

Feeding behaviour of *Paracentrotus lividus* in the presence of *Caulerpa taxifolia* introduced in the Mediterranean Sea

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Caulerpenyne

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ABSTRACT

Experiments in aquaria have shown that the feeding behaviour of the sea urchin *Paracentrotus lividus* in presence of the green alga *Caulerpa taxifolia* varies during the year. In summer, the alga is strongly avoided and is not consumed. In winter and spring, although the alga is only moderately appreciated, its consumption is significant, but it is hardly or not at all absorbed. These results are confirmed by content analysis of the digestive tract of *P. lividus* sampled *in situ* in an area colonized by *C. taxifolia*. The sea urchins' feeding behaviour is linked to the presence of toxic and repellent secondary metabolites of *C. taxifolia*, caulerpenyne in particular.

RÉSUMÉ

Comportement alimentaire de l'oursin *Paracentrotus lividus* en présence de *Caulerpa taxifolia* introduite en Méditerranée.

Des expériences en aquarium montrent que le comportement alimentaire de l'oursin *Paracentrotus lividus* en présence de l'algue verte *Caulerpa taxifolia* varie au cours de l'année. En été, l'algue est fortement évitée et n'est pas consommée. En hiver et au printemps, bien que moyennement appréciée, l'algue est consommée de façon significative mais elle est peu ou pas absorbée. Ces résultats sont confirmés par les analyses du contenu du tube digestif de *P. lividus* prélevé *in situ* dans une zone colonisée par *C. taxifolia*. Le comportement alimentaire des oursins est lié à la présence des métabolites secondaires toxiques et répulsifs de *C. taxifolia*, et en particulier à celle de la caulerpenyne.

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INTRODUCTION

The tropical alga *Caulerpa taxifolia* (Vahl) C. Agardh (Chlorophyta, Ulvophyceae, Caulerpales) was introduced into the Mediterranean Sea in 1984 (Meinesz and Hesse, 1991). Its rapid expansion on a variety of substrates and across a wide depth range has been noted (Meinesz *et al.*, 1993). In tropical regions the development of *C. taxifolia* is not invasive, whereas in the Mediterranean this alga can form extremely dense beds and may account for more than

80 % of the benthic vegetation over an area of several hectares (Meinesz *et al.*, 1993). In certain stations that have been invaded for several years, it has thus become the major plant food resource. Because of the invasive nature of this introduced species, its impact on the fauna and flora of the Mediterranean requires investigation.

Caulerpa taxifolia is now in competition with benthic plants. A decrease of the biomass and diversity of algae has been observed, particularly among the hard substrate

sciaphilic and photophilic communities (Boudouresque *et al.*, 1992; Verlaque and Fritayre, 1994). Similarly, changes in the structure of *Posidonia oceanica* (L.) Delile beds have been recorded (Villele and Verlaque, 1994). The silting of the substrate engendered by the dense beds of *C. taxifolia* cause major alterations in the composition of the invertebrate fauna. Some Molluscs are more abundant, while the numbers and diversity of several Crustaceans have declined (Bellan-Santini *et al.*, 1994). The first findings from investigations of the fish fauna show that invasion of the sea bottom by *C. taxifolia* alters the structure of the populations (Francour *et al.*, 1994).

The species of the genus *Caulerpa* have developed an efficient strategy against grazers and epiphytes by synthesizing toxic or repellent secondary metabolites (Norris and Fenical, 1982; Capon *et al.*, 1983; Faulkner, 1984; Paul and Fenical, 1986). Evidence has been provided of the presence of numerous terpenes in *C. taxifolia* introduced into the Mediterranean (Guerriero *et al.*, 1992 and 1993). To determine whether these metabolites do in fact have a repellent effect on Mediterranean grazers, we investigated the feeding behaviour of the common sea urchin *Paracentrotus lividus* (Lamarck, 1816) in the presence of *C. taxifolia*. This sea urchin is one of the main Mediterranean herbivores, and its selective grazing pressure has a strong influence on the structure of benthic phytocenoses (Verlaque, 1987). Consequently, *P. lividus* feeding behaviour when faced with a *C. taxifolia*-based diet is well worth investigation.

We have measured the consumption and absorption rate of *C. taxifolia* and also determined the situation of this alga among the food preferences of *P. lividus*. The results of these *in vitro* experiments are compared with those concerning the feeding behaviour of *P. lividus in situ*.

MATERIALS AND METHODS

For the aquarium experiments, we used 20 to 30 dm³ tanks equipped with an air diffuser and an internal filter. A framed grid was placed 2 cm above the bottom of the aquaria to prevent the sea urchins from reingesting their own faeces (Knoepffler-Peguy *et al.*, 1987). The artificial sea water (Instant Ocean sea salt) was renewed at three-day intervals. The sea water temperatures of the aquaria, the diameter and the site of sampling of sea urchins are given in Table 1. *C. taxifolia* thalli were always sampled at Cap Martin (Alpes Maritimes, France).

Table 1

Temperatures of aquaria sea water, diameters and sites of sampling of sea urchins used in the feeding behaviour experiments.

Experiments	Sea water temperatures	Diameters of sea urchins	Site of sampling of sea urchins
Absorption rate	Spring: 13 to 17 °C Summer: 19 to 21 °C	45 to 48 mm	Samena (Bouches-du-Rhône)
Food preferences	Winter: 12 to 16 °C Spring: 18 to 20 °C Summer: 21 to 25 °C	40 to 54 mm	Samena (Bouches-du-Rhône) Banyuls (Pyrénées-orientales)

Consumption and absorption rates of *Caulerpa taxifolia*

Experiments were carried out in spring (March, April and May) and summer (July) 1994. The duration of the experiments varied from one to four weeks. Ten sea urchins were placed in each aquarium, in the presence of a single alga. The control algae used as food were *Cystoseira compressa* (Esper) Gerloff and Nizmuddin or *Halopteris scoparia* (L.) Sauvageau (ex *Stypocaulon scoparium* (L.) Kützinger). The overall mass of each alga was weighed and made up to 50 g (wet weight) every day to estimate the sea urchins' daily food intake. This quantity of plant matter in each aquarium was non-limiting. The wet weight of plants was measured after standardized centrifugation. For the calculation of food intake, aquaria containing the algae without the presence of echinoderms were used to deduce the variations in mass due to the growth or decay of algae. For each of the three species, a dw/ww conversion rate was established giving the following results: 11 % for *C. taxifolia*, 16 % for *C. compressa* and 29 % for *H. scoparia*. In each aquarium, the faeces were collected, dried (70 °C for 24 h) and weighed. In control experiments, 10 sea urchins were kept without algae to quantify the faeces deposited in total absence of food. The absorption rate corresponds to the percentage of material ingested which penetrates the intestinal wall. It is estimated by means of the following formula:

$$TA = ((Mi - Mr) * 100) * Mi^{-1}$$

TA = absorption rate;

Mi = dry weight ingested;

Mr = dry weight defecated.

We have also studied ingestion of *C. taxifolia* previously cultivated for a month in aquarium conditions and ingestion of *C. taxifolia* and *Cystoseira compressa* previously subjected to a lethal thermal shock (40 °C). These experiments were carried out in summer.

The situation of *Caulerpa taxifolia* among the food preferences of *Paracentrotus lividus*

Experiments were carried out in summer, winter and spring from summer 1992 to spring 1994. Five sea urchins were placed in each aquarium, and were exposed to two plants, one of which was always *C. taxifolia*. The quantity of plant matter in each aquarium was non-limiting (75 g wet weight per plant and per aquarium) and was checked daily to avoid any imbalance in the resources available for the sea urchins. The wet weight of plants was measured after stan-

standardized centrifugation. Each experiment lasted five days, which is longer than the time necessary to renew the digestive content of sea urchins under normal feeding conditions (Frantzis, 1992; Kempf, 1962; Nédélec, 1982). The authorities of the plants used in the food preference experiments are given in Table 2.

To determine the feeding behaviour of *P. lividus* in presence of *C. taxifolia*, we have confronted this alga with different plants according to their situation among the food preferences of the sea urchins. For instance, the most strongly preferred species are *Rissoella verruculosa*, *Cystoseira amantacea* var. *stricta* and *Dictyota dichotoma*; the moderately preferred species are *Corallina elongata*, *Codium fragile* and *Jania rubens* and the avoided plants are *Asparagopsis armata*, *Codium bursa* and *Ceramium clavulatum* (Knoepfler-Peguy *et al.*, 1987; Odile *et al.*, 1988; Rico, 1989; San Martin, 1987). With regard to *Posidonia oceanica*, a species moderately preferred by *P. lividus*, all the leaf shoots used were sampled at 6 m depth. We used the intermediate leaves in growth phase (leaves whose petiole is not yet developed) which are generally free of macroscopic epiphytes. *Posidonia oceanica* was sampled at Cap Ferrat (Alpes-Maritimes, France) and at Marseille (Bouches-du-Rhône, France) for the reference areas, and at Cap Martin specimens were collected in meadows invaded by *C. taxifolia*. After five days of experimentation, the digestive content was removed and analysed under a light microscope by the "contact method" described by Jones (1968) and Nédélec (1982). Analysis of 100 contacts, each one corresponding to a specified alga fragment, gives a good estimation of the digestive content of a sea urchin (Paul *et al.*, 1983), especially if its diet offers little variety (here a choice between two algae). In order to determine whether there is really a choice in the diet of *P. lividus*, we used the χ^2 test (differences significant at the threshold of 5 %) to compare the observed distribution pattern with the theoretical distribution pattern (50 % of each alga).

Feeding behaviour of *Paracentrotus lividus* in situ

Sea urchins were collected on 12/02/93, 10/03/93 and 11/05/93 at Cap Martin. This station is in the Mediterranean area that has been most intensively invaded by *C. taxifolia* (Meinesz *et al.*, 1993). On each of the collection dates, five sea urchins were sampled in the *Posidonia oceanica* beds colonized by *C. taxifolia* and five on rocky substrata. The analysis of five specimens was adequate as a basis for a satisfactory assessment of dietary habits (Nédélec, 1982). Analyses of gut contents were carried out using the contact method. Examination of 100 contacts for the sea urchins taken in the *P. oceanica* beds was sufficient, whereas 400 may be required for specimens taken on rocky substrata (Nédélec, 1982). But the detailed composition of all the species present in the digestive tract is not being studied. Therefore, for the purposes of our experiments, 100 contacts were sufficient to determine the dietary habit of urchins on rocky substrata. We have specified five classes of contact: "*C. taxifolia*", "*Dictyota dichotoma*", "*Posidonia oceanica*", "other algae" and "sand or remains of calcareous algae".

RESULTS

Consumption rate and absorption rate

In spring (Fig. 1), *C. taxifolia* was both poorly consumed (26 mg/d/indiv.) and poorly absorbed (2 %) by *P. lividus*.

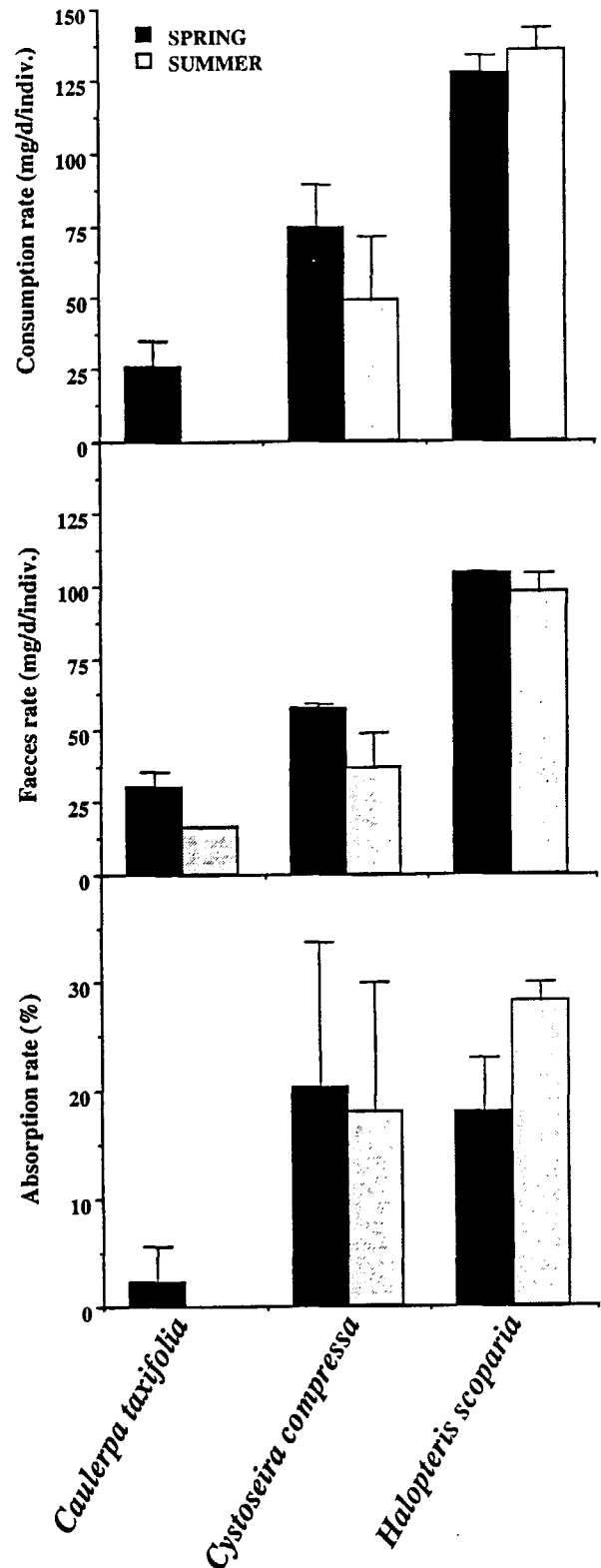


Figure 1

Consumption rate, faeces deposited and absorption rate of *Paracentrotus lividus* feeding on *Caulerpa taxifolia*, *Halopteris scoparia* and *Cystoseira compressa* in spring and summer experiments (means \pm SE).

Among the controls, *Halopteris scoparia* was the most strongly consumed, followed by *Cystoseira compressa*. When experiments lasted longer than two weeks, consumption of the control alga varied over time. Daily food intake was always lower at the beginning of the experiment, then increased progressively to reach a consumption rate 2 to 2.5 times higher than in the first period. The absorption rates were 22 % for *Cystoseira compressa* and 23 % for *Halopteris scoparia*.

In summer (Fig. 1), the sea urchins did not consume *C. taxifolia*, and mean consumption for controls were 49 mg dry weight/d/indiv. for *Cystoseira compressa* and 136 mg/d/ind. for *Halopteris scoparia*. Mean absorption rates were respectively 18 % and 28 %. When *C. taxifolia* was maintained for one month in the aquarium before the beginning of the experiment (Fig. 2), consumption rate was 62 mg/d/indiv. and the absorption rate was 33 %. In the same way, consumption rates and faeces production were higher for algae exposed to a lethal thermal shock (Fig. 2). In these experiments, absorption rates of *C. taxifolia* and *C. compressa* were respectively 15 and 47 %.

The control algae were moderately preferred species for *P. lividus*. Nevertheless, we show that *H. scoparia* is the most strongly consumed (two to three times more than *C. compressa* in spring and winter).

Situation of *Caulerpa taxifolia* among the food preferences of *Paracentrotus lividus*

During each set of experiments carried out in the aquarium, when a plant was tested several times, the results did not significantly differ (Mann-Whitney non-parametric tests; $p < 0.01$). We therefore calculated the overall means for each of the plants tested (Tab. 2).

In winter, *C. taxifolia* was among the plants moderately preferred by *P. lividus*. The consumption rate of this alga varied according to the degree of preference for the plant

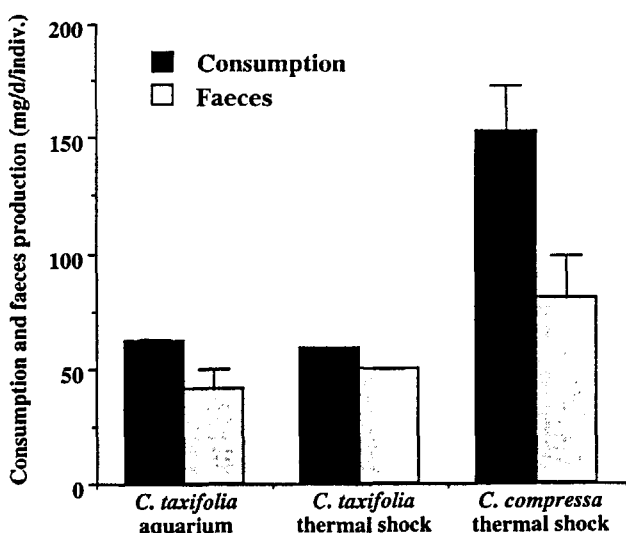


Figure 2 Consumption rate and faeces deposited of *Paracentrotus lividus* feeding on *Caulerpa taxifolia* previously cultivated for a month in aquarium and on *C. taxifolia* and *Cystoseira compressa* previously subjected to a lethal thermal (40 °C) shock in summer period (means \pm SE).

with which it was coupled: the higher the degree of preference for the other plant, the lower the amount of *C. taxifolia* present in the digestive tract. The sea urchins' most strongly preferred species were always more consumed than *C. taxifolia*. The animals showed no differences in the

Table 2

Authorities, sites of sampling and percentages of plants in the digestive content of *Paracentrotus lividus* during food preference experiments against *Caulerpa taxifolia* (* indicated $p < 0.01$ for χ^2 test).

Species	Authorities	% of algae	Sites of sampling
WINTER			
Preferred species			
<i>Cystoseira amantacea</i> var <i>stricta</i>	(Montagne) Sauvageau	99*	Marseille
<i>Cystoseira compressa</i>	(Esper) Gerloff et Nizamuddin	76*	Cap Martin
<i>Plocamium cartilagineum</i>	(L.) P. Dixon	84*	Marseille
<i>Bryopsis muscosa</i>	Lamouroux	77*	Cap Martin
<i>Dilophius spiralis</i>	(Montagne) Hamel	75*	Marseille
Moderately preferred species			
<i>Posidonia oceanica</i>	(L.) Delile	71*	Marseille
<i>Dictyota dichotoma</i>	(Hudson) Lamouroux	67*	Cap Martin
<i>Jania rubens</i>	(L.) Lamouroux	66*	Marseille
<i>Ceramium ciliatum</i>	(Ellis) Ducluzeau	65*	Marseille
<i>Codium vermilara</i>	(Olivieri) Delle Chiaje	62*	Cap Martin
<i>Corallina elongata</i>	Ellis et Solander	61*	Cap Martin
Indifferent species			
<i>Halopteris scoparia</i>	(L.) Sauvageau	55*	Cap Martin
<i>Posidonia oceanica</i>	(L.) Delile	55	Cap Ferrat
<i>Corallina elongata</i>	Ellis et Solander	47*	Marseille
<i>Posidonia oceanica</i>	(L.) Delile	45	Cap Martin
Avoided species			
<i>Laurencia undulata</i>	Yamada	38*	Cap Martin
<i>Ulva rigida</i>	C. Agardh	28*	Marseille
<i>Asparagopsis armata</i>	Harvey	21*	Marseille
<i>Codium bursa</i>	(L.) C. Agardh	11*	Cap Martin
SPRING			
Preferred species			
<i>Cystoseira compressa</i>	(Esper) Gerloff et Nizamuddin	97*	Cap Martin
<i>Posidonia oceanica</i>	(L.) Delile	94*	Cap Martin
<i>Posidonia oceanica</i>	(L.) Delile	90*	Cap Ferrat
<i>Enteromorpha compressa</i>	(L.) Greville	87*	Marseille
Avoided species			
<i>Asparagopsis armata</i>	Harvey	31*	Marseille
<i>Codium bursa</i>	(L.) C. Agardh	18*	Marseille
SUMMER			
Preferred species			
<i>Rissoella veruculosa</i>	(Bertoloni) J. Agardh	100*	Banyuls
<i>Corallina elongata</i>	Ellis et Solander	98*	Banyuls
<i>Codium fragile</i>	(Suringar) Hariot	96*	Banyuls
<i>Posidonia oceanica</i>	(L.) Delile	85*	Banyuls
<i>Codium vermilara</i>	(Olivieri) Delle Chiaje	81*	Banyuls
Moderately preferred species			
<i>Ceramium clavulatum</i>	(C. Agardh) Montagne	71*	Banyuls

consumption of *C. taxifolia* and the consumption of *Posidonia oceanica*, *Halopteris scoparia* and *Corallina elongata*. These species are generally moderately preferred algae for *P. lividus*. Finally, four species were significantly avoided in favour of *C. taxifolia*: *Asparagopsis armata*, *Codium bursa*, *Laurencia undulata* and *Ulva rigida*. Only the classification of *Ulva rigida* among the avoided species may seem surprising. In fact, this species has often been considered as preferred (Kempf, 1962; San Martin, 1987; Odile, 1988; Rico, 1989). Nevertheless, for Knoepffler-Peguy *et al.* (1987), *Ulva rigida* is preferred to *Codium fragile* (which is avoided) in August, and the order of classification is reversed in January.

In spring, *C. taxifolia* was poorly selected by *P. lividus*. The percentage of this alga in the digestive tract content decreased significantly in comparison with winter when it was offered at the same time as *Cystoseira compressa* (Mann-Whitney non-parametric test; $p < 0.01$) or *Posidonia oceanica* ($p < 0.01$). For *Asparagopsis armata* and *Codium bursa*, there was also a decrease, but it was not significant.

In summer, *C. taxifolia* was less preferred than in spring and was strongly avoided by *P. lividus*. When coupled to a strongly avoided alga (*Ceramium clavulatum*), *C. taxifolia* accounted for 30 % of the digestive contents of *P. lividus*. Similarly, *Posidonia oceanica*, which is considered to be only moderately preferred by sea urchins, accounted for 85 % of the digestive content.

What was apparent was a seasonal variation in the situation of *C. taxifolia* in the food preference ranking of *P. lividus*. The alga was moderately appreciated in winter, little appreciated in spring and strongly avoided in summer.

In situ feeding behaviour of *Paracentrotus lividus*

Analysis of the digestive content of *P. lividus* sampled *in situ* (Fig. 3) in a dense *C. taxifolia* bed confirms *in vitro* experiments. Results from the three experiments showed that undetermined contacts were always less than 2 %. On rocky substrate, *C. taxifolia* accounted for about 60 % of the diet of the sea urchins in winter, and less than 20 % in spring. The rest of the digestive content was made up of *Posidonia oceanica* and calcareous encrusting algae (high percentage of sand). The percentage of calcareous algae increased in spring, as did the proportion of other algae. In winter, in a *P. oceanica* bed invaded by *C. taxifolia*, the sea urchins consumed both plants indiscriminately. In spring, consumption of *C. taxifolia* dropped in favour of consumption of *P. oceanica*.

DISCUSSION

Seasonal variations of consumption

Taken as a whole, the *in vitro* results provide evidence of strong variations in the feeding behaviour of *P. lividus* in presence of *C. taxifolia*.

In summer, this alga was clearly avoided in the food preference assays and the sea urchins did not graze it. In winter and spring, *C. taxifolia* was moderately appreciated by the

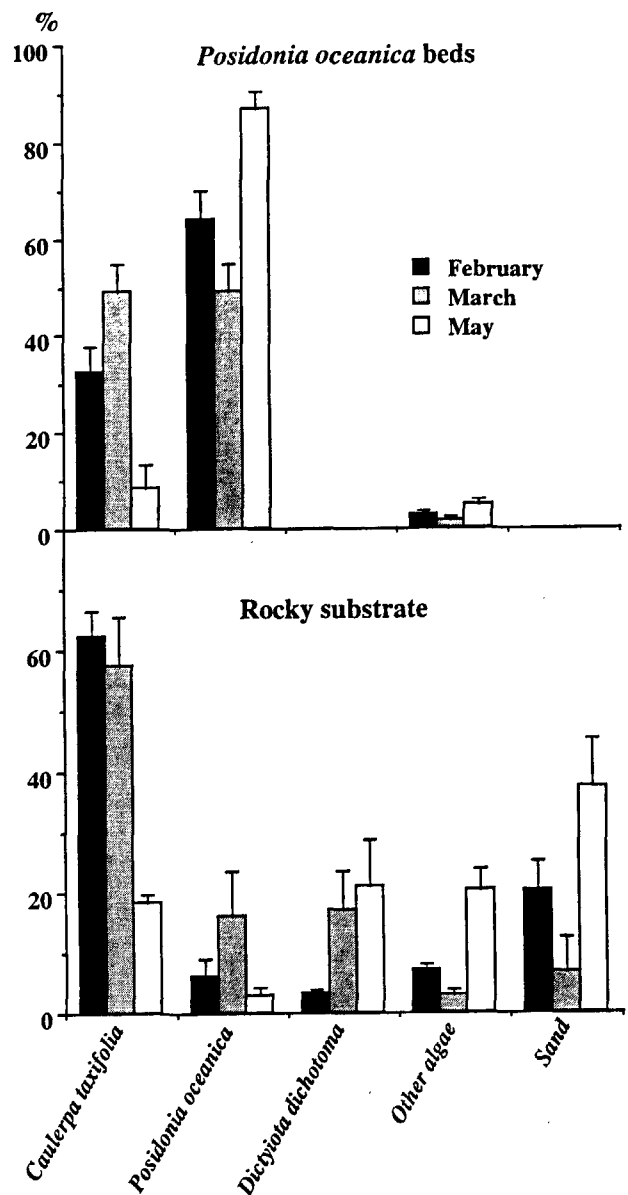


Figure 3

In situ feeding behaviour of *Paracentrotus lividus* at Cap Martin (Alpes-Maritimes, France). Percentage of algae, *Posidonia oceanica* and sand in digestive contents of sea urchins sampled on rocky substrate and in *Posidonia oceanica* beds on 12/02, 10/03 and 11/05/1993 (means \pm SE).

sea urchins. The alga was consumed, but it was not at all absorbed.

We already have such results of long-term experiments, where the consumption and the faeces production of *P. lividus* feeding on *C. taxifolia* were compared to those feeding on control algae (Lemée *et al.*, 1994 a). During the summer and autumn period, the consumption of *C. taxifolia* was very low, and decrease from 0.45 g wet weight/d/indiv. in the first weeks of experiments to nothing after three months. The consumption of *C. taxifolia* was then 3 to 30 times lower than that of the control alga. In the winter and spring periods, the consumption of *C. taxifolia* was low at the beginning of the experiment; but it reached the control consumption level after two months of experiment (Lemée *et al.*, 1994 a).

Physiological disorder of *Paracentrotus lividus* when feeding on *Caulerpa taxifolia*

Lemée *et al.* (1994 *b*) have found that physiological disorders occurred in *P. lividus* when given a diet exclusively composed of *C. taxifolia*. Whatever the period of the year, these urchins showed marked loss of spines and long righting times when compared to the controls. In the most reproductive period (winter and spring), the sea urchins feeding on *C. taxifolia* have a gonosomatic ratio 6 to 7 times lower than that of control urchins. Moreover, some urchins died after three months of experiment in the summer and autumn periods, and presented marked sublethal signs in the winter and spring periods. In the present study, we have also observed mortality of *P. lividus* (after 18 days of experiments) when *C. taxifolia* was the sole potential food source. This occurred in the spring food preference experiments. Summer experiments were certainly too short (one week) to observe any mortality.

The physiological disorders and ultimate death of the sea urchins exclusively in presence of *C. taxifolia* may be due to the action of toxic substances present in the alga and/or an insufficient nutritional intake.

Nutritional intake

The mean amounts of algae ingested by *P. lividus* vary according to the species, but daily intake rates for controls are comparable to those described in the literature. Consumption rates recorded *in vitro* by Mastaller (1974) – 66 to 146 mg dry weight of Fucophyceae/day/individ. for small-sized animals (4 cm diameter) are comparable to our findings. In July, Frantzis (1992) and Frantzis and Grémare (1992) obtained consumption rates of 95 and 38 mg/d/individ. for *Halopteris scoparia* and *Cystoseira compressa* respectively. These values do not differ significantly from our summer results (Mann-Whitney U test, $p < 0.05$). All these values are nonetheless lower than those of the consumption rates recorded *in situ* in Corsica (France) in a *Cystoseira balearica* community (Verlaque, 1987): 250 to 313 mg dry weight/day/individ. of 5 cm diameter.

Whatever the seasons, the sea urchins in presence of *C. taxifolia* have evident insufficient nutritional input. In winter (Lemée *et al.*, 1994 *a*), *P. lividus* consumes *C. taxifolia* as much as the control food and in spring (present study), this consumption is less than in winter and two to five times lower than the control algae. But the alga is not absorbed. In summer, the sea urchins refused to graze *C. taxifolia*.

This insufficient nutritional intake could explain the physiological disorders observed, but the original cause may be the presence of secondary metabolites in *C. taxifolia*. These substances could act either as repellent molecules and/or as toxic agents, and the seasonal variations of the concentrations of such substances in the algae may explain the variations in the consumption of *C. taxifolia* by *P. lividus*.

Evidence for chemical defence in *Caulerpa taxifolia*

The species of the genus *Caulerpa* synthesize toxic or repellent secondary metabolites as a defence mechanism

against herbivores and epiphytes (Doty and Aguilar-Santos, 1966; Aguilar-Santos and Doty, 1968, 1971; Capon *et al.*, 1983; Faulkner, 1984; Paul and Fenical, 1986; Norris and Fenical, 1982).

The first to be discovered was caulerpin, a di-indolopentacyclic compound probably derived from tryptophane (Maiti and Thomson, 1977, 1978). This substance is not, however, considered as a bioactive secondary metabolite (MacConnell *et al.*, 1982; Meyer and Paul, 1992; Paul *et al.*, 1987; Vidal *et al.*, 1984). Caulerpin is present in *Caulerpa taxifolia* (Maiti and Thomson, 1977).

The second is caulerpenyne, an acetylinic sesquiterpene described by Amico *et al.* (1978) in *Caulerpa prolifera* and detected in *C. taxifolia* by Paul and Fenical (1986, 1987), Scharztz *et al.* (1990) and Guerriero *et al.* (1992, 1993). The latter authors have also offered evidence of the presence of other terpenes in *C. taxifolia* introduced into the Mediterranean, which are the precursors or derivatives of caulerpenyne.

These terpenes are active on several toxicological models (Fischell *et al.*, 1994; Lemée *et al.*, 1993; Pesando *et al.*, 1994; Parent-Massin *et al.*, 1996) such as mice and mammal cells in culture (fibroblasts, melanocytes, hematopoietic cells and tumor cells). They are also toxic on marine models: sea-urchin eggs, microalgae (Lemée *et al.*, 1993, 1994 *c*), ciliates (Dini *et al.*, 1994) and bacteria (Giannotti *et al.*, 1994).

Lemée *et al.* (1993) have offered evidence of an annual variation in the toxicity of methanolic extracts of *C. taxifolia* in the Mediterranean, which reflects a variation in the concentration of active secondary metabolites. Amade *et al.* (1996) have confirmed these findings by studying the annual fluctuations in caulerpenyne content (the most abundant secondary metabolite in the alga). The fronds of *C. taxifolia* collected at Cap Martin at 5 m depth contain more than 13 % of caulerpenyne (relative to the dry weight of the alga) in August and less than 0.2 % in February.

Paracentrotus lividus therefore consumes *C. taxifolia* solely when the caulerpenyne content is very low, and so it appear that this molecule may be largely responsible for the chemical defence of the plant against grazers. It has already been established that caulerpenyne inhibits grazing by certain herbivores, such as the tropical sea urchin *Lytechinus variegatus* (MacConnel *et al.*, 1982).

The low absorption rates of *C. taxifolia* observed in the spring experiments may result from the absence of specific enzymes required to digest the alga, but Prim and Lawrence (1975) have shown that the symbiotic bacteria in the intestine of *P. lividus* are capable of digesting a wide range of algae.

Caulerpenyne and/or other *C. taxifolia* secondary metabolites could inactivate the intestinal flora or digestive enzymes of *P. lividus*, since these substances are toxic towards some bacteria (Giannotti *et al.*, 1994) and some proteins (Mayer *et al.*, 1993; Schwartz *et al.*, 1990).

Influence of the abiotic factors on chemical defence

Abiotic factors, temperature in particular, play an important role in determining the caulerpenyne content in

C. taxifolia (Amade *et al.*, 1996). Lemée *et al.* (1993) have also shown a decrease in the toxicity of the methanolic extracts of *C. taxifolia* when it is kept in an aquarium. A drop in the active secondary metabolite content might explain why the sea urchins consume *C. taxifolia* that has spent one month in the aquarium, whereas they refuse to graze on freshly collected thalli (summer experiments). Similarly, the sea urchins consume more of the alga when it has undergone lethal thermal shock of 40 °C, probably resulting in degradation or destruction of the caulerpenyne.

A similar result may be observed for the thalli of *Cystoseira compressa* subjected to the same thermal shock. In the Phaeophyceae, the chemical defence mechanism appears to be linked to the presence of polyphenols (Geiselman and MacConnell, 1981; Norris and Fenical, 1982). *Cystoseira compressa* contains 23 % of these compounds (in relation to the total dry weight) and *Halopteris scoparia* only 2 % (Frantzis and Grémare, 1992). This might explain why the latter species is more strongly consumed than *C. compressa* in our experiments.

Similarly, we have made an experiment in winter for food preference assays with *Asparagopsis armata*. This alga had been kept in an aquarium for a week before given to sea urchins. The consumption rate obtained were three times higher than those of experiments made with freshly collected algae. In fact, this species synthesizes brominated substances (Codomier *et al.*, 1977) and is generally avoided by *P. lividus* (Rico, 1989). *A. armata* may have lost some of its repellent secondary metabolites when kept in the aquarium. We have not taken the results of this experiment into account for the calculation of the mean consumption.

The aquaria conditions (temperature, light, artificial sea water) appeared to be factors capable of inducing variations in algae secondary metabolite contents. Only freshly collected thalli should be used for food preference or content measurement experiments.

In the same way, variations in chemical defence metabolite contents in algae collected in different areas could explain why Frantzis (1992) and Frantzis and Grémare (1992) obtained absorption rate values consistently higher than ours for *Halopteris scoparia* and *Cystoseira compressa*. This fact could also explain the great variability in the absorption rate results for *C. compressa* in the experiments here presented.

The *Posidonia oceanica* sampled at Cap Martin presents a greater number of tannin cells because they react to *C. taxifolia* invasion by an increase in phenolic acid synthesis (Cuny *et al.*, 1994). Nevertheless, the *P. oceanica* from Cap Martin and from the reference area (Cap Ferrat) are consumed at the same rate in winter and spring food experiments.

Ecological impact of chemical defence

Analysis of the digestive content of sea urchins sampled *in situ* shows that even though *C. taxifolia* is not a preferred species for *P. lividus*, it is nevertheless consumed in relatively large quantities in winter. For specimens sampled in the *Posidonia oceanica* bed, digestive content analysis

confirmed the findings of experiments on the food preferences of sea urchins: the two species were consumed without any preference in winter and the consumption rate of *C. taxifolia* dropped in spring. On rocky substrate, *C. taxifolia* is the most abundant plant, and *P. lividus* is certainly obliged to consume it in winter. In spring, this alga is less appreciated. This change of diet may reflect either the increase in the defensive secondary metabolites content, with a consequent increase in the repellent effect of the alga, or the greater availability of other seasonal algae. These species develop at the beginning of spring and provide *P. lividus* with a new preferred food source. The first hypothesis would appear to be the more logical, since an increase in the sand content in the digestive tract is also observed (which reflects the absorption of encrusting algae) although this sea urchin is not considered to be a scraper (Verlaque and Nédélec, 1983). It may however adopt this behaviour in areas that are poor in macrophytes (Verlaque, 1987) or, as we have shown, in areas that have been invaded by *C. taxifolia*. After consuming the upright plant-bearing stratum, the sea urchins attack the encrusting stratum. In addition, Ruitton and Boudouresque (1994) have provided evidence of the migration of *P. lividus* towards the *Posidonia oceanica* beds in areas that have been intensively colonized by *C. taxifolia*. This displacement confirms the repellent effect of the secondary metabolites of the alga. Finally, the overgrazing of endemic algae and *P. oceanica* encourage the spread of *C. taxifolia*.

The physiological disorder and the low gonadosomatic ratio of specimens that consume *C. taxifolia*, as well as the antimetabolic effect of the caulerpenyne on the eggs of *P. lividus* (Lemée *et al.*, 1993), may have an impact on the recruitment and density of sea urchins in areas that have been invaded by *C. taxifolia*. This is in agreement with the 77 % drop in numbers of *P. lividus* that was recorded between February and December 1993 at Cap Martin (Ruitton and Boudouresque, 1994).

CONCLUSION

The feeding behaviour of *Paracentrotus lividus* in presence of *C. taxifolia* varies during the year. In summer, *C. taxifolia* is strongly avoided and the quantity of secondary metabolites in the alga is so high that sea urchins prefer to starve to death rather than consume it. In winter and spring, the secondary metabolite level drops. *C. taxifolia* then becomes an alga that is moderately appreciated by *P. lividus*. The sea urchins consume it but it is not absorbed. The result is a general weakening of the animals that may culminate in death. In sites that have been invaded for several years, *C. taxifolia* tends to replace all the native plants in the sublittoral zone. But this new trophic resource cannot be exploited by the herbivores because of the presence of toxic and repellent secondary metabolites, and consequently *C. taxifolia* cannot be considered as a replacement food. The decrease of sea urchin numbers in areas invaded by *C. taxifolia* has already been observed, with the predictable consequence of a major alteration of the food web in the sublittoral zone.

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