rich, dark locations.

•**Origin:** Native to the Pacific Ocean, the algae most likely traveled here in the ballast water of lar docked at the Port of Palm Beach.

•**Local sightings:** Found last year on coral reefs off Juno Beach and in the Indian River Lagoon. nine-month hiatus, it was spotted recently on a Martin County artificial reef.