

# Effects of *Caulerpa taxifolia* secondary metabolites on the embryogenesis, larval development and metamorphosis of the sea urchin *Paracentrotus lividus*

*Caulerpa taxifolia*  
Larval development  
*Paracentrotus lividus*  
Toxicity test

*Caulerpa taxifolia*  
Développement de larve  
*Paracentrotus lividus*  
Test de toxicité

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## ABSTRACT

The biological activity of a methanolic extract of the introduced species *Caulerpa taxifolia* and caulerpenyne (main secondary metabolite of the alga) was tested on embryogenesis, pluteus and metamorphosis stages of the sea urchin *Paracentrotus lividus*. The bioassays revealed different levels of toxicity according to the egg and larval development stages. Larval development was the most sensitive phase, followed by gastrulation. Methanolic extracts at a concentration of  $1.0 \mu\text{g ml}^{-1}$  and caulerpenyne at a concentration of  $100 \text{ ng ml}^{-1}$  ( $300 \text{ nM}$ ) tested on young larvae (4-arm stage) cause death within four hours. The same concentrations of methanolic extract, tested on eggs, blocked cleavage up to early blastula stage, whereas a double concentration of caulerpenyne was necessary to obtain the same results. After exposure to  $0.2$  and  $0.6 \mu\text{g ml}^{-1}$  of methanolic extract, larvae at 8-arm stage (when echinus rudiment develops inside the body) displayed abnormal development and high mortality. When larvae became competent for metamorphosis, the presence of  $4.0 \mu\text{g ml}^{-1}$  of methanolic extract and  $400 \text{ ng ml}^{-1}$  of caulerpenyne did not alter the success of this process. Therefore, the methanolic extract expressed as caulerpenyne equivalent is more active than pure caulerpenyne, suggesting that other secondary metabolites in the alga are also responsible for the toxicity.

## RÉSUMÉ

Effets des métabolites secondaires de l'algue verte *Caulerpa taxifolia* sur l'embryogenèse, la phase larvaire et la métamorphose de l'oursin comestible *Paracentrotus lividus*.

L'activité biologique des extraits méthanoliques de *Caulerpa taxifolia* introduite en Méditerranée, et de la caulerpényne (métabolite secondaire le plus abondant dans l'algue) a été étudiée sur l'embryogenèse, la phase larvaire et la métamorphose de *Paracentrotus lividus*. Les résultats montrent que la sensibilité aux toxines varie selon la phase du développement ontogénique. Le développement larvaire est la phase la plus sensible, suivi par la gastrulation. L'extrait brut à une concentration de  $1,0 \mu\text{g ml}^{-1}$  et la caulerpényne à  $100 \text{ ng ml}^{-1}$  ( $300 \text{ nM}$ ) testés

sur de jeunes larves (stade à quatre bras) provoquent la létalité larvaire en quatre heures. Le développement embryonnaire ne va pas au-delà du début du stade blastula pour les mêmes concentrations d'extrait, tandis que pour la caulerpényne il faut une concentration double (200 ng ml<sup>-1</sup>). Il existe des anomalies du rudiment et une forte mortalité des larves exposées aux concentrations de 0,2 et 0,6 µg ml<sup>-1</sup> d'extrait méthanolique pendant la formation du rudiment échinien (stade huit bras). Lorsque que les larves deviennent compétentes à la métamorphose la présence de 4,0 µg ml<sup>-1</sup> d'extrait méthanolique et de 400 ng ml<sup>-1</sup> de caulerpényne n'affecte pas le passage à la vie benthique. L'extrait brut, exprimé en équivalent caulerpényne, est plus actif que la caulerpényne pure, ce qui suppose que d'autres métabolites secondaires dans l'algue soient aussi responsables de la toxicité.

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## INTRODUCTION

*Caulerpa taxifolia* (Vahl) C. Agardh (Ulvophycées, Caulerpales) is a pantropical alga introduced accidentally into the Mediterranean Sea in 1984 (Meinesz and Hesse, 1991). In northern Mediterranean waters, the development of *C. taxifolia* is more important and its growth is more rapid than in the original tropical area. Its coverage reaches 100 % over extensive areas between depth of 5 and 25 m and over all types of substrates (Meinesz *et al.*, 1993). The population appears to compete with the indigenous flora and so damages the infralittoral communities (Boudouresque *et al.*, 1992; Verlaque and Fritayre, 1994). These characteristics, combined with the rapid extension of *C. taxifolia* from the Italian to the Spanish coasts (Meinesz *et al.*, 1993), permit its introduction to be qualified as biological pollution (Rueness, 1989).

An ecological risk is that this alga could replace the seagrass of *Posidonia oceanica* (L.) Delile as a source of food and refuge for several benthic species, including sea urchins (Meinesz et Hesse, 1991; Boudouresque *et al.*, 1992; Villele et Verlaque, 1994). Another risk to sea urchins lies in the fact that *Caulerpa taxifolia*, like the other species of *Caulerpa*, contains toxic or repulsive compounds which can be potentially harmful to marine organisms (Norris and Fenical, 1982; Paul and Fenical, 1986). The sesquiterpene caulerpényne is a major metabolite in *C. taxifolia* and is present in larger amounts in Mediterranean than in tropical populations (Guerriero *et al.*, 1992; Amade *et al.*, 1995). In addition, *C. taxifolia* synthesizes other potentially toxic terpenes (Guerriero *et al.*, 1992; 1993). Caulerpenyne can be released into sea water (Amade *et al.*, 1994). In this environment this metabolite is very unstable; nevertheless the products of its degradation also showed toxic properties (Amade and Lemée, unpub.).

A first evaluation of the toxicity of secondary metabolites of *C. taxifolia* in the Mediterranean has been made both for potential risk to humans (Pesando *et al.*, 1994) and for ecological risks. Methanolic and aqueous extracts of *C. taxifolia*, caulerpenyne and three of the four minor terpenes isolated by Guerriero *et al.* (1992; 1993) were active on several toxicological models, such as urchin eggs (Lemée *et al.*, 1993). Seasonal differences in the toxicity of methanolic extracts of *C. taxifolia* were visible with the different models and suggested a variation in the production of

secondary metabolites during the year with a maximum during summer (Lemée *et al.*, 1993).

During spawning, sea urchins produce millions of eggs which develop into pelagic larvae. These larvae remain in the water column for about one month before metamorphosis and subsequent settlement on the bottom. Larval losses during this period are due principally to natural mortality, predation and dispersal by currents (Thorson, 1966; Pedrotti and Fenaux, 1992). However, the metabolic activity of certain species of algae could also modify the quality of the natural environment and thereby influence spawning, pelagic development and settling. This has been demonstrated for certain microplanktonic algae (Wilson, 1981).

The aim of the present work is to assess the potential biological activity of the secondary metabolites produced by *Caulerpa taxifolia* and to determine their effects upon the embryogenesis, larval survival, development and metamorphosis of *Paracentrotus lividus* (Lamarck), the most abundant sea urchin present in the northeastern part of the western Mediterranean Sea. How do susceptibilities to toxicity change during larval development? Different concentrations of methanolic extract of *C. taxifolia* and of caulerpenyne were examined in experiments at cleavage and gastrula, pluteus and metamorphosis stages. Effects of toxicity were measured by differences in cleavage and in the formation of gastrula, in the rates of mortality, in the transfer into different larval stages, in the duration of larval development and by differences in successful metamorphosis. A toxic effect is considered when delay or failure in development, or mortality, were superior to 50 %. Aptitude for benthic life was considered to be successful if the metamorphosis rate was greater than 80 %.

## METHODS

*Caulerpa taxifolia* was collected at Cap Martin (Alpes-Maritimes, France) at 5 m depth in September, during the most toxic season (Lemée *et al.*, 1993). After collection, thalli (fronds, stolons and rhizoids) were rinsed, dried on absorbent paper and then ground with methanol. After filtration to remove non soluble material, the methanol extract was dried *in vacuo*. Caulerpenyne was purified and measured according to Amade *et al.* (1994). The methanolic extract and

caulerpenyne were suspended in ethanol and kept at  $-20^{\circ}\text{C}$ . The highest concentration of ethanol used in the experiments (0.5 %) did not affect eggs and larval development; 0.5 % of ethanol in sea water was used as a control.

Adults of *Paracentrotus lividus* were collected in the Bay of Villefranche-sur-Mer (Alpes-Maritimes, France) at a depth of 5-10 m. Eggs and sperm were extracted and tested as soon as possible. Eggs were rinsed several times with fresh sea water, counted and used if they had previously shown a successful fertilization rate of 97 %. Each experiment was performed in triplicate at  $20^{\circ}\text{C}$  with the eggs of three to five females pooled together. The experiments with eggs and larvae were repeated two or three times for each concentration of metabolite.

### Effects on embryogenesis

During embryogenesis experiments, eggs were diluted to a concentration of two eggs per millilitre, placed in jars and maintained in suspension using motor-driven paddles. Fertilization was effected by adding 20  $\mu\text{l}$  of sperm diluted in 50 ml of sea water. Thirty seconds after fertilization, different concentrations of toxins were added to the suspension: from 2.0  $\text{ng ml}^{-1}$  to 4.0  $\mu\text{g ml}^{-1}$  for methanolic extract and from 10 to 200  $\text{ng ml}^{-1}$  for caulerpenyne. At intervals of 30 min, 60 min, 1 h 30, 2 h 30, 3 h, 6 h, 12 h and 24 h, a sample of 50 ml (approximately 100 eggs) was removed from each flask, and cell division was measured relative to controls.

### Effects on larval survival rate and development

Larvae were reared from winter to spring 1994 in 1L jars at a concentration of two larvae per millilitre. The rearing techniques were from Pedrotti and Fenaux (1993). Larvae were incubated in sea water during four hours with different concentrations of toxins which varied from 0.2  $\mu\text{g ml}^{-1}$  to 2  $\mu\text{g ml}^{-1}$  for methanolic extract, and from 10 to 200  $\text{ng ml}^{-1}$  for caulerpenyne. Three experiments were performed at different stages of larval development: 1) In a first batch of larvae, toxins were added at the 4-arm stage (48 hours after fertilization) and at the 8-arm stage when echinus rudiment develops inside the body (8 and 11 days after fertilization for experiments with methanolic extract and caulerpenyne respectively). 2) In another batch, toxins (only for methanolic extract) were added from the 8-arm stage (8 days after the fertilization). 3) In the last experiment, toxins were added at the moment when larvae reached competence for metamorphosis.

After four hours treatment, larvae were rinsed and placed in jars with sea water filtered through a 0.45  $\mu\text{m}$  Millipore membrane. Every other day, each larval stage was supplied with a volume of *Cricosphaera elongata* (Droop) Braarud considered to be the optimal concentration for development (Fenaux *et al.*, 1985) and 80 % of the rearing sea water was changed. Larvae were maintained in suspension throughout the experiment by motor-driven rotation. Controls were run simultaneously. Experiments were carried out with the same photoperiod as in the field. Different larval stages were identified by the appearance of new ske-

letal structures. Larval survival was calculated by counting the entire contents of each jar. Larval development was calculated by the time of passage of the larvae into the subsequent developmental stage and the metamorphosis rate was noted.

### Effects on metamorphosis

Time to metamorphosis was examined by placing three replicates of 30 competent larvae (larvae able in 24 hours to metamorphose) in different treatments. Controls were prepared by placing larvae in dishes covered with a bacterial film substratum (which is considered to induce metamorphosis) containing sea water filtered through a 0.45  $\mu\text{m}$  filter, and in sterile dishes containing only filtered sea water. For each experimental series, a batch of larvae was placed in dishes covered with a bacterial film substratum containing sea water with different concentrations of toxins, and in sterile dishes containing filtered sea water with different concentrations of toxins. The different concentrations of toxins varied from 0.2  $\mu\text{g ml}^{-1}$  to 4.0  $\mu\text{g ml}^{-1}$  for methanolic extract and from 50 to 400  $\text{ng ml}^{-1}$  for caulerpenyne. Twenty-four and 48 hours later, post-larvae fixed were counted.

## RESULTS

Experiments showed that alterations in embryogenesis and larval development depend on the concentration of toxins and also on the stages of the larvae to which the treatment was applied.

### Effects on eggs

In experiments with methanolic extracts at concentrations below 0.8  $\mu\text{g ml}^{-1}$ , no activity was observed and the development of eggs was the same as in the controls. At the level of 1  $\mu\text{g ml}^{-1}$ , eggs underwent abnormal development; cell division was delayed, with 64 % of first cleavage occurring about five hours after fertilization and subsequent development inhibited at early blastula. Higher concentrations of toxins inhibited cell division or caused abnormal embryonic development (Fig. 1).

Caulerpenyne, at concentrations between 10 and 40  $\text{ng ml}^{-1}$  (30 to 120 nM), exhibited no effect on cellular division. With 50  $\text{ng ml}^{-1}$  of caulerpenyne, the first cleavage and the formation of the gastrula stage was delayed; however, the subsequent development was not altered. So, 48 hours after fertilization, the 4-arm stage larvae was formed. At 100  $\text{ng ml}^{-1}$  (300 nM), development was blocked at the gastrula stage and at 200  $\text{ng ml}^{-1}$ , development did not go beyond the early blastula stage (Fig. 1).

### Effects on larval survival rate and development

Survival rate in the control was greater than 74 % in all the experiments. Active compounds in the methanolic extract at a concentration of 1.0  $\mu\text{g ml}^{-1}$  tested on young larvae (4-arm stage) caused larval death within four hours (Fig. 2 a). Survival

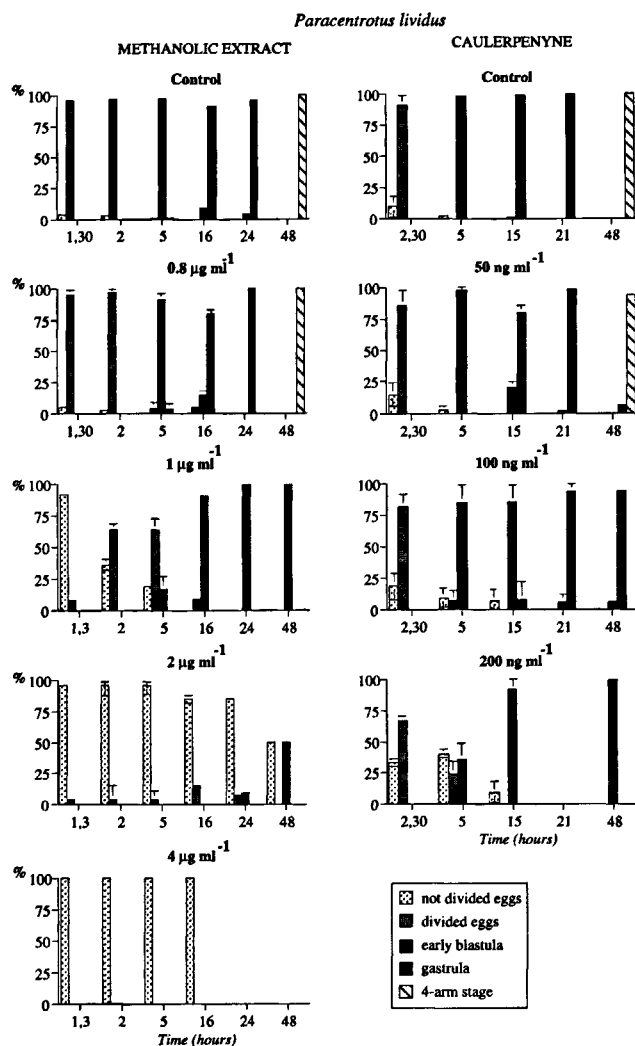


Figure 1

Effects of different concentrations of methanolic extract of *Caulerpa taxifolia* and *caulerpenyne* on the embryonic development of *Paracentrotus lividus*. Values were means  $\pm$  SD of three replicates.

rates were very low for young larvae treated with  $0.6 \mu\text{g ml}^{-1}$  of methanolic extract. Five days after fertilization, there remained only 7.1 % of the initial number of larvae (Fig. 2 a). Despite the higher mortality, the few remaining larvae recovered development with a prolonged 4-arm stage, a rapid transit to the 6-arm stage; however, the rate of metamorphosis was low. Fourteen days after fertilization, only 5 % of larvae had metamorphosed whereas in the control, the rate of metamorphosis was 100 % (Fig. 3). For young larvae exposed to a concentration of  $0.2 \mu\text{g ml}^{-1}$  of methanolic extract, the survival rate was higher (70 %) up to 8 days after fertilization; it then decreased after a second four-hour exposure to toxins at 8-arm stage (Fig. 2 a). Larval development, however, was similar to that observed in the controls; nevertheless the metamorphosis rate was 74 %, 14 days after fertilization (Fig. 3).

Active compounds in the methanolic extract at a concentration of  $1.0 \mu\text{g ml}^{-1}$  tested from 8-arm stage, when echinus rudiment develops inside the body (8 days after fertilization) caused larval death within four hours (Fig. 2 a). Methanolic extract at concentrations of  $0.6$  and  $0.2 \mu\text{g ml}^{-1}$

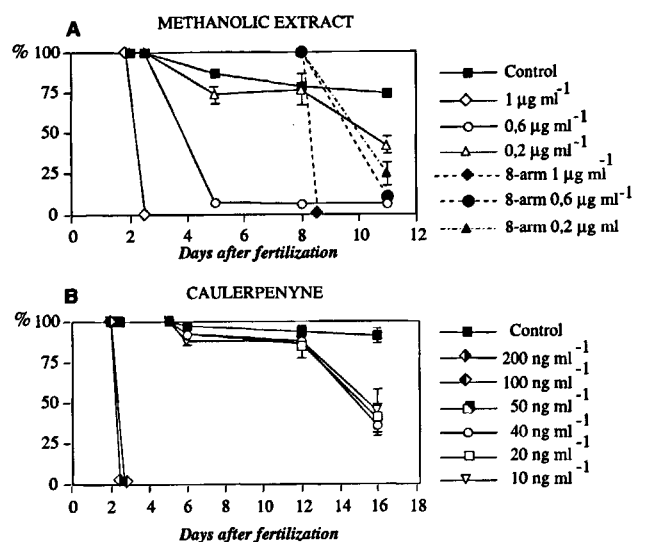


Figure 2

Survival rate of *Paracentrotus lividus*. A) In a first batch of larvae, different concentrations of methanolic extract of *Caulerpa taxifolia* were added 2 and 8 days after fertilization (respectively at the 4- and 8-arm stages). In a second batch of larvae, toxins were added from the 8-arm stage (8 days after fertilization), B) larvae were exposed to different concentrations of *caulerpenyne* at 2 and 12 days after fertilization (respectively at the 4- and 8-arm stages). Values were means  $\pm$  SD of three replicates.

caused abnormal development and high mortality. One day after experiment, 90 % and 36 % of larvae treated with  $0.6$  and  $0.2 \mu\text{g ml}^{-1}$  respectively of methanolic extract showed reduction in the larval skeleton and regression of larval tissues. Eleven days after fertilization, there were respectively 10 % and 12.7 % of the initial number of larvae (Fig. 2 a). For larvae treated with  $0.6 \mu\text{g ml}^{-1}$ , the metamorphosis rate was nil 14 days after fertilization; the rate was respectively 14 % and 21 %, 15 and 18 days after fertilization. For larvae treated with  $0.2 \mu\text{g ml}^{-1}$ , the metamorphosis rate was 34 % 14 days after fertilization; the rate was respectively 46 % and 100 %, 15 and 18 days after fertilization.

In experiments using *caulerpenyne*, controls showed 100 % metamorphosis, 17 days after fertilization. *Caulerpenyne* at a concentration of 200 and  $100 \text{ng ml}^{-1}$  (600 and 300 nM) tested on young larvae (4-arm stage) caused larval death within four hours (Fig. 2 b). A concentration of  $50 \text{ng ml}^{-1}$  of *caulerpenyne* produced high mortality and abnormal development; however, experiments were stopped at the 6-arm stage due to the high mortality of controls. At concentrations of 40, 20, and  $10 \text{ng ml}^{-1}$  of *caulerpenyne*, survival rate and development were the same as in controls up to 12 days after fertilization (at 8-arm stage), when larvae were exposed to a second four-hour experiment. At this time, survival decreased below 50 % in all concentrations tested (Fig. 2 b and Fig. 3).

Methanolic extracts used in our experiments contained 10 % of *caulerpenyne* (quantified by HPLC method; Amade *et al.*, 1994). When results of toxicity were expressed in equivalent *caulerpenyne*, we observed that methanolic extract was more active than pure *caulerpenyne* during the first cleavage, in the morula stage, in the forma-

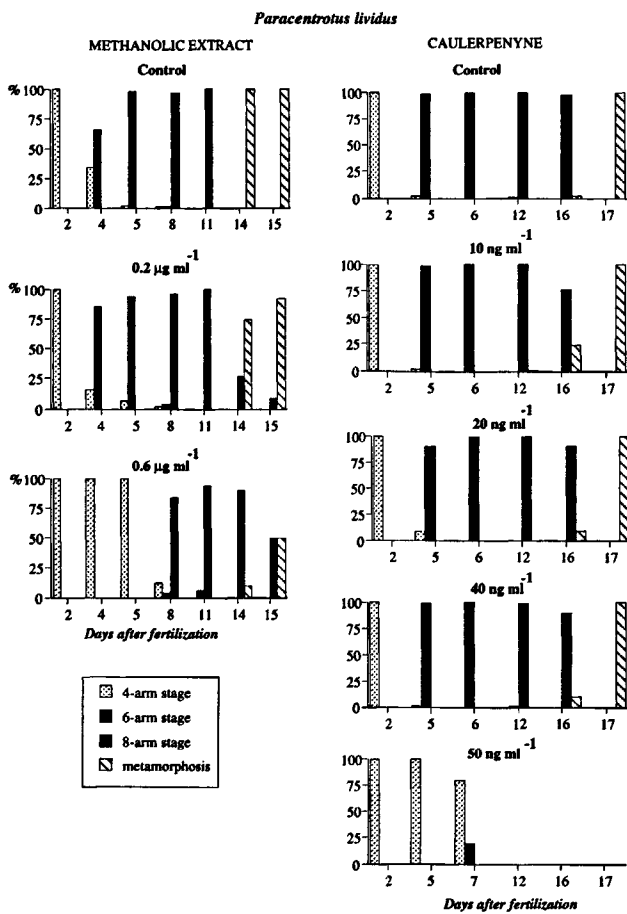


Figure 3

Effects of different concentrations of methanolic extract of *Caulerpa taxifolia* and caulerpenyne on the stage-specific development of *Paracentrotus lividus*.

tion of the 6-arm stage and in the acquisition of competence for metamorphosis (Fig. 4).

Effects on metamorphosis

Control larvae kept for 24 hours in filtered sea water did not undergo metamorphosis. The same results were observed with larvae placed in filtered sea water with different concentration of toxins (Tab. 1). This result shows that toxins alone were not able to induce metamorphosis. When, a substratum was offered to larvae, 24 hours later 100 % of metamorphosis was observed. Control larvae placed in dishes containing a substratum displayed, 24 hours later, 100 % metamorphosis. For larvae placed in a dish containing substratum with different concentration of toxins, the metamorphosis rate was greater than 80 %, except for 200 ng ml<sup>-1</sup> of caulerpenyne, where the rate of metamorphosis was 67.6 %. Two days later, 100 % metamorphosis was observed in all treatments (Tab. 1).

DISCUSSION

Sea-urchin eggs have been used as an ecotoxicological model that permits us rapidly to determine the concentrations at which toxic substances prevent embryo development into the first larval stage (Bougis, 1967; Bougis *et al.*, 1979; Kobayashi, 1971; Okubo and Okubo, 1962; Kobayashi, 1977; Kobayashi, 1981). Even if this test provides information about the effects of toxic substances during embryogenesis, we can not presume that larval development will be successful. The application of a second test using pelagic larvae may show the activity of toxic com-

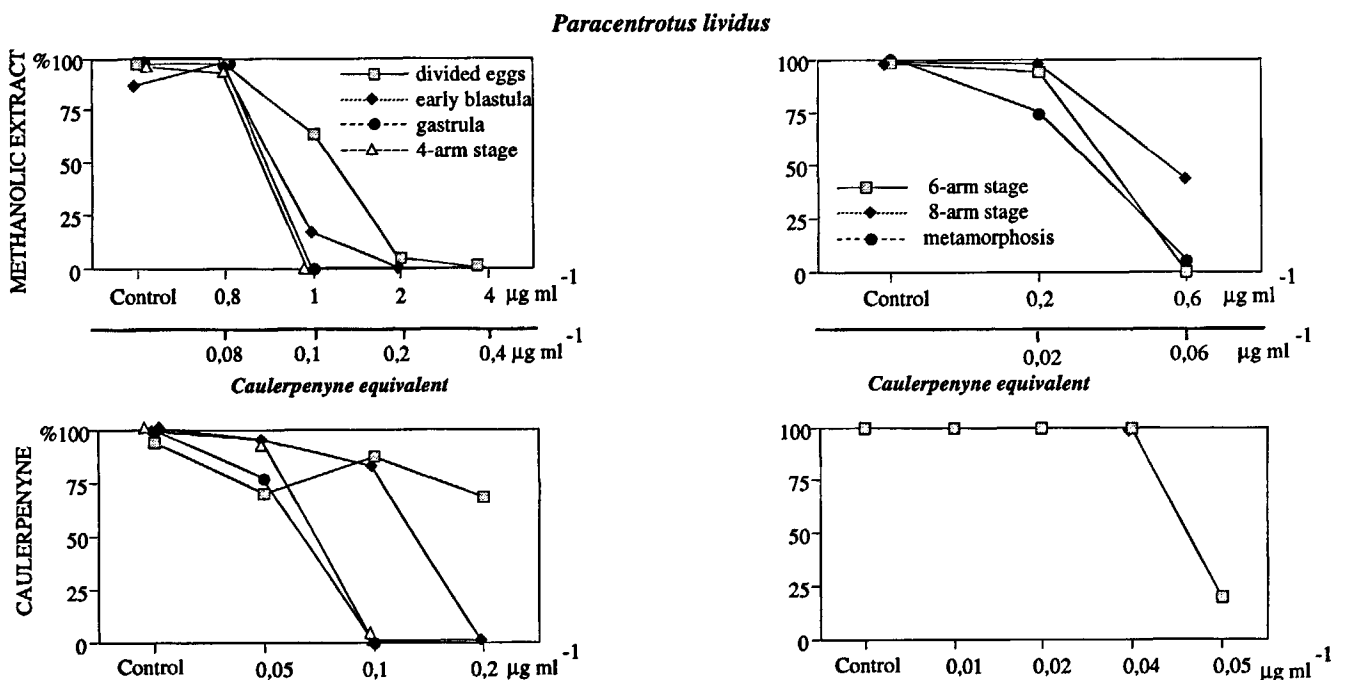


Figure 4

Effects of different concentrations of methanolic extract of *Caulerpa taxifolia* expressed as caulerpenyne equivalent (10 %) and caulerpenyne on A) embryogenesis and B) larval development of *Paracentrotus lividus*.

Table 1

Percentage of metamorphosis of *Paracentrotus lividus* larvae. A) With different concentrations of methanolic extract. B) With different concentrations of caulerpenyne. Experiments took place at 20 °C. For each experimental series, three replicates of 30 larvae were placed in sterile dishes with sea water filtered through a 0,45 µm membrane and in dishes containing sea water with a bacterial and algal substratum which is considered to induce metamorphosis. Time to metamorphosis were examined during the first 48 hours. Value are means ± SD of 90 larvae.

A Methanol. extract	sea water 24 h (%)	substratum 24 h (%)	substratum 48 h (%)
Control	0*	100	100
4 µg ml <sup>-1</sup>	0*	83.5 ± 4.9	98.2 ± 0.4
2 µg ml <sup>-1</sup>	0*	96.5 ± 4.9	100
1.5 µg ml <sup>-1</sup>	0*	100	100
1.0 µg ml <sup>-1</sup>	0*	100	100
0.6 µg ml <sup>-1</sup>	0*	99.5 ± 0.7	100
0.2 µg ml <sup>-1</sup>	0*	99.0	100
B Caulerpenyne	sea water	substratum	substratum
Control	0 *	100	100
400 ng ml <sup>-1</sup>	0 *	92.3 ± 5.0	100
200 ng ml <sup>-1</sup>	0 *	67.6 ± 17	100
100 ng ml <sup>-1</sup>	0 *	86.6 ± 8.5	100
50 ng ml <sup>-1</sup>	0 *	91.3 ± 7.5	100

\* after 24 hours larvae were placed in a dish with a substratum and 100 % metamorphosis was observed

pounds (tested at concentrations close to that found in the sea water) on the larval development up to metamorphosis. The effect of the quality of the natural environment on larval development is a recent approach (Boidron-Metairon, 1988; Fenaux *et al.*, 1994) it can therefore account for successful recruitment.

All species of the *Caulerpa* produce toxic secondary metabolites; toxins serve as a chemical defence against herbivores and epiphytes (Norris and Fenical, 1982; Paul and Fenical, 1986, 1987). Some studies on the ecological role of these compounds showed that they are toxic or deterrent toward bacteria, fungi, sea-urchin eggs and larvae and herbivorous fishes (Paul and Fenical, 1986, 1987; Giannotti *et al.*, 1994). Toxicity of the secondary metabolites synthesized by *C. taxifolia* also modifies the development of ciliates (Dini *et al.*, 1994) and the proliferation of microalgae (Lemée *et al.*, 1994). Paul and Fenical (1986) found that caulerpenyne, at concentrations ranging from 2 to 8 µg ml<sup>-1</sup>, was active or toxic for eggs and larvae of two species of sea urchins (*Lytechinus pictus* and *Echinometra mathaei*).

In our experiments, we demonstrate that the active compounds of methanolic extract and caulerpenyne affect embryogenesis and larval development of *Paracentrotus lividus*. Toxicity or an activity of secondary metabolites were detected, depending on the ontogenetic development of eggs and larvae and on the concentration of toxins. The strongest concentration tested in our experiments (4.0 µg ml<sup>-1</sup> of methanolic extract), inhibited first cleavage. At lower concentrations of toxins, we observe either an inhibition of embryonic development or a blockage at the gastrula or early blastula stages, followed by an anomalous

development of cells. However, sensitivity to toxicity seems to be more important during larval development. We determined the lethal doses of methanolic extract (1.0 µg ml<sup>-1</sup>) of *Caulerpa taxifolia* and caulerpenyne (100 ng ml<sup>-1</sup>) for larvae of the sea urchin *Paracentrotus lividus*. Other tested doses are capable of disturbing larval development. Compounds were principally toxic to young larvae (4-arm stage) and during the formation of the echinus rudiment (at the 8-arm stage). As larvae became competent to metamorphose, results showed that the presence of different concentrations of toxins has little effect on the metamorphosis rate and consequently on the transfer from planktonic to benthic life. Competent larvae are more resistant to higher concentrations of toxins than eggs and 4-, 6- and 8-arm stage larvae. Even at concentrations four times higher (4.0 µg ml<sup>-1</sup>) than that which causes larval death, the metamorphosis rate is not affected. We observed that the presence of toxins in sea water without substrata is not conducive to metamorphosis, and that the quality of substrata is not altered by the presence of toxic products. Two-day old post-larvae did not show mortality; however, juvenile survival remains to be tested. Despite the higher mortality induced by the presence of certain concentrations of toxins, the remaining larvae were able to recover ontogenetic development. This plasticity in the life cycle reflects a morphological or functional adaptation developed by larvae in response to variations in the environmental conditions (Boidron-Metairon, 1988; McEdward, 1985; Strathmann *et al.*, 1992; Pedrotti and Fenaux, 1993). The ability to develop after exposure to toxins also suggests genetic variation among larvae in their susceptibility to the toxins.

Generally, caulerpenyne accounts for 0.2 % of the wet weight of the alga (Guerriero *et al.*, 1992). However, in summer the amount of this compound in the algae can reach 1.3 % of the wet weight. The methanolic extract used in our experiments comes from algae collected in Cap Martin in September, the season when *Caulerpa taxifolia* is at its most toxic (Lemée *et al.*, 1993) and contains 10 % of caulerpenyne. When methanolic extract is expressed in equivalent caulerpenyne, we observe that it is more active than pure caulerpenyne. This permits us to suggest that there may exist some other secondary metabolites in the alga responsible for the toxicity.

Experiments performed in an aquarium containing thalli of *C. taxifolia* showed that caulerpenyne can be released in sea water at a level of 2 ng ml<sup>-1</sup> (Amade *et al.*, 1994). In our experimental conditions, the lower effective concentration of secondary metabolites on larvae was 10 ng ml<sup>-1</sup> of caulerpenyne. In the field, concentrations of organisms are inferior than those used in laboratory experiments, and the active concentration of toxic compounds is certainly lower than 10 ng ml<sup>-1</sup>. Furthermore, *P. lividus* larvae spend from 15 to 30 days in the water column, where they are scattered by currents before recruitment on the bottom (Fenaux *et al.*, 1992; Pedrotti and Fenaux, 1992). So, during transport in areas colonized by *C. taxifolia*, the larvae could be exposed to toxins for more than four hours. The influence of *C. taxifolia* secondary metabolites on larval development in the field could then be taken into account.

The development of *C. taxifolia* in Mediterranean affects benthic organisms, but due to the high biomass of this alga over extended areas and the large amount of secondary metabolites released in the environment, the planktonic compartment could also be affected.

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