

## SEASONAL EFFECTS OF *CAULERPA TAXIFOLIA* (VAHL.) C. AGARDH ON THE GROWTH OF *PHAEODACTYLUM TRICORNUTUM* BOHLIN

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Caulerpacean algae synthesize many toxic secondary metabolites as a defense against grazers and epiphytes (PATTERSON *et al.*, 1984). Recently introduced populations of *Caulerpa taxifolia* (Vahl) C. Agardh in the Mediterranean sea also produce these substances, some of them (caulerpenyn) even in higher concentration than in the tropical ones (GUERRIERO *et al.*, 1992). This toxicity is liable to seasonal variations, as shown by toxicity experiments against mice, urchins and mammalian cells (LEMEE *et al.*, 1993). Since the mediterranean populations of *C. taxifolia* are extremely dense, an undesirable impact may be produced on both planktonic and benthic microalgae. As they are the first step in the food web and play an important ecological role in the sea, if microalgae population are affected by *C. taxifolia*, the whole ecosystem will be.

Our goal is to check whether *C. taxifolia* is toxic for some marine microalgae growing in laboratory culture conditions and to verify the existence of seasonal differences in the *C. taxifolia* effect on them. We will show the results obtained for *Phaeodactylum tricornutum* Bohlin a pennate diatom common in supralittoral rock pools, and extensively used in laboratory work and aquacultural systems to feed the invertebrate juveniles. *C. taxifolia* was collected at days 20/11/93, 28/2/94, 22/5/94 and 1/9/94 on the French coasts of Cap Martin at 9 m depth, and immediately transported to the laboratory in aerated opaque containers. Each toxicity test were started the day after. Unialgal cultures of *P. tricornutum* were supplied by Dr. Lubian, ICMA (CSIC), Cadiz. Cultures were maintained in artificial seawater (ADSA-Micro) enriched with f/2 Guillard's medium (GUILLARD & RYTHER, 1962) in a growth chamber set at 20°C on an alternating 12:12 LD cycle at 100  $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  cool-white fluorescent lighting. Subcultures were previously acclimated for a period of 2 weeks at the temperature at which the experiment was to be conducted (the same registered in the sea when *C. taxifolia* were collected: 15°C, 12°C, 17°C and 25°C respectively). Six culture vessels containing 200 ml sterile medium were inoculated to give initial population densities around  $10^4$  cells  $\text{ml}^{-1}$ . Simultaneously, two fragments consisting on both blades and stolons of *C. taxifolia* (2 g each) were added simultaneously to 4 of them. One millilitre aliquots from thoroughly mixed cultures were withdrawn daily for a period of 27 days (25 days for winter experiment), and preserved with a drop of formaldehyde. Cell counts were performed with a Coulter Multisizer-2.

	Autumn		Winter		Spring		Summer	
	Cont.	Caul.	Cont.	Caul.	Cont.	Caul.	Cont.	Caul.
Max. Growth Rate	0.84	0.29	0.72	0.57	1.75	0.56	0.58	0.15
Max. Cell Concentration	28	11	15	2.6	4.8	1.4	4.3	1.3

Table I. Growth rate (div./day) and maximum cell concentration (cells/ml  $\times 10^5$ ) for both control (Cont.) and test (Caul.) experiments.

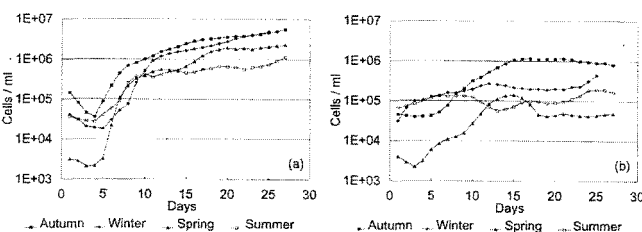


Fig. 1 Growth curves for control (a) and test cultures (b) in the four seasonal experiments.

Smoothed growth curves (fig. 1) showed a less growth in test cultures than in controls, in every season. Slightly different profiles among control growth curves could be explained by temperature and initial cell concentration. Obviously, test cultures were also affected by these two factors, but differences among the control and test curves were too large for these factors to account for them. There were observed different behaviours in the growth of test cultures among the different seasons. A lag-phase was present in the autumn and spring test cultures but not in the other seasons. The slope and the length of the exponential growth phase changed in the different seasons, and so the number of cells at the end of the exponential growth changed too. When maximum growth rates and maximum cell concentrations were considered it appeared that, in all the seasons, they both were lower in test cultures than in controls (Table I). Maximum growth rate pointed to summer and spring test cultures as the most different to the controls (3.85 and 3.12 times lower) (Table I). Maximum cell concentration for spring and summer experiment were 3.54 and 3.22 times lower in test cultures than in controls (Table I) and although winter experiment showed the highest difference between test and control cultures (5.77 times lower) this result, due to a ciliate proliferation had to be discarded. The ciliate growth could be the cause of the inhibition of the diatom growth instead of the *C. taxifolia* effect. Moreover, in this season collected *C. taxifolia* was strongly epiphyted. That fact together with the ciliate growth might point out that the macroalga was less toxic (LEMEE *et al.*, 1993; DINI *et al.*, 1992).

Then, we can conclude that, in the experimental conditions described, control/test culture ratios of the maximum growth rate and maximum cell concentration at the end of the exponential phase were the best features to compare the effect of *C. taxifolia* in different seasons and that *P. tricornutum* is highly affected by *C. taxifolia*, specially in summer and spring. Anyway, the extrapolation of these results to the natural environment is to be considered carefully since culture conditions in the laboratory differ from the environment where *C. taxifolia* develops.

### REFERENCES

- DINI F., GUERRIERO A., GIUBBILINI P., MEINESZ A., PESANDO D., DURAND-CLEMENT M., PIETRA F., 1992. Ciliates as a probe for biological pollution produced by the seaweed *Caulerpa taxifolia*. Third Asian Conference on ciliate..., Shenzhen, China, July 1-6, 8.1
- GUERRIERO A., MEINESZ A., D'AMBROSIA M. & PIETRA F., 1992. Isolation of toxic and potentially toxic sesqui- and monoterpenes from tropical green seaweed *Caulerpa taxifolia* which has invaded the region of Cap Martin and Monaco. *Helvet. Chim. Acta*, 75: 689-695
- GUILLARD R.L.L. & RYTHER J.H., 1962. Studies on marine planktonic diatoms. I. *Cyclotella nana* Hust. and *Detonula confervacea* (Cl.) Gran. *Can. J. Microbiol.*, 8: 229-239
- LEMEE R., PESANDO D., DURAND-CLEMENT M., DUBREUIL A., MEINESZ A., GUERRIERO A. & PIETRA F., 1993. Preliminary survey of toxicity of the green alga *Caulerpa taxifolia* introduced into the Mediterranean. *J. Appl. Phycol.*, 5: 485-493
- PATTERSON G.M.L., NORTON T.R., FURUSAWA E., FURUSAWA S., KASHIWAGI M. & MOORE R.E., 1984. Antineoplastic Evaluation of Marine Algal Extracts. *Bot. Mar.*, 27: 485-488.